



# Clarifying the effect of gut microbiota on allergic conjunctivitis risk is instrumental for predictive, preventive, and personalized medicine: a Mendelian randomization analysis

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## Abstract

**Background** Allergic conjunctivitis is an ocular immune disease which affects the conjunctiva, eyelids, and cornea. Growing evidence implicates the gut microbiota in balancing and modulating immunity response, and in the pathogenesis of allergic disease. As a result, gut microbial imbalance could be a useful indicator for allergic conjunctivitis. From the perspective of predictive, preventive, and personalized medicine (PPPM), clarifying the role of gut microbial imbalance in the development of allergic conjunctivitis could provide a window of opportunity for primary prediction, targeted prevention, and personalized treatment of the disease.

**Working hypothesis and methodology** In our study, we hypothesized that individuals with microbial dysbiosis may be more susceptible to allergic conjunctivitis due to an increased inflammatory response. To verify the working hypothesis, our analysis selected genetic variants linked with gut microbiota features ( $N = 18,340$ ) and allergic conjunctivitis (4513 cases, 649,376 controls) from genome-wide association studies. The inverse-variance weighted (IVW) estimate, Mendelian randomization (MR)-Egger, weighted median estimator, maximum likelihood estimator (MLE), and MR robust adjusted profile score (MR.RAPS) were employed to analyze the impact of gut microbiota on the risk of allergic conjunctivitis and identify allergic conjunctivitis-related gut microbes. Ultimately, these findings may enable the identification of individuals at risk of allergic conjunctivitis through screening of gut microbial imbalances, and allow for new targeted prevention and personalized treatment strategies.

**Results** Genetic liability to *Ruminococcaceae\_UCG\_002* (OR, 0.83; 95% CI, 0.70–0.99;  $P = 4.04 \times 10^{-2}$ ), *Holdemanella* (OR, 0.78; 95% CI, 0.64–0.96;  $P = 2.04 \times 10^{-2}$ ), *Catenibacterium* (OR, 0.69; 95% CI, 0.56–0.86;  $P = 1.09 \times 10^{-3}$ ), *Senegali-massilia* (OR, 0.71; 95% CI, 0.55–0.93;  $P = 1.23 \times 10^{-2}$ ) genus were associated with a low risk of allergic conjunctivitis with IVW. Besides, we found suggestive associations of a genetic-driven increase in the *Oscillospira* (OR, 1.41; 95% CI, 1.00–2.00;  $P = 4.63 \times 10^{-2}$ ) genus with a higher risk of allergic conjunctivitis. Moreover, MLE and MR.RAPS show consistent results with IVW after further validation and strengthened confidence in the true causal associations. No heterogeneity and pleiotropy was detected.

**Conclusions** Our study suggests that gut microbiota may play a causal role in the development of allergic conjunctivitis and provides new insights into the microbiota-mediated mechanism of the disease. Gut microbiota may serve as a target for future predictive diagnostics, targeted prevention, and individualized therapy in allergic conjunctivitis, facilitating the transition from reactive medical services to PPPM in the management of the disease.

**Keywords** Gut microbiota · Allergic conjunctivitis · Predictive, preventive, personalized medicine · Mendelian randomization · Casual association

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## Introduction

### Allergies conjunctivitis as a major challenge of healthcare

Allergic conjunctivitis is one of the most common allergic diseases affecting 10–20% of the population [1]. They are induced by the ocular reaction to environmental allergens and come in many forms. Allergic conjunctivitis usually causes itching, redness, and swelling symptoms, impacting the quality of life and being an economic burden [2]. Some severe forms can negatively impact vision when corneal scarring and pannus develop. As a T helper 2 (Th2) paradigm disease, the allergen reaches the conjunctiva and causes a response in persons with allergic conjunctivitis: Th2 cells secrete cytokines that activate immunoglobulin E (IgE), which binds to the membranes of mast cells and allergen and stimulates the release of inflammatory mediators [3]. Currently, the management of allergic conjunctivitis begins with non-pharmacological treatments and progresses to pharmacological treatments, such as antihistamines, mast cell stabilizers, corticosteroids, and other types of immune-therapy. Identifying the immunological factors associated with allergic conjunctivitis and developing advanced interventions is crucial. This can lead to the discovery of specific immune-related indicators for allergic conjunctivitis, which can strongly encourage the transition from reactive to predictive, preventive, and personalized medicine (PPPM) [4].

### Gut microbiota serves as the hub in holistic PPPM approach

Recently, the human gut microbiota's role as a host partner has been acknowledged [5]. The microbiota in the gut, which are thought to constitute a new organ, influence metabolism and other organs such as the brain, skin, lungs, and liver, by the microbiota and their metabolites [6]. It has been shown that eubiosis has a beneficial effect on human health and has an anti-inflammatory nature. In contrast, microbial dysbiosis may trigger an inflammatory response through Th1, Th2, and Th17 cells [7]. There have been new studies between microbial dysbiosis with allergic diseases. Infants who were given antibiotics in the first 6 months of their lives were more likely to develop allergies later in childhood. This included a greater risk of developing food allergy, food allergy, anaphylaxis, asthma, atopic dermatitis, allergic rhinitis, allergic conjunctivitis, urticaria, contact dermatitis, medication allergy, and other allergies [8]. However, additional allergy symptoms, such as allergic conjunctivitis, may be

mitigated by administering a probiotic supplement containing the *Lactobacillus rhamnosus* GG in the form of an extensively hydrolyzed casein formula [9]. Moreover, probiotics supplementation is believed to prevent allergic disease via improving intestinal barrier mechanisms, modulating the immune system towards anti-inflammatory response, and synthesizing beneficial anti-inflammatory metabolites [10]. Thus far, several studies have established and analyzed the difference in gut microbiota in some allergic diseases compared with healthy population, such as allergic asthma and allergic dermatitis [11, 12]. Nonetheless, there is no study elucidate the genus associated with allergic conjunctivitis by predictive, preventive, and personalized approach.

### Mendelian randomization method helps to implement PPPM

Microorganisms of all shapes and sizes, including bacteria, eukarya, archaea, viruses, and parasites, are found in the human gut microbiota, with the greatest number of microbes in the human body. Gut microbiota composition and function may be altered by various host factors, including but not limited to food, age, delivery method, geographical impacts, and drug [13]. Therefore, small observational studies make it difficult to interpret and compare the microbiota between healthy individuals and disease states. Mendelian randomization (MR) is a widely used method to assess causal relationships by exploiting genetic variants as instrumental exposure variables, which overcomes the bias due to most acquired confounding factors [14]. Some host genetic variations have been shown in recent studies to affect the gut microbiota's composition and function [15]. Evidence linking gut microbiota to immunological and inflammatory diseases such as osteoarthritis, systemic lupus erythematosus, and chronic widespread pain has been derived using MR [16–18]. This study aims to establish a causal relationship between specific gut microbiota and allergic conjunctivitis using MR analysis. Through the lens of PPPM, understanding the role of gut microbial imbalance in the development of allergic conjunctivitis and identifying allergic conjunctivitis-related gut microbiota can aid in early screening of high-risk individuals, timely implementation of targeted prevention, and personalized clinical therapy to alleviate inflammatory responses and allergic symptoms, improve quality of life, and reduce economic burden.

### Working hypothesis in the framework of PPPM

In this study, we hypothesize that individuals with microbial dysbiosis may have a higher incidence of allergic conjunctivitis due to the triggering of an inflammatory response and

the induction of an imbalance in the immune system, resulting in the activation of Th2 cells with varying levels of severity. Additionally, our analysis aims to identify specific gut microbial genera that may contribute to the onset of allergic conjunctivitis, and potentially provide new indicators for this condition. To test our hypothesis, our analysis selected genetic variants associated with gut microbiota features ( $N = 18,340$ ) and allergic conjunctivitis (4513 cases, 649,376 controls). We performed a two-sample MR to assess the impact of gut microbiota on the risk of developing allergic conjunctivitis.

## Methods

### Ethics approval

The published or publicly available data were not involved in primary studies. Nonetheless, each study’s ethical clearance may be found in corresponding primary articles. Furthermore, the 1975 Declaration of Helsinki was adhered to throughout all research projects to ensure ethical conduct.

### Assessment of assumptions

The valid genetic instrumental variables (IVs) in the MR study must fulfill the 3 assumptions [19] (Fig. 1): (1) The IVs must be associated with gut microbiota. (2) The IVs must not be associated with factors that confound the relationship between gut microbiota and allergic conjunctivitis. (3) The IVs are only associated with allergic conjunctivitis through gut microbiota.

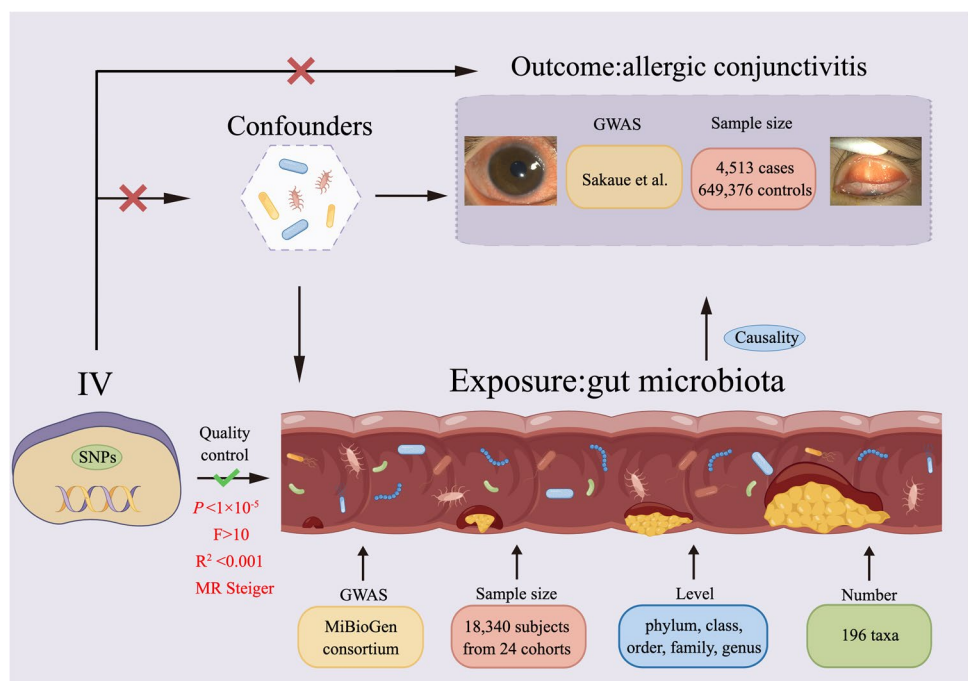
### The data source for gut microbiota

Genome-wide association studies (GWAS) by Kurilshikov et al. provided the gut microbiota genetic variations used here [20]. The MiBioGen consortium was used for this GWAS investigation, and it included 18,340 participants from 24 cohorts in the USA, Canada, Israel, South Korea, Germany, Denmark, the Netherlands, Belgium, Sweden, Finland, and the UK. By comparing GM taxa variation across several populations, the GWAS investigation uncovered a total of 122,110 variant sites representing 211 taxa across five taxonomic levels (phylum, class, order, family, genus), whereas 15 bacterial taxa of these 211 were unknown. Thus, we analyzed the 196 taxa which were already known. We used this massive GWAS to get IVs of GM taxa; further information about the GM data could be obtained in the corresponding original articles.

### Selection of genetic instruments

The gut microbiota-related single nucleotide polymorphisms (SNPs) were screened to get the required IVs. First, there was a locus-wide significance ( $P < 5 \times 10^{-5}$ ) for the SNPs related to the gut microbiota. Additionally, SNPs with linkage disequilibrium (LD) were removed ( $R^2 < 0.001$ ) in the LD data from the 1000 Genomes Project [21]. Furthermore, we eliminated SNPs that included palindromes. The F-statistic was then utilized to exclude low-powered IVs ( $F < 10$ ). Finally, all SNPs were analyzed using MR Steiger, and the SNPs potentially leading to causation were included.

**Fig. 1** Acyclic graph interpretation of Mendelian randomization analysis



## The data source for allergic conjunctivitis

We got the summary statistics for allergic conjunctivitis from the GWAS conducted by Sakaue et al [22]. This GWAS data came from 475,270 people in Europe and 178,619 people in East Asia. There were 4513 cases of allergic conjunctivitis (613 European origin cases and 3,900 East Asian origin cases) and 649,376 controls (474,657 European origin controls and 174,719 East Asian origin controls) used for the analysis, with the data normalized by age, sex, genetic relatedness, genotyping batch, and the first 10 principal components.

## Sensitivity analysis

We applied sensitivity analysis to test and correct for horizontal pleiotropy and heterogeneity and examine the robustness of the MR estimates. Unbalanced pleiotropic effects on the result were first evaluated using MR-Egger's intercept values [23]. The closer the intercept to zero, the lower the unbalanced pleiotropic was considered. Meanwhile, SNPs with anomalies were filtered out using MR-Pleiotropy RESidual Sum and Outlier (MR-PRESSO), a method for assessing the pleiotropy of causative SNPs [24]. To determine heterogeneity, however, we employed Cochran's  $Q$  test [25]. After completing the analysis, the leave-one-out test was used to evaluate the results' robustness further [16].

## Statistical method

Both the "Two Sample MR" and "MR-PRESSO" packages in R software (version 1.4.1717) were used to estimate the MR and sensitivity findings. A causal impact of individual SNPs was evaluated using the Wald ratio. Inverse variance weighting (IVW) was the main method to obtaining a global estimate as long as sensitivity analysis was successful. Additionally, the findings of IVW were analyzed using the weighted median (WM) estimator and the MR-Egger. The WM estimator provided a consistent estimate when over 50% of the weight came from valid IVs [26]. For the MR-Egger, it allows for little evidence of a violation of the IV3 assumption (instrumental variable assumption 3), which means permitting a low degree of evidence of the pleiotropic effects and still obtaining an unbiased causal estimate [27]. However, its statistical power decreased. Thus, it is generally believed that the results of IVW are the most meaningful when passing the sensitivity analysis. Additionally, we employed the maximum likelihood estimator (MLE) [28] and MR robust adjusted profile score (MR.RAPS) [29] to validate MR results. MLE assumes the linear correlation of allergic conjunctivitis and each gut microbiota taxa with normal distribution and allows for uncertainty in both

gene-gut microbiota taxa and gene-allergic conjunctivitis associations. MR.RAPS was calculated for the causal estimate to provide higher statistical power if weak IVs existence. Consequently, all valid SNPs' combined MR estimates were shown as a circular map. The MR findings were presented using odds ratio (OR) and confidence interval (CI). Assuming a significance level of  $P < 0.05$ , we inferred the possibility of a causal relationship.

## Results

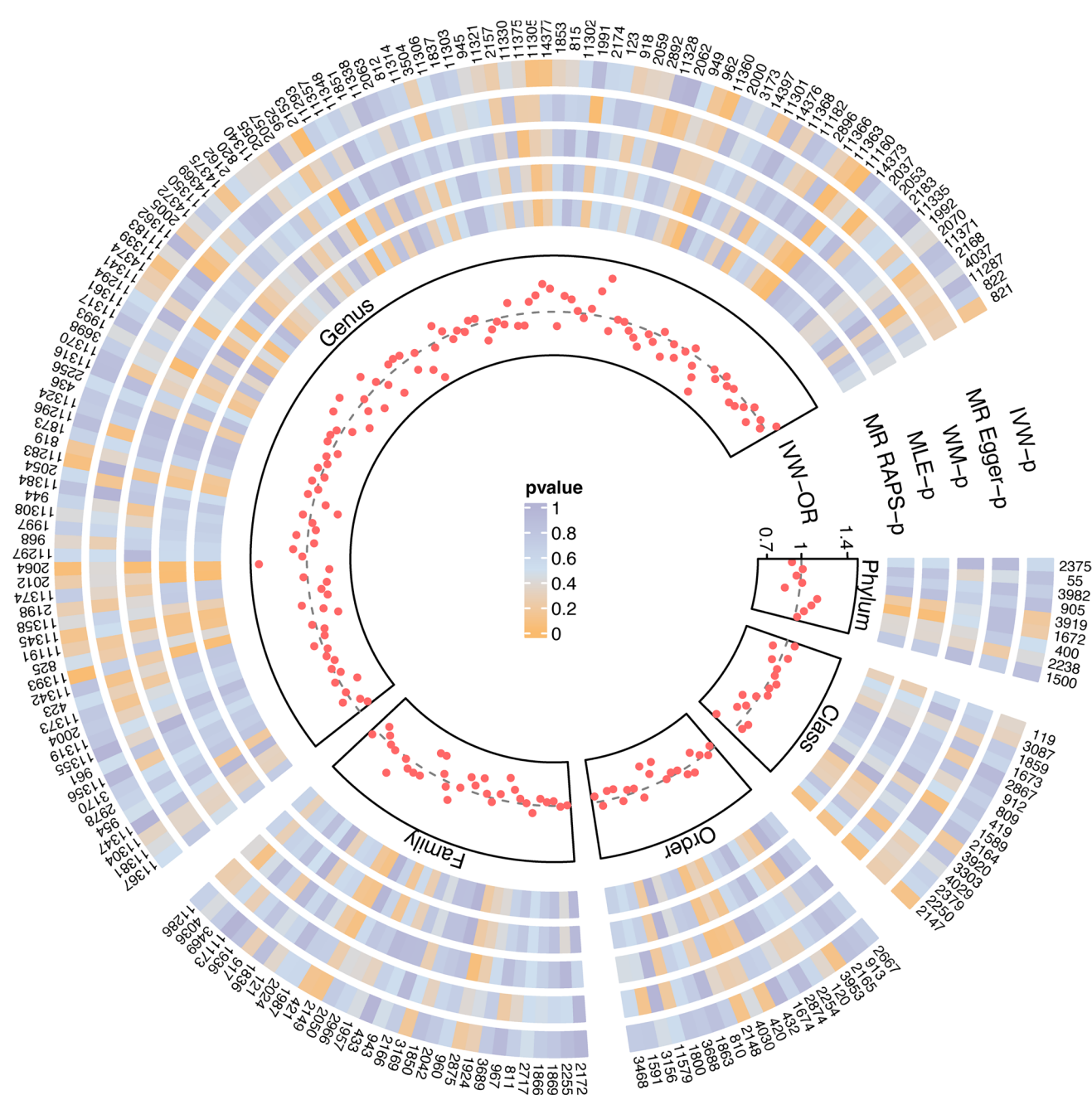
### Instrumental variable selection

Using the MiBioGen consortium, we identified 2166 SNPs as IVs linked with 196 bacterial taxa for allergic conjunctivitis ( $P < 1 \times 10^{-5}$ ); quality control processes via LD effects and palindromic were performed to ensure their accuracy. There were 9 phyla, 16 classes, 20 orders, 32 families, and 119 genera. The  $F$  values for each SNP varied from 14.59 to 88.43, demonstrating that they all had sufficient validity. Supplementary Table 1 displays the most crucial data for all IVs.

### Causal associations of gut microbiota with allergic conjunctivitis

Figure 2 and Supplementary Table 2 show the impact of changes in 196 bacterial taxa abundance on allergic conjunctivitis risk based on data from the GWAS of the Sakaue et al.'s study. After MR analysis, the overall estimate calculated with IVW revealed that 5 genera of gut microbiota were genetically predicted for the causal association with allergic conjunctivitis. Our results suggest the decrease of allergic conjunctivitis risk could attribute to the increase of *Ruminococcaceae\_UCG\_002* (OR, 0.83; 95% CI, 0.70–0.99;  $P = 4.04 \times 10^{-2}$ ), *Holdemanella* (OR, 0.78; 95% CI, 0.64–0.96;  $P = 2.04 \times 10^{-2}$ ), *Catenibacterium* (OR, 0.69; 95% CI, 0.56–0.86;  $P = 1.09 \times 10^{-3}$ ), and *Senegalimassilia* (OR, 0.71; 95% CI, 0.55–0.93;  $P = 1.23 \times 10^{-2}$ ) genus, whereas the host-genetic-driven increases in the *Oscillospira* genus were potentially related to a higher risk of allergic conjunctivitis (OR, 1.41; 95% CI, 1.00–2.00;  $P = 4.63 \times 10^{-2}$ ) (Fig. 2). The results of weight median also provide the evidence of a protective effect of the genetic-driven increase in *Holdemanella* (OR, 0.83; 95% CI, 0.63–1.11;  $P = 0.21$ ), and *Ruminococcaceae\_UCG\_002* (OR, 0.86; 95% CI, 0.66–1.13;  $P = 0.29$ ) on the risk of allergic conjunctivitis. MR-Egger had not shown a causal association between the five genera and allergic conjunctivitis ( $P \geq 0.21$ ). Furthermore, MR.RAPS and MLE were employed to confirm the IVW results, and





**Fig. 2** SNPs influence the causal-effect with five MR methods. Each red dot represents the causal effect on allergic conjunctivitis of each SNP with IVW, and each region corresponds to a different level of gut microbiota, including phylum, class, order, family, and genus. The gray dashed line represents OR=1. Circles from outside are the P-value of IVW, the P-value for MR-Egger, the P-value for WM, the

P-value for MLE, and the P-value for MR-RAPS. The outermost circle is the ID of each gut microbiota, which corresponds to the bacterial taxon name in Supplementary Table 1. Abbreviations: OR, odds ratio; IVW, inverse-variance-weighted estimate; WM, weighted median estimator; MLE, maximum likelihood estimator; MR.RAPS, MR robust adjusted profile score

the results of these methods evidenced that *Ruminococcaceae\_UCG\_002*, *Holdemanella*, *Catenibacterium*, *Senegalimassilia*, and *Oscillospira* genus are significantly associated with allergic conjunctivitis development ( $P \leq 0.043$ ), which strengthened the confidence of the causal associations (Table 1).

**Sensitivity analyses**

The results of all sensitivity assessments are then tallied and shown in Fig. 3. Cochrane’s Q reveals no heterogeneity for the SNPs that genetically predict allergic conjunctivitis, and the MR-Egger regression intercept indicated no unbalanced

**Table 1** MR estimate for the association between gut microbiota and allergic conjunctivitis

Exposure level	Microbiota	MR method	OR	95% CI	P
Genus	<i>Catenibacterium</i>	IVW	0.69	0.56–0.86	$1.09 \times 10^{-3}$
		WM	0.70	0.52–0.94	$1.93 \times 10^{-2}$
		MR-Egger	0.28	0.03–2.45	0.33
		MLE	0.69	0.61–0.78	$2.46 \times 10^{-3}$
		MR-RAPS	0.69	0.61–0.79	$4.08 \times 10^{-3}$
Genus	<i>Senegalimassilia</i>	IVW	0.71	0.55–0.93	$1.24 \times 10^{-2}$
		WM	0.67	0.48–0.95	$2.35 \times 10^{-2}$
		MR-Egger	0.50	0.19–1.32	0.25
		MLE	0.77	0.69–0.85	$1.18 \times 10^{-2}$
		MR-RAPS	0.77	0.69–0.85	$1.28 \times 10^{-2}$
Genus	<i>Holdemanella</i>	IVW	0.78	0.64–0.96	$2.05 \times 10^{-2}$
		WM	0.83	0.63–1.11	0.21
		MR-Egger	0.79	0.27–2.31	0.68
		MLE	0.83	0.75–0.91	$4.01 \times 10^{-2}$
		MR-RAPS	0.82	0.74–0.90	$4.32 \times 10^{-2}$
Genus	<i>Ruminococcaceae_UG_002</i>	IVW	0.83	0.70–0.99	$4.04 \times 10^{-2}$
		WM	0.86	0.66–1.13	0.29
		MR-Egger	0.72	0.43–1.19	0.21
		MLE	0.83	0.77–0.90	$2.56 \times 10^{-2}$
		MR-RAPS	0.83	0.76–0.90	$2.61 \times 10^{-2}$
Genus	<i>Oscillospira</i>	IVW	1.42	1.01–2.00	$4.63 \times 10^{-2}$
		WM	1.63	1.06–2.51	$2.69 \times 10^{-2}$
		MR-Egger	2.37	0.37–15.43	0.40
		MLE	1.34	1.17–1.55	$3.57 \times 10^{-2}$
		MR-RAPS	1.34	1.16–1.55	$4.20 \times 10^{-2}$

OR odds ratio, IVW inverse-variance-weighted estimate, WM weighted median estimator, MLE maximum likelihood estimator, MR.RAPS MR robust adjusted profile score

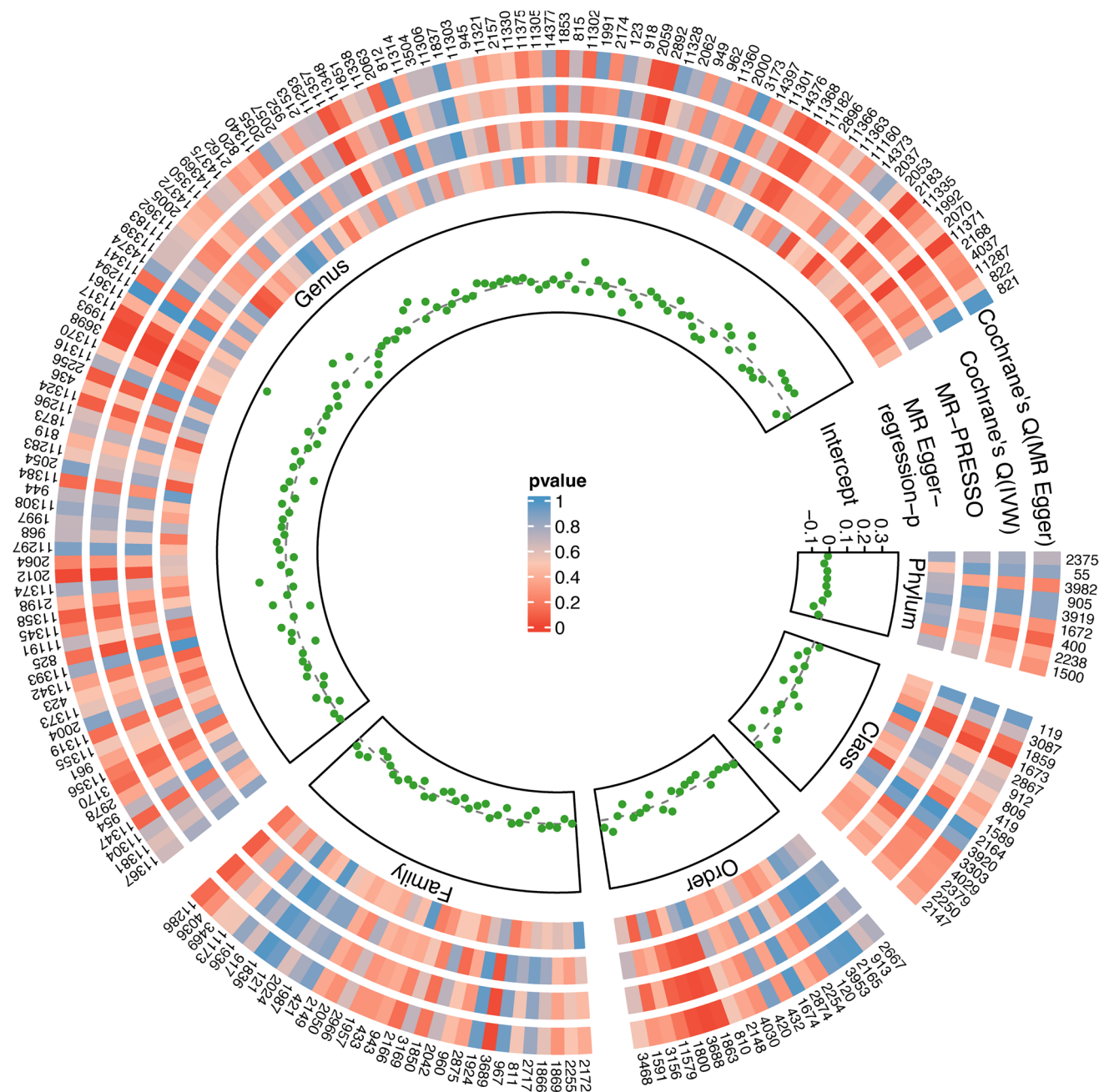
pleiotropy for risk of allergic conjunctivitis (all  $P > 0.05$ ; Table 2). Additionally, horizontal pleiotropy, which was not rectified by MR-Egger regression, was tested using MR-PRESSO (Table 2). No single SNP was found to notably impact our MR findings using the “leave-one-out” evaluation, as indicated in Supplementary Figure 1. Thus, the IVW, MLE, and MR.RAPS should be considered the main results, given that no substantial heterogeneity or unbalanced pleiotropy is present for allergic conjunctivitis risk. Therefore, the risk of allergic conjunctivitis may be strongly linked to the presence of the bacteria of the genera *Ruminococcaceae\_UG\_002*, *Holdemanella*, *Catenibacterium*, *Senegalimassilia*, and *Oscillospira*, as shown by the MR power calculation (Fig. 3 and Table 1).

## Discussion

### Implementing PPPM for prevention and management of ocular diseases

Severe non-communicable disorders pose a significant social and economic burden on healthcare and society. This has

prompted a shift towards implementing PPPM medicine, which focuses on preventative measures rather than reactive treatment [30]. Recently, PPPM provides a new approach to manage the ocular related diseases. One important example is the highlighting of protein profiles in tears and the exemplification of corresponding biomarkers for several relevant pathologies, such as dry eye disease and diabetic retinopathy. These biomarkers could predict disease development, target preventive measures, and facilitate the creation of treatment algorithms tailored to individual patient profiles [31]. Another prominent example is the strong recommendation to apply the “Flammer Syndrome Phenotype” questionnaire to select potential Sjögren’s syndrome patients, who can be detected early in life during the reversible phase of health adverse effects. This can lead to cost-effective targeted prevention [32]. In the context of PPPM, identifying reliable risk factors is crucial for effective disease management and control. In this direction, we can evaluate the risk factors and enforce effective interventions to suppress the disease initiation and progression. Previous researches have reported parental history of allergic disease, city residence at birth, regular meat, margarine consumption, and passive smoking are risk factors for allergic conjunctivitis



**Fig. 3** Sensitivity analysis results of all SNPs. Each green dot represents the intercept of MR-Egger, and each region corresponds to a different level of gut microbiota, including phylum, class, order, family, and genus. The gray dashed line represents intercept = 0. Circles from outside are the *P*-values for Cochrane’s Q (MR-Egger), Cochrane’s Q (IVW), and the *P*-values for the MR-PRESSO, the

*P*-values for the MR-Egger regression. The outermost circle is the ID of each gut microbiota, which corresponds to the bacterial taxon name in Supplementary Table 1. Abbreviations: IVW, inverse-variance-weighted estimate; MR, Mendelian randomization. MR-PRESSO, MR-Pleiotropy RESidual Sum and Outlier

[33, 34]. However, the current treatment of allergic conjunctivitis, which includes both non-pharmacological and pharmacological measures, is primarily reactive in nature and does not focus on preventative measures. Non-pharmacological measures for managing allergic conjunctivitis involve taking specific actions to reduce exposure to environmental allergens. Topical

measures, such as non-steroidal anti-inflammatory agents and corticosteroids, as well as systemic pharmacological measures and immunotherapy, are also commonly used [2]. Therefore, it is need to seek special risk factors could also guide subsequent precise and individualized treatment for allergic conjunctivitis. In this study, the causal associations comprehensively

**Table 2** Sensitivity analyses of MR analyses of gut microbiota on allergic conjunctivitis

Microbiota	Pleiotropy		Heterogeneity	
	MR-Egger intercept <i>P</i> -value	MR-PRESSO <i>P</i> -value	Cochrane's Q <i>P</i> -value	Cochrane's Q <i>P</i> -value
<i>Catenibacterium</i>	0.47	0.77	0.69	0.66
<i>Senegalimassilia</i>	0.50	0.23	0.34	0.28
<i>Holdemanella.id.</i>	0.99	0.94	0.87	0.80
<i>Ruminococcaceae_UCG_002</i>	0.55	0.60	0.35	0.31
<i>Oscillospira</i>	0.60	0.39	0.27	0.22

MR Mendelian randomization, MR-PRESSO MR-Pleiotropy RESidual Sum and Outlier

and deeply between gut microbiota and allergic conjunctivitis based on publicly available genetic databases in multi-populations were investigated for the first time, and we established 5 distinct microbial genera are pivotal in the development of allergic conjunctivitis.

### Allergic conjunctivitis reflects systemic impairments

The World Health Organization has reported that individuals with allergies often experience a suboptimal quality of life, which can lead to an adverse economic impact [35]. The World Allergy Organization has expressed concern over the increasing global burden of allergic diseases, especially in children and developing countries [36]. Allergic conjunctivitis refers to a group of heterogeneous ocular inflammatory conditions that are modulated by mast cells activation and can affect the conjunctiva, eyelids, and cornea. The most prevalent symptoms are ocular itching and blurred vision. Recently, the Ocular Allergy group of the European Academy of Allergy and Clinical Immunology revised the classification of allergic conjunctivitis, distinguishing between two types of ocular surface hypersensitivity disorders: ocular allergy and ocular non-allergic hypersensitivity. Ocular allergy is divided into IgE-mediated or non-IgE-mediated mechanisms. The IgE-mediated type includes seasonal allergic conjunctivitis (SAC), perennial allergic conjunctivitis (PAC), vernal keratoconjunctivitis (VKC), and atopic keratoconjunctivitis (AKC). The non-IgE-mediated forms include contact blepharconjunctivitis, VKC, and AKC. Included in the category of ocular non-allergic hypersensitivity are various conditions such as giant papillary conjunctivitis, irritative conjunctivitis, irritative blepharitis, and other mixed or borderline forms. Currently, approximately 20% of the world population is affected by some form of allergy, with up to 40–60% of allergic patients experiencing ocular symptoms [37, 38]. Furthermore, allergic conjunctivitis is often associated with other allergic symptoms and diseases, such as asthma, rhinitis, and atopic dermatitis, indicating an abnormal immune system response to certain substances that leads to systemic inflammation and

tissue damage [34]. For instance, SAC and PAC are the ocular forms of a systemic allergic disorder (a type 1 IgE-dependent hypersensitivity), which is typically manifested in the respiratory system as allergic rhinitis and/or asthma [39]. Additionally, VKC children have a higher prevalence of Ig deficiency and vitamin D deficiency, and 15–60% of affected children may also present with other atopic diseases [40, 41]. Moreover, AKC involves IgE-mediated, Th1-mediated allergy, and delayed-type hypersensitivity mechanisms, and can cause more severe ocular symptoms and signs, as well as a higher prevalence of atopic dermatitis and asthma [40]. Numerous studies have demonstrated that ocular disorders can indicate systemic impairments in the holistic PPPM approach [42, 43], such as diabetic retinopathy serving as an early event and a reliable predictor of severe complications associated with diabetes mellitus [44]. Therefore, allergic conjunctivitis may serve as an early indicator and reliable predictor of systemic dysfunction, making it crucial for predicting and preventing associated systemic pathologies.

### The systemic impact of gut microbiota

The gut microbiota is established before birth, and seven bacterial classes—Firmicutes, Bacteroidetes, Actinobacteria, Fusobacteria, Proteobacteria, Verrucomicrobia, and Cyanobacteria—tend to thrive in this environment [45]. Recent research has demonstrated that the mammalian gut microbiota positively impacts the host, including food provision, catabolism of indigestible chemicals, suppression of opportunistic pathogen colonization, and even participation in the growth and development of gut structures [13]. In addition, pattern recognition receptors—detection of pathogen-associated molecular patterns, antigen exposure and presentation, and epigenetic modification through metabolic by gut microbiota's products such as short-chain fatty acids (SCFAs)—mediated interactions between gut commensal bacteria and the developing characteristics and functions of the human immune system [46]. It also contributes to the enhancement of the immune system, plays a vital role in



digestion and metabolism, regulates epithelial cell proliferation and differentiation, modifies insulin resistance, and affects its secretion [47]. Furthermore, the gut microbiota influences brain-gut communication, impacting the mental and neurological functions of the host [48]. Therefore, the gut microbiota plays a significant role in maintaining normal gut physiology and overall health. Tachalov and colleagues recently demonstrated the crucial role of maintaining optimal oral hygiene practices for improving individual outcomes and reducing morbidity during the COVID-19 pandemic, within the context of PPPM. Their suggested pathomechanisms consider potential preferences in the interaction between the viral particles and the host microbiota including oral cavity, and the respiratory and gastrointestinal tracts [49]. It is known that alterations to the innate immune system, which may result from gut microbiota dysbiosis, have been linked to various illnesses, including allergies and autoimmune disorders [50]. Hence, from the perspective of PPPM, correcting gut microbiota imbalance should be taken into consideration when developing targeted interventions and preventative measures for immunity-related diseases, such as allergic conjunctivitis [51].

### Link between gut microbiota and allergic disorders

Food allergy, atopic dermatitis, eczema, asthma, hay fever, allergic rhinitis, and allergic conjunctivitis are among the many types of allergic illnesses affecting an ever-increasing number of individuals throughout the globe. Strachan proposed the “hygiene hypothesis” in 1989, which states that the incidence of allergy diseases is correlated with decreasing exposure to environmental microbes [52]. Researchers back up this hypothesis with data from animal research and many epidemiological investigations [53]. An IgE-mediated Th2 immune response is significantly linked to allergic disorders. When allergens stimulate antigen-presenting cells, they deliver allergens to Th0 cells, which then develop into Th2 cells in response to cytokines like IL-4. Th2 cells, on the other hand, secrete cytokines, including interleukin-4 (IL-4), interleukin-5 (IL-5), and interleukin-13 (IL-13), which encourage B cells to make IgE and attach to the surface of sensitized effector cells like mast cells and basophils. Cell-mediated immunity, notably against intracellular bacterial and viral infections, autoimmunity, and tumor defense, were linked instead to Th1 responses. Furthermore, Th1-derived cytokines such as IFN- $\gamma$  may stifle the differentiation of Th2 cells. As a bonus, IL-23 signaling may activate Th17, characterized by IL-17A production. So far, we have known the dynamic cross regulation between the mediators directing lymphocyte polarization exists, and dominant Th2 cell differentiation is one of the immunological bases of IgE-mediated allergic reactions [54]. Based on these evidences, it is not hard to conclude the factors that affect the immunity system homestasis, and

enhanced IL4/IL13-driven response is the essential for allergy. Recently, genetic predisposition associated with a preponderance of type 2 immunity was confirmed contribute to the development of allergic disorders [55]. Further, the microbiota in one’s digestive tract may have a role in developing allergic disorders [56]. In light of these facts, we suggest that a hereditary predisposition to the effects of altered gut flora contributes to allergic disorders. In addition, Delday et al. [57] found fewer species of the genus *Bacteroides* and more species of the genus Firmicutes in infants with atopic dermatitis. Similarly, Stockholm et al. [11] found that children who developed asthma had reduced relative abundances of *Lachnospiraceae* (including *Lachnospiraceae incertae sedis* and *Roseburia*) and *Ruminococcaceae* (including *Ruminococcus* and *Faecalibacterium*). However, the mechanism connecting certain gut microbiota with allergic conjunctivitis has not been explored till now and warrants more study.

### Immunomodulatory functions of *Ruminococcaceae*\_UCG\_002, *Holdemanella*, *Catenibacterium*, and *Oscillospira* genera

Changes in Firmicutes and Bacteroidetes are particularly significant in many pathological states since these two constitute the vast majority of the bacterial population. Trans-differentiation of Th17 cells into regulatory T cells (Tregs) is connected with the ratio of Firmicutes to Bacteroidetes, which could be used as a disease predictor [58]. Our investigation revealed that the genera *Ruminococcaceae*\_UCG\_002, *Holdemanella*, *Catenibacterium*, and *Oscillospira*, all of the phylum Firmicutes, are associated with the onset of allergic conjunctivitis. *Ruminococcaceae*\_UCG\_002 can convert certain primary bile acids into secondary bile acids since it is a member of the bile salt hydrolase and 7-dehydroxylase-active family *Ruminococcaceae* [59]. As we know, bile acids exert hormone-like functions by activating FXR and TGR5 and the dysregulated bile acid pool may lead to perturbations in multiple pathological processes, such as immune regulation and lipid and glucose homeostasis. In addition, prior research has linked the *Ruminococcaceae*\_UCG\_002 genera to immune regulation and shown an inverse association between their level and C-reactive protein [59, 60]. *Holdemanella* biformis is inversely correlated with C-reactive protein levels [61]. Several researchers discovered that *Holdemanella* could create a high quantity of C18-3OH, and mice treated orally with C18-3OH were less likely to develop colitis after being given dextran sulfate sodium [62]. Thus, it was hypothesized that *Holdemanella*, through C18-3OH, may influence the host’s immunological and neuroendocrine communication pathways. Recurrent oral and vaginal ulcers, skin lesions, and uveitis are classic symptoms of Behçet’s illness, a Th1 paradigm disease. Behçet’s disease patients have significantly

greater levels of *Catenibacterium* than the general population [63]. Due to its prevalence, *Catenibacterium* was likely linked to Th1 trans-differentiation, which IL-6 controls. These results suggest that the bacterial genera *Ruminococcaceae\_UCG\_002*, *Holdemanella*, and *Catenibacterium* are all engaged in immunomodulation via regulating lymphocyte polarization and affecting the dynamic cross-regulation of immune cells.

Comparatively, the functions of the three other genera of gut microbes that demonstrate pathogenic potential are consistent except for the *Oscillospira* genus. Consistent with our findings, Canani et al. [64] reported that after treatment with *Lactobacillus rhamnosus GG*, the only species substantially different between tolerant and allergic babies with cow's milk allergy was *Oscillospira* and that there was a large quantity of *Oscillospira* in the allergic samples. *Oscillospira* overabundance was also positively correlated with the onset of diabetes and inflammation in animals with obesity, type 2 diabetes mellitus, and dextran sulfate sodium-induced ulcerative colitis [65]. Recent research has shown that *Oscillospira* may create a wide variety of SCFAs, with butyrate being the most abundant and strongly correlated to inflammatory disorders [66]. As described previously, early intestinal dysbiosis activates pro-allergic processes and increases the risk of allergy by the mechanisms disrupting the balance between Th1 and Th2 cells. Taken together, suppressing Th17 cells, enhancing IL-10 production, and activating the Treg/Th2 are the essential factors which induce the immune system balance, and might be the pathology mechanics of enriched *Oscillospira* and impoverished *Ruminococcaceae\_UCG\_002*, *Holdemanella*, and *Catenibacterium* in allergic conjunctivitis. Future molecular and clinical studies are needed to understand the underlying processes better.

### Study limitations

Although large-scale GWAS studies allow for more solid inferences, they are not without flaws. For one, the majority of participants in the GWAS were of European ancestry, the causal relationship between gut microbiota alteration and allergic conjunctivitis might be limited in others ethnic group. Secondly, we selected the GWAS data of exposure (gut microbiota) and outcome (allergic conjunctivitis) from publicly available summary data; however, it is impossible to establish whether overlapping subjects were engaged in the two samples' MR analyses. Third, due to the limitation of the database, we could not distinguish different forms of allergic conjunctivitis. Further research is needed to test the gut microbiota alteration in different types of allergic conjunctivitis, aiming to promote a PPM in patients with allergic conjunctivitis. Fourth, we did not account for multiple testing because

a rigorous multiple testing correction may neglect potential strains that are causally related to allergic conjunctivitis.

## Conclusions and expert recommendations

To summarize, our results give an insight into the gut microbiota alteration role in the pathophysiology of allergic diseases and broaden our knowledge of allergic conjunctivitis and gut microbiota. Our study is the first study to comprehensively assess the causal effects of the gut microbiota on allergic conjunctivitis and identified that the reduction of *Ruminococcaceae\_UCG\_002*, *Holdemanella*, *Catenibacterium*, and *Senegalimassilia* genus made a causal contribution to allergic conjunctivitis, whereas the genera *Oscillospira* are potentially increased the occurrence of allergic conjunctivitis, which could provide new indicators for early prediction of allergic conjunctivitis.

### Innovative screening and targeted interventions

Stool samples can be obtained noninvasively, making them an idea source for analyzing the human gut microbial. With the advent of high-throughput sequencing technologies targeting the 16S rDNA gene, it is now possible to quickly and conveniently analyze the microbial communities in the gut using stool samples [67]. Knowing the change of gut microbial is crucial for predictive diagnosis and targeted prevention. Moreover, allergic conjunctivitis may serve as an early indicator and reliable predictor of systemic dysfunction, such as atopic dermatitis and asthma. Effective early identification of allergic conjunctivitis can provide targeted protection for patients to avoid the impact of allergic conjunctivitis and potential systemic diseases that may exist. Therefore, 16S rDNA high-throughput sequencing is strongly recommended to select individuals at high risk, and targeted preventive measures can be triggered early in life, which conforms to the claim of primary prevention. Individuals who are at risk of a decrease in *Ruminococcaceae\_UCG\_002*, *Holdemanella*, *Catenibacterium*, and *Senegalimassilia* and an increase in *Oscillospira* levels should be a priority for healthcare providers. Early aggressive treatment to correct microbial dysbiosis, such as dietary changes, taking probiotic and prebiotic supplements, and targeted antibiotics, should be considered [68]. For example, direct addition of *Ruminococcaceae\_UCG\_002* probiotics can be given to boost its levels, while targeted antibiotics can be used to suppress the growth of *Oscillospira*. In addition, high-throughput sequencing of 16S rDNA can be a routine examination to identify changes in gut microbial in patients with allergic conjunctivitis. The novel personalized treatments such as detecting the gut microbiota individually, designing person-related probiotic strains, changing diet, and taking

specific antibiotics may help ameliorate the inflammatory response and symptoms, and improve the quality of life in the early stages of allergic conjunctivitis. This is important for secondary prevention of the disease.

### Future directions for research on gut microbiota and allergic conjunctivitis

Our understanding of the role of gut microbiota in balancing and modulating the immune response, particularly in the pathophysiology of autoimmune and allergic diseases, has advanced significantly in recent years. As a result, specific gut microbial dysbiosis can induce immune system dysregulation, leading to the activation of Th2 cells and the development of allergic conjunctivitis. Therefore, a comprehensive understanding of the biomolecular modifications responsible for gut microbial dysbiosis is crucial in the discovery of effective preventive and predictive measures. Further research is necessary not only to verify specific allergic conjunctivitis-related gut microbes, but also to confirm the mechanism and causal association in animal models and clinical trials. Ultimately, this will lead to the transition from reactive medical services to PPPM in the management of allergic conjunctivitis.

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**Author contribution** Conceptualization, J.Z.; methodology, Y.-J.C.; investigation, K.-C. L.; writing—original draft, J.Z.; writing—review and editing, J.Z.; resources, R.-L. Y.; and supervision, K.S.

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**Data availability** All data used for this study are publicly available and their original studies are cited from (<https://mibiogen.gcc.rug.nl/> and <https://www.nature.com/articles/s41588-021-00931-x>).

**Code availability** All software applications used are included in this article.

### Declarations

**Ethics approval** The published or publicly available data were not involved in primary studies. Nonetheless, each study's ethical clearance may be found in corresponding primary articles. Furthermore, the 1975 Declaration of Helsinki was adhered to throughout all research projects to ensure ethical conduct.

**Consent to participate** Not applicable.

**Consent for publication** Not applicable.

**Conflict of interest** The authors declare no competing interests.

### References

- Warner JO, Kaliner MA, Crisci CD, et al. Allergy practice worldwide: a report by the World Allergy Organization Specialty and Training Council. *Int Arch Allergy Immunol*. 2006;139(2):166–74. <https://doi.org/10.1159/000090502>.
- Bielory L, Delgado L, Katelaris CH, Leonardi A, Rosario N, Vichyanoud P. ICON: Diagnosis and management of allergic conjunctivitis. *Ann. Allergy Asthma Immunol: official publication of the American College of Allergy, Asthma, & Immunology*. 2020;124(2):118–34. <https://doi.org/10.1016/j.anai.2019.11.014>.
- Bridgewood C, Newton D, Bragazzi N, Wittmann M, McGonagle D. Unexpected connections of the IL-23/IL-17 and IL-4/IL-13 cytokine axes in inflammatory arthritis and enthesitis. *Semin Immunol*. 2021;101520. <https://doi.org/10.1016/j.smim.2021.101520>.
- Golubnitschaja O, Kubatka P, Mazurakova A, et al. Systemic effects reflected in specific biomarker patterns are instrumental for the paradigm change in prostate cancer management: a strategic paper. *Cancers*. 2022;14(3) <https://doi.org/10.3390/cancers14030675>.
- Riccio P, Rossano R. The human gut microbiota is neither an organ nor a commensal. *FEBS Lett*. 2020;594(20):3262–71. <https://doi.org/10.1002/1873-3468.13946>.
- Simonyté Sjödin K, Hammarström ML, Rydén P, et al. Temporal and long-term gut microbiota variation in allergic disease: a prospective study from infancy to school age. *Allergy*. 2019;74(1):176–85. <https://doi.org/10.1111/all.13485>.
- Gomaa EZ. Human gut microbiota/microbiome in health and diseases: a review. *Antonie van Leeuwenhoek*. 2020;113(12):2019–40. <https://doi.org/10.1007/s10482-020-01474-7>.
- Koidl L, Untersmayr E. The clinical implications of the microbiome in the development of allergy diseases. *Expert Rev Clin Immunol*. 2021;17(2):115–26. <https://doi.org/10.1080/1744666x.2021.1874353>.
- Nocerino R, Bedogni G, Carucci L, et al. The impact of formula choice for the management of pediatric cow's milk allergy on the occurrence of other allergic manifestations: the Atopic March cohort Study. *J Pediatr*. 2021;232:183–91.e3. <https://doi.org/10.1016/j.jpeds.2021.01.059>.
- Qamer S, Deshmukh M, Patole S. Probiotics for cow's milk protein allergy: a systematic review of randomized controlled trials. *Eur J Pediatr*. 2019;178(8):1139–49. <https://doi.org/10.1007/s00431-019-03397-6>.
- Stokholm J, Blaser MJ, Thorsen J, et al. Maturation of the gut microbiome and risk of asthma in childhood. *Nat Commun*. 2018;9(1):141. <https://doi.org/10.1038/s41467-017-02573-2>.
- Park DH, Kim JW, Park HJ, Hahm DH. Comparative analysis of the microbiome across the gut-skin axis in atopic dermatitis. *Int J Mol Sci*. 2021;22(8) <https://doi.org/10.3390/ijms22084228>.
- Adak A, Khan MR. An insight into gut microbiota and its functionalities. *Cell Mol Life Sci*. 2019;76(3):473–93. <https://doi.org/10.1007/s00018-018-2943-4>.
- Bowden J, Holmes MV. Meta-analysis and Mendelian randomization: a review. *Res Synth Methods*. 2019;10(4):486–96. <https://doi.org/10.1002/jrsm.1346>.
- Goodrich JK, Davenport ER, Clark AG, Ley RE. The relationship between the human genome and microbiome comes into view. *Annu Rev Genet*. 2017;51:413–33. <https://doi.org/10.1146/annurev-genet-110711-155532>.

16. Xiang K, Wang P, Xu Z, et al. Causal effects of gut microbiome on systemic lupus erythematosus: a two-sample mendelian randomization study. *Front Immunol.* 2021;12:667097. <https://doi.org/10.3389/fimmu.2021.667097>.
17. Yu XH, Yang YQ, Cao RR, Bo L, Lei SF. The causal role of gut microbiota in development of osteoarthritis. *Osteoarthr Cartil.* 2021;29(12):1741–50. <https://doi.org/10.1016/j.joca.2021.08.003>.
18. Freidin MB, Stalteri MA, Wells PM, et al. An association between chronic widespread pain and the gut microbiome. *Rheumatology (Oxford, England).* 2021;60(8):3727–37. <https://doi.org/10.1093/rheumatology/keaa847>.
19. König IR, Greco FMD. Mendelian randomization: progressing towards understanding causality. *Ann Neurol.* 2018;84(2):176–7. <https://doi.org/10.1002/ana.25293>.
20. Kurilshikov A, Medina-Gomez C, Bacigalupe R, et al. Large-scale association analyses identify host factors influencing human gut microbiome composition. *Nat Genet.* 2021;53(2):156–65. <https://doi.org/10.1038/s41588-020-00763-1>.
21. Auton A, Brooks LD, Durbin RM, et al. A global reference for human genetic variation. *Nature.* 2015;526(7571):68–74. <https://doi.org/10.1038/nature15393>.
22. Sakaue S, Kanai M, Tanigawa Y, et al. A cross-population atlas of genetic associations for 220 human phenotypes. *Nat Genet.* 2021;53(10):1415–24. <https://doi.org/10.1038/s41588-021-00931-x>.
23. Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. *Int J Epidemiol.* 2015;44(2):512–25. <https://doi.org/10.1093/ije/dyv080>.
24. Verbanck M, Chen CY, Neale B, Do R. Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. *Nat Genet.* 2018;50(5):693–8. <https://doi.org/10.1038/s41588-018-0099-7>.
25. Hemani G, Bowden J, Davey SG. Evaluating the potential role of pleiotropy in Mendelian randomization studies. *Hum Mol Genet.* 2018;27(R2):R195–r208. <https://doi.org/10.1093/hmg/ddy163>.
26. Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent estimation in Mendelian randomization with some invalid instruments using a weighted median estimator. *Genet Epidemiol.* 2016;40(4):304–14. <https://doi.org/10.1002/gepi.21965>.
27. Bowden J, Del Greco MF, Minelli C, Davey Smith G, Sheehan NA, Thompson JR. Assessing the suitability of summary data for two-sample Mendelian randomization analyses using MR-Egger regression: the role of the I2 statistic. *Int J Epidemiol.* 2016;45(6):1961–74. <https://doi.org/10.1093/ije/dyw220>.
28. Burgess S, Scott RA, Timpson NJ, Davey Smith G, Thompson SG. Using published data in Mendelian randomization: a blueprint for efficient identification of causal risk factors. *Eur J Epidemiol.* 2015;30(7):543–52. <https://doi.org/10.1007/s10654-015-0011-z>.
29. Zhao Q, Chen Y, Wang J, Small DS. Powerful three-sample genome-wide design and robust statistical inference in summary-data Mendelian randomization. *Int J Epidemiol.* 2019;48(5):1478–92. <https://doi.org/10.1093/ije/dyz142>.
30. Golubnitschaja O, Baban B, Boniolo G, et al. Medicine in the early twenty-first century: paradigm and anticipation - EPMA position paper 2016. *EPMA J.* 2016;7(1):23. <https://doi.org/10.1186/s13167-016-0072-4>.
31. Zhan X, Li J, Guo Y, Golubnitschaja O. Mass spectrometry analysis of human tear fluid biomarkers specific for ocular and systemic diseases in the context of 3P medicine. *EPMA J.* 2021;12(4):449–75. <https://doi.org/10.1007/s13167-021-00265-y>.
32. Kunin A, Polivka J Jr, Moiseeva N, Golubnitschaja O. "Dry mouth" and "Flammer" syndromes-neglected risks in adolescents and new concepts by predictive, preventive and personalised approach. *EPMA J.* 2018;9(3):307–17. <https://doi.org/10.1007/s13167-018-0145-7>.
33. Mpairwe H, Nkurunungi G, Tumwesige P, et al. Risk factors associated with rhinitis, allergic conjunctivitis and eczema among schoolchildren in Uganda. *Clin Exp Allergy: J British Soc Allergy Asthma Clin Immunol.* 2021;51(1):108–19. <https://doi.org/10.1111/cea.13769>.
34. Vazirani J, Shukla S, Chhawchharia R, Sahu S, Gokhale N, Basu S. Allergic conjunctivitis in children: current understanding and future perspectives. *Curr Opin Allergy Clin Immunol.* 2020;20(5):507–15. <https://doi.org/10.1097/aci.0000000000000675>.
35. Pawankar R. Allergic diseases and asthma: a global public health concern and a call to action. *World Allergy Organ J.* 2014;7(1):12. <https://doi.org/10.1186/1939-4551-7-12>.
36. Nappi E, Paoletti G, Malvezzi L, et al. Comorbid allergic rhinitis and asthma: important clinical considerations. *Expert Rev Clin Immunol.* 2022;18(7):747–58. <https://doi.org/10.1080/1744666x.2022.2089654>.
37. Patel N, Venkateswaran N, Wang Z, Galor A. Ocular involvement in atopic disease: a review. *Curr Opin Ophthalmol.* 2018;29(6):576–81. <https://doi.org/10.1097/icu.0000000000000532>.
38. Leonardi A, Bogacka E, Fauquert JL, et al. Ocular allergy: recognizing and diagnosing hypersensitivity disorders of the ocular surface. *Allergy.* 2012;67(11):1327–37. <https://doi.org/10.1111/all.12009>.
39. O'Brien TP. Allergic conjunctivitis: an update on diagnosis and management. *Curr Opin Allergy Clin Immunol.* 2013;13(5):543–9. <https://doi.org/10.1097/ACI.0b013e328364ec3a>.
40. Bozkurt B, Artac H, Ozdemir H, Ünü A, Bozkurt MK, Irkeç M. Serum vitamin D levels in children with vernal keratoconjunctivitis. *Ocul Immunol Inflamm.* 2018;26(3):435–9. <https://doi.org/10.1080/09273948.2016.1235714>.
41. Bozkurt B, Artac H, Arslan N, et al. Systemic atopy and immunoglobulin deficiency in Turkish patients with vernal keratoconjunctivitis. *Ocul Immunol Inflamm.* 2013;21(1):28–33. <https://doi.org/10.3109/09273948.2012.723110>.
42. Zhang Q, Wang N, Rui Y, Xia Y, Xiong S, Xia X. New insight of metabolomics in ocular diseases in the context of 3P medicine. *EPMA J.* 2023;14(1):53–71. <https://doi.org/10.1007/s13167-023-00313-9>.
43. Kropp M, De Clerck E, Vo TKS, Thumann G, Costigliola V, Golubnitschaja O. Short communication: unique metabolic signature of proliferative retinopathy in the tear fluid of diabetic patients with comorbidities - preliminary data for PPPM validation. *EPMA J.* 2023;14(1):43–51. <https://doi.org/10.1007/s13167-023-00318-4>.
44. Kropp M, Golubnitschaja O, Mazurakova A, et al. Diabetic retinopathy as the leading cause of blindness and early predictor of cascading complications-risks and mitigation. *EPMA J.* 2023;14(1):21–42. <https://doi.org/10.1007/s13167-023-00314-8>.
45. Bäckhed F, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI. Host-bacterial mutualism in the human intestine. *Science (New York, N.Y.).* 2005;307(5717):1915–1920. <https://doi.org/10.1126/science.1104816>.
46. Akira S, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. *Cell.* 2006;124(4):783–801. <https://doi.org/10.1016/j.cell.2006.02.015>.
47. Mills S, Stanton C, Lane JA, Smith GJ, Ross RP. Precision nutrition and the microbiome, part I: current state of the science. *Nutrients.* 2019;11(4) <https://doi.org/10.3390/nu11040923>.
48. Borgmann D, Ciglieri E, Biglari N, et al. Gut-brain communication by distinct sensory neurons differently controls feeding and glucose metabolism. *Cell Metab.* 2021;33(7):1466–82.e7. <https://doi.org/10.1016/j.cmet.2021.05.002>.
49. Tachalov VV, Orekhova LY, Kudryavtseva TV, et al. Making a complex dental care tailored to the person: population health in focus of predictive, preventive and personalised (3P) medical approach. *EPMA J.* 2021;12(2):129–40. <https://doi.org/10.1007/s13167-021-00240-7>.



50. Round JL, Mazmanian SK. The gut microbiota shapes intestinal immune responses during health and disease. *Nat Rev Immunol*. 2009;9(5):313–23. <https://doi.org/10.1038/nri2515>.
51. Bubnov RV, Spivak MY, Lazarenko LM, Bomba A, Boyko NV. Probiotics and immunity: provisional role for personalized diets and disease prevention. *EPMA J*. 2015;6(1):14. <https://doi.org/10.1186/s13167-015-0036-0>.
52. Bach JF. The hygiene hypothesis in autoimmunity: the role of pathogens and commensals. *Nat Rev Immunol*. 2018;18(2):105–20. <https://doi.org/10.1038/nri.2017.111>.
53. Lambrecht BN, Hammad H. The immunology of the allergy epidemic and the hygiene hypothesis. *Nat Immunol*. 2017;18(10):1076–83. <https://doi.org/10.1038/ni.3829>.
54. Annunziato F, Romagnani C, Romagnani S. The 3 major types of innate and adaptive cell-mediated effector immunity. *J Allergy Clin Immunol*. 2015;135(3):626–35. <https://doi.org/10.1016/j.jaci.2014.11.001>.
55. Johansson E, Mersha TB. Genetics of Food Allergy. *Immunol Allergy Clin N Am*. 2021;41(2):301–19. <https://doi.org/10.1016/j.iaac.2021.01.010>.
56. McKenzie C, Tan J, Macia L, Mackay CR. The nutrition-gut microbiome-physiology axis and allergic diseases. *Immunol Rev*. 2017;278(1):277–95. <https://doi.org/10.1111/imr.12556>.
57. Delday M, Mulder I, Logan ET, Grant G. *Bacteroides thetaiotaomicron* ameliorates colon inflammation in preclinical models of Crohn's disease. *Inflamm Bowel Dis*. 2019;25(1):85–96. <https://doi.org/10.1093/ibd/izy281>.
58. Vomhof-DeKrey EE, Stover A, Basson MD. Microbiome diversity declines while distinct expansions of Th17, iNKT, and dendritic cell subpopulations emerge after anastomosis surgery. *Gut Pathogens*. 2021;13(1):51. <https://doi.org/10.1186/s13099-021-00447-z>.
59. Eetemadi A, Tagkopoulos I. Methane and fatty acid metabolism pathways are predictive of Low-FODMAP diet efficacy for patients with irritable bowel syndrome. *Clin Nutr (Edinburgh, Scotland)*. 2021;40(6):4414–21. <https://doi.org/10.1016/j.clnu.2020.12.041>.
60. Lee KH, Guo J, Song Y, et al. Dysfunctional gut microbiome networks in childhood IgE-mediated food allergy. *Int J Mol Sci*. 2021;22(4) <https://doi.org/10.3390/ijms22042079>.
61. Chen Y, Meng P, Cheng S, et al. Assessing the effect of interaction between C-reactive protein and gut microbiome on the risks of anxiety and depression. *Mol Brain*. 2021;14(1):133. <https://doi.org/10.1186/s13041-021-00843-1>.
62. Pujo J, Petitfils C, Le Faouder P, et al. Bacteria-derived long chain fatty acid exhibits anti-inflammatory properties in colitis. *Gut*. 2021;70(6):1088–97. <https://doi.org/10.1136/gutjnl-2020-321173>.
63. Yasar Bilge NS, Pérez Brocal V, Kasifoglu T, et al. Intestinal microbiota composition of patients with Behçet's disease: differences between eye, mucocutaneous and vascular involvement. *The Rheuma-BIOTA study. Clinical and experimental rheumatology*. 2020;38(Suppl 127(5)):60–8.
64. Berni Canani R, Sangwan N, Stefka AT, et al. Lactobacillus rhamnosus GG-supplemented formula expands butyrate-producing bacterial strains in food allergic infants. *ISME J*. 2016;10(3):742–50. <https://doi.org/10.1038/ismej.2015.151>.
65. Gophna U, Konikoff T, Nielsen HB. *Oscillospira* and related bacteria - from metagenomic species to metabolic features. *Environ Microbiol*. 2017;19(3):835–41. <https://doi.org/10.1111/1462-2920.13658>.
66. Jiao N, Baker SS, Nugent CA, et al. Gut microbiome may contribute to insulin resistance and systemic inflammation in obese rodents: a meta-analysis. *Physiol Genomics*. 2018;50(4):244–54. <https://doi.org/10.1152/physiolgenomics.00114.2017>.
67. Johnson JS, Spakowicz DJ, Hong BY, et al. Evaluation of 16S rRNA gene sequencing for species and strain-level microbiome analysis. *Nat Commun*. 2019;10(1):5029. <https://doi.org/10.1038/s41467-019-13036-1>.
68. Holscher HD. Dietary fiber and prebiotics and the gastrointestinal microbiota. *Gut Microbes*. 2017;8(2):172–84. <https://doi.org/10.1080/19490976.2017.1290756>.

### PPPM Innovation Highlights

#### Working hypothesis in the framework of PPPM

In this study, we hypothesize that individuals with microbial dysbiosis may have a higher incidence of allergic conjunctivitis due to the triggering of an inflammatory response and the induction of an imbalance in the immune system, resulting in the activation of Th2 cells with varying levels of severity. Additionally, our analysis aims to identify specific gut microbial genera that may contribute to the onset of allergic conjunctivitis, and potentially provide new indicators for this condition. To test our hypothesis, our analysis selected genetic variants associated with gut microbiota features ( $N = 18,340$ ) and allergic conjunctivitis (4513 cases, 649,376 controls). We performed a two-sample MR to assess the impact of gut microbiota on the risk of developing allergic conjunctivitis.

#### Predictive approach, targeted prevention, and personalization of medical services

Stool samples can be obtained noninvasively, making them an idea source for analyzing the human gut microbial. With the advent of high-throughput sequencing technologies targeting the 16S rDNA gene, it is now possible to quickly and conveniently analyze the microbial communities in the gut using stool samples. Knowing the change of gut microbial is crucial for predictive diagnosis and targeted prevention. Moreover, allergic conjunctivitis may serve as an early indicator and reliable predictor of systemic dysfunction, such as atopic dermatitis and asthma. Effective early identification of allergic conjunctivitis can provide targeted protection for patients to avoid the impact of allergic conjunctivitis and potential systemic diseases that may exist. Therefore, 16S rDNA high-throughput sequencing is strongly recommended to select individuals at high risk, and targeted preventive measures can be triggered early in life, which conforms to the claim of primary prevention. Individuals who are at risk of a decrease in *Ruminococcaceae\_UCG\_002*, *Holdemanella*, *Catenibacterium*, *Senegalimassilia*, and an increase in *Oscillospira* levels should be a priority for healthcare providers. Early aggressive treatment to correct microbial dysbiosis, such as dietary changes, taking probiotic and prebiotic supplements, and targeted antibiotics, should be considered. For example, direct addition of *Ruminococcaceae\_UCG\_002* probiotics can be given to boost its levels, while targeted antibiotics can be used to suppress the growth of *Oscillospira*. In addition, high-throughput sequencing of 16S rDNA can be a routine examination to identify changes in gut microbial in patients with allergic conjunctivitis. The novel personalized treatments such as detecting the gut microbiota individually, designing person-related probiotic strains, changing diet, and taking specific antibiotics may help ameliorate the inflammatory response and symptoms, and improve the quality of life in the early stages of allergic conjunctivitis. This is important for secondary prevention of the disease.

### Advantages of gut microbiota served as a target to diagnose and predict allergic conjunctivitis in the framework of PPPM

In the field of allergic conjunctivitis, detecting changes in gut microbial improves the prediction and early diagnosis, which advantage over other approach. First, analyzing gut microbial is a non-invasive method that reflects systemic changes in the immune system. This provides a convenient approach for early screening of potential allergic risks in a larger population. Second, gut microbiota with preventive potential including *Ruminococcaceae\_UCG\_002*, *Holdemanella*, *Catenibacterium*, and *Senegalimassilia* genus could be used to prevent implement targeted interventions to correct dysbiosis and prevent the development of allergic conjunctivitis and related systemic diseases, saving medical resources. Third, analyzing gut microbial can also aid in the development of new therapies for allergic conjunctivitis. Probiotics have been shown to have a positive

effect on the immune system and reduce inflammation, making them a potential treatment option for allergic conjunctivitis. This personalized approach to treatment has the potential to improve outcomes for patients with allergic conjunctivitis.

Therefore, gut microbiota could serve as a target for predictive diagnostics, targeted prevention, and individualized therapy in allergic conjunctivitis, facilitating the transition from reactive medical services to PPPM in the management of the disease.

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