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Soluble receptor for advanced glycation end products (sRAGE) and asthma: Mendelian randomisation study

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To the Editor:

Of the many immune components involved in asthma pathobiology, recent studies have focused on the potential roles of receptor for advanced glycation end products (RAGE).[1] RAGE is a member of the immunoglobulin superfamily, which serves as a pattern-recognition receptor for many glycated and non-glycated ligands.[1] Membrane-bound RAGE (mRAGE), through binding with ligands, activates various downstream inflammatory pathways.[1] By contrast, the cleaved form—soluble RAGE (sRAGE)—functions as a decoy receptor through binding proinflammatory ligands destined for mRAGE, and hence offers anti-inflammatory effects.[1] These RAGE pathways are involved in a range of lung diseases (e.g., acute respiratory distress syndrome) and are a candidate for targeted therapies.[1]

Epidemiological studies have reported associations of lower circulating sRAGE levels with incident asthma[2] and higher disease severity.[1] For example, a multicenter cohort analysis of bronchiolitis has shown that infants with lower sRAGE levels have a higher risk of developing recurrent wheeze and asthma in childhood.[2] Genome-wide association studies (GWAS) have also found that polymorphisms in RAGE-coding gene (*AGER*) are associated with natural variations of lung function in Europeans.[1] Additionally, its missense variant (rs2070600) has been associated with lower levels of sRAGE, more airflow limitation, and higher asthma severity.[1,3] Notwithstanding these epidemiological *associations*, the *causal* role of sRAGE in asthma (and the role of potential RAGE-targeted therapies) remains unclear. To address the knowledge gap, we performed a Mendelian randomisation study to examine the causal relationship of sRAGE with asthma, its major phenotypes, and risk factors.

This is a two-sample Mendelian randomisation study using GWAS summary statistics from two large studies—the SCALLOP consortium[4] and UK Biobank.[5–8] Details of the Methods are available in the **Online Repository**. Briefly, Mendelian randomisation can provide unbiased causal estimates from observational data because the genetic polymorphisms associated with the exposure (sRAGE) are allocated randomly at conception and its causal inference is less susceptible to confounding and reverse causation.[9] Of 15 cohorts that comprise the SCALLOP consortium, 13 cohorts conducted a genome-wide protein quantitative trait loci (pQTL) analysis of plasma proteins in

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21,758 individuals of European ancestry.[4] The UK Biobank is a prospective cohort study that enrolled approximately 500,000 individuals, and longitudinally measured comprehensive phenotypic data and performed genome-wide genotyping. The current analysis restricted the sample to subjects of European ancestry (46,799 cases with asthma and 347,457 controls) to minimize population stratification bias. The current analysis has complied with all ethical regulations according to UK Biobank policy[5] and was approved by the institutional review board of Massachusetts General Hospital.

As the genetic instruments, we identified independent *cis*-acting variants strongly associated with plasma sRAGE levels ($P_{GWAS} < 1 \times 10^{-5}$, r^2<0.001, 500kb from *AGER*; **Table S1**) in the SCALLOP consortium.[4] To ensure no confounding for variants, we investigated Bonferroni-corrected associations with potential confounders—alcohol intake, physical activity and smoking status—in the UK Biobank. We also assured that variants affect outcomes solely through sRAGE by examining pleiotropy in Ensembl, GWAS catalog, and PhenoScanner. Separately, using the UK Biobank data, we computed the GWAS statistics for asthma (primary outcome) and six major asthma phenotypes (secondary outcomes): 1) childhood, 2) adult-onset, 3) allergic, 4) non-allergic, 5) obese, and 6) non-obese asthma, as previously described.[6–8] We weighted the magnitude of association of each variant with outcomes by that with sRAGE, and estimated the combined effect of sRAGE on each outcome by inverse-variance weighted method with a fixed-effects model. In the sensitivity analysis, we repeated the analysis by selecting a variant only from *AGER* coding-region ($P_{GWAS} < 1 \times 10^{-5}$, r^2<0.001). We also examined the role of potential asthma risk factors (atopic dermatitis, total IgE level, blood eosinophil and neutrophil counts, body mass index [BMI]) using 2-step Mendelian randomisation method.

The analysis identified two genetic instruments (rs204993, rs9267536) strongly associated with plasma sRAGE levels. These variants had an *F*-statistic of > 10, without significant association with the potential confounders (**Table S1**). Higher genetically-instrumented sRAGE levels were associated with a significantly lower risk of overall asthma (OR per one standard deviation [SD] increment in inverse-rank normalized sRAGE level, 0.78; 95%CI, 0.66-0.91; P=0.002; **Figures 1** and **S1**). In the stratified analysis, with limited statistical power, the association appeared consistent across

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the asthma phenotypes (**Figure 1**)—e.g., allergic (OR, 0.75; 95%CI, 0.60-0.93; P=0.008) and obese (OR, 0.69; 95%CI, 0.52-0.92; P=0.01) asthma. The sensitivity analysis using a variant in *AGER* coding-region (rs3134940) also demonstrated a consistent result—a negative relationship between sRAGE and overall asthma risk (OR, 0.74; 95%CI, 0.58-0.95; P=0.02; **Figure S2**). In the 2-step Mendelian randomisation analysis (**Figure 2** and **Table S2**), higher sRAGE levels were associated with a significantly lower total IgE level (SD difference, −0.60; 95%CI, −0.92 to −0.28; P<0.001) and BMI (SD difference, −0.073; 95%CI, −0.12 to −0.029; P=0.001). Both of these risk factors were also associated with a significantly higher risk of overall asthma. Similarly, a higher total IgE level was significantly associated with an increased risk of allergic asthma (OR, 1.27; 95%CI, 1.01-1.62; P=0.04), while it was not associated with non-allergic asthma (OR, 1.16; 95% CI 0.92-1.47; P=0.21; **Table S3** and **Figure S3**).

The current Mendelian randomisation analysis demonstrated that the genetically-instrumented sRAGE level is negatively associated with the asthma risk and that the causal association is consistent across a wide range of asthma phenotypes. Our results are concordant with previous epidemiological findings that lower circulating sRAGE levels are associated with greater risks of incident asthma[2] and higher disease severity, both in children and adults.[1] Prior research also has shown that higher levels of sRAGE ligands (e.g., danger-associated molecular patterns [DAMPs], including HMGB1) are associated with increased blood IgE level and asthma severity.[1] In the present analysis, the validity is buttressed by the use of Mendelian randomisation approach. This approach has the major advantage that it can address unmeasured confounding and reverse causation that occur in traditional observational studies.[9] Our analysis meets the three assumptions of the Mendelian randomisation design:[9] 1) the genetic instruments are strongly correlated with sRAGE (the relevance assumption); 2) they do not share common causes with the outcome (the independence assumption); and 3) they affect the outcome only though their effect on sRAGE (the exclusion restriction assumption). The present analysis, using large cohort data, builds on earlier reports, and extends them by robustly examining causal effects of sRAGE on asthma, its phenotypes, and risk factors.

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Our observations suggest that sRAGE plays a fundamental role in the pathobiology of asthma and its phenotypes. The exact mechanism(s) underlying the observed relationships is beyond the scope of current study and merits further investigations. Lower levels of sRAGE (as a decoy receptor) reduce binding of numerous RAGE ligands, thereby leading to upregulated signalling of mRAGE—which are most abundantly-expressed in type I alveolar cells—and downstream inflammatory (e.g., NFκB, JAK/STAT) pathways in the lungs.[1] Additionally, experimental models have shown that environmental allergen exposures induce alarmins in an mRAGE-dependent fashion, inducing interleukin (IL)-4/IL-13 production and T2 inflammation.[1,10] Furthermore, research also reported that administration of sRAGE diminishes LPS-induced T1 and T17 cytokines.[11] Taken together, there is a growing rationale for targeting the RAGE signalling pathways in asthma.

Several potential limitations should be noted. Firstly, misclassification of risk factors and asthma outcomes in the GWAS datasets is possible. Yet, it is unrelated to the measured sRAGE levels in the current two-sample design; thus, this independent nondifferential misclassification would have biased the inferences toward the null. Secondly, the effects of genetic instruments may be mitigated by developmental processes. Yet, such mechanisms would have decreased the genetic effects, also biasing the inferences toward the null. Finally, to minimize the population stratification bias, we restricted the study samples to individuals of European ancestry. Therefore, the inferences should be cautiously generalized to other racial/ethnic populations.

To conclude, the current Mendelian randomisation analysis demonstrated that a higher genetically-instrumented sRAGE level is causally associated with a lower risk of asthma. The observation was consistent across major asthma phenotypes. Our inferences should encourage further research into identifying patients with specific molecular phenotype(s) in which the RAGE pathways play major pathobiological roles.

Key words: soluble form of receptor for advanced glycation end products; asthma; phenotypes; *AGER*; Mendelian randomisation; GWAS; UK Biobank

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FIGURE LEGENDS

Figure 1. Mendelian randomisation estimates for the effect of soluble receptor for advanced glycation end products (sRAGE) on asthma and its phenotypes

By using the inverse variance weighted method, the combined causal effect of sRAGE on asthma (overall) and six asthma phenotypes was estimated. The odds ratios were estimated per one standard deviation increment in the inverse-rank normalized sRAGE level.

Abbreviation: CI, confidence interval; sRAGE, soluble receptor for advanced glycation end products

Figure 2. Two-step Mendelian randomisation estimates for the effect of soluble receptor for advanced glycation end products (sRAGE) on risk factors and their effect on asthma

The numbers indicate estimated odds ratios (ORs) or regression coefficients with 95% CIs from the Mendelian randomisation analyses. For the effect of the plasma sRAGE level on the binary risk factor (atopic dermatitis), an OR per one standard deviation (SD) increment in the inverse-rank normalized sRAGE level was estimated. For the effect of sRAGE on the continuous risk factors (plasma total IgE level, blood eosinophil and neutrophil count, and body mass index), coefficients in their SD scale per one SD increment in the inverse-rank normalized sRAGE level were estimated

For the effect of the binary risk factor on asthma risk, an OR was estimated by comparing the patients with the risk factor to those without. For the effect of the continuous risk factors, ORs per one SD increment in the corresponding risk factors were estimated. Arrows indicate causal directions in the 2-step Mendelian randomisation analyses. *, $P<0.05$; **, $P<0.001$.

Abbreviations: CI, confidence interval; IgE, immunoglobulin E; sRAGE, soluble receptor for advanced glycation end products

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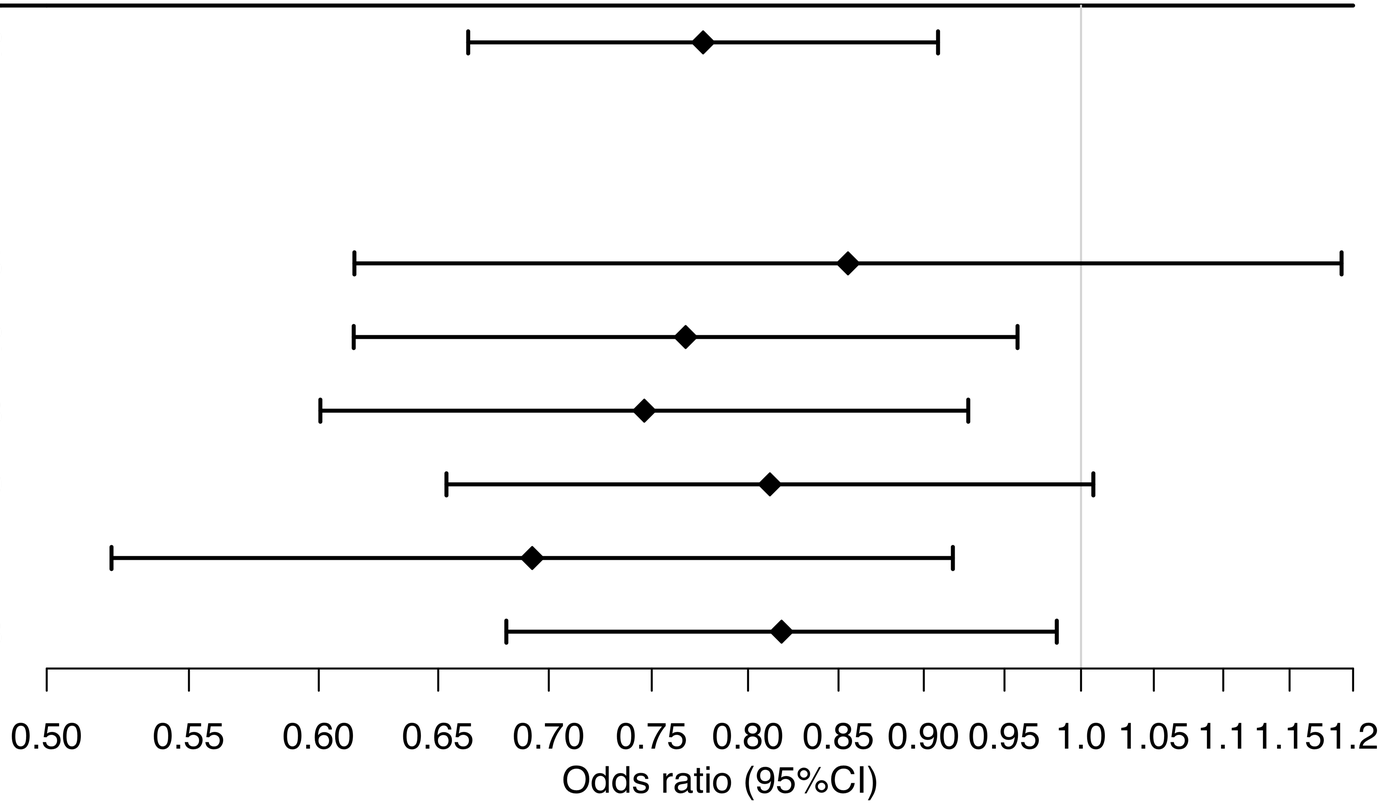
Asthma outcomes	Cases	Odds ratio (95% CI)	P-value
Asthma (overall)	46799	0.78 (0.66-0.91)	0.002
Asthma phenotypes			
Childhood asthma	9676	0.86 (0.61-1.19)	0.35
Adult-onset asthma	22294	0.77 (0.61-0.96)	0.02
Allergic asthma	23183	0.75 (0.60-0.93)	0.008
Non-allergic asthma	23616	0.81 (0.65-1.01)	0.059
Obese asthma	13550	0.69 (0.52-0.92)	0.01
Non-obese asthma	33095	0.82 (0.68-0.98)	0.033



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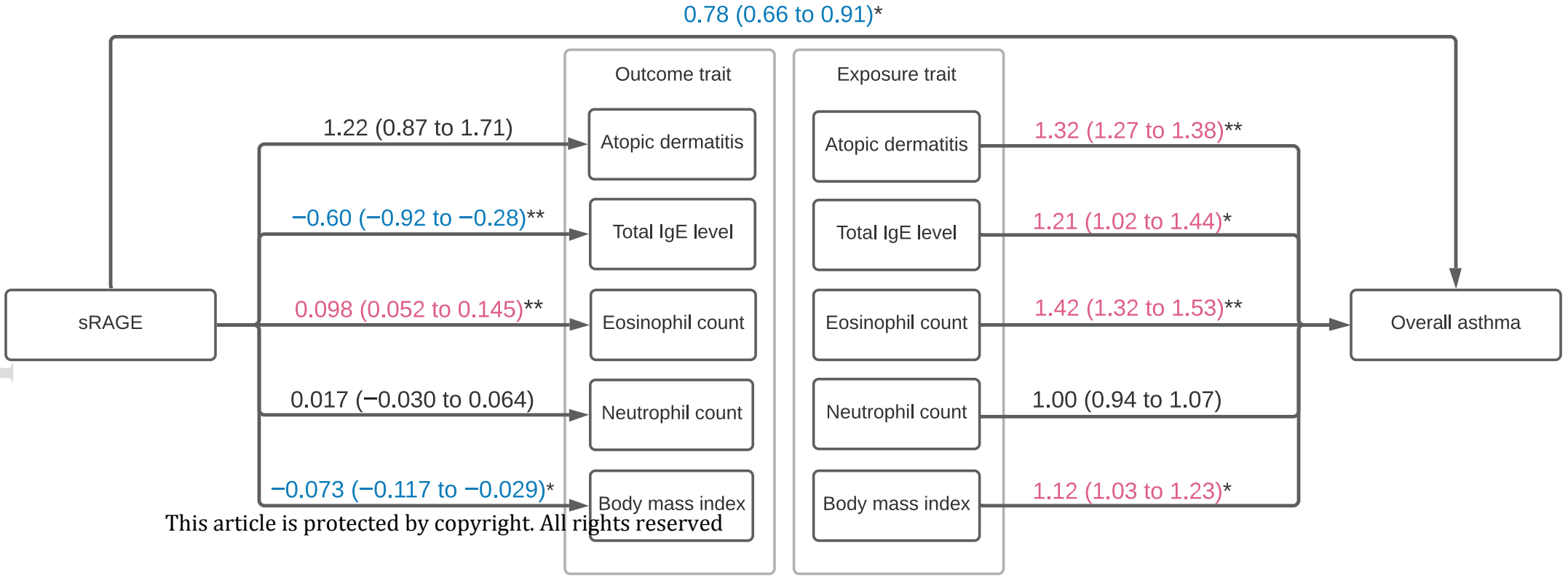

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ONLINE SUPPLEMENT

Soluble receptor for advanced glycation end products (sRAGE) and asthma: Mendelian randomisation study

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CONTENTS

Supplemental Methods……..……..……...…...……2

Supplemental References……..…….....……..6

Table S1. Characteristics of the selected variants strongly associated with soluble receptor for advanced glycation end products (sRAGE) level……..……10

Table S2. Results of two-step Mendelian randomisation analyses for the effect of soluble receptor for advanced glycation end products (sRAGE) on risk factors and their effect on asthma……..……..11

Table S3. Two-step Mendelian randomisation analyses for the effect of risk factors on allergic asthma and non-allergic asthma……..……12

Figure S1. Mendelian randomisation estimates for the effect of soluble receptor for advanced glycation end products (sRAGE) on overall asthma outcome……13

Figure S2. Mendelian randomisation estimates for the effect of soluble receptor for advanced glycation end products (sRAGE) on overall asthma outcome using a different for selecting a variant…….……14

Figure S3. Two-step Mendelian randomisation estimates for the effect of risk factors on allergic asthma and non-allergic asthma……15

SUPPLEMENTAL METHODS

Data Summary, Quality Control, and Imputation of Genotyping Data

The SCALLOP consortium

Detailed information of the SCALLOP consortium was described elsewhere.[1,2] In brief, of the 15 cohorts that comprise the SCALLOP consortium (n=30,931), 13 cohorts conducted a genome-wide protein quantitative trait loci (pQTL) analysis of 90 plasma proteins in 21,758 adults of European ancestry. Plasma protein levels were measured by the use of Olink platform.[2] The mean inter-plate coefficients of variation averaged across proteins was 16.6% (range 11%-26%). Protein levels with Normalized Protein eXpression (relative quantification) on the $\log_2$ scale were transformed using rank-based, inverse-normal transformation and/or standardized to unit variance. For the genome-wide association analyses, additive model regression adjusting for population-structure and study-specific parameters was used. After quality control (QC) for genotyping, 90 proteins in up to 21,758 participants remained for the genome-wide meta-analysis and 451 pQTLs for 85 proteins were finally identified. The summary statistics data are publicly available at https://zenodo.org/record/2615265.

The UK Biobank

The details of genotyping procedures in the UK Biobank were described on the UK Biobank website (http://biobank.ctsu.ox.ac.uk/) and in previous research.[3,4] In summary, approximately 500,000 participants aged 40-69 years were enrolled in 2006-2010. The study has collected extensive phenotypic (e.g., demographics, medical history, anthropomorphic measures) and genotypic data. After QC, the current UK Biobank data contain approximately 800,000 markers that passed SNP QC in at least one batch. The genotype imputation was performed using the UK10k+1000 Genome Phase 3 panels; genotype imputation procedure provided over 90 million variants.[5,6] The detailed genotype imputation information can be found in the UK Biobank website (https://www.ukbiobank.ac.uk/wp-content/uploads/2018/03/UKB-Genotyping-and-Imputation-Data-Release-FAQ-v3-2.pdf). The current study restricted the sample to the subjects of European ancestry to minimize population stratification bias. All participants from this study provided UK Biobank-

acquired informed consent and provided data according to the UK Biobank protocol. The current study has complied with all ethical regulations according to UK Biobank policy. This research was approved and conducted using the UK Biobank under application numbers 16549 and 45052 and approved by the institutional review board of Massachusetts General Hospital.

Ascertainment of Asthma and its Phenotypes

First, according to previous research,[4,7,8] we identified all UK Biobank participants with asthma by using the data fields 6152 (self-reported physician-diagnosis of asthma), 20002 (non-cancer illness disease code), 41202 (*International Classification of Diseases, Tenth Revision, Clinical Modification* [*ICD-10-CM*] primary diagnosis in the hospital), and 41204 (*ICD-10-CM* secondary diagnosis in the hospital), with the use of the *ICD-10-CM* diagnosis codes of J45. We excluded individuals with chronic obstructive pulmonary disease, emphysema or chronic bronchitis (self-reports or International Statistical Classification of Diseases and Related Health Problems, 10th Revision codes) from cases with asthma and controls. In the current study, the primary outcome was (overall) asthma (n=46,799) and the secondary outcomes were six major asthma phenotypes: 1) childhood asthma (defined as age of onset ≤12 years; n=9,676)[8], 2) adult-onset asthma (defined as age of onset ≥26 years; n=22,294)[8], 3) allergic asthma (defined as asthma with an allergic disease—eczema, food allergy, and/or allergy rhinitis [identified by data fields 6152, 20002, 41202, 41204]; n=23,183)[4,7], 4) non-allergic asthma (defined as asthma without any allergic disease; n=23,616), 5) obese asthma (defined as BMI of ≥30 kg/m^2; n=13,550), and 6) non-obese asthma (defined as BMI of <30 kg/m^2; n=33,095). Young adult-onset asthma (age of onset 12.1–25 years) was not included in the genetic analysis due to its higher heterogeneity.[8–10] We also identified shared controls (n=347,457) with high-quality genotyping and complete phenotype/covariate data for GWAS association analysis. To account for relatedness, the association test between imputed variants and asthma traits in UK Biobank data and was carried out using BOLT-linear mixed model (LMM). The output of BOLT-LMM linear regression was transformed into log odds ratio (log odds ratio) for HBP binary phenotype.[8,11]

GWAS Summary Statistics for Two-step Mendelian Randomisation Study

Atopic dermatitis

We used GWAS summary statistics from the EArly Genetics & Lifecourse Epidemiology (EAGLE) eczema consortium.[12] Detailed information of the EAGLE consortium was described elsewhere.[12] Briefly, a meta-analysis of >15 million genetic variants in 21,399 cases and 95,464 controls from populations of European, African, Japanese and Latino ancestry were conducted, followed by replication in 32,059 cases and 228,628 controls from 18 studies. We used GWAS summary statistics of the European ancestry to minimize population stratification bias—18,900 cases with atopic dermatitis and 84,166 controls. The summary statistics data are publicly available from https://grasp.nhlbi.nih.gov/FullResults.aspx.

Plasma total IgE

We used GWAS summary statistics from the INTERVAL study.[13] Detailed information of the INTERVAL study was described elsewhere.[13] Briefly, approximately 50,000 adult blood donors were recruited at 25 centres of England's National Health Service Blood and Transplant from 2012 to 2014. For proteomic profiling, randomly selected two non-overlapping subcohorts of 2,731 and 831 participants of European ancestry were enrolled. The levels of 2,994 plasma proteins, including total IgE, were measured by the use of SomaLogic assays, a validated high-throughput and aptamer-based method.[14,15] The summary statistics data are publicly available at http://www.phpc.cam.ac.uk/ceu/proteins/.

Blood eosinophil and neutrophil counts

We used GWAS summary statistics of the samples of 173,480 European ancestry adults from three large-scale studies—INTERVAL (n=40,521),[13] UK BiLEVE (n=45,694)[16] and UK Biobank (n=87,265).[3] Detailed information of the study was described elsewhere.[17] Briefly, 29.5 million genetic variants for association with 36 red cell, white cell, and platelet properties were tested. The summary statistics data are publicly available at https://gwas.mrcieu.ac.uk/.

Body mass index

We used GWAS summary statistics from the GIANT consortium.[18] Detailed information of the GIANT consortium was described elsewhere.[18] Briefly, the genetic testing for BMI were conducted up to 339,224 individuals from 125 studies, 82 with GWAS results (n=236,231) and 43 with results from Metabochip (n=103,047). We use GWAS summary statistics of the European ancestry to minimize population stratification bias (n=322,154). The summary statistics data are publicly available at https://gwas.mrcieu.ac.uk/.

Statistical Analysis

As genetic instruments, we first identified independent *cis*-acting variants strongly associated with plasma sRAGE levels ($P_{GWAS} < 1 \times 10^{-5}$, $r^2 < 0.001$, 500kb from *AGER*: **Table S1**) in the SCALLOP consortium.[1] To ensure no confounding for variants (the independence condition), we investigated their Bonferroni-corrected associations with potential confounders—alcohol intake, physical activity, and smoking status—in the UK Biobank.[3] We also assured that variants affect outcomes solely through sRAGE (the exclusion restriction condition) by examining the variants in three curated genetic cross-reference systems—Ensembl,[19] GWAS catalog,[20] and PhenoScanner.[21] To investigate the association of variants with outcomes, we used GWAS summary statistics of the UK Biobank. We weighted the magnitude of association of each variant with outcomes by that with sRAGE, and estimated the combined causal effect of sRAGE on each outcome by inverse-variance weighted method with a fixed-effects model,[22–24] using R *MendelianRandomization* package.[25]

In the sensitivity analysis, to examine the consistency, we also repeated the analysis by selecting a variant only from the *AGER* coding-region ($P_{GWAS} < 1 \times 10^{-5}$, $r^2 < 0.001$). We also examined the role of potential asthma risk factors (atopic dermatitis, total IgE level, blood eosinophil and neutrophil counts, BMI) using a 2-step Mendelian randomisation method. As genetic instruments for the risk factors, we identified independent variants strongly associated with each of the risk factors from the corresponding summary statistics data—1) $P_{GWAS} < 5 \times 10^{-8}$ and $r^2 < 0.001$, and 2) F-statistic> 10 for total IgE level. To estimate the causal effect of the risk factors on the asthma outcome with more than five variants, we used MR Pleiotropy RESidual Sum and

Outlier (MR-PRESSO) test.[21] MR-PRESSO test[21] detects violation of the restriction exclusion criterion assumption and provides corrected estimates by removing variants which exhibit pleiotropy. We conducted two-step Mendelian randomisation using R *TwoSampleMR* package[26] and MR-PRESSO using *MRPRESSO* package.[21] We analysed the data using R version 3.5.1 (R foundation for Statistical Computing, Vienna, Austria).

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Table S1. Characteristics of the selected variants strongly associated with soluble receptor for advanced glycation end products (sRAGE) level

Variant	Consequence	Effect allele	Association with sRAGE		*F*-statistic	Association with three potential confounders in the UK Biobank (Bonferroni-corrected P-value)		
			Coefficient	$\log_{10}$(P-value)		Alcohol intake	Physical activity	Smoking status
rs204993	Non-coding	A	0.15	-34.9	156.6	0.99	0.99	0.99
rs9267536	Non-coding	A	0.14	-7.02	28.5	0.08	0.99	0.44

Abbreviation: sRAGE, soluble form of advanced glycation end products

Table S2. Results of two-step Mendelian randomisation analyses for the effect of soluble receptor for advanced glycation end products (sRAGE) on risk factors and their effect on asthma

Exposure	Risk factor	Method	Number of variants	Coefficient	Standard error	P-value	P_{GWAS} threshold	LD threshold	*F*-statisitc threshold
sRAGE	Atopic dermatitis	Inverse variance weighted (fixed effects)	2	0.20	0.17	0.24	1×10^{-5}	0.001	
sRAGE	Total IgE level	Inverse variance weighted (fixed effects)	2	-0.60	0.16	<0.001	1×10^{-5}	0.001	
sRAGE	Eosinophil count	Inverse variance weighted (fixed effects)	2	0.098	0.024	<0.001	1×10^{-5}	0.001	
sRAGE	Neutrophil count	Inverse variance weighted (fixed effects)	2	0.017	0.024	0.48	1×10^{-5}	0.001	
sRAGE	Body mass index	Inverse variance weighted (fixed effects)	2	-0.073	0.022	0.001	1×10^{-5}	0.001	
Risk factor	**Outcome**	**Method**	**Number of variants**	**Coefficient**	**Standard error**	**P-value**	**P_{GWAS} threshold**	**LD threshold**	***F*-statisitc threshold**
Atopic dermatitis	Asthma (overall)	MR-PRESSO	13	0.28	0.022	<0.001	5×10^{-8}	0.001	
Total IgE level	Asthma (overall)	Wald ratio	1	0.19	0.088	0.03		0.001	10
Eosinophil count	Asthma (overall)	MR-PRESSO	171	0.35	0.036	<0.001	5×10^{-8}	0.001	
Neutrophil count	Asthma (overall)	MR-PRESSO	144	0.0019	0.033	0.95	5×10^{-8}	0.001	
Body mass index	Asthma (overall)	MR-PRESSO	59	0.12	0.045	0.01	5×10^{-8}	0.001	

Abbreviations: GWAS, genome-wide association studies; IgE, immunoglobulin E; LD, linkage disequilibrium; MR-PRESSO, MR Pleiotropy RESidual Sum and Outlier test; sRAGE, soluble receptor for advanced glycation end products

Table S3. Two-step Mendelian randomisation analyses for the effect of risk factors on allergic asthma and non-allergic asthma

Risk factor	Outcome	Method	Number of variants	Coefficient	Standard error	P-value	P_{GWAS} threshold	LD threshold	*F*-statisitc threshold
Atopic dermatitis	Allergic asthma	MR-PRESSO	13	0.46	0.043	<0.001	5×10^{-8}	0.001	
Total IgE level	Allergic asthma	Wald ratio	1	0.24	0.12	0.04		0.001	10
Eosinophil count	Allergic asthma	MR-PRESSO	171	0.45	0.047	<0.001	5×10^{-8}	0.001	
Neutrophil count	Allergic asthma	MR-PRESSO	144	-0.012	0.042	0.77	5×10^{-8}	0.001	
Body mass index	Allergic asthma	MR-PRESSO	59	0.10	0.058	0.085	5×10^{-8}	0.001	
Risk factor	**Outcome**	**Method**	**Number of variants**	**Coefficient**	**Standard error**	**P-value**	**P_{GWAS} threshold**	**LD threshold**	***F*-statisitc threshold**
Atopic dermatitis	Non-allergic asthma	MR-PRESSO	13	0.10	0.025	0.002	5×10^{-8}	0.001	
Total IgE level	Non-allergic asthma	Wald ratio	1	0.15	0.12	0.21		0.001	10
Eosinophil count	Non-allergic asthma	MR-PRESSO	171	0.33	0.040	<0.001	5×10^{-8}	0.001	
Neutrophil count	Non-allergic asthma	MR-PRESSO	144	0.0094	0.036	0.80	5×10^{-8}	0.001	
Body mass index	Non-allergic asthma	MR-PRESSO	59	0.10	0.065	0.12	5×10^{-8}	0.001	

Abbreviations: GWAS, genome-wide association studies; IgE, immunoglobulin E; LD, linkage disequilibrium; MR-PRESSO, MR Pleiotropy RESidual Sum and Outlier test

Figure S1. Mendelian randomisation estimates for the effect of soluble receptor for advanced glycation end products (sRAGE) on overall asthma outcome

The size of the squares is proportional to the weight of the Mendelian randomisation estimate for each variant, with the horizontal lines indicating their 95% confidence intervals. The centre of the diamond represents the combined Mendelian randomisation point estimate with the lateral tips indicating its 95% confidence interval, estimated by the inverse variance weighted method. The odds ratios were estimated per one standard deviation increment in the inverse-rank normalized sRAGE level.

Abbreviations: CI, confidence interval; IVW, inverse variance weighted; sRAGE, soluble receptor for advanced glycation end products

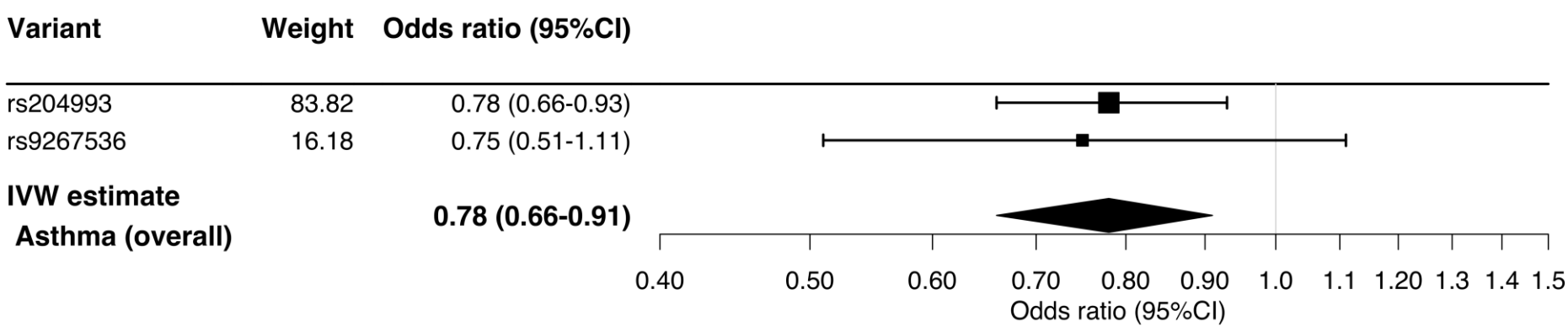


Figure S2. Mendelian randomisation estimates for the effect of soluble receptor for advanced glycation end products (sRAGE) on overall asthma outcome using a different for selecting a variant

This sensitivity analysis used one variant selected from the *AGER* coding-region with the use of P_{GWAS} ($P_{GWAS}<1\times10^{-5}$) and linkage disequilibrium ($r^2<0.001$) thresholds. The size of the squares is proportional to the weight of the Mendelian randomisation estimate for the variant, with the horizontal lines indicating their 95% confidence intervals. The odds ratios were estimated per one standard deviation increment in the inverse-rank normalized sRAGE level.

Abbreviations: CI, confidence interval; IVW, inverse variance weighted; sRAGE, soluble receptor for advanced glycation end products

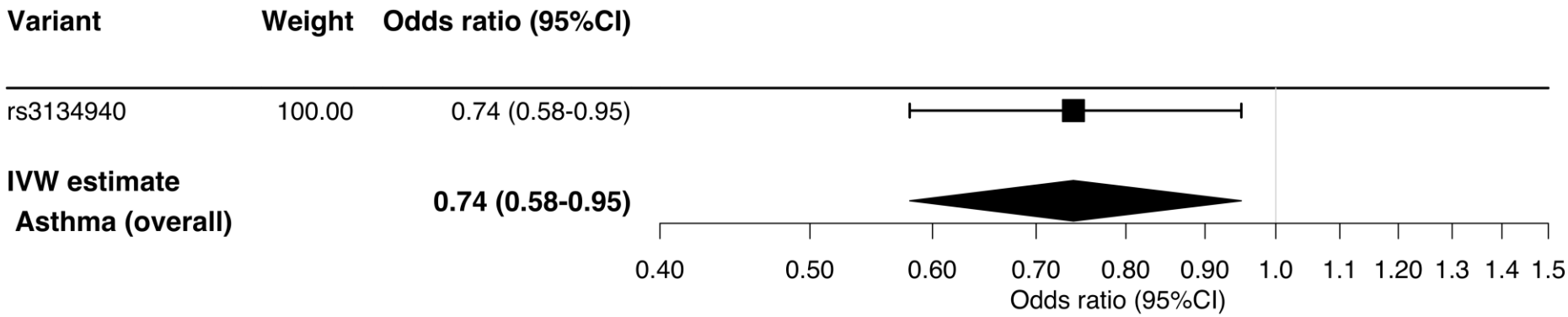
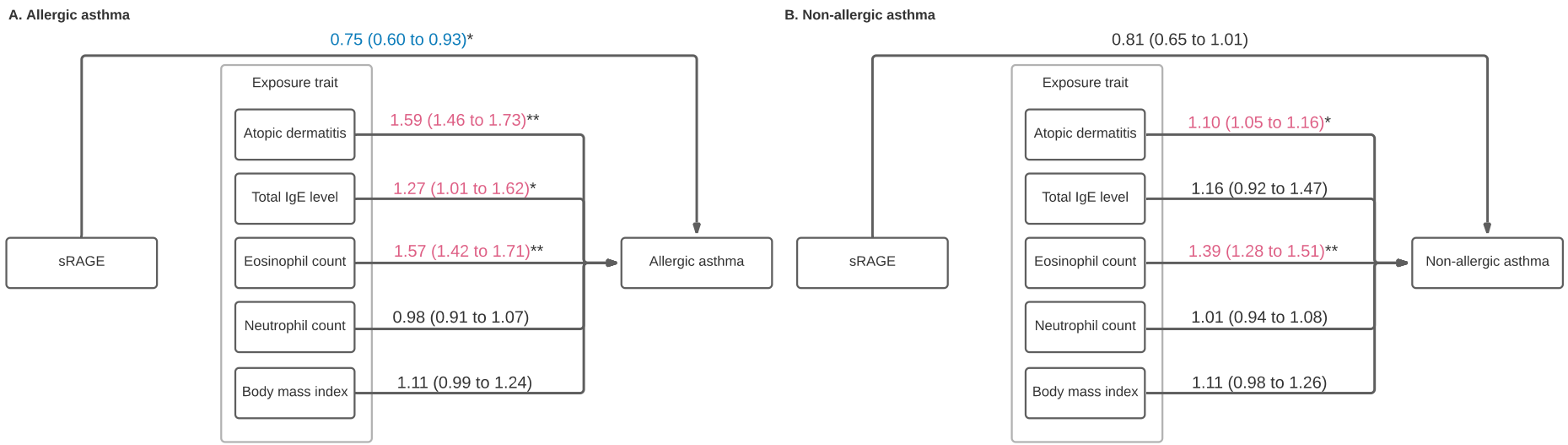


Figure S3. Two-step Mendelian randomisation estimates for the effect of risk factors on allergic asthma and non-allergic asthma

A. Allergic asthma outcome

B. Non-allergic asthma outcome