Exploring the Effect of the Gut Microbiome on the Risk of Age-Related Macular Degeneration From the Perspective of Causality

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Purpose. To explore the mechanisms relating the gut microbiome (GM) to age-related macular degeneration (AMD), as they remain unclear. GM taxa that appear to act within the gut–retina axis may affect the risk of AMD.

Methods. Single-nucleotide polymorphisms (SNPs) of 196 GM taxa were obtained from the MiBioGen consortium, and a Mendelian randomization (MR) study was carried out to estimate the causality between GM taxa and AMD (defined as an endpoint based on ICD-9 and ICD-10). Using the data from the FinnGen consortium (6157 patients and 288,237 controls), we explored the GM taxa for causality and verified the results at the replication stage based on the MRC-IEU consortium (3553 cases and 147,089 controls). Inverse variance weighting (IVW) was the main method used to analyze causality, and the MR results were verified using heterogeneity tests and pleiotropy tests.

Results. According to the MR results, order *Rhodospirillales* ($P = 3.38 \times 10^{-2}$), family *Victivallaceae* ($P = 3.14 \times 10^{-2}$), family *Rikenellaceae* ($P = 3.58 \times 10^{-2}$), genus *Slackia* ($P = 3.15 \times 10^{-2}$), genus *Faecalibacterium* ($P = 3.01 \times 10^{-2}$), genus *Bilophila* ($P = 1.11 \times 10^{-2}$), and genus *Candidatus Soleaferrea* ($P = 2.45 \times 10^{-2}$) were suggestively associated with AMD. In the replication stage, only order *Rhodospirillales* ($P = 0.03$) passed validation. The heterogeneity ($P > 0.05$) and pleiotropy ($P > 0.05$) tests in two stages confirmed the robustness of the MR results.

Conclusions. We confirmed that order *Rhodospirillales* influenced the risk of AMD based on the gut–retina axis, providing new impetus for the development of the GM as an intervention to prevent the occurrence and development of AMD.

Keywords: gut–retina axis, age-related macular degeneration (AMD), gut microbiome, Mendelian randomization, causality

As a degenerative disease caused by many factors, age-related macular degeneration (AMD) is one of the main causes of severe vision loss.[1] Because of the increased aging of the world's population, the incidence rate of AMD is on the rise. The number of people with AMD may even exceed 280 million by 2040.[2] Therefore, identifying the risk factors of AMD can help intervene in the incidence of AMD as soon as possible and alleviate the burden of AMD on public health. The mechanism of AMD involves a complex combination of genetic susceptibility, inflammation, environmental factors, and other risk factors.[3] Through clinical imaging technology (such as optical coherence tomography and indocyanine green angiography), researchers have revealed some intraocular risk factors (such as extracellular deposits).[4–6] However, localized mechanistic studies in the eye have struggled to fully elucidate the impact of the risk of developing AMD. Due to the complex pathogenesis, interventions to reduce the risk of AMD have been limited.

Studies have found that dietary patterns and nutrition may affect the risk of AMD.[7] For example, some researchers have proposed that lipids from the diet are processed by retinal pigment epithelial cells and are an important component of drusen.[8] In addition to local risk factors of the eye, systemic factors may also explain the pathogenesis of AMD from another aspect. The gut ecosystem is significantly shaped by the intestine itself and includes a vast network of the gut microbiome (GM) taxa.[9] The interactions among nutrients, the GM, and intestinal organs maintain a dynamic balance. When the GM is ecologically disturbed, it may affect the digestion and absorption of nutrients, causing immune and metabolic diseases.[10] Crosstalk between GM taxa and the brain has been well established. The relationship between GM taxa and ophthalmic diseases is still in the preliminary exploration stage. Rowan et al.[11] first confirmed the internal relationship between the GM taxa of wild-type aged mice and the retina through untargeted metabolomics

and proposed the concept of the gut–retina axis. There is a huge difference in the intestinal microbial composition between mice and humans, and further research is required to confirm this view. Zinkernagel et al.[12] sequenced the gut metagenomes of 12 patients with neovascular AMD and found that enrichment of the GM taxa differed from the control group.

Notably, because of the influence of diet habits and daily life, there may be large individual differences in the abundance of each GM taxon. Considering the high cost of sequencing technology, it is difficult for most researchers to conduct large-sample, randomized controlled studies to reduce the error caused by individual differences. Mendelian randomization (MR) evaluates the causal relationships of genetic variations from a large sample through publicly available de-identified data to obtain more reliable conclusions.[13] This method simulates the random allocation of samples to the control and experimental groups of a randomized controlled trial, making it possible to analyze the relationship of the gut–retina axis.

In this study, we hoped to further clarify the causality between GM taxa and AMD through the use of human GM genomics research to provide a more powerful basis for further research on mechanisms of the gut–retina axis. In addition, it is hoped that GM taxa with an association with AMD will serve as new targets for intervention.

Methods

Ethics Statement

The genome-wide association study (GWAS) data used for analysis consisted of de-identified public data and were searched from among published studies. Ethics committees of all original institutions approved all of the GWASs following the tenets of the Declaration of Helsinki. The summary statistics for genetic associations with AMD can be found at the FinnGen study and the IEU OpenGWAS project (GWAS ID: ukb-b-17194) at https://gwas.mrcieu.ac.uk/. The MR analysis code can be found at https://mrcieu.github.io/TwoSampleMR/articles/index.html.

Genetic Instrumental Variables of the Gut Microbiome

Independent GM taxon genetic variant loci were identified from the MiBioGen consortium, which is the largest study to investigate the genetics of the human GM from 24 population-based cohorts.[14] In the MiBioGen consortium, 16S rRNA data of 18,340 subjects were obtained from European, Asian, Hispanic, Middle Eastern, and African ancestries. A total of seven fecal DNA extraction methods were used to obtain the GM taxa data, which were then adjusted for age, principal genetic components, technical covariates, and gender. With the use of direct taxonomic binning, 122,110 variables in 211 taxa of the GM (containing 15 unknown taxa) were divided into five levels (phylum, class, order, family, and genus). A detailed description of the GM taxa GWASs is available in the study by Kurilshikov et al.[14] To obtain more accurate results, we removed 15 unknown GM taxa.

AMD GWAS Dataset in the Discovery Stage

To discover the impact of GM taxa on AMD, we used summary-level data composed of 6157 AMD patients and 288,237 controls. The dataset was obtained from the FinnGen study (https://r7.finngen.fi/) in the discovery stage.[15] AMD is defined as an endpoint based on *International Classification of Disease*, Ninth Revision (ICD-9; 3625A and 3625B) and ICD-10 (H35.30). In the FinnGen consortium, all subjects were Finnish, and 16,962,023 single-nucleotide polymorphisms (SNPs) were analyzed. The first 10 principal components (PCs), genetic factors, age, and sex were adjusted.

AMD GWAS Dataset in the Replication Stage

We verified significant GM taxa identified in the discovery stage using the MR database.[16] This database contains 42,334 GWASs involving 244,792,068,559 genetic associations (as of September 12, 2022). We chose GWAS ID: ukb-b-17194 as the replication outcome of AMD (ICD-9), which originated from the Medical Research Council Integrative Epidemiology Unit (MRC-IEU) consortium based on the UK Biobank study.[17] The MRC-IEU consortium recruited 150,642 European samples (involving 9,851,867 genetic associations) between 2006 and 2010[18] (Supplementary Fig. S1). In the replication stage, this GWAS includes 3553 AMD cases and 147,089 controls. In the analysis, sex, chip, and first 10 PCs were adjusted.

IV Quality Control

For the MR analysis, three assumptions had to be satisfied[19] (Fig. 1) (terms appearing in the manuscript are explained in the Supplementary Materials): (1) independent instrumental variables (IVs) were associated with each GM taxon and were not associated with AMD; (2) independent IVs associated with each GM taxon were not associated with confounders; and (3) effects of the IVs were associated with AMD only through each GM taxon without other factors.

All of the genetic variables were quality controlled to obtain valid IVs. As with the current criteria for GM studies, we chose $P < 1 \times 10^{-5}$ screening IVs.[20] The presence of a weak IV was assessed using *F*-values ($\frac{R_2(n-k-1)}{k(1-R^2)}$). For $F <$ 10, the IV was defined as weak and was removed. To make the SNPs independent from each other, a linkage disequilibrium was carried out based on the European-based 1000 Genomes Project.[21] The threshold R^2 for identifying independent SNPs was set to 0.001, and the clumping distance was 10,000 kb. Finally, we performed Steiger filtering[22] to remove the IVs that might cause inversion of causality.

Statistical Analysis

R 4.1.1 (R Foundation for Statistical Computing, Vienna, Austria) and the R package TwoSampleMR were used for the data analysis. For causal effect assessment using a single IV, the Wald ratio test was used to estimate the association between a single IV and each GM taxon. The inverse variance weighted (IVW) test was the main method used for calculations without horizontal pleiotropy,[23] which occurs when the IV has an effect on AMD outside of its effect on the GM in MR. If the number of SNPs with pleiotropy was less than half, the weighted median (WM) estimator[24] was used as an additional method. In addition, MR-Egger

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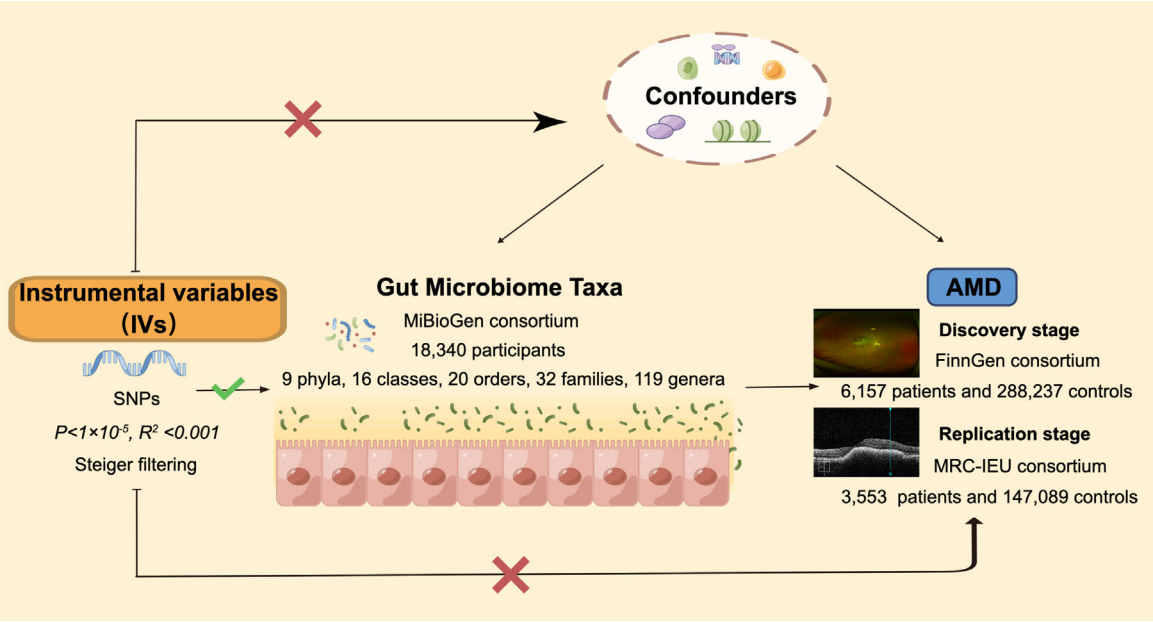

Figure 1. Directed acyclic graph composed of the IVs (each GM taxon-related SNP from five levels), exposure (196 GM taxa from five levels), and outcome (AMD in two stages).

regression[25] could provide valid results even when all IVs were invalid.

Sensitivity analysis was necessary for the MR results. Cochran's *Q* method was used to examine the heterogeneity of the results. MR-Egger regression tested the pleiotropy of the results. If $P > 0.05$, heterogeneity and pleiotropy were assumed not to exist. In addition, for the GM taxa with causality, we performed further pleiotropy tests using MR-PRESSO (Mendelian Randomization Pleiotropy RESidual Sum and Outlier) and removed the outliers.

Table 1. IVW Results for GM Taxa and AMD in the Discovery Stage

Level	GM Taxa	OR	95% CI	*P*
Order	*Rhodospirillales*	1.16	1.01–1.32	3.38×10^{-2}
Family	*Victivallaceae*	1.12	1.01–1.25	3.14×10^{-2}
Family	*Rikenellaceae*	0.82	0.68–0.99	3.58×10^{-2}
Genus	*Slackia*	0.81	0.67–0.98	3.15×10^{-2}
Genus	*Faecalibacterium*	1.31	1.10–1.57	3.01×10^{-3}
Genus	*Bilophila*	1.27	1.06–1.53	1.11×10^{-2}
Genus	*Candidatus Soleaferrea*	0.80	0.69–0.92	2.45×10^{-3}

Results

In this study, we first obtained effective IVs through quality control. Then, MR was conducted using IVs to evaluate the causal relationship between 196 GM taxa and AMD in the discovery stage (based on the FinnGen consortium). Finally, the causal relationship was further verified in the replication stage (based on the MRC-IEU consortium). For all MR results, we conducted sensitivity analyses to evaluate heterogeneity (Cochran's *Q*) and pleiotropy (MR-Egger regression and MR-PRESSO) (Fig. 1).

Gut Microbiota and AMD IVs

We conducted quality control and identified 2075 SNPs in the discovery stage (Supplementary Table S1) and 1580 SNPs in the replication stage (Supplementary Table S2) as IVs, which were associated with 196 GM taxa (including 119 genera, 32 families, 20 orders, 16 classes, and nine phyla) for AMD. These IVs were effective and independent ($P < 1 \times 10^{-5}$; $R^2 < 0.001$). In the discovery stage (FinnGen consortium), the number of IVs for each GM taxon ranged from three to 19, and the *F* statistics varied from 14.59 to 88.43 (Supplementary Table S1). In the duplicated stage, the number of IVs for each GM taxon ranged from two to 15, and the *F* statistics varied from 17.68 to 88.43 (Supplementary Table S2). In the two stages, all of the *F* statistics were greater than 10, indicating that there was no weak IV. Additional information about the GM taxon genetic IVs in the AMD GWAS dataset can be found in Supplementary Tables S1 and S2.

Causal Association Based on MR Results in the Discovery Stage

Based on IVW results, six GM taxa were suggestively associated with AMD (Table 1). At the order level, the IVW results confirmed that the order *Rhodospirillales* was an influential factor in AMD risk (odds ratio [OR] = 1.16; $P = 3.38 \times 10^{-2}$) (Fig. 2, Table 1). At the family level, the family *Victivallaceae* (OR = 1.12; $P = 3.14 \times 10^{-2}$) and family *Rikenellaceae* (OR = 0.82; $P = 3.58 \times 10^{-2}$) had a significant impact on AMD risk (Fig. 2, Table 1). At the genus level, it can be genetically expected that *Slackia* (OR = 0.81; $P = 3.15 \times 10^{-2}$), *Faecalibacterium* (OR = 1.31; $P = 3.01 \times 10^{-2}$), *Bilophila* (OR = 1.27; $P = 1.11 \times 10^{-2}$), and *Candidatus Soleaferrea* (OR = 0.80; $P = 2.45 \times 10^{-2}$) could significantly affect AMD risk (Fig. 2, Table 1). At the phylum and class levels, no statistically significant association was found between the GM taxa and the AMD risk (Fig. 2, Supplementary Tables S3 and S4).

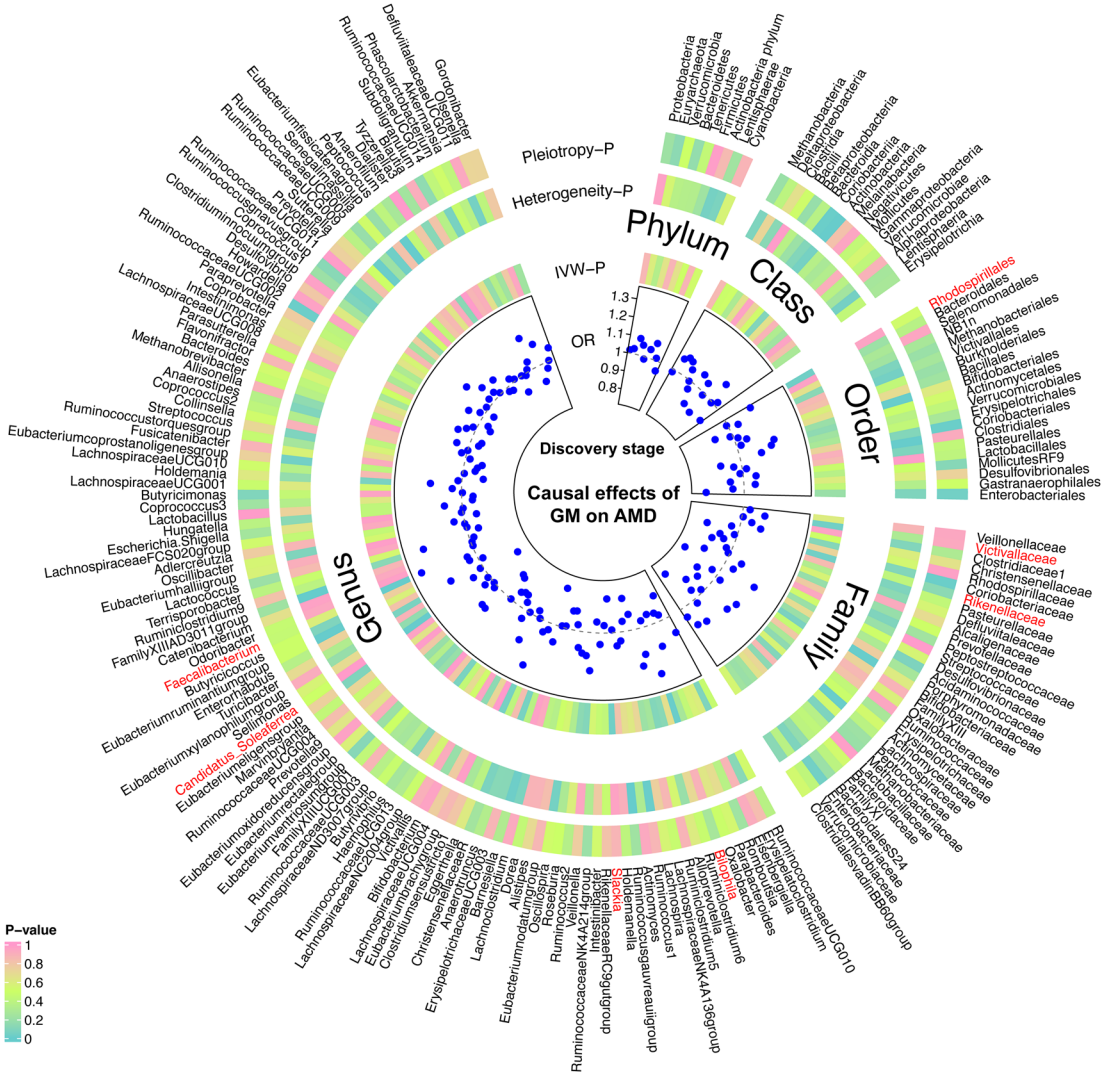

Figure 2. Causal analyses and sensitivity analyses of each gut microbiome taxon from five levels and AMD based on IVW results ($P < 1 \times 10^{-5}$) in the discovery stage (FinnGen consortium). From the outside to the inside, they are, respectively, the GM taxa name, the *P* value of the pleiotropy test (MR-Egger regression), the *P* value of the heterogeneity test (Cochran's *Q*), the *P* value based on the IVW results, and the OR based on the IVW results. The color corresponding to the *P* value is based on the RGB color ($P = 0$, #66CCCC; $P = 0.5$, #CCFF66; $P = 1$, #FF99CC).

The MR results of 196 GM taxa based on WM and MR-Egger are presented in Supplementary Tables S3 and S4.

Sensitivity Analyses in the Discovery Stage

The results of the sensitivity analyses in the discovery stage for 196 GM taxa are displayed in Figure 2 and Supplementary Tables S3 and S4. No heterogeneity (IVW: $P \geq 0.30$; MR-Egger: $P \geq 0.23$) or horizontal pleiotropy ($P \geq 0.13$) were observed in *Rhodospirillales*, *Victivallaceae*, *Rikenellaceae*, *Slackia*, *Faecalibacterium*, *Bilophila*, or *Candidatus Soleaferrea* (Fig. 2, Table 2). The MR-PRESSO results further confirmed the absence of horizontal pleiotropy in six GM taxa ($P \geq 0.32$) (Fig. 2, Table 2). Among those sensitivity analyses, the IVW results were more reliable and further validated the accuracy of the *Rhodospirillales*, *Victivallaceae*, *Rikenellaceae*, *Slackia*, *Faecalibacterium*, *Bilophila*, and *Candidatus Soleaferrea* causal effects on AMD.

Causal Association Based on MR Results in the Replication Stage

Based on MRC-IEU, we investigated causal relationships between 196 GM taxa and AMD (Fig. 3, Supplementary Tables S5 and S6). Note that only the effect of *Rhodospirillales* on the risk of AMD was further validated ($P = 0.03$) (Fig. 3, Supplementary Table S5). However, the effects of *Victivallaceae*, *Rikenellaceae*, *Slackia*, *Faecalibacterium*, *Bilophila*, and *Candidatus Soleaferrea* on the AMD risk were not significant at the replication stage ($P > 0.05$) (Fig. 3, Supplementary Tables S5 and S6). The results of WM and MR-Egger analysis of the GM taxa and AMD can be found in Supplementary Tables S5 and S6.

Sensitivity Analyses in the Replication Stage

To ensure the accuracy of the causality at the replication stage, we performed a sensitivity analysis on the results

Table 2. Sensitivity Analyses of MR Results Between GM Taxa and AMD in the Discovery Stage

		Heterogeneity (Cochran's *Q*), *P*		Pleiotropy, *P*	
Level	**GM Taxa**	**IVW**	**MR-Egger**	**MR-Egger Regression**	**MR-PRESSO**
Order	*Rhodospirillales*	0.95	0.94	0.52	0.32
Family	*Victivallaceae*	0.30	0.23	0.93	0.38
Family	*Rikenellaceae*	0.41	0.52	0.13	0.98
Genus	*Slackia*	0.96	0.90	0.88	0.97
Genus	*Faecalibacterium*	0.59	0.56	0.44	0.63
Genus	*Bilophila*	0.89	0.93	0.26	0.91
Genus	*Candidatus Soleaferrea*	0.99	0.99	0.50	0.76

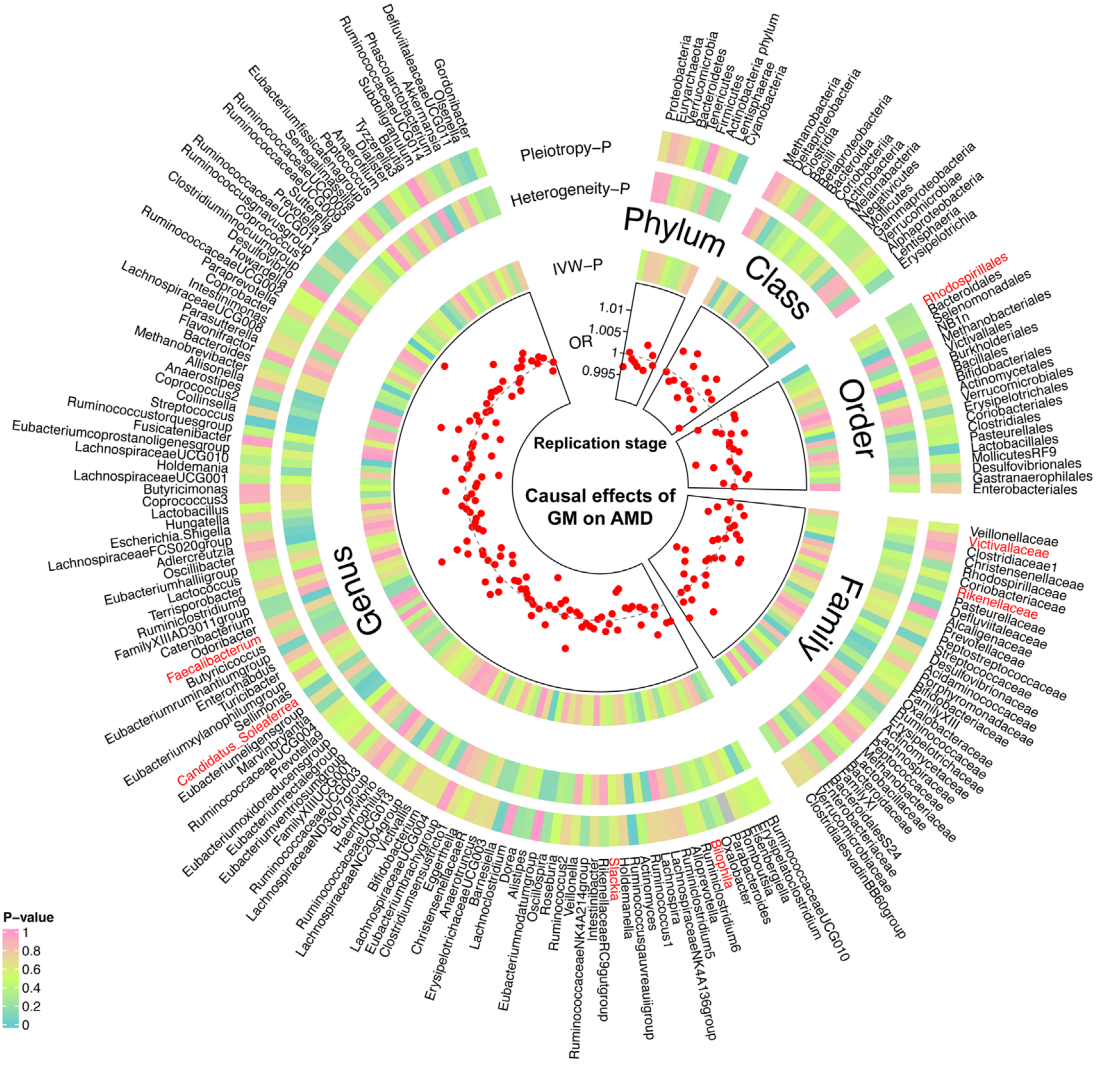

Figure 3. Causal analyses and sensitivity analyses of each gut microbiome taxon from five levels and AMD based on IVW results ($P < 1 \times 10^{-5}$) in the replication stage (MRC-IEU consortium). From the outside to the inside, they are, respectively, the GM taxa name, the *P* value of the pleiotropy test (MR-Egger regression), the *P* value of the heterogeneity test (Cochran's *Q*), the *P* value based on the IVW results, and the OR based on the IVW results. The color corresponding to the *P* value is based on the RGB color ($P = 0$, #66CCCC; $P = 0.5$, #CCFF66; $P = 1$, #FF99CC).

for 196 GM taxa (Fig. 3, Supplementary Tables S5 and S6). If horizontal pleiotropy was detected, the results of these GM taxa were corrected through MR-PRESSO and PhenoScanner. For *Rhodospirillales*, *Victivallaceae*, *Rikenellaceae*, *Slackia*, *Faecalibacterium*, *Bilophila*, and *Candidatus Soleaferrea*, the final results confirmed that there was no heterogeneity ($P > 0.05$) or horizontal pleiotropy ($P > 0.05$) (Table 3). Overall, the combined results of the two stages suggest that *Rhodospirillales* impacts AMD risk.

Table 3. Sensitivity Analyses of MR Results Between GM Taxa and AMD in the Replication Stage

		Heterogeneity (Cochran's *Q*), *P*		Pleiotropy, *P*	
Level	**GM Taxa**	**IVW**	**MR-Egger**	**MR-Egger Regression**	**MR-PRESSO**
Order	*Rhodospirillales*	0.66	0.71	0.27	0.69
Family	*Victivallaceae*	0.53	0.43	0.85	0.70
Family	*Rikenellaceae*	0.60	0.66	0.23	0.72
Genus	*Slackia*	0.55	0.43	0.58	0.47
Genus	*Faecalibacterium*	0.25	0.27	0.36	0.06
Genus	*Bilophila*	0.45	0.52	0.23	0.33
Genus	*Candidatus Soleaferrea*	0.16	0.57	0.09	0.20

Discussion

Studies continue to reveal the mechanisms of nutrition and other environmental factors in the pathogenesis of AMD.[26] As a factor that affects nutritional intake, the association between the GM and AMD is not yet clear. Compared with the current treatment methods, the relative accessibility of the GM taxa opens up new opportunities for interventions to modify the risk of AMD. In clinical research, many confounders interfere with exploring causal relationships between GM taxa and retinal diseases. For this reason, we studied GM taxa and identified associations with ICD codes. We choose to use MR for causal association analysis, minimized the interference of confounding factors through quality control, and obtained more reliable causal associations using IVs from large sample sources. Based on the largest GWAS of human microbiology, which was conducted by Kurilshikovet al.,[14] we confirmed that the GM taxa of one order (*Rhodospirillales*), two families (*Victivallaceae* and *Rikenellaceae*), and four genera (*Slackia*, *Faecalibacterium*, *Bilophila*, and *Candidatus Soleaferrea*) had an impact on AMD risk. During the replication stage, only the effect of *Rhodospirillales* passed verification. The causal effect between other GM taxa and AMD still must be further investigated. Based on the principle of MR, the direction of these causal relationships is determined—that is, not reversed. This means that our results confirm that GM taxa are involved in the pathogenesis of AMD, which provides theoretical support for the study of the mechanism of the gut–retina axis.

Zhang et al.[27] used a laser-induced mouse model that presented some features of AMD (neovascularization and inflammation) and compared the differences between germ-free (GF) mice (the gold standard for microbiome studies) and specific pathogen-free (SPF) mice under a normal diet. They found that, compared with SPF mice, the GF mice model had reduced neovascularization and peripheral microglia infiltration, which confirmed that some feature changes of AMD are regulated by the GM, indicating a connection between the gut–retina axis. Rowan et al.[28] compared the GM composition of mice with and without the AMD phenotype through 16S rDNA sequencing and found significant differences in the GM taxa mediated by diet. In addition, they also found that *Clostridia* and *Bacilli* were risk factors for AMD. In our MR study, we explored the existence of a causal relationship between *Rhodospirillales* and AMD. Although different taxa were found, this may be due to the fact that the subjects of the study by Rowan et al.[28] were mice (the subjects of the outcomes in our study were humans). It is undeniable that our study supports the idea of an association within the gut–retina axis. In addition to animal studies, the control study of Kiang et al.[29] revealed that 89 patients with AMD and 49 healthy subjects had significant differences in their GMs. In that study, the GM taxa related to AMD were found to be enriched in an immunoglobulin A (IgA)-bound fraction and participate in immune regulation.[29] Zinkernagel et al.[12] found that *Anaerotruncus*, *Ruminococcus torques*, and *Eubacterium ventriosum* were enriched in AMD patients. These three bacterial genera belong to the *Firmicutes* phylum, *Clostridia* class, *Clostridiales* order, and *Ruminococcaceae* family, which had the same classification as *Faecalibacterium* and *Candidatus Soleaferrea* in our results. However, these results have not been verified in the replication phase, and a larger sample is required for further exploration.

Gastaldello et al.[7] reported that a Mediterranean diet lowers the odds of developing AMD and also decreases the risk of progression to more advanced stages of the disease. Dietary factors and GMs can also interact. Rowan et al.[28] showed that high fat intake can exacerbate AMD in a manner dependent on the GM. The Age-Related Eye Disease Study 2 found that nutritional supplements can delay the progression of AMD, and these supplements may play a role by regulating GM homeostasis.[30] The GM is rich in genes involved in amino acid metabolism pathways such as arginine and glutamic acid. Studies in patients with AMD have found a decrease in the genes involved in these amino acid metabolic pathways.[12,31] Therefore, GM involvement in the development of AMD is partially influenced by genetic and nutritional factors. This means that the intestinal microbiome may become a potential target for the prevention and treatment of AMD. In addition, clarifying specific taxa of the GM is of great value for the identification of AMD targets.

To the best of our knowledge, our study is the first to identify the role of *Rhodospirillales* in eye diseases, although the specific pathogenic mechanism for AMD has not been reported. Luo et al.[32] tested the GM of hypoxia-induced pulmonary hypertension (PH) mice and found changes in *Rhodospirillales*, including reversal of the increase in disease-associated *Rhodospirillales* and a decrease in antiinflammatory and immunomodulatory functional *Bacteroidaceae* in the mesenchymal stem cell–treated group. The mRNA expression of interleukin-1β (IL-1β) and IL-6 was increased in the PH mice. *Rhodospirillales* was considered a biomarker of PH mice. The abundance change of *Rhodospirillales* also revealed the same trend in the study by Ma et al.[33] In the colon cancer mice model, the abundance of *Bacteroidetes*, *Rhodospirillales*, and *Muribaculateae* was reduced. After treatment with *Lactiplantibacillus plantarum*-12, the change in GMs in colon cancer mice was reversed, and the levels of proinflammatory factors (tumor necrosis factor-alpha [TNF-α] and IL-1β) were reduced. Based on the role of *Rhodospirillales* in pulmonary hypertension and colon cancer, it can be found that red

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Rhodospirillales is involved in the regulation of inflammatory responses. It is well known that inflammation plays an integral role in the mechanisms of AMD. Some AMD-related studies have found significant changes in the levels of proinflammatory factors (IL-1β and TNF-α) in patients with AMD.[34–36] Studies have confirmed that changes in diet affect the composition of the GM and increase local inflammation, thus increasing the risk of AMD.[37] These inflammatory mediators have similar effects in the pathogenesis of PH, colon cancer, and AMD. Therefore, we speculate that the effect of *Rhodospirillales* on AMD might be similar to the mechanisms of pulmonary hypertension and colon cancer, and this connection can be extended to the retina through proinflammatory factors. Furthermore, *Rhodospirillales* can be considered an AMD biomarker.

The order of *Rhodospirillales* belongs to the *Proteobacteria* phylum and the *Alphaproteobacteria* class. *Proteobacteria* is a Gram-negative bacterium, and its metabolites, such as lipopolysaccharide and ethanol production, are involved in inflammation, immune escape, and other mechanisms.[38] Sookoian et al.[39] suggested that *Proteobacteria* mainly come from the intestine and can affect the risk of vascular disease through inflammatory injury. Sun et al.[40] found that a deficiency of angiogenin will increase the abundance of *Alphaproteobacteria*, leading to the disruption of homeostasis and thus inducing colitis. The impact of *Proteobacteria* on disease risk depends on the combined effects of all the orders belonging to this phylum in the intestine. *Rhodospirillales*, as an order of the *Proteobacteria* phylum, is bound to participate in the occurrence and development of the *Proteobacteria* phylum in diseases. According to the research by Andriessen et al.,[37] when the GM is altered or even dysregulated in homeostasis, it will alter pathogen-associated molecular pattern molecules (PAMPs) and subsequently make the intestinal permeability increase, which elevates ocular and systemic inflammation and enhances pathological neovascularization. Intestinal permeability is influenced by a combination of the GM and the mucosal immune system.[41] Increased intestinal permeability enhances PAMP translocation.[42] PAMPs affect proinflammatory signaling through pattern recognition receptors and induce inflammation, thus allowing intestinal-derived PAMPs to induce retinal inflammatory disease. Inflammation is considered to play a significant role in AMD pathogenesis.[43] Singh et al.[44] compared the difference in the frequency of immune cells between patients with AMD and normal subjects and found that the frequency of Th1 cells and CXCR3 CD4 T cells in patients with AMD was significantly reduced. Interestingly, studies have found that the GM can affect the homeostasis of microglia through metabolites[45] and activate retinal-specific T cells.[46] Horai et al.[46] demonstrated that T-cell receptors can receive GM-derived activation signals and regulate autopathogenic T cells that cause diseases in distal tissues (such as the retina). These systemic inflammatory factors enhance the local inflammatory response in the eye, enhancing the secretion of vascular endothelial growth factor (VEGF) and triggering neovascularization.[47] Due to the stimulation of local inflammatory factors, the retinal pigment epithelium becomes degenerated and the photoreceptor cells are gradually destroyed, forming irregular pigmentation.[48] Therefore, GM taxa are associated with the pathogenesis of AMD through inflammation-related immune mechanisms, a relationship that to some extent reveals the intrinsic correlation of the gut–retina axis. This implies not only that AMD may be a local ophthalmic disease but also that its pathological mechanism may involve systemic factors, whether immune responses or the GM. It is undeniable that our research has confirmed the role of *Rhodospirillales* in AMD risk. Therefore, we believe that inflammation, as an important link in the pathogenesis of AMD, may connect *Rhodospirillales* and AMD through the gut–retina axis.

This MR study provides evidence for the direct causal effects of *Rhodospirillales* on the development of AMD. The limitation of the study is that the sample size in the replication stage was small, and the statistical power of the IVs may affect the validation effect. GWAS research based on large-sample sources would provide a greater theoretical basis for researchers to evaluate relationships between GM taxa and AMD. In future research, further analysis of AMD and GWASs based on optical coherence tomography images is needed to obtain more accurate conclusions from as much cohort information as possible while ensuring patient privacy. In addition, GM taxa are affected by diet, body shape, and other confounders, and it is difficult to completely balance these factors. The European population was selected for the two stages of the study. Considering that there may be racial differences in the GM taxa, we should be alert to this problem when evaluating our conclusions. The study of the gut–retina axis complements studies of retinal nutrition. Many studies have explored the effects of nutrition on the retina,[8] but the specific GM taxa impact on the retina is not yet clear. We did not use overly conservative multiple corrections in order not to omit intestinal GM taxa with potential causal relationships.

In conclusion, our findings reinforced the impact of the GM on AMD, particularly the effects of specific taxa. Our study provides new impetus for development of the GM as an intervention to prevent the occurrence and development of AMD. New interventions may be able to reduce the risk of AMD.

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