Causal Relationship Between Gut Microbiome and Allergic Asthma: A Two-Sample Mendelian Randomization Study

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Research Article

Keywords: allergic asthma, allergic diseases, causal relationship, gut microbial gener, Mendelian randomization

Posted Date: May 19th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2945061/v1

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Abstract

Background

Growing evidence has well documented the close association between the gut microbiome and allergic respiratory disease, which has been notably represented by allergic asthma. However, it is unclear whether this association is a causal link. Therefore, we investigated the potential causal associations between the gut microbiome and allergic asthma or other allergic diseases.

Methods

In this study, we performed two-sample Mendelian randomization (MR) analyses by using the publicly available genome-wide association study (GWAS) summary data. Single-nucleotide polymorphisms (SNPs) that significantly correlated were selected as instrumental variables. The inverse variance weighted (IVW) method was used to examine the potential causal gut microbial genera for allergic asthma and other allergic diseases. The robustness of the primary findings of the MR analyses was ensured by using different sensitivity analyses.

Results

Combining the findings from multiple analyses, the host genetic-driven increases in *Butyricimonas* at the genus level were positively correlated with the risk of allergic asthma. In addition, phylum *Bacteroidetes*, class *Bacteroidia*, order *Bacteroidales* was also found to have negative associations with the risk of allergic asthma; genus *Slackia* was identified as having potential causal effects with allergic asthma. No clear evidence of pleiotropy and heterogeneity was observed in *Butyricimonas*. *Butyricimonas* was also found to have an association with allergic rhinitis, but not with other allergic diseases.

Conclusion

Our findings indicate that there are new gut microbial genera that were causally associated with the risk of allergic asthma and other allergic diseases, and offer novel insights into the pathogenesis of allergic respiratory diseases.

Introduction

Allergic asthma is the most common phenotype of asthma. Patients repeatedly inhale allergens from the environment, leading to bronchial anaphylaxis, which is the main cause of allergic asthma [1]. Compared with non-allergic asthma, bronchospasms are reported to occur more frequently and be more severe in patients with allergic asthma [2]. Allergic asthma is often accompanied by allergic rhinitis and conjunctivitis [3]. Genetic, environmental and stress factors interact in susceptible individuals, resulting in changes in their immune system and enhanced airway responsiveness [4–6]. Presently, the treatments for allergic asthma mainly include environmental control measures, allergen immunotherapy, and glucocorticoids. Among them, glucocorticoids are widely used as anti-inflammatory drugs in allergic
asthma of all grades. However, long-term use of glucocorticoids may lead to loss of calcium and infection susceptibility. A meta-analysis involving 17 RCTs found that gut probiotics could significantly reduce the risk of eczema, but they had no obvious effect on allergic asthma or allergic rhinitis [7]. The guidelines issued by the World Allergy Organization (WAO) in 2015 also indicated that supplementation of gut probiotics is beneficial to allergic diseases, but the evidence supporting this is still insufficient [8]. Therefore, the supplementation of gut microbiota may be a promising strategy to prevent allergic asthma and other allergic diseases. However, this causal association has not been fully confirmed.

A growing body of evidence suggests that the ecological imbalance of gut microbiota may affect the development and severity of allergic asthma [9–11]. Short-chain fatty acids (SCFAs) are metabolites produced by symbiotic bacteria in the gastrointestinal tracted, maintained by fermentation of indigestible digestive products and intestinal amino acids, and they can be absorbed into the circulatory system [12,13]. In both ovalbumin (OVA)- and house dust mite (HDM)-induced models of allergic asthma, SCFAs have been documented to reduce inflammation [12,14]. However, the human gut microbiome can also produce proinflammatory metabolites, such as histamine [15]. This reflects the complexity of bacterial immune regulation. Unlike non-allergic asthma, which can repeatedly find the same gut microbiota, the status of gut microbiota of allergic asthma is heterogeneous. Therefore, it is unclear whether there is a causal relationship between gut microbiota and allergic asthma.

Mendelian randomization (MR) uses instrumental variables (IVs) and is a common method that is used to examine whether there is a causal association between exposure and complex outcomes (16). The results of MR analysis mainly depend on the aggregated data of the genome-wide association studies (GWAS) database, thus greatly reducing the impact of confounding factors. Therefore, in this study we used the most up-to-date GWAS database to conduct an MR analysis to investigate the potential causal associations between gut microbial genera and the risk of allergic asthma.

Materials and Methods

Study Design

The overall flowchart for this study is illustrated in Fig. 1. We conducted a two-sample MR study to investigate the causal relationships between host genetic-driven gut microbiota and allergic asthma. For significant results, we then explored whether associations exist among the gut microbiota, lung functions (FEV1, FVC, and FEV1/FVC ratio), and five allergic diseases, namely, allergic rhinitis, allergic purpura, allergic conjunctivitis, allergic contact dermatitis, and allergic urticaria.

Data Sources and SNP Selection

Summary genetic association estimates for the human gut microbiome were obtained from the largest, genome-wide meta-analysis, the MiBioGen consortium study [17], which consisted of 18,340 individuals from 24 population-based cohorts of European (n = 13266), Hispanic, Middle Eastern, Asian and African...
ancestries. In addition, the GWAS study was adjusted for sex, age, genetic principal components, and cohort-specific potential microbiome batch effects.

GWAS summary statistics for allergic asthma and five other allergic diseases were drawn from the FinnGen consortium round 8 databases (Table S1) after adjusting for sex, age, 10 principal components, and genotyping batch. FinnGen is a large public-private partnership aiming to collect and analyze genome and health data from 500,000 Finnish biobank participants. The diagnoses were based on ICD-10 and the following study IDs were used to obtain the outcome data: allergic asthma (ALLERG_ASTHMA, 8525 cases and 193,857 controls), allergic rhinitis (ALLERG_RHINITIS, 9707 cases and 331,173 controls), allergic purpura (D3_ALLERGPURPURA, 780 cases and 337,408 controls), allergic conjunctivitis (H7_ALLERGICCONJUNCTIVITIS, 18,321 cases and 324,178 controls), allergic contact dermatitis (L12_ALLERGICCONTACT, 3846 cases and 306,909 controls), and allergic urticaria (L12_URTICA_ALLERG, 2112 cases and 331,270 controls).

For lung function, we focused on forced expiratory volume in 1-second (FEV1), forced vital capacity (FVC), and FEV1/FVC ratio (Table S1). The related summary statistics were extracted from UK Biobank, comprising a sample size of 344,267 participants of European ancestry, which were then adjusted for age, age*age, gender, height, and smoking status.

Based on the previously published studies and getting more acceptable instrumental variables [18], the SNPs association threshold was set to be p < 1.0 x 10^-5, but it was not associated with the outcome (p > 5.0 x 10^-8). A total of five levels, namely, phylum, class, order, family, and genus, of bacterial taxa were analyzed in our study. Then, to retain the independent instrumental variables, SNPs were clumped and discarded at linkage disequilibrium r^2 < 0.001 within a 10,000 kilobase pairs window, which was based on reference data of European ancestry from the 1000 Genomes Project [19]. Subsequently, weak instrumental variables were removed using the F statistic (F < 10). Finally, after harmonizing the exposure and the outcome data, the palindromic SNPs (A/T, G/C) were also excluded from the MR analysis.

**Mendelian Randomization Analysis and Sensitivity Analyses**

A two-sample MR analysis was conducted using the inverse-variance weighted (IVW) method as the primary analysis. IVW is classically used in combination with the Wald ratio estimates to obtain an unbiased estimate [20]. To provide robust evidence of results, four additional methods were employed to validate the findings, namely, the maximum likelihood method [21], weighted median [22], simple median [23], and MR–Egger method [24]. The MR–Egger regression analysis allows free evaluation of the non-zero intercept value as an estimated value of average pleiotropic bias, and it gives a consistent estimate even if all of the instrumental variables are invalid. If some instrumental variables were valid, an unbiased estimate of causality could be provided by the weighted median method and maximum likelihood method.
To ensure the robustness of MR findings, The MR–Egger intercept test and Cochrane’s Q heterogeneity method were conducted to detect any potential horizontal pleiotropy [25]. We also performed the Pleiotropy Residual Sum and Outlier (MR–PRESSO) test to find and correct the outlier instrumental variables reflecting pleiotropic biases [26]. Additionally, we applied the leave-one-out method to rule out the possibility of the influence of a single SNP on the causality.

**Statistical Analysis**

All of the analyses were conducted using the TwoSampleMR package and MR–PRESSO package in R software (version 4.1.2). The MR results are presented as odds ratios (ORs) with 95% confidence intervals (CIs). The significant threshold for each level was corrected based on the Bonferroni method (p < 0.05/n). A value of p < 0.05 but above the Bonferroni-corrected significance threshold was considered suggestive of evidence for a potential causal effect.

**Results**

Under the suggestive significance threshold of p < 1.0 x 10^{-5}, a total of 1958 independent SNPs from 175 bacterial taxa (excluding the unknown taxa) were identified after a series of quality control. The 175 bacterial taxa consisted of 11 classes, 26 families, 117 genera, 12 orders, and 9 phyla. To account for multiple testing, we implemented the Bonferroni-corrected method to examine for associations with allergic asthma risks using the IVW model, and the results are listed as follows: class p < 4.5 x 10^{-3} (0.05/11), family p < 1.9 x 10^{-3} (0.05/26), genus p < 4.2 x 10^{-4} (0.05/117), order p < 4.2 x 10^{-3} (0.05/12), and phylum p < 5.6 x 10^{-3} (0.05/9).

**Causal Associations of Gut Microbiota on Allergic Asthma**

Based on the IVW model, the host genetic-driven increases in *Butyricimonas* at the genus level were positively associated with a higher risk of allergic asthma (OR = 1.279, 95% CI = 1.12–1.46, p = 2.87 x 10^{-4}, Table 1). The conclusion was confirmed by the results of the maximum likelihood method (OR = 1.283, 95% CI = 1.12–1.47, p = 3.24 x 10^{-4}), weighted median method (OR = 1.31, 95% CI = 1.098–1.563, p = 2.74 x 10^{-3}) and simple median method (OR = 1.27, 95% CI = 1.053–1.532, p = 1.24 x 10^{-2}). In addition, four suggestive causal effects were identified, within the phylum *Bacteroidetes* (OR = 0.8, 95% CI = 0.673–0.951, p = 1.14 x 10^{-2}), class *Bacteroidia* (OR = 0.819, 95% CI = 0.695–0.965, p = 1.69 x 10^{-2}), order *Bacteroidales* (OR = 0.819, 95% CI = 0.695–0.965, p = 1.69 x 10^{-2}) and genus *Slackia* (OR = 1.169, 95% CI = 1.021–1.34, p = 2.42 x 10^{-2}). The details on instrumental variables are summarized in Table S2.

In the sensitivity analysis, no clear evidence of pleiotropy (MR–Egger intercept = 0.009, MR–Egger p = 0.654, and MR–PRESSO Global Test p = 0.952) and heterogeneity (Cochran Q = 6.485, p = 0.927) was observed in genus *Butyricimonas* (Table 1). The results of leave-one-out sensitivity analysis revealed that no single SNP drives the causal associations between the genus *Butyricimonas* and allergic asthma.
Furthermore, no evidence of heterogeneity or pleiotropy bias was noted among the phylum Bacteroidetes, class Bacteroidia, order Bacteroidales, and genus Slackia as well (Fig. 2).

Causal Association of Gut Microbiota with Lung Function

Decreased lung function is one of the important characteristics necessary for asthma diagnosis, with the FEV1, FVC, and FEV1/FVC ratio being particularly important. Therefore, we assessed the associations between the gut microbiota related to allergic asthma and lung function. The five methods of MR analysis suggested that the genus Butyricimonas has no causal effect on FEV1 (OR = 0.981, 95% CI = 0.954–1.008, PIVW = 0.174), FVC (OR = 0.986, 95% CI = 0.961–1.011, PIVW = 0.278) or FEV1/FVC ratio (OR = 0.982, 95% CI = 0.954–1.012, PIVW = 0.234). No evidence was detected indicating a causal relationship between the other four potential gut microbiota and lung function (Fig. 3, Table S3). In addition, the sensitivity analysis results supported the robustness of the MR analysis (Table S4-5).

Causal Association of Gut microbiota with Other Allergic Diseases

The prevalence of allergic diseases is increasing worldwide and affects respiratory, digestive, skin, and other systems. However, gut microbiota share a common central role in the risk factors, mechanism, and therapy of these allergic diseases. We evaluated the causal relationship between the five gut microbiota and other allergic diseases, namely, allergic rhinitis, allergic purpura, allergic conjunctivitis, allergic contact dermatitis, and allergic urticaria.

The genus Butyricimonas was associated with allergic rhinitis (OR = 1.192, 95% CI = 1.022–1.392, PIVW = 0.0256, Fig. 4), but not with allergic purpura (OR = 1.247, 95% CI = 0.815–1.908, PIVW = 0.31), allergic conjunctivitis (OR = 1.109, 95% CI = 0.987–1.246, PIVW = 0.0806), allergic contact dermatitis (OR = 0.981, 95% CI = 0.782–1.23, PIVW = 0.867), and allergic urticaria (OR = 0.97, 95% CI = 0.748–1.257, PIVW = 0.816). Additionally, no significant causality between the other four gut microbiota and other allergic diseases was found in all of the models (Fig. 4, Table S6). Neither pleiotropy bias (MR–Egger p > 0.1, Table S7) or heterogeneity (Cochran's Q p > 0.1, Table S8) were found.

Discussion

This MR study found that the genus Butyrismimonas was significantly positively associated with a high risk of allergic asthma. In addition, the phylum Bacteroidetes, class Bacteroidia, and order Bacteroidales was also found to have negative associations with the risk of allergic asthma. Genus Slackia was identified as having suggestive causal effects with allergic asthma, but more genome-wide data are needed to confirm this result. Therefore, our findings further indicate that gut microbiota has a causal association with allergic asthma, and suggest the regulatory role of gut microbial genera in allergic asthma.
The main metabolite of the genus Butyrisimonas is butyrate, which is an important energy source for colon cells. Most of the butyrate is metabolized into energy by those cells. Butyrate is believed to be beneficial to the gut, and it helps to promote the body's anti-inflammatory and immune response. The severity of allergic asthma seems to be related to butyrate produced in the gut. The evidence showed that in the OVA-induced airway inflammation mouse model, supplementing butyrate bacteria from the feces of infants can reduce the airway inflammation [27]. The phylum Bacteroides, class Bacteroidia and order Bacteroidales are colonized bacteria present in the gut of healthy adults. The observational research suggested that the decrease of genus Bacteroides is related to the development of allergic diseases and the increase of the risk of sensitization [28–30]. The phylum Bacteroides, class Bacteroidia and order Bacteroidales has been previously shown to promote the cellular and physical maturation of the developing immune system through its ability to guide the development of CD4+T cells, thus inducing the differentiation of Th1 lineage and the correction of Th1/Th2 imbalance [31]. Interestingly, contrary to the conclusions of observational studies and systematic review [10,32], the results of this MR study show that there is a causal association between gut microbiota and allergic asthma or allergic rhinitis. In particular, increasing the proportion of the genus Butyrisimonas may reduce its benefits and increase the risk of allergic asthma and allergic rhinitis. The significant change in the proportion of gut microbiota and the increasing risk of allergic asthma seems to occur at the same time.

When establishing the causal effects, multi-levels of research evidence should be considered, but observational studies under different conditions are susceptible to confounding factors, thus reducing the accuracy of conclusions. Therefore, the relevance of observational studies cannot be equated with causal correlations. MR avoids the influence of confounding factors through genetic instrumental variables, and it can carry out accurate causal assessment. However, the development of allergic diseases is a long-term process, and more data are needed to support our findings.

Our study is the first MR analysis of gut microbiota, allergic asthma, and multiple allergic diseases. It has several advantages: First, compared with the inherent limitations of observational studies, MR studies are unlikely to be affected by reverse causality and confounding factors. Second, extensive GWAS sample data, two sets of independent IVs, and different methods are applied to causal association assessments to improve reliability. It must be acknowledged that this study also has the following limitations: First, this study is limited to individuals of European descent, and the difference in dietary habits lead to diversity of gut microbiota among different populations, so it may be limited to extrapolate the research findings to other ethnic groups. Second, this study is unable to determine whether overlapping participants were included in the exposure and outcome GWAS used in the two-sample MR analysis. Lastly, this study cannot further answer the different role the genus Butyrisimonas played in this MR study and other observational studies.

In conclusion, this MR study shows there are causal associations between gut microbiota and allergic asthma in European populations, as well as a potential causal association between gut microbiota and allergic rhinitis. General gut microbiota were found to be pathogenic or protective factors of allergic asthma, thus suggesting that previously reported associations in observational studies may be incorrect.
Our research findings do not support the benefit of supplementing with gut probiotics from the genus *Butyrisimonas*, to prevent allergic asthma or allergic rhinitis, but instead indicate the potential harm. With this research we hope to strengthen the understanding of the characteristics of gut microflora.

**Declarations**

**Acknowledgements**

We want to acknowledge the participants and investigators of the FinnGen and the UK Biobank study. We also thank the members of the MiBioGen consortium for sharing summary-level data publicity available. We also Than Dr. Junhao Tu for his outstanding advice in this manuiscript.

**Author contributions**

WWan, YQiu, and C-PYang designed the research. WWan, YQiu, Y-X Ren and X-Y Huang collected and analyzed the data. YQiu, Y-X Ren and X-Y Huang performed the literature search. WWan and C-PYang drafted the article. YQiu and A-D Peng supervised the study. All authors were involved in writing the paper. All authors contributed to the article and approved the submitted version.

**Funding**

This research was supported by National Natural Science Foundation of China (No.82160211).

**Date availability statement**

The original contributions presented in the study are included in the article/Supplementary Material, without undue reservation.

**Ethics statement**

All summary statistics from large GWASs were publicly available in this study, and ethical approval for each GWAS was performed in accordance with the declaration of Helsinki and was approved by the ethics committee of the corresponding institution.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

**References**


### Table

**Table 1.** MR results of gut microbiota on allergic asthma

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<th>Outcome</th>
<th>Method</th>
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### Figures
Figure 1

The overview flowchart of hypothesis and study design. This study was a two-sample Mendelian randomization analysis testing the causal effects between gut microbiota and allergic asthma. There are three assumptions required for a valid genetic instrument: the SNPs were significantly correlated with gut microbiota, they were independent of confounders, and the allergic asthma was only related through gut microbiota.
Figure 2

MR sensitivity analysis for gut microbiota on allergic asthma. Scatterplot of five MR tests between the genus Butyricimonas (A), phylum Bacteroidetes (B), class Bacteroidia (C), order Bacteroidales (D), and genus Slackia (E) and allergic asthma. MR leave-one-out sensitivity analysis are included for the genus Butyricimonas (F), phylum Bacteroidetes (G), class Bacteroidia (H), order Bacteroidales (I) and genus Slackia (J) on allergic asthma.
### Figure 3

Forest plot of causal relationships estimated for five gut microbiota and lung functions (forced expiratory volume in 1-second (FEV1), forced vital capacity (FVC), and FEV1/FVC ratio) using the inverse variance weighted method.
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**Figure 4**

Forest plot of causal relationships estimated for five gut microbiota and other allergic diseases (allergic rhinitis, allergic purpura, allergic conjunctivitis, allergic contact dermatitis, and allergic urticaria) using the inverse variance weighted method.

**Supplementary Files**
This is a list of supplementary files associated with this preprint. Click to download.

- Tables.xlsx