



Smoking behavior might affect allergic rhinitis and vasomotor rhinitis differently: A mendelian randomization appraisal

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ABSTRACT

Background: Allergic rhinitis and vasomotor rhinitis are harassing numerous patients and their risk factors have not been well investigated. Here, we try to identify their risk factors and distinguish these 2 diseases.

Methods: A two-sample Mendelian randomization (MR) study was implemented to discover the risk factors of allergic and vasomotor rhinitis. Based on previous studies, we selected 15 potential risk factors and the genome-wide summary statistics were extracted from the non-FinnGen consortium. The genome-wide summary statistics of rhinitis were obtained from the FinnGen consortium. Both univariable MR and multivariable MR analyses were performed to identify the causal risk factors. The Cochrane's Q value was calculated to appraise the heterogeneity. MR-Egger intercept and MR-RPESSO were utilized to appraise the pleiotropy.

Results: In the univariable model, the number of cigarettes per day can decrease the risk of allergic rhinitis (IVW OR = 0.29[0.18, 0.47], p-value = 2.70×10^{-7}) while increasing the risk of vasomotor rhinitis (IVW OR = 1.30[1.04, 1.62], p-value = 0.022). Besides, no other risk factors could affect the risk of either allergic or vasomotor rhinitis. After adjusting for age of smoking initiation and alcohol intake, the cigarettes per day could still decrease the risk of allergic rhinitis (IVW OR = 4.66×10^{-3} [1.99×10^{-4} , 0.11], p-value = 0.003) while not affecting the risk of vasomotor rhinitis (IVW OR = 0.92[0.44, 1.96], p-value = 0.834).

Conclusion: Smoking can affect the risk of allergic and vasomotor rhinitis differently where it decreases the risk of allergic rhinitis and increases the risk of vasomotor rhinitis.

Keywords: Allergic rhinitis, Vasomotor rhinitis, Mendelian randomization, Risk factors

INTRODUCTION

The incidence of rhinitis has been reported to harass 14% of adults and 13% of children in the United States.¹ Furthermore, rhinitis can coexist

with asthma and usually is a strong risk factor for new-onset asthma, exerting a heavy burden on society.² Thus, it is necessary to identify the risk factors for rhinitis and control them. It has been

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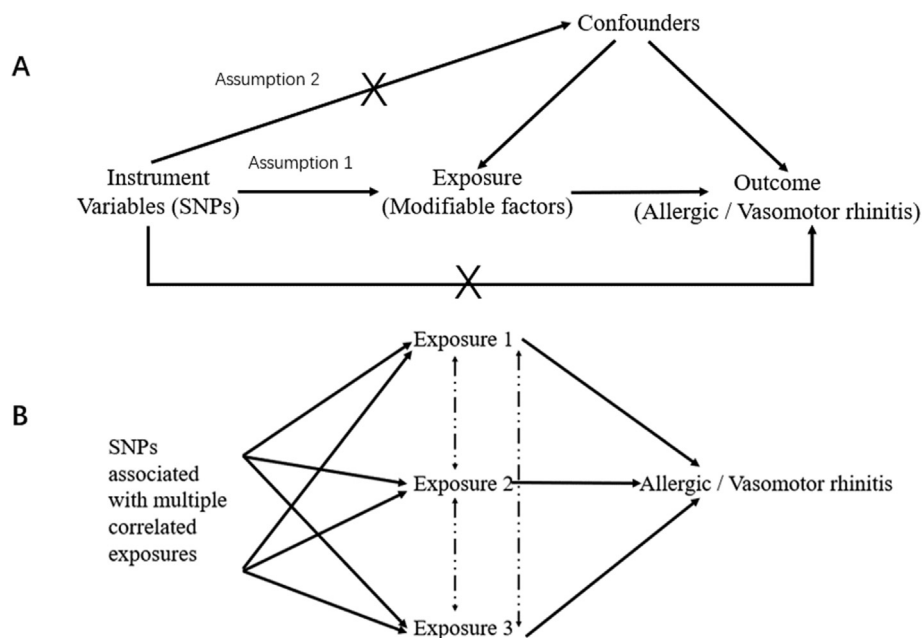


Fig. 1 The basic principles of two-sample Mendelian randomization and multivariable Mendelian randomization

reported that genetic factors might play an important role in the pathogenesis of rhinitis, together with those environmental factors.³ For example, single nucleotide polymorphism (SNP) located in the interleukin, chemokine, and receptor coding genes might affect the pathogenesis of rhinitis and so do the epigenetic factors.³ A recent cohort study, consisting of 11 506 adults in Northern Europe, found that smoking and living in new buildings could increase the risk of rhinitis.⁴ However, Skaaby et al reported that smoking might lower the risk of hay fever and allergic sensitization.⁵ Besides, obesity was reported to be associated with allergic rhinitis where the impact of obesity on allergic rhinitis is not clear.⁶ Considering the association between environmental risk factors and rhinitis tends to be biased by the unknown confounders such as socioeconomic status, it is necessary to overcome the unknown confounders and reverse causation when attempting to identify causal risk factors for rhinitis.

Mendelian randomization (MR) is a method for causal inference using genetic variants as the instrumental variables and has achieved great success in many fields.⁷ Since the gametes are randomly allocated at conception, the SNP's different effect on the exposure can simulate the

different levels of the exposure and they can divide the population into different groups the same as the randomized controlled trial (RCT).⁸ Nowadays, MR studies based on the two-sample setting are developing rapidly thanks to the accumulation of summary statistics derived from publicly available genome-wide association studies (GWAS). Meanwhile, a few MR studies focused on allergic rhinitis and hay fever have been reported and they added evidence to observational studies. Feng et al supported that serum 25-hydroxyvitamin D could not affect the risk of allergic rhinitis, allergic sensitization, and non-allergic rhinitis, and Skaaby et al also found the null association between vitamin B12 and allergic rhinitis, together with folate concentrations.^{9,10} Meanwhile, Tea et al and Frederikke et al ruled out the causal relationship between alcohol intake and allergic rhinitis.¹¹

However, recent MR studies are only focused on a small number of risk factors and the main outcome is allergic rhinitis and no study has treated vasomotor rhinitis as the outcome yet. It is known that vasomotor rhinitis is a kind of rhinitis with high heterogeneity and many aspects of it are still under investigation.¹² Thus, we hope to perform a comprehensive MR study to identify

modifiable risk factors for rhinitis, including both allergic rhinitis and vasomotor rhinitis.

METHODS

Mendelian randomization design

This study consisted of 2 parts including univariable and multivariable MR analyses. Initially, the potential causal risk factors of rhinitis were identified and we further judged whether these factors independently affected the risk of rhinitis. This study is reported mainly based on "STrengthening the Reporting of OBservational studies in Epidemiology" (STROBE).¹³

Univariable MR analysis

The Mendelian randomization analysis is performed based on the following 3 principles:¹ The genetic variant is closely associated with the exposure;² The genetic variant is not associated with potential confounders;³ The genetic variant is not associated with the outcome except via the way of the exposure⁷ (Fig. 1A). Usually, the first assumption can be well satisfied using the GWAS summary statistics and the last 2 assumptions sometimes cannot be well tested. As the genetic variants (SNP) are closely associated with the exposure, they would affect the exposure firstly. Also, the only pathway is "genetic variants → exposure → outcome" if we observed the significant results since the genetic variants are not directly associated with potential confounders and outcomes. Thus, the observed associations from MR should be causal.

Initially, we extracted SNPs associated with the exposure from the GWAS summary statistics if reaching the genome-wide significance (p -value $< 5 \times 10^{-8}$). Then, we removed the SNP with its minor allele frequency less than 0.01. The SNPs in close linkage disequilibrium (LD) with the most significant one in the given region would be removed as well. The palindromic SNPs (A/T or C/G) were also excluded. Before MR analysis, we also appraise the weak instrument bias using the F statistics ($F = \beta^2 / \text{se}^2$) for each SNP and calculate a general F statistic for all SNPs.¹⁴ The SNP with less statistical power would be excluded. To avoid the bias caused by the violation of assumption 2, we conducted a phenome-wide association study to find the SNPs associated

with potential confounders and removed these SNPs.¹⁵ A Bonferroni correction p -value was adopted to define SNP not associated with the outcome (outcome p -value $> 0.05/\text{number of SNPs}$).

Multivariable Mendelian randomization analysis

The multivariable MR (MVMR) analysis would be performed only if the exposure-outcome association is significant in the univariable MR analysis (Fig. 1B). We will include other exposures closely associated with the significant one in the multivariable regression model. Before MVMR analysis, we would also calculate the MVMR F statistics for each exposure, and the exposure would be removed if its F statistic is less than 10.

DATA SOURCE DESCRIPTION

Genetic instrumental variables for modifiable risk factors

We extracted the instrumental variables from GWAS results and removed SNPs in high linkage disequilibrium ($r^2 < 0.01$) to get independent instrumental variables using 1000 genome Phase 3 European samples as the reference panel (<https://www.internationalgenome.org/>). All instrumental variables reached the genome-wide significance (GWAS p -value $< 5 \times 10^{-8}$) and each minor allele frequency was more than 0.01. Genetic variants associated with the number of cigarettes smoked per day (CPD, a continuous variable) were all obtained from the recent GWAS result derived from GWAS & Sequencing Consortium of Alcohol and Nicotine use (GSCAN).¹⁶ Meanwhile, the IVs for the age of smoking initiation and alcoholic drinks per week were also obtained from the GSCAN. We obtained genetic variants associated with BMI, waist circumference, hip circumference, and waist-to-hip ratio (WHR) from the Genetic Investigation of ANthropometric Traits (GIANT) consortium.^{17,18} The instrumental variables for lipidemic traits, including HDL, LDL, total cholesterol, and triglycerides, were extracted from the Global Lipids Genetics Consortium (GLGC).¹⁹ We obtained instrumental variables for HbA1c²⁰ and fasting glucose²¹ from the Meta-Analyses of Glucose and Insulin-related traits Consortium (MAGIC). The genetic variants associated with systolic blood pressure (SBP) and

diastolic blood pressure (DBP) were extracted from a GWAS meta-analysis in over 1 million individuals from UK Biobank (UKB) and International Consortium for Blood Pressure (ICBP).²²

Genetic instrumental variables for allergic and vasomotor rhinitis

The GWAS results of allergic and vasomotor rhinitis were obtained from the FinnGen Consortium (<https://r4.finngen.fi/>). The definitions of these 2 diseases are based on the international code of diseases (ICD) where allergic rhinitis included participants diagnosed by ICD-10 J301, J302, J303, J304 in ICD-10 and 477 in ICD-9. As for vasomotor rhinitis, it includes participants diagnosed by J300 in ICD-10. The samples were genotyped with Illumina (Illumina Inc., San Diego, CA, USA) and Affymetrix arrays (Thermo Fisher Scientific, Santa Clara, CA, USA). The individuals with ambiguous gender, high genotype

missingness (>5%), excess heterozygosity ($\pm 4SD$), and non-Finnish ancestry were excluded. Meanwhile, variants with high missingness (>2%), low HWE *P*-value ($<1e-6$), and low minor allele count (<3) were excluded. The Sequencing Initiative Suomi (SiSu) was used as the reference panel for genotype imputation (<http://sisuproject.fi/>). The genotype imputation was conducted using the software Beagle 4.1.²³ The GWAS was performed under the mixed model logistic regression provided by the SAIGE software, adjusting for sex, age, 10 PCs, genotyping batch, and genetic relatedness. The allergic rhinitis samples consisted of 4300 cases and 171,926 controls and there were 744 cases and 174,935 controls in the samples of vasomotor rhinitis.

Each GWAS study was performed under the license of its corresponding Independent Ethics Committee and related information can be founded in the original GWAS publications. All GWAS

Exposure	Consortium	NSNP	Sample size	R2(%)	F
Cigarettes per day	GSCAN	25	842,717	4.32	1521.92
Age of smoking initiation	GSCAN	2	842,717	0.01	42.14
Alcoholic drinks per day	GSCAN	13	842,717	0.03	19.45
BMI	GIANT	42	344,369	2.65	223.17
Waist circumference	GIANT	23	344,369	1.78	271.32
Hip circumference	GIANT	26	344,369	1.75	235.90
WHR	GIANT	9	344,369	1.64	637.96
HDL	GLGC	57	188,578	7.54	269.71
LDL	GLGC	39	188,578	5.31	271.10
TC	GLGC	42	188,578	9.60	476.70
TG	GLGC	31	188,578	6.21	402.71
HbA1c	MAGIC	20	159,940	1.23	99.57
Fasting glucose	MAGIC	25	151,188	1.34	82.12
SBP	UKB + ICBP	313	757,601	4.64	117.72
DBP	UKB + ICBP	349	757,601	4.73	107.73

Table 1. A summary of GWAS summary statistics for different risk factors. Notes: (1) Exposure: the risk factors; BMI = body mass index; WHR = waist-to-hip ratio; HDL = high-density lipoprotein; LDL = low-density lipoprotein; TC = total cholesterol; TG = triglycerides; HbA1c = glycated hemoglobin; SBP = systolic blood pressure; DBP = diastolic blood pressure. (2) Consortium: The name of GWAS consortium. (3) NSNP: the number of SNPs in the MR analysis. (4) Sample size: the sample size of each GWAS. (5) R2(%): the genetic explanation of each risk factor. (5) F: the general F statistic for each risk factor

summary statistics are freely downloadable and can be used without restriction.

Statistical methods

The inverse-variance weighted (IVW) method was adopted to combine each IV's effect size, and MR-Egger and median-based methods were used as the complement to the IVW. We calculated Cochrane's Q value to assess the heterogeneity. As for horizontal pleiotropy, the MR-Egger intercept and MR-PRESSO methods were utilized.^{24,25} If the outliers were detected, they would be removed and we would reassess the MR causal estimation. If heterogeneity still exists, the median-based estimation will be adopted as the main effect size. If there is horizontal pleiotropy, the MR-Egger estimation will be the main effect size.

In the univariable MR analysis, we tested the causal relationship between 15 exposures and 2

kinds of rhinitis (allergic rhinitis and vasomotor rhinitis). The exposure-outcome association is defined to be significant if its Bonferroni corrected p-value is less than 0.05. The Bonferroni corrected p-value is calculated using the following formula: corrected p-value = $p * (1/n)$ where p is the original MR p-value and n is the number of statistical tests ($n = 14$). The univariable MR analysis was performed using the R packages "TwoSampleMR" and "MendelianRandomization". The MR-PRESSO was conducted using the R package "MRPRESSO".²⁵ The IVW model was the main method in MVMR analysis and the MR-Egger method was the complementary method. The MVMR was performed using the R packages "MendelianRandomization" and "MVMR".

The mRnd was used to calculate the statistical power for Mendelian randomization (<https://>

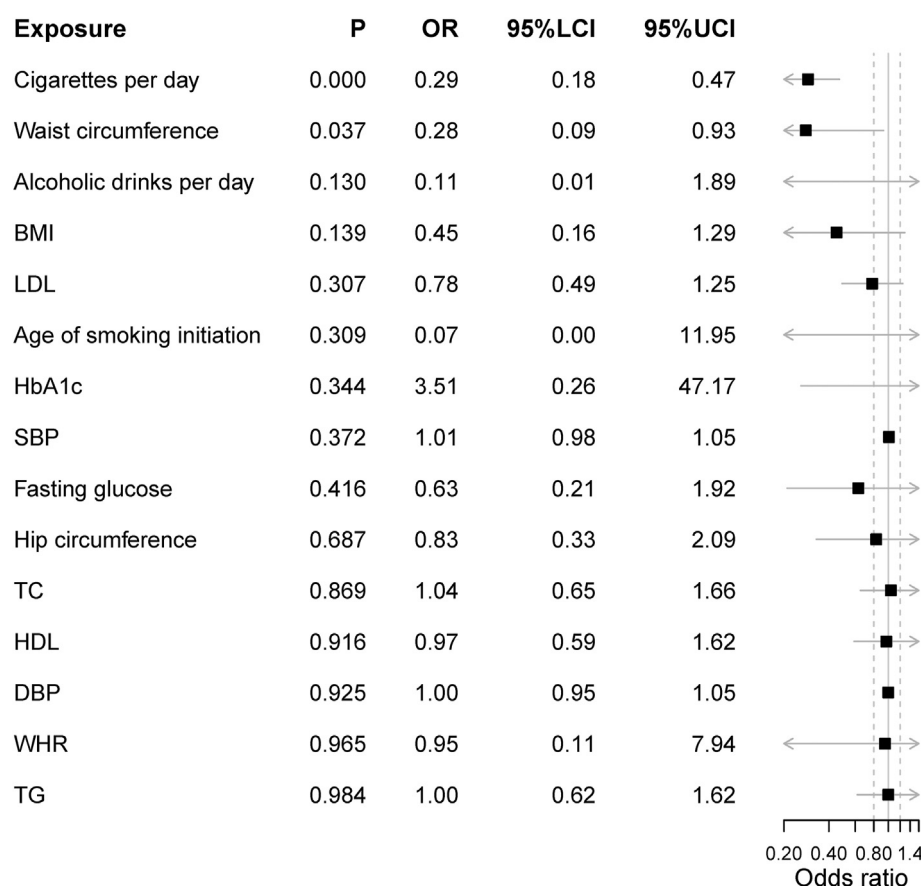


Fig. 2 The forest plot of different risk factors' effect on allergic rhinitis. Notes: (1) Exposure: the risk factors; BMI = body mass index; WHR = waist-to-hip ratio; HDL = high-density lipoprotein; LDL = low-density lipoprotein; TC = total cholesterol; TG = triglycerides; HbA1c = glycated hemoglobin; SBP = systolic blood pressure; DBP = diastolic blood pressure. (2) P: the p-value of each MR result. (3) OR: the odds ratio. (4) 95%LCI: the lower limit of 95% confidence interval. (5) 95%UCI: the upper limit of 95% confidence interval

cnsgenomics.shinyapps.io/mRnd/). All statistical analyses and data visualization were performed in R software 3.4.0 (www.r-project.org).

RESULTS

Valid IVs

Generally, we included 15 risk factors as the exposures. The number of genetic variants varies between 2 and 349, explaining 0.01%–6.21% of the exposure (Table 1). The genetic variants for each exposure were valid IVs.

In the univariable MR analysis, the F statistics for each IV are much greater than the empirical threshold 10 and the general F statistics for each exposure were greater than 10 as well (Supplementary Table 1–15).

In the multivariable MR analysis, the F statistics of CPD is 50.52 and the p-value of Q is 0.815, suggesting sufficient statistical power and validity.

ALLERGIC RHINITIS

Smoking and drinking

Of these 15 risk factors, only CPD was causally associated with allergic rhinitis after Bonferroni correction and it might be a protective factor (IVW OR = 0.29[0.18, 0.47], p-value = 2.70×10^{-7} , Bonferroni p-value = 4.05×10^{-6}) (Fig. 2). Therein, the risk of allergic rhinitis would reduce 71% if increasing per SD of CPD. However, the alcohol-intake seemed not to affect the allergic rhinitis (IVW OR = 0.11[0.01, 1.89], p-value = 0.130). After adjusting for the age of smoking initiation and alcohol intake, the CPD could still decrease the risk of allergic rhinitis (IVW OR = 4.66×10^{-3} [1.99×10^{-4} , 0.11], p-value = 0.003). The p-value of Cochrane's Q and MR-Egger intercept is greater than 0.05, suggesting no significant heterogeneity or pleiotropy.

Anthropometric traits

Besides, a higher waist circumference could also lower the risk of allergic rhinitis suggestively (IVW OR = 0.28[0.09, 0.93], p-value = 0.037, Bonferroni p-value = 0.560). However, the waist-to-hip ratio (WHR) might not affect it (IVW OR = 0.95[0.11,

7.94], p-value = 0.965), and so did the hip circumference (IVW OR = 0.83[0.33, 2.09], p-value = 0.687). These results suggested that waist circumference and hip circumference were 2 different indicators for the risk of allergic risk. Meanwhile, the BMI was not associated with the risk of allergic rhinitis (IVW OR = 0.45[0.16, 1.29], p-value = 0.139).

Blood lipids

We failed to observe any causal association between blood lipids and allergic rhinitis. Three kinds of cholesterol could not affect the risk of allergic rhinitis, including HDL (IVW OR = 0.97 [0.59, 1.62], p-value = 0.916), LDL (IVW OR = 0.78 [0.49, 1.25], p-value = 0.307) and total cholesterol (IVW OR = 1.04[0.65, 1.66], p-value = 0.869). Meanwhile, the triglycerides cannot affect it as well (IVW OR = 1.00[0.62, 1.62], p-value = 0.984).

Glycemic traits

Here, we included 2 glycemic traits, including HbA1c and fasting glucose. The HbA1c level cannot affect the risk of allergic rhinitis (IVW OR = 3.51[0.26, 47.17], p-value = 0.344) and the fasting glucose cannot affect it either (IVW OR = 0.63[0.21, 1.92], p-value = 0.416).

Blood pressure

Both kinds of blood pressure were not associated with the risk of allergic rhinitis, including SBP (IVW OR = 1.01[0.98, 1.05], p-value = 0.372) and DBP (IVW OR = 1.00[0.95, 1.05], p-value = 0.924).

VASOMOTOR RHINITIS

Smoking and drinking

No risk factor was associated with vasomotor rhinitis after Bonferroni correction. However, it was suggestive that CPD could increase the risk of vasomotor rhinitis (IVW OR = 1.30[1.04, 1.62], p-value = 0.022, Bonferroni p-value = 0.325) (Fig. 3). After adjusting for the age of smoking initiation and alcohol intake, the CPD cannot affect the risk of vasomotor rhinitis (IVW OR = 2.40[0.45, 12.76], p-value = 0.316). In this study, the alcoholic drinks per day cannot affect the risk of

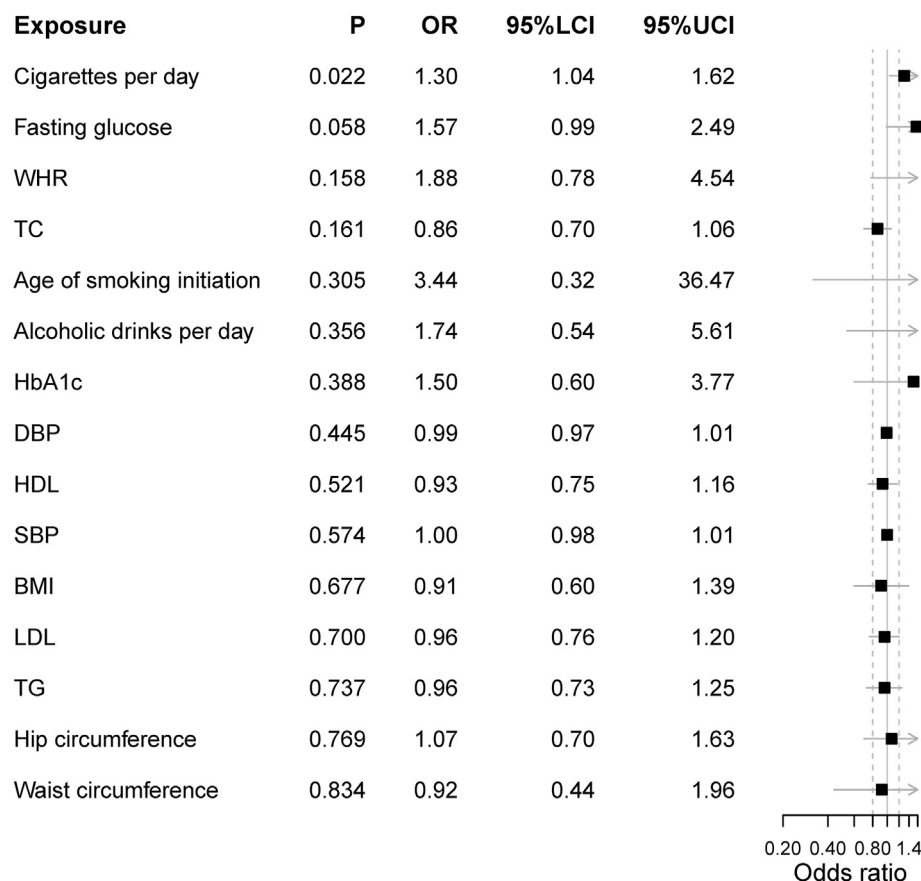


Fig. 3 The forest plot of different risk factors' effect on vasomotor rhinitis. Notes: (1) Exposure: the risk factors; BMI = body mass index; WHR = waist-to-hip ratio; HDL = high-density lipoprotein; LDL = low-density lipoprotein; TC = total cholesterol; TG = triglycerides; HbA1c = glycated hemoglobin; SBP = systolic blood pressure; DBP = diastolic blood pressure. (2) P: the p-value of each MR result. (3) OR: the odds ratio. (4) 95%LCI: the lower limit of 95% confidence interval. (5) 95%UCI: the upper limit of 95% confidence interval

vasomotor rhinitis (IVW OR = 1.74[0.54, 5.61], p-value = 0.356).

Anthropometric traits

Unlike allergic rhinitis, the vasomotor rhinitis cannot be affected by the waist circumference (IVW OR = 0.92[0.44, 1.96], p-value = 0.834). Besides, no other anthropometric traits are causally associated with vasomotor rhinitis, including BMI (IVW OR = 0.91[0.60, 1.39], p-value = 0.677), hip circumference (IVW OR = 1.07[0.70, 1.63], p-value = 0.769) and waist-to-hip ratio (IVW OR = 1.88[0.78, 4.54], p-value = 0.158).

Blood lipids

No causal association was observed between blood lipids and vasomotor rhinitis. Four blood lipids cannot influence the risk of vasomotor rhinitis,

including HDL (IVW OR = 0.93[0.75, 1.16], p-value = 0.521), LDL (IVW OR = 0.96[0.76, 1.20], p-value = 0.700), total cholesterol (IVW OR = 0.86[0.70, 1.06], p-value = 0.161), and triglycerides (IVW OR = 0.96[0.73, 1.25], p-value = 0.737).

Glycemic traits

Here, we included 2 glycemic traits but either can affect the risk of vasomotor rhinitis, including HbA1c (IVW OR = 1.50[0.60, 3.77], p-value = 0.388) and fasting glucose (IVW OR = 1.57[0.99, 2.49], p-value = 0.058).

Blood pressure

Two kinds of blood pressure were not associated with the risk of allergic rhinitis, including SBP (IVW OR = 0.99[0.98, 1.01], p-value = 0.574) and DBP (IVW OR = 0.99[0.97, 1.01], p-value = 0.445).

Sensitivity analysis and power calculation

A leave-one-out sensitivity analysis was performed for each exposure-outcome association. The outliers were removed in the further analysis and no IV could drive the results. The statistical power for CPD is 100%.

DISCUSSION

To summarize, we identified smoking might be the only common risk factor and it could affect the risk of allergic rhinitis and vasomotor rhinitis in the opposite direction. The CPD might decrease the risk of allergic rhinitis while increasing the risk of vasomotor rhinitis suggestively. Meanwhile, a higher waist circumference could marginally decrease the risk of allergic rhinitis. Besides, no other risk factors were causally associated with the risk of either allergic or vasomotor rhinitis. However, this study has its limitations¹: The study population is limited to the Europeans and the conclusion cannot expand to other ethnicities easily;² Different risk factors might affect the risk of allergic and vasomotor rhinitis differently based on the age and sex, and immunity and clinical/functional outcomes are also important but we failed to consider them due to data limitations;³ The statistical power for cigarettes per day is sufficient while relatively low for other traits and we cannot avoid the false negative results;⁴ We failed to fully replicate our findings due to data limitations.

The recent consensus pertaining to the impact of smoking on allergic rhinitis is still controversial.²⁶ On one hand, smoking can increase the synthesis of immunoglobulin E and stimulate airway hyperresponsiveness and it can also hamper the immune functions, suggesting 2 opposite effects of smoking on the pathogenesis of allergic rhinitis. Our results lent strong support to it that smoking could lower the risk of allergic rhinitis.⁵ Several reasons can account for it:¹ Patients with allergic rhinitis tend to quit smoking and thus it may introduce reversal causality;^{5,2} Smoking might have a strong immunosuppressive effect smoking and thus decrease the risk of allergic rhinitis.²⁷ However, Holm et al reported that respiratory diseases, including asthma, wheeze, hay fever, and chronic bronchitis,

could not significantly predict smoking cessation.²⁸ Our MR indicated smoking can decrease the risk of AR, suggesting its effect on suppressing immunity might be a major one. Thus, we deemed that the immunosuppressive effect of smoking might be the main mechanism that could explain the significant MR results.

Recently, Elisia et al reported that smoking can induce inflammation and compromise our innate immunity since higher CRP, fibrinogen, IL-6, IL-8, and leukocytes were observed in smokers.²⁹ Additionally, the smokers had lower NK cells and higher Tregs than non-smokers, indicating that smoking may disturb our immune function.²⁹ Groneberg et al reported that chronic exposure to smoking could elevate the levels of vasoactive intestinal peptide and neuropeptide tyrosine.³⁰ The vasoactive intestinal peptide is an inhibitory noncholinergic neurotransmitter that can cause bronchodilation and vasodilatation, and further lead to hypersecretion.³¹ Thus, chronic exposure to smoking might cause vasodilation and further contribute to vasomotor rhinitis.

As per the abovementioned, we deemed that smoking could affect the risk of allergic rhinitis and vasomotor rhinitis via 2 different mechanisms where the former might be explained by the immunosuppressive effect of smoking and the latter by the vasodilation effect of smoking.

In our MR analysis, we did not find that alcohol intake could increase the risk of allergic/vasomotor rhinitis and this was in concordance with previous studies. For instance, A MR study did not observe the causal relationship between prenatal alcohol exposure and allergic rhinitis³² and Skaaby et al further ruled out the causal relationship between alcohol intake and allergic sensitization.¹¹

There is a lack of evidence on whether waist circumference could affect the risk of allergic rhinitis and Liu et al reported there was no difference in waist circumference between allergic rhinitis and controls.³³ However, Choi et al found that waist circumference and body mass index were greater among women without chronic rhinitis than among those with chronic rhinitis,³⁴ partly consistent with our findings. Here, that higher waist circumference could reduce the risk

of allergic rhinitis might be explained by the activated inflammation and compromised immunity caused by obesity since obesity could trigger the chronic mild systemic inflammation.³⁵ This was similar to the mechanism that smoking can suppress allergic rhinitis via its immunosuppressive effect. As for vasomotor rhinitis, no related studies have been reported. An up-to-date meta-analysis suggested that obesity/overweight might be associated with a higher risk of allergic rhinitis in children.³⁶ Obesity is measured by BMI and we hypothesized that different anthropometric traits might have different impacts on rhinitis. Also, we deemed that obesity's impact on rhinitis might be affected by age and it should be further investigated.

Our study did not suggest that blood lipids could serve as indicators for the risk of allergic/vasomotor rhinitis. However, Sheha et al found that TC (total cholesterol) was positively correlated with the severity of allergic rhinitis and dyslipidemia might play a potential role in the severity of allergic rhinitis symptoms and impairment of patients' quality of life via the interleukin-17A (IL-17A).³⁷ Meanwhile, Vinding et al discovered that high levels of HDL could lower the risk of allergic rhinitis while the triglycerides could elevate the risk of it.³⁸ These previous studies all indicated that blood lipids were associated with allergic rhinitis while failing to point out whether such association was causal. Although MR is an effective method for causal inference, it might suffer from some biases and the high false negative rate is one of them. Since the IV selection process was very strict and should meet the basic 3 principles, sometimes the statistical power could not be well satisfied. However, considering that the statistical power in our study was sufficient, we preferred to rule out the possibility that blood lipids could affect the risk of allergic/vasomotor rhinitis.

Similar to blood lipids, we did not find that glycemic traits could affect the risk of allergic or vasomotor rhinitis. However, Hashimoto et al reported that the prevalence of allergic rhinitis is lower in subjects with higher levels of fasting plasma glucose,³⁹ and this conclusion was further confirmed by Hwang et al.⁴⁰ Considering the

relatively low statistical power, we could not rule out the possibility that HbA1c and fasting glucose affect the risk of allergic or vasomotor rhinitis. The observational results might be biased by potential confounders or the reversal causation.

As for blood pressure, Sakalliglu et al demonstrated that blood pressure was not associated with rhinitis and should not be followed up⁴¹ while Hwang et al found a lower prevalence of allergic rhinitis in the high blood pressure group.⁴⁰ Our MR results supported the former conclusion. Li et al did not observe the relationship between allergic rhinitis and blood pressure in adults but suggested an association in young women aged 20-39.⁴² Thus, we hypothesized that the blood pressure's effect on rhinitis might be affected by ethnicity and age.

Overall, our MR analysis discovered that smoking can lower the risk of allergic rhinitis while increasing the risk of vasomotor rhinitis. Besides, a higher waist circumference could marginally lower the risk of allergic rhinitis. No other causal relationship was observed. Generally, we think our study has several strengths¹: The sample size for each risk factor is large and guarantees the statistical power;² We strictly followed the basic principles of MR and the results can be convincing;³ The outcomes include the allergic rhinitis and vasomotor rhinitis, and this is the first MR analysis to investigate the risk factors of vasomotor rhinitis;⁴ Our results can be informative in distinguishing 2 rhinitis. However, the chances of both codes of AR and VMR coexistence are hard to give due to the unavailability of individual-level data. Intuitively, the coexistence chances should be low so that we obtained that the smoking should affect AR and VMR differently, even in the opposite direction. Besides, the generalizability of results should be interpreted with caution as we only tested them in European participants.

CONCLUSION

Smoking can lower the risk of allergic rhinitis while elevating the risk of vasomotor rhinitis. This result might help to distinguish 2 rhinitis in clinical practice.

Abbreviations

MR = Mendelian randomization; IVW = inverse-variance weighted; SNP = single nucleotide polymorphism; RCT = randomized controlled trials; GWAS = genome-wide association study; IV = instrumental variable; LD = linkage disequilibrium; MVMR = multivariable Mendelian randomization; OR = odds ratio; SD = standard deviation; BMI = body mass index; WHR = waist-to-hip ratio; HDL = high-density lipoprotein; LDL = low-density lipoprotein; TC = total cholesterol; TG = triglycerides; HbA1c = glycated hemoglobin; SBP = systolic blood pressure; DBP = diastolic blood pressure.

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Authors' consent for publication

All authors have given consent for publication.

Author contributions

A.Y contributed to the study design, supervised the data analysis process, and mainly revised the manuscript. S.W and L.Q were responsible for data acquisition, statistical analysis, and data visualization; and S.W drafted the original manuscript. H.W and L.Q read and revised the original manuscript and gave substantial suggestions on statistics. All authors have approved the publication of this study. A.Y takes responsibility for the integrity of the data and the accuracy of the data analysis.

Ethics approval

All GWAS consortia have approved by corresponding ethical committees.

Availability of data and materials

GWAS summary statistics of smoking and drinking can be accessed via GSCAN (<https://conservancy.umn.edu/handle/11299/201564>).

GWAS summary statistics of smoking and drinking can be accessed via GIANT (http://portals.broadinstitute.org/collaboration/giant/index.php/GIANT_consortium_data_files).

GWAS summary statistics of blood lipids can be accessed via GLGC (<http://lipidgenetics.org/#data-downloads-title>).

GWAS summary statistics of glycemic traits can be accessed via MAGIC (<https://magicinvestigators.org/downloads/>).

GWAS summary statistics of glycemic traits can be accessed via the GWAS catalog (<https://www.ebi.ac.uk/gwas/>).

GWAS summary statistics of rhinitis can be accessed via FinnGen (<https://r4.finngen.fi/>).

Declaration of competing interest

No conflicts of interest to be disclosed.

Acknowledgments

We thank all GWAS consortia to make their GWAS summary statistics publicly available, including GSCAN, GIANT, GLGC, MAGIC, and FinnGen.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.waojou.2022.100630>.

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Supplemental Methods

Clinical Data Acquisition

The skin prick test used 10 common inhalant allergens, including *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, outdoor mold mixture, cockroach, dog hair, early spring tree pollen mixture, mid spring tree pollen mixture, grass pollen mixture, ragweed, and mugwort. If any one of them was positive, it was assessed as atopy. FeNO measurements were performed according to the ATS/ERS guideline using NIOX MINO[®] (Aerocrine AB, Solna, Sweden) before spirometry. Presence of nasal polyps was evaluated through rhinoscopic examination. Serum samples were collected from the study subjects and analyzed for levels of cytokines, including interleukin (IL)-4, IL-5, IL-8, IL-10, IL-13, IL-17, interferon-gamma (IFN- γ), and basic fibroblast growth factor (bFGF), using multiplex cytokine analysis kits (R&D systems, Minneapolis, MN, USA) and data were collected using Luminex 100 (Luminex, Austin, TX, USA). Concentrations of transforming growth factor beta (TGF β), periostin, and chitinase were measured in the serum samples by enzyme-linked immunosorbent assay (Thermo Scientific Inc., Rochester, NY, USA).

QCT Imaging Variables

We extracted the airway structural variables, including luminal wall thickness (WT) and hydraulic diameter (D_h), at five large airways and five grouped-segmental airways (**Figs. 1A and 1B**). Regions of the five large airways included the trachea, right main bronchus (RMB), Bronint (intermediate bronchus), left main bronchus (LMB), and trifurcation of left lower bronchus (TriLLB). Regions of the five grouped-segmental airways were defined and averaged in the respective lobes as sRUL (RB1, RB2, and RB3), sRML (RB4 and RB5),

sRLL (RB6, RB7, RB8, RB9, and RB10), sLUL (LB1, LB2, LB3, LB4, and LB5), and sLLL (LB6, LB9, LB9, and LB10). These quantities of WT and D_h contain inter-subject variability due to sex, age, and height and hence were normalized by equations predicted from healthy subjects.¹ The normalized quantities were expressed as WT^* and D_h^* . An increase in WT^* and decrease in D_h^* could be considered as airway wall thickening and luminal narrowing, respectively.

Lung parenchymal variables, including functional small airway disease lung (fSAD, **Fig. 1C**) and emphysematous lung (Emph, **Fig. 1D**), were further extracted. To differentiate the portion of emphysema from air-trapping, we used the parametric response map (PRM) that requires the usage of image registration method.² To minimize density variations in the CT scans, a fraction-threshold method was also adopted.³ This method uses a fixed threshold of air fraction (β_{air}) to determine the percentage fSAD (fSAD%) and percentage Emph (Emph%) in a voxel rather than using the Hounsfield unit-based threshold of -950 and -856 for inspiration and expiration images, respectively. The criteria used for the voxel classification were as follows: if $\beta_{air} > 98.5\%$ at inspiration (Emph voxel); if $\beta_{air} > 98.5\%$ at inspiration and $\beta_{air} > 90\%$ at expiration (fSAD voxel); if otherwise a normal voxel. The counted number of voxels for Emph and fSAD was divided by number of voxels in the total lung or five lung lobes and was denoted by Emph% and fSAD%, respectively. The fSAD was calculated by the subtraction of the emphysema portion at inspiration from air-trapping portion at expiration.

Statistical Analysis

The imaging and clinical variables acquired by thoracic radiologists and clustering method were compared between groups using Kruskal–Wallis and chi-square tests for continuous and categorical variables, respectively. Dunn’s and Bonferroni post hoc tests were applied after pre-hoc tests were performed for continuous and categorical variables, respectively, to confirm the significant relationship between each group. *P* values < 0.05 were considered statistically significant.

Supplemental Results

QCT Imaging-based Clustering

To obtain clustering-based groups comparable to the grouping by thoracic radiologists, we included only the four imaging-based metrics obtained at post-bronchodilator, among which two metrics were airway structures (WT^* and D_h^*) and the other two were parenchymal metrics (fSAD% and Emph%). The respective airway structural metrics contain 10 regional values (2×10), while the lung parenchymal metrics contain a total of five lobar regional values (2×6). Therefore, we fed 32 imaging-based metrics to the clustering analysis. A principal component analysis (PCA) was performed along with a parallel analysis in order to derive statistically uncorrelated and reduced variables (**Fig. S3A**).⁴ The parallel analysis suggested the number 5 as an optimal number of the principal components (PC) that explained 71% of the entire data (**Table S1**). The first PC most significantly correlated with Emph% (Total, LUL); the second PC mostly correlated with fSAD% (Total, LUL) and D_h^* (sLUL); the third PC was mostly correlated with WT^* (Bronint, sRUL); the fourth PC correlated with D_h^* (LMB) and fSAD% (LLL and RLL); and the fifth PC most significantly correlated with WT^* (trachea, RMB) and D_h^* (trachea).

The reduced five variables derived from PCA were used to test the two clustering methods of *K*-means and hierarchical. For evaluating the stability of clustering membership, we performed a non-parametric bootstrapping analysis⁵ by changing the number of clustering membership from three to five. This analysis was conducted 200 times using the data resampled from the original data. We found that the repeatability of *K*-means with four clusters was the best, thus demonstrating that the Jaccard mean remained > 0.8 for all clusters (**Fig. 3B**). We projected the imaging metrics on two-dimensional projected coordinates using the multidimensional scaling technique⁶ and further confirmed that *K*-means with four membership provided better grouping with lesser overlapping between clusters (**Figs. 3C and 3D**). More details of the current clustering membership algorithm can be found in the paper reported by Choi et al.⁷

Quantitative Assessment of Visually Perceived CT Phenotypes

We compared airway dimensions, such as WT^* and D_h^* , and parenchymal variables, such as fSAD% and Emph%, among the three groups (**Table 2**). The asthma group could be compared with the means of healthy subjects, which were selected according to the propensity score matching method. Although the three groups demonstrated similar values of WT^* , the values of D_h^* measured at the segmental bronchi were significantly decreased in LA or SA type when compared to those in NN type. The value of D_h^* was the most prominently reduced in the lower lobar bronchi (sRLL and sLLL, $P < 0.01$) when compared with that in the upper lobar bronchi. Emph% was noticeably greater in SA type than in LA and NN types, but LA type also demonstrated a moderate increase over NN type. The SA type also displayed an increase in fSAD% compared to that of NN type or LA type. There was no difference in

the airway and parenchymal responsiveness between pre- and post-bronchodilator across the groups (not reported here).

Clinical Features of Groups Classified by Radiologists

No significant differences were observed in age, onset age of disease, disease duration, body mass index (BMI), and presence of sinusitis or nasal polyps among the three groups. All subjects in the SA group were men with smoking history, while those in other groups were predominantly women and non-smokers. The proportions of patients with severe asthma in the NN, LA, and SA types were 36.8%, 77.8%, and 100%, respectively ($P < 0.005$). This was consistent with the features such as lower use of controller medication and oral corticosteroid use in NN type than in the other types. Reductions in the ratio of FEV₁ to FVC and FEV₁ were more prominent in the LA and SA groups than in the NN group ($P < 0.001$), with consistency seen in both pre- and post-bronchodilators. A fixed airflow obstruction, defined as FEV₁ $< 70\%$ and FEV₁/FVC $< 70\%$ despite bronchodilator application, was never observed in the NN type; whereas, it was more frequent in the LA and SA types ($P < 0.001$). Subjects in the SA type exhibited a greater bronchial hyper-reactivity to methacholine than subjects in the other types ($P < 0.05$). The percentage of sputum eosinophils was significantly increased in the LA type when compared to that in other types, especially NN type ($P < 0.05$). However, other laboratory findings such as FeNO, serum total IgE, and several cytokine levels, including IL-4, IL-5, IL-8, IL-10, IL-13, IL-17, bFGF, IFN- γ , TGF β , periostin, and chitinase, were similar between these groups. Finally, 5 years after the study, the number of

114 acute asthma exacerbations per year was significantly lower in patients with NN type, but
115 highest in those with SA type (**Table 3**).

116

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136 **Supplemental Figure Legends**

137 Supplemental Figure S1. Flow chart of enrolled and excluded subjects

138 Supplemental Figure S2. Representative images of each phenotype determined through CT:

139 NN type (A), LA type (B), and SA type (C).

140 Supplemental Figure S3. Screen plot of eigenvalues according to the number of principal

141 components (A), cluster stability analysis using bootstrapping (B), clustering membership of

142 K-means clustering (C) and hierarchical clustering (D) on 2D projected coordinates

Supplemental Table S1. Standardized loadings of five principal components (PCs) based on correlation matrix

		PC 1	PC 2	PC 3	PC 4	PC 5
	Proportion of variation	25%	22%	11%	7%	6%
	Cumulative variation	25%	47%	58%	65%	71%
WT*	Trachea	−0.097	0.284	0.176	−0.062	0.428*
	RMB	0.070	0.251	0.088	0.041	0.472*
	Bronint	−0.353	0.174	0.542*	−0.385	0.239
	LMB	0.342	0.447	0.029	0.118	0.108
	TriLLB	−0.426	0.115	0.315	−0.089	0.425
	sRUL	−0.399	0.398	0.500*	−0.333	−0.074
	sRML	−0.381	0.515	0.410	−0.222	0.007
	sRLL	−0.460	0.496	0.473	−0.287	−0.235
	sLUL	−0.527	0.584	0.240	−0.270	−0.239
	sLLL	−0.600	0.364	0.372	−0.292	0.032
D_h^*	Trachea	−0.164	0.327	−0.313	0.214	0.401*
	RMB	−0.389	0.574	−0.351	0.159	0.342
	Bronint	−0.348	0.425	0.135	0.232	0.537
	LMB	−0.219	0.494	−0.296	0.451*	0.145
	TriLLB	−0.626	0.461	−0.117	0.218	0.114
	sRUL	−0.404	0.533	−0.145	0.312	−0.367
	sRML	−0.458	0.504	−0.094	0.192	−0.127
	sRLL	−0.553	0.596	0.002	0.178	−0.315
	sLUL	−0.457	0.644*	−0.153	0.186	−0.358
	sLLL	−0.610	0.432	−0.242	0.261	−0.109
Emph%	RUL	0.542	0.323	0.443	0.404	−0.140
	RML	0.635	0.309	0.305	0.073	−0.075
	RLL	0.658	0.335	0.465	0.236	−0.027
	LUL	0.739*	0.356	0.469	0.283	−0.010
	LLL	0.718	0.327	0.486	0.130	0.035
	Total	0.729*	0.362	0.494	0.277	−0.024
fSAD%	RUL	0.607	0.609	−0.344	−0.108	−0.033
	RML	0.545	0.527	−0.374	−0.266	0.028
	RLL	0.500	0.550	−0.309	−0.413*	0.023
	LUL	0.551	0.621	−0.424	−0.147	0.015
	LLL	0.396	0.583	−0.286	−0.457*	−0.007
	Total	0.581	0.643*	−0.394	−0.285	0.004

145 Values are presented as the Pearson correlation coefficients and statistical significance ($p <$
146 0.05) is denoted in bold by *.

147 Abbreviations: WT^* , normalized luminal wall thickness; D_h^* , normalized hydraulic diameter;
148 Emph, emphysema; fSAD, functional small airway disease; RMB, right main bronchus;
149 Bronint, intermediate bronchus; LMB, left main bronchus; TriLLB, trifurcation of left lower
150 bronchus; sRUL, averaged in segmental airways of right upper lobe; sRML, averaged in
151 segmental airways of right middle lobe; sRLL, averaged in segmental airways of right lower
152 lobe; sLUL, averaged in segmental airways of left upper lobe; sLLL, averaged in segmental
153 airways of left lower lobe

154 **Supplemental Table S2. Comparison of normalized wall thickness (WT^*) and normalized hydraulic diameter (D_h^*) between clusters at**
 155 **post-bronchodilator**

Variable (region)		Cluster 1 (N = 7)	Cluster 2 (N = 33)	Cluster 3 (N = 16)	Cluster 4 (N = 5)	P value	Healthy subjects
WT^*	Trachea	1.034 (0.055)	1.016 (0.096)	0.997 (0.045)	1.039 (0.037)	0.096	0.970 (0.093)
	RMB	0.966 (0.100)	0.937 (0.159)	0.901 (0.152)	1.017 (0.046)	0.354	0.857 (0.143)
	Bronint	0.688 (0.055)	0.700 (0.060)	0.661 (0.044)	0.669 (0.032)	0.173	0.671 (0.061)
	LMB	0.806 (0.140)	0.701 (0.053)	0.708 (0.117)	0.889 (0.164)	<0.01 †//¶	0.724 (0.105)
	TriLLB	0.653 (0.051)	0.696 (0.067)	0.634 (0.031)	0.629 (0.018)	<0.001 ‡//	0.632 (0.041)
	sRUL	0.609 (0.054)	0.608 (0.042)	0.575 (0.029)	0.590 (0.023)	<0.05 ‡	0.573 (0.037)
	sRML	0.602 (0.056)	0.587 (0.041)	0.522 (0.031)	0.581 (0.033)	<0.001 †‡¶	0.551 (0.038)
	sRLL	0.598 (0.054)	0.590 (0.036)	0.539 (0.028)	0.561 (0.009)	<0.001 †‡	0.552 (0.041)
	sLUL	0.586 (0.031)	0.559 (0.024)	0.524 (0.022)	0.533 (0.022)	<0.001 †‡§	0.526 (0.039)
	sLLL	0.612 (0.045)	0.612 (0.035)	0.560 (0.029)	0.564 (0.020)	<0.001 †‡§//	0.572 (0.039)
	Total**	0.601 (0.046)	0.591 (0.028)	0.544 (0.016)	0.566 (0.013)	<0.001 †‡	0.555 (0.032)
D_h^*	Trachea	1.081 (0.122)	0.993 (0.082)	0.963 (0.083)	0.971 (0.059)	0.072	0.916 (0.089)
	RMB	0.921 (0.083)	0.815 (0.052)	0.759 (0.055)	0.785 (0.045)	<0.001 *†‡§	0.766 (0.087)
	Bronint	0.659 (0.038)	0.619 (0.039)	0.581 (0.056)	0.586 (0.075)	<0.005 †§	0.579 (0.056)

	LMB	0.743 (0.086)	0.666 (0.052)	0.619 (0.051)	0.667 (0.035)	<0.005 ††	0.615 (0.065)
	TriLLB	0.518 (0.055)	0.501 (0.045)	0.396 (0.053)	0.416 (0.022)	<0.001 ††\$//	0.441 (0.058)
	sRUL	0.352 (0.038)	0.311 (0.040)	0.270 (0.042)	0.271 (0.034)	<0.001 ††\$	0.282 (0.038)
	sRML	0.314 (0.034)	0.277 (0.029)	0.214 (0.031)	0.250 (0.032)	<0.001 ††\$	0.257 (0.039)
	sRLL	0.336 (0.042)	0.293 (0.039)	0.220 (0.021)	0.240 (0.018)	<0.001 ††\$//	0.267 (0.044)
	sLUL	0.285 (0.021)	0.240 (0.022)	0.201 (0.020)	0.221 (0.022)	<0.001 *††\$	0.232 (0.030)
	sLLL	0.360 (0.017)	0.319 (0.037)	0.261 (0.053)	0.252 (0.016)	<0.001 *††\$//	0.290 (0.040)
	Total**	0.329 (0.020)	0.288 (0.022)	0.233 (0.025)	0.247 (0.018)	<0.001 *††\$//	0.266 (0.031)
Emph%	RUL	0.7 (0.4)	1.6 (4.7)	1.4 (1.7)	12.9 (9.6)	<0.005 \$//¶	0.7 (1.4)
	RML	1.7 (1.0)	1.9 (1.9)	2.4 (2.2)	6.2 (1.3)	<0.05 \$//¶	1.9 (3.0)
	RLL	0.7 (0.5)	1.1 (1.7)	1.0 (0.9)	10.4 (7.8)	<0.01 \$//¶	0.8 (0.013)
	LUL	1.1 (1.0)	1.6 (2.4)	2.2 (2.8)	15.8 (7.1)	<0.005 \$//¶	1.1 (0.020)
	LLL	1.0 (0.7)	1.4 (1.6)	1.8 (2.7)	11.5 (4.7)	<0.01 \$//¶	1.0 (1.4)
	Total	1.0 (0.7)	1.4 (1.9)	1.7 (1.8)	12.2 (5.8)	<0.005 \$//¶	1.0 (1.6)
fSAD%	RUL	27.2 (13.7)	5.2 (5.7)	8.7 (8.6)	38.3 (13.9)	<0.001 *†//¶	10.9 (13.9)
	RML	42.9 (10.2)	15.6 (12.3)	20.8 (13.9)	47.5 (10.7)	<0.001 *†//¶	19.9 (17.7)
	RLL	14.7 (16.3)	1.7 (2.1)	4.1 (5.6)	19.5 (11.3)	<0.001 */¶	3.9 (7.2)

LUL	32.6 (14.9)	6.3 (6.4)	11.6 (10.5)	34.2 (9.9)	<0.001 ^{*/†//¶}	12.1 (14.9)
LLL	16.6 (17.9)	2.8 (4.5)	4.2 (5.5)	20.5 (13.6)	<0.001 ^{*/†//¶}	3.3 (6.2)
Total	25.1 (12.4)	4.8 (4.3)	8.6 (6.7)	30.1 (11.0)	<0.001 ^{*/†//¶}	8.7 (10.5)

Values are presented as mean (and standard deviation, SD). Kruskal–Wallis tests with Dunn’s post hoc tests were performed for “populations: Cluster 1 vs. Cluster 2 vs. Cluster 3 vs. Cluster 4.” ^{*}P < 0.05 for Cluster 1 vs. Cluster 2. [†]P < 0.05 for Cluster 1 vs. Cluster 3. [‡]P < 0.05 for Cluster 2 vs. Cluster 3. [§]P < 0.05 for Cluster 1 vs. Cluster 4. ^{//}P < 0.05 for Cluster 2 vs. Cluster 4. [¶]P < 0.05 for Cluster 3 vs. Cluster 4. Reference values of healthy subjects were balanced for age, sex, body mass index, history of smoking, and pack-years with asthma cohort using propensity score matching methods.

^{**} Total means average of segmental airways.

Abbreviations: WT^{*}, normalized luminal wall thickness; D_h^{*}, normalized hydraulic diameter; Emph, emphysema; fSAD, functional small airway disease; RMB, right main bronchus; Bronint, intermediate bronchus; LMB, left main bronchus; TriLLB, trifurcation of left lower bronchus; sRUL, averaged in segmental airways of right upper lobe; sRML, averaged in segmental airways of right middle lobe; sRLL, averaged in segmental airways of right lower lobe; sLUL, averaged in segmental airways of left upper lobe; sLLL, averaged in segmental airways of left lower lobe

Supplemental Table S3. Airway and parenchymal responsiveness of pre- and post-bronchodilators

Variable (Region)	Cluster 1 (N = 7)	Cluster 2 (N = 33)	Cluster 3 (N = 16)	Cluster 4 (N = 5)	<i>P</i> value
$\Delta D_h^*/D_h^*$ (post)					
Trachea	0.071 (0.048; 0.088)	0.038 (0.011; 0.070)	0.020 (0.008; 0.045)	0.018 (0.004; 0.043)	0.259
RMB	0.043 (0.024; 0.084)	0.044 (0.020; 0.079)	0.031 (0.006; 0.076)	0.044 (−0.005; 0.049)	0.451
Bronint	0.023 (0.017; 0.044)	0.034 (0.010; 0.053)	0.018 (0.008; 0.029)	−0.002 (−0.040; 0.011)	0.143
LMB	0.054 (0.016; 0.079)	0.042 (0.011; 0.084)	0.017 (−0.003; 0.047)	0.047 (0.013; 0.050)	0.233
TriLLB	0.048 (0.032; 0.056)	0.080 (0.031; 0.153)	0.061 (0.019; 0.107)	0.024 (0.015; 0.098)	0.648
sRUL	0.059 (0.003; 0.098)	0.108 (0.026; 0.156)	0.075 (−0.012; 0.096)	0.012 (0.011; 0.083)	0.322

sRML	0.041 (0.026; 0.132)	0.117 (0.063; 0.146)	0.042 (−0.052; 0.137)	0.078 (−0.017; 0.091)	0.223
sRLL	0.133 (−0.012; 0.196)	0.113 (0.043; 0.170)	0.009 (−0.056; 0.054)	0.058 (0.023; 0.088)	<0.05 [‡]
sLUL	0.065 (0.033; 0.245)	0.117 (0.036; 0.158)	0.004 (−0.055; 0.101)	0.066 (−0.023; 0.130)	<0.05
sLLL	0.101 (0.014; 0.156)	0.157 (0.065; 0.201)	0.009 (−0.057; 0.084)	0.017 (−0.020; 0.182)	<0.05 [‡]
Total [§]	0.082 (0.068; 0.101)	0.118 (0.065; 0.160)	0.040 (−0.023; 0.073)	0.016 (0.003; 0.121)	<0.01 [‡]
Δ fSAD%					
RUL	8.3 (4.8; 14.8)	−0.3 (−1.8; 0.8)	−1.5 (−8.4; 0.1)	1.9 (−1.4; 2.7)	<0.01 ^{*†}
RML	9.2 (3.4; 23.9)	−1.1 (−3.7; 6.5)	−4.3 (−10.8; 0.7)	1.7 (0.2; 2.8)	<0.05 [†]

RLL	1.9 (-1.5; 5.3)	-0.1 (-1.1; 0)	-2.3 (-7.4; -0.2)	-1.1 (-2.7; 0)	0.07
LUL	16.8 (3.8; 19.3)	-0.3 (-3.3; 0.8)	-2.2 (-6.5; 0.2)	-1.7 (-2.3; 4.6)	<0.05 ^{*†}
LLL	2.3 (-5.8; 5.8)	-0.2 (-1.5; 0.1)	-1.5 (-3.2; -0.4)	-0.9 (-1.9; -0.4)	0.24
Total	8.4 (2.2; 15.1)	-0.8 (-1.8; 0.7)	-2.0 (-4.8; -1.0)	-0.9 (-2.0; 1.5)	<0.05 [†]

Values are presented as median (interquartile ranges). Kruskal–Wallis tests with Dunn’s post hoc tests were performed for “populations: Cluster 1 vs. Cluster 2 vs. Cluster 3 vs. Cluster 4.” ^{*}P < 0.05 for Cluster 1 vs. Cluster 2. [†]P < 0.05 for Cluster 1 vs. Cluster 3. [‡]P < 0.05 for Cluster 2 vs. Cluster 3.

[§] Total means average of segmental airways.

Abbreviations: WT^{*}, normalized luminal wall thickness; D_h^* , normalized hydraulic diameter; Emph, emphysema; fSAD, functional small airway disease; RMB, right main bronchus; Bronint, intermediate bronchus; LMB, left main bronchus; TriLLB, trifurcation of left lower bronchus; sRUL, averaged in segmental airways of right upper lobe; sRML, averaged in segmental airways of right middle lobe; sRLL, averaged in segmental airways of right lower lobe; sLUL, averaged in segmental airways of left upper lobe; sLLL, averaged in segmental airways of left lower lobe

Supplemental Table S4. Comparison of serum cytokine levels of the four CT imaging-based clusters

Serum cytokines	Cluster 1 (N = 7)	Cluster 2 (N = 33)	Cluster 3 (N = 16)	Cluster 4 (N = 5)	<i>P</i> value
IL-4 (pg/mL)	2.3 [1.4; 3.3]	0.3 [0.1; 0.4]	0.5 [0.0; 2.1]	0.0 [0.0; 0.1]	0.318
IL-5 (pg/mL)	1.3 [1.1; 1.6]	1.3 [1.1; 1.8]	1.5 [1.2; 2.4]	1.2 [1.1; 1.2]	0.412
IL-8 (pg/mL)	14.0 [10.1; 21.6]	10.0 [6.2; 14.7]	11.0 [9.1; 17.9]	16.0 [15.2; 24.6]	0.142
IL-10 (pg/mL)	5.5 [3.9; 12.2]	6.9 [4.8; 13.3]	8.9 [6.4; 16.0]	7.8 [6.1; 9.3]	0.63
IL-13 (pg/mL)	2.3 [0.8; 12.6]	2.6 [0.7; 15.2]	1.7 [0.6; 5.6]	1.1 [1.0; 2.3]	0.827
IL-17 (pg/mL)	16.0 [12.0; 30.8]	23.0 [11.8; 104.5]	10.0 [7.6; 23.8]	30.0 [27.5; 35.3]	0.222
bFGF (pg/mL)	24.0 [17.3; 52.9]	47.0 [18.0; 76.4]	24.0 [15.3; 38.9]	20.0 [9.3; 32.1]	0.471
IFN- γ (pg/mL)	28.0 [12.6; 40.1]	43.0 [30.8; 89.1]	28.0 [28.8; 67.2]	28.0 [23.4; 35.6]	0.146
TGF β (pg/mL)	2.1 [1.5; 4.0]	1.5 [0.9; 2.6]	1.2 [0.9; 2.3]	3.6 [2.4; 4.4]	0.359
Periostin (pg/mL)	89.0 [86.0; 101.8]	79.0 [65.3; 94.8]	66.0 [55.6; 106.3]	58.0 [52.0; 67.1]	0.335
Chitinase (pg/mL)	45.0 [20.9; 81.5]	28.0 [13.7; 51.0]	28.0 [17.2; 67.4]	32.0 [14.6; 51.3]	0.751

Values are presented as median (interquartile ranges). Kruskal–Wallis tests were performed.

Abbreviations: IL, interleukin; bFGF, basic fibroblast growth factor; IFN- γ , interferon-gamma; TGF β , transforming growth factor beta

74 asthma patients with informed consent were enrolled between April to July 2013

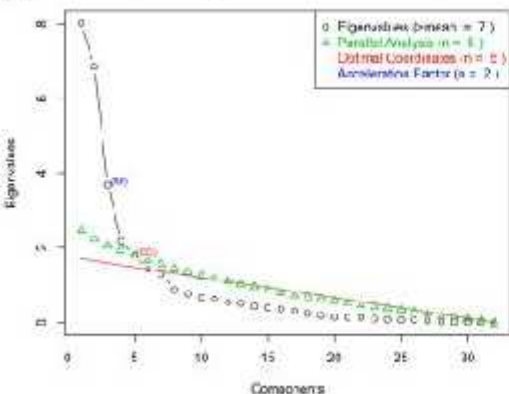
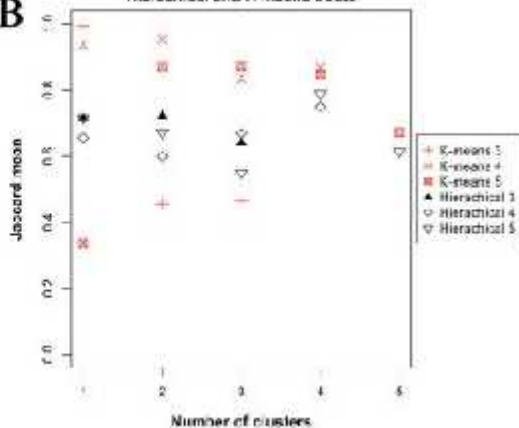
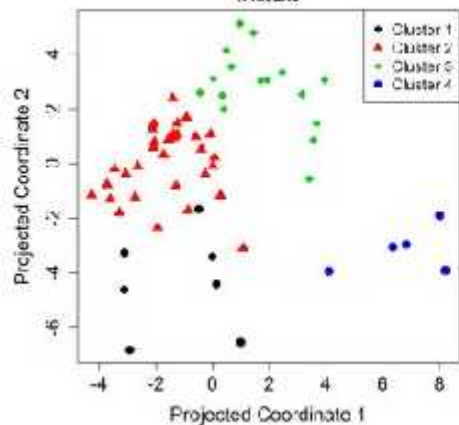
- In each subject, FeNO, pre-BD PFT/CT scan, post-BD PFT/CT scan, skin prick test, induced sputum analysis, rhinoscopy, and blood sampling for cytokine measurement were performed on the same day

13 subjects were excluded as follows:

- Imaging was not performed at post-bronchodilator (N=1)
- Lack of patient compliance for breathing coaching (air volume difference between TLC and FRC ≤ 250 mL) (N=10)
- Right middle lobe segmentation was failed (N=1)
- Imaging quality was poorer due to high BMI (N=1)

61 subjects were included in the current analysis



A**Non Graphical Solutions to Screen Test****B****Hierarchical and K-means boots****C****k means****D****Hierarchical**