• Meta-Analysis •

Investigating the causal link between gut microbiota and dry age-related macular degeneration: a bidirectional Mendelian randomization study

Hai–Yan Huang[1], *Jing Wang*[1], *Bo Qin*[1], *Yao Tan*[2,3]

[1]Clinical Medical College of Guizhou Medical University, Guiyang 550004, Guizhou Province, China
[2]Postdoctoral Station of Clinical Medicine, The Third Xiangya Hospital, Central South University, Changsha 410000, Hunan Province, China
[3]The Third Xiangya Hospital, Central South University, Changsha 410000, Hunan Province, China
Co-first authors: Hai-Yan Huang and Jing Wang
Correspondence to: Yao Tan. Department of Ophthalmology, The Third Xiangya Hospital, Central South University, No. 138 Tongzipo Road, Yuelu District, Changsha 410000, Hunan Province, China. tanyao2000@qq.com
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Abstract

● **AIM:** To assess the causal link between 211 gut microbiota (GM) taxa and dry age-related macular degeneration (dAMD) risk.

● **METHODS:** Mendelian randomization using instrumental factors taken from a genome-wide association study (GWAS) were used. Inverse variance weighted (IVW) analysis and sensitivity analysis were performed on the FinnGen project, which included 5095 cases and 222 590 controls.

● **RESULTS:** The IVW analysis showed substantial genus- and family-level relationships between GM taxa and dAMD risk. Specifically, the family *Peptococcaceae* (P=0.03), genus *Bilophila* (P=3.91×10^{-3}), genus *Faecalibacterium* (P=6.55×10^{-3}), and genus *Roseburia* (P=0.04) were linked to a higher risk of developing dAMD, while the genus *Candidatus Soleaferrea* (P=7.75×10^{-4}), genus *Desulfovibrio* (P=0.04) and genus *Eubacterium ventriosum group* (P=0.04) exhibited a protective effect against dAMD. No significant causal relationships were observed at higher taxonomic levels. Additionally, in the reverse IVW analysis, no meaningful causal effects of the 7 GM taxa.

● **CONCLUSION:** These findings give support for the gut-retina axis participation in dAMD and shed light on putative underlying processes. Investigations on the connection between GM and dAMD have not yet revealed the underlying mechanism.

● **KEYWORDS:** dry age-related macular degeneration; gut microbiota; mendelian randomization; gut-retina axis; genome-wide association study
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INTRODUCTION

One of the main causes of vision impairment, especially in industrialized nations, is age-related macular degeneration (AMD), which is expected to impact 196 million people globally by 2020[1]. AMD is categorized into dry and wet types, with dry age-related macular degeneration (dAMD) representing 85%-90% of cases[2-3]. AMD development is primarily triggered by age-induced transformations in retinal pigment epithelium (RPE) cells, with other contributing factors including genetics, lipid metabolism, oxidative stress, and aging[4-5]. Various therapeutic interventions have targeted distinct pathobiological aspects of dAMD, including photobiomodulation aimed at enhancing mitochondrial function in RPE cells, complement inhibition to reduce the inflammatory cascade, and neuroprotection strategies to preserve neuronal integrity[6]. Anti-inflammatory therapies address the chronic inflammation characteristic of dAMD, while cell-based therapies, visual cycle modulation, gene therapy, and prosthetic devices offer innovative solutions to mitigate symptoms and progression of the disease[7-8]. Despite the application of these diverse methods, the pathophysiological mechanisms underlying dAMD remain largely elusive, indicating a gap between therapeutic application and a deep understanding of disease pathology.

The human gut microbiota (GM) significantly influences human health, as it constitutes the largest microecosystem within the body, playing a crucial role in maintaining various physiological processes[9]. Links between genetic modifications and human health and disease states were evidenced, with

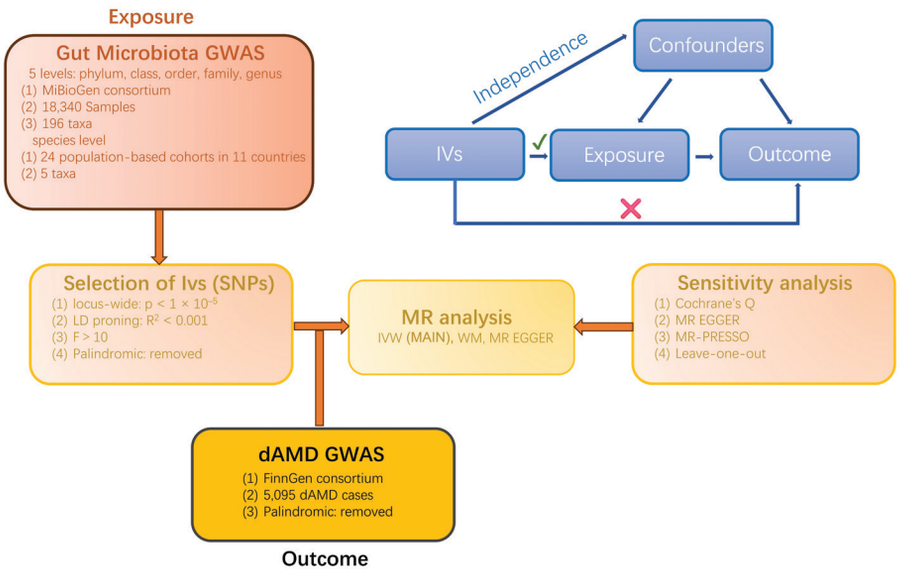

Figure 1 An introduction to the process and key assumptions of MR analysis GWAS: Genome-wide association study; IVs: Instrumental variables; SNPs: Single nucleotide polymorphisms; LD: Linkage disequilibrium; IVW: Inverse variance weighted; WM: Weighted median; dAMD: Dry age-related macular degeneration; MR: Mendelian randomization.

these modifications impacting neural, endocrine, inflammatory, and immune communication *via* the gut-brain axis[10]. Studies had suggested a correlation between GM and ocular diseases, including dAMD[11]. GM dysbiosis contributes to the onset or progression of long-term retinal illnesses such as AMD, diabetic retinopathy, or retinitis pigmentosa[12]. Recent studies have suggested that the progression of AMD can be attributed to chronic inflammation caused by gut permeability and dysbiosis[13]. The GM has been unequivocally recognized as a pivotal factor in the development of systemic low-grade inflammation[14]. The multifactorial nature of dAMD, influenced by diet, lifestyle, and physical activity, complicates the understanding of GM's impact, making it a complex yet vital area of study.

Mendelian randomization (MR) is an advanced statistical technique that uses genetic variations as instrumental variables (IVs) to establish causal relationships between risk factors and health outcomes[15]. While MR had been used to investigate the causal relationship between GM and various conditions, its used in unraveling the mechanisms of dAMD was yet unexplored[16-18]. This work used MR to examine the causal link between GM taxa and dAMD, potentially revealing complex processes underlying the illness. Understanding the correlation between certain GM and dAMD could lead to the identification of unique biomarkers and the creation of innovative treatment and diagnostic approaches.

MATERIALS AND METHODS

Ethical Approval The data analyzed in this study were collated at a summary level, procured from publicly accessible sources, and thoroughly de-identified, ensuring privacy and confidentiality. As the analysis involved publicly available and anonymized data, this study did not require ethical approval by an ethics committee or institutional review board. Each dataset used in the analysis was collected under the ethical standards of the Declaration of Helsinki and had previously received approval by the relevant institutional ethics committee.

Study Design In our survey, double-sample MR analysis was implemented to decipher the causal relationship between the potential transgenic group and dAMD (Figure 1). We harnessed IVs derived from the most comprehensive GM genetics study available. To evade sample overlap, summary-level data regarding dAMD was sourced from the FinnGen project (https://r8.finngen.fi/)[19]. FinnGen project has set its sights on scrutinizing the genetic makeup and health records of 500 000 Finnish citizens, with a particular focus on identifying disease markers. This initiative is poised to yield significant insights. In compliance with the STROBE-MR guidelines, our study design, data collection, and analytical methods have been structured to enhance the clarity and reproducibility of our findings[20].

Sources of Exposure to Genetically Modified Taxa Our exposure data for GM taxa was derived from the comprehensive GWAS report by the MiBioGen consortium[21]. In order to investigate how host genetics contribute to the make-up of intestinal microbiome, the MiBioGen alliance carefully compiled and scrutinized genome-wide genotypes and 16S faecal microbiome information from 18 340 people across 24 cohorts. Utillising 18 340 samples of 16S rRNA gene sequencing from 24 population cohorts in 11 countries/regions representing various progenitors, this study was the most cited of its kind. We extracted independent variables of GM taxa at five distinct levels through an exhaustive GWAS analysis.

Outcome Sources of Dry Age-Related Macular Degeneration The FinnGen project served as the source for the dAMD GWAS summary data (https://r8.finngen.fi/). The diagnosis of dAMD was performed in accordance with the recommendations established by the 10th revision of the International Classification of Diseases. After controlling for age, gender, genetic association, genotyping cohort, and the first 10 principal components, our dAMD analysis incorporated 227 685 Finnish individuals, including 5095 dAMD cases and 222 590 controls. We supplemented our data with information from the International AMD Genomics Consortium, which included 33 976 individuals (16 144 with dAMD and 17 832 controls)[22].

Mendelian Randomization Analysis R program was used to carry out all statistical calculations (Version 4.2.3). Using the "TwoSampleMR" R package (Version 0.5.6), we performed a causality analysis of GM taxa and dAMD. We concluded a causal relationship when the *P*-value was less than 0.05[23]. To deem MR analysis as valid, the IV must meet three criteria: 1) It should be associated with the exposure, 2) ideally, it wouldn't rely on the result when taking into account the exposure and confounding variables related to the exposure-outcome relationship, 3) it should be independent of any other factors that could potentially confuse the connection between the variable being studied and outcome. Through the MR analysis, we utilized summary-level GWAS data of GM as the exposure variable. Single nucleotide polymorphisms (SNPs) with *P*-values less than the significance criterion were chosen as IVs ($P<1\times10^{-5}$) and considered linkage disequilibrium effects using a parameter of 0.001 and a genetic separation of 10 000 kb. Using the PhenoScanner website (http://www.phenoscanner.medschl.cam.ac.uk/)[24], we evaluated relationships between IVs and putative confounders and eliminated SNPs linked to confounders to reduce horizontal pleiotropy. The F-statistic evaluates the robustness of the correlation between the exposure and the IVs. Calculated as $F=(R^2(n-k-1))/(k(1-R^2))$, where R^2 is the proportion of variance in the exposure explained by the IVs, *n* is the total sample size in the GWAS, and k is the number of IVs. An F-statistic below 10 typically indicates weak IVs, potentially undermining the reliability of the results[25]. A higher R^2 value indicates a stronger IV, enhancing the validity of the MR analysis.

We utilized the Wald ratio (WR), the inverse variance weighted (IVW), the weighted median (WM), and the MR-Egger techniques for the purpose of performing causal estimations. When there was no horizontal pleiotropy, the IVW test gave accurate values, using fixed or random effects models based on heterogeneity. We measured magnitude of the effect with odds ratios (OR) and 95% confidence intervals (CI). The WM method was used if SNPs with heterogeneity extruded 50%, and MR-Egger results were considered valid provided that SNPs with pleiotropy extruded 50%[26]. Additionally, we applied the MR-RAPS approach to adjust for potential weak instrument bias and provide more robust causal estimates.

Sensitivity Analysis Heterogeneity was evaluated using the IVW and MR-Egger techniques, and outcomes were quantified using Cochran's *Q* statistics. Multi-effectiveness was ruled out using MR-Egger intercept testing. The MR-PRESSO approach was used to find outliers and then reanalyze them. The "leave-one-out" strategy assessed the genetic causes of abnormal SNPs and their impact on MR estimates. Outliers causing changes in MR estimates were removed, and the analysis were repeated. The significance threshold was set at $P=0.05$.

Seeking Causation and Genetic Correlation To ensure the accuracy of our MR results, we conducted additional studies to minimize potential biases arising from genetic correlations between the variables being studied. Although we made thorough efforts to remove SNPs tied to dAMD during the IV selection process, there remains a risk that some SNPs, which aren't obviously connected, could impact the genetic basis of dAMD. The Linkage Disequilibrium Score (LDSC) regression, a robust statistical technique, measures the degree of genetic coinheritance between two traits by utilizing Chi-squared statistics related to SNPs[27-28]. We employed LDSC to identify any genetic links between the carefully screened GM and dAMD. This analysis was crucial to ensure that any genetic overlap between exposure and outcome didn't obscure true causal relationships.

It is crucial to determine whether GM directly affect the onset of dAMD, or if, conversely, the development of dAMD influences the composition of GM. To address this, we used reverse MR to avoid the pitfalls of reverse causality bias, enabling us to gain a clearer understanding of the direction and strength of the causal relationship.

RESULTS

Instrumental Variables Selection with Gut Microbiota Relevance Following quality control measures for LD effects and palindromic sequences, a total of 2033 SNPs (derived from the MiBioGen consortium) were found to be associated with dAMD ($P<1\times10^{-5}$), serving as IVs for 196 bacterial taxa. Out of the original 211 GM, 15 taxa remain unnamed, resulting in a total of 196 identified taxa. These bacterial taxa belong to 9 different phyla (accounting for 103 SNPs), 16 classes (179 SNPs), 20 orders (217 SNPs), 32 families (340 SNPs), and 119 genera (1194 SNPs). All SNPs exhibited satisfactory validity, with a range between 20.35 and 27.85, and all *F*-values >10.

Mendelian Randomization Analysis Results Based on data from the FinnGen project, the risk of dAMD was

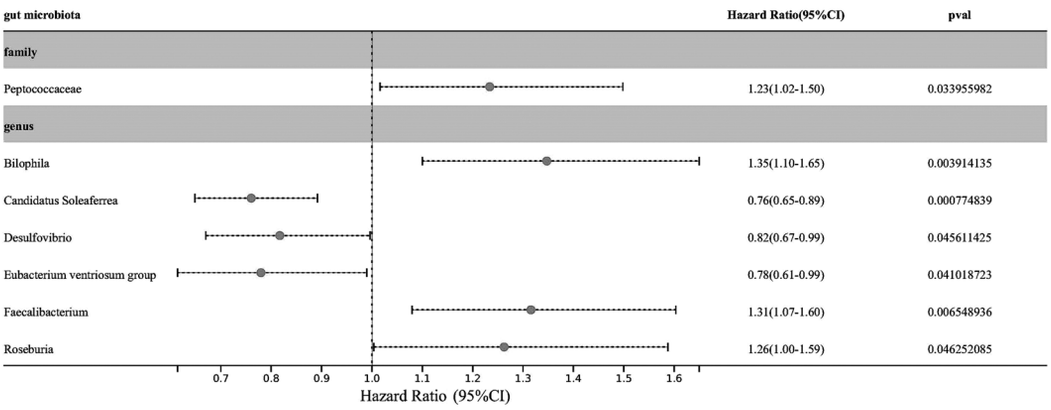

Figure 2 MR results of GM taxa with a causal relationship to dAMD CI: Confidence intervals; MR: Mendelian randomization; GM: Gut microbiota; dAMD: Dry age-related macular degeneration.

evaluated by examining the abundance of 196 bacterial taxa. At the level of the family, there are indications that an augmented presence of *Peptococcaceae* resulted in raised susceptibility to dAMD (95%CI, 1.02–1.50; P=0.03; Figure 2). A sufficient amount of power is available to examine the causal impact of *Peptococcaceae* on dAMD. Based on the IVW analysis conducted at the genus level, the study identified the following taxa as risk factors for dAMD: *Bilophila* (95%CI, 1.10-1.65; $P=3.91\times10^{-3}$), *Faecalibacterium* (95%CI, 1.07–1.60; $P=6.55\times10^{-3}$), and *Roseburia* (95%CI, 1.00–1.59; P=0.04; Figure 2). Conversely, the following taxa were found to exhibit a protective effect against dAMD: *Candidatus Soleaferrea* (95%CI, 0.65-0.89; $P=7.75\times10^{-4}$), *Desulfovibrio* (95%CI, 0.67-0.99; P=0.04) and *Eubacterium ventriosum group* (95%CI, 0.61-0.99; P=0.04; Figure 2). The statistical power to evaluate causality between the six genera and dAMD was limited. It is important to note that the remaining 189 GMs did not demonstrate statistical significance in their association with dAMD in this study.

Sensitivity Analysis To verify the precision of MR outcomes for 1 family and 6 genera related to dAMD, 196 GM taxa were subjected to heterogeneity and pleiotropy studies using information from the FinnGen project. This analysis aimed to assess the variability and potential confounding effects of genetic variants on the associations between these microbial taxa and dAMD. The results showed no significant heterogeneity or pleiotropy for any of 1 family and 6 genera. There was no statistically significant horizontal pleiotropy between these microbial species and dAMD, according to the Cochran's Q statistic and the intercept in the MR Egger regression (P>0.05; Figure 3). Therefore, we primarily relied on the IVW estimates. A leave-one-out study' findings and their visual representation indicated that it was determined that many SNPs acting together, rather than one single SNP, were responsible for the causal relationship between these microbial taxa and dAMD (Figure 4). Using the MR-RAPS method, we further validated the robustness of our findings. Specifically, *Bilophila* and *Faecalibacterium* genera showed associations with increased risk of dAMD, while the *Candidatus Soleaferrea*, and *Eubacterium ventriosum* group showed a protective effect.

Reverse MR Results for Causal Effects of GM Taxa on dAMD Furthermore, the IVW analysis revealed no meaningful causal effects of the 7 GM taxa: *Bilophila* (P=0.19), *Faecalibacterium* (P=0.21), *Roseburia* (P=0.71), *Candidatus Soleaferrea* (P=0.33), *Desulfovibrio* (P=0.79), *Peptococcaceae* (P=0.18), and *Eubacterium ventriosum* group (P=0.43) on dAMD.

We applied LDSC analysis to estimate the SNP-based heritability of GM taxa used as IVs in our MR analysis. The results revealed heritability for some GM taxa, with h^2 values as follows: genus *Faecalibacterium* (0.0247), genus *Roseburia* (0.0273), and genus *Bilophila* (0.0297). Unfortunately, heritability could not be determined for some GM taxa, including family *Peptococcaceae*, genus *Eubacterium ventriosum* group, genus *Candidatus Soleaferrea*, and genus *Desulfovibrio*, due to insufficient data. Furthermore, we examined the genetic correlation between these GM taxa and dAMD, finding that none of the GM taxa demonstrated significant genetic correlation with dAMD (P>0.05). This outcome suggests minimal confounding due to overlapping genetic architectures and supports the robustness of our MR results.

Validation with Independent Dataset In validating our findings with an independent dataset from the International AMD Genomics Consortium, we employed multiple MR methods. The IVW method provided initial estimates, which we then cross-validated using WM, MR-Egger, Simple Mode, and Weighted Mode approaches. Notably, significant results were obtained for the genus *Faecalibacterium* and *Bilophila* with the IVW method (P<0.05), supporting the robustness of our findings.

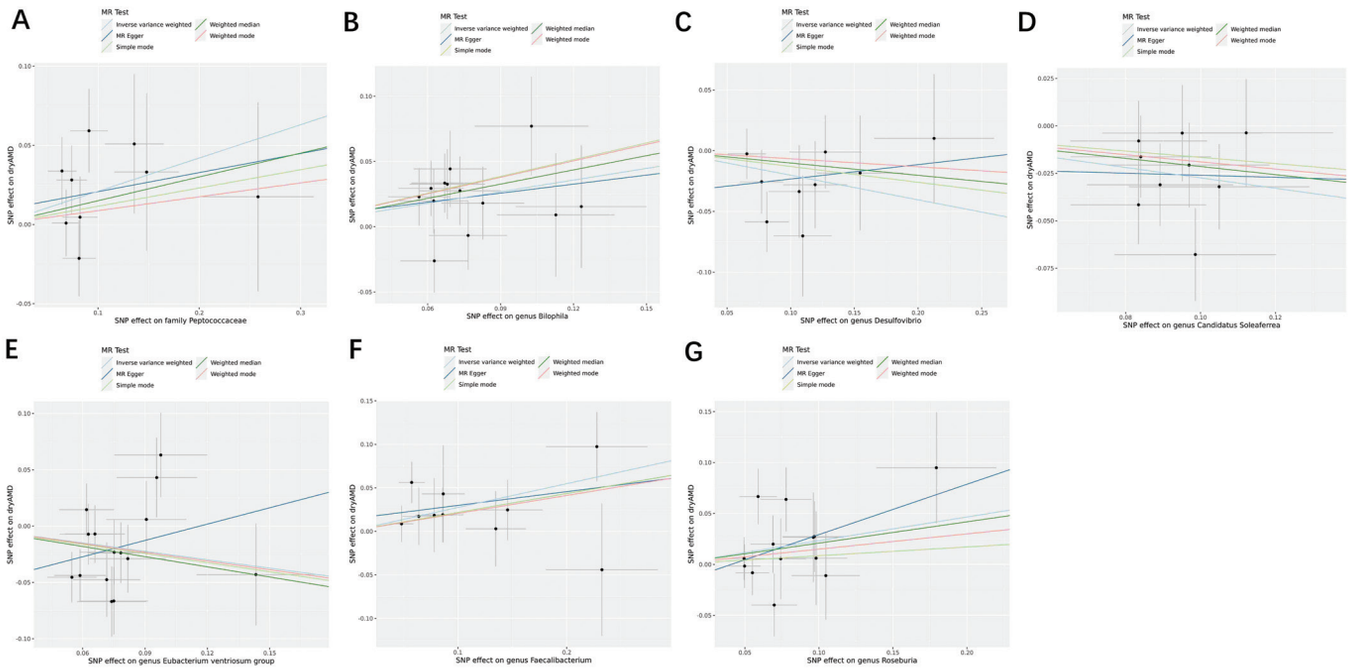

Figure 3 Scatter plots depicting associations of metabolites with dAMD A: Family *Peptococcaceae*; B: Genus *Bilophila*; C: Genus *Desulfovibrio*; D: Genus *Candidatus soleaferrea*; E: Genus *Eubacterium*; F: Genus *Faecaliabacterium*; G: Genus *Roseburia*. The causal estimates for different approaches, with each line representing the gradient. The scatter plot in the background illustrates the relationship between the SNP's effect on the outcome (represented by a point and a vertical line) and its effect on the exposure (represented by a point and a horizontal line). dAMD: Dry age-related macular degeneration.

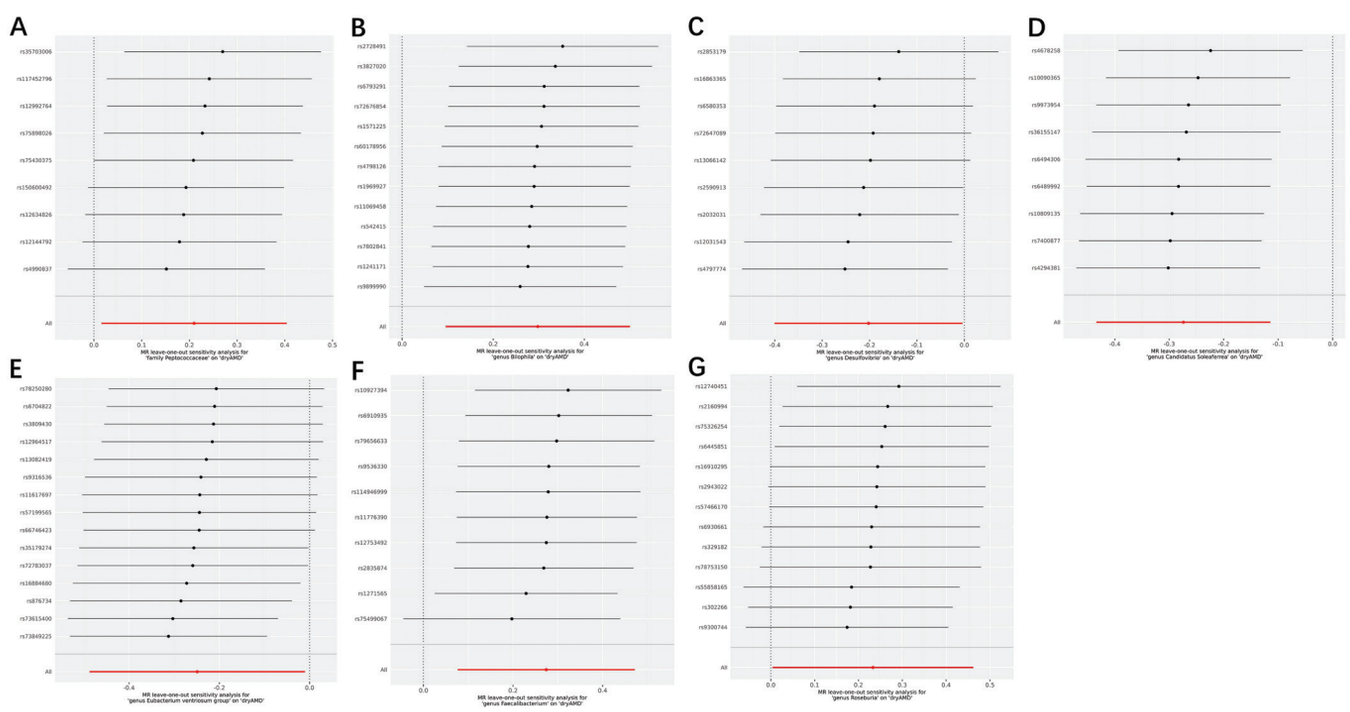

Figure 4 The leave-one-out analysis results for GM and dAMD It includes the following panels: A: Family *Peptococcaceae*; B: Genus *Bilophila*; C: Genus *Desulfovibrio*; D: Genus *Candidatus soleaferrea*; E: Genus *Eubacterium*; F: Genus *Faecaliabacterium*; G: Genus *Roseburia*. Each panel represents the leave-one-out analysis for the corresponding entity on dAMD. GM: Gut microbiota; dAMD: Dry age-related macular degeneration.

DISCUSSION

Our research harnesses MR to elucidate the role of specific GM, particularly *Faecalibacterium* and *Bilophila*, in the pathogenesis of dAMD. This study is novel in its focus, leveraging genetic data to parse the causal relationships between GM and a major ocular condition, providing a unique contribution to the burgeoning field of the gut-retina axis. The gut microbiome plays a significant role in various

physiological processes, with its dysregulation contributing to chronic inflammation, a key factor in the pathogenesis of dAMD[29-31]. By examining specific gut microbial taxa, we gain insights into their impact on retinal health.

The occurrence of GM dysbiosis results in various disorders related to neurology, immunity, and metabolism as indicated by studies[29-32]. The gut-retina axis, akin to the well-documented gut-brain axis, posits that systemic metabolic products from GM can impact retinal health[33]. Previous research has established significant insights into the impact of GM on systemic inflammation and its potential influence on chronic diseases, including AMD. Rinninella *et al*[13]. discuss the modulation of the GM by diet and micronutrients and its correlation with the risk and progression of AMD, providing a comprehensive view of the gut-retina axis and suggesting a potential role for diet in AMD management. Furthermore, Fu *et al*[34] emphasize the emerging focus on GM across various eye diseases, including AMD, underlining the importance of GM in ocular health. Cao *et al*[35] also highlight the association of dietary patterns and probiotics with AMD, pointing out that specific nutrients and probiotic interventions could modulate the GM in a way that might reduce AMD risk. Nonetheless, the role of GM dysbiosis in the pathogenesis of dAMD remains unclear.

Our findings that certain gut bacteria such as *Faecalibacterium* and *Bilophila* are associated with dAMD risk align with this theory. In our study, *Faecalibacterium*, generally known for its systemic anti-inflammatory effects, is unexpectedly associated with an increased risk of dAMD. This genus produces short-chain fatty acids (SCFAs), such as butyrate, which are typically beneficial due to their anti-inflammatory properties and roles in maintaining gut barrier integrity[36]. However, our findings suggest that in the context of dAMD, these SCFAs may exert complex, and potentially detrimental, effects on the immune responses within the retina. This observation aligns with recent evidence presented by Mao *et al*[37], who through MR analysis, identified *Faecalibacterium* as a risk factor for AMD, thereby supporting the notion that its influence on AMD may be mediated by mechanisms that complicate typical anti-inflammatory responses. On the other hand, *Bilophila* has been implicated in pro-inflammatory processes, particularly associated with high-fat diets, which could exacerbate inflammatory pathways involved in dAMD[13]. The presence of *Bilophila* in higher concentrations in individuals at risk of dAMD suggests a potential mechanistic pathway where diet and microbiome-induced inflammation converge on the retinal environment, exacerbating or precipitating degenerative changes[38].

Unlike prior studies that have typically addressed AMD as a uniform disease, our research meticulously focuses on dAMD, which accounts for 85%-90% of all AMD cases and exhibits distinct pathophysiological traits and therapeutic targets compared to wet AMD[37,39]. This specificity is essential for tailoring interventions more effectively towards the predominant form of the disease. We have employed advanced statistical methodologies such as bidirectional MR, LDSC, and MR-RAPS. These techniques are critical for refining the accuracy of our causal inferences, particularly in complex traits like dAMD, where traditional observational approaches may be confounded by numerous external factors. Our use of a large, independent validation dataset from the International AMD Genomics Consortium not only corroborates our initial findings but also enhances the generalizability of our results across broader and more diverse populations[22]. Further, we have expanded the discussion to include a detailed comparison with referenced studies, emphasizing our methodological enhancements and the specific contributions of our work in isolating the effects of individual microbial genera like *Faecalibacterium* and *Bilophila* on dAMD[37,39]. This approach not only fills a critical gap in understanding but also paves the way for potential targeted therapeutic strategies that could modulate these specific microbial populations to manage or prevent dAMD.

The concept of the gut-retina axis has gained traction as more research points to the influence of GM on various physiological processes, including immune response and inflammation[40]. Our study suggests that microbial metabolites, such as those produced by *Faecalibacterium* and *Bilophila*, could travel from the gut to the retina, affecting its health. In particular, these metabolites might influence the inflammatory pathways involved in dAMD. Recent studies have shown that gut dysbiosis can lead to increased permeability and inflammation, which could trigger or worsen the retinal degenerative processes characteristic of dAMD[41]. The implications of our study suggest that interventions targeting the GM could offer new therapeutic avenues for managing or preventing dAMD. Modifying diet or using prebiotics and probiotics to alter the abundance of specific microbial taxa might positively impact the progression of dAMD[42]. This aligns with earlier studies that found links between dietary patterns and AMD risk, suggesting that GM modulation could play a crucial role in the prevention of retinal diseases. Further research is needed to unravel the specific mechanisms through which these microbial taxa influence the retinal environment. Longitudinal studies and randomized controlled trials focusing on dietary interventions could shed light on how changes in GM affect dAMD risk and progression.

However, there are several limitations to consider. MR studies involving GM cannot completely exclude weak instrumental bias, even when the assumptions of MR are met. Additionally, the estimation of causal effects in our MR analysis was based

on summary-level data from the MiBioGen consortium, which includes results from 24 population-based cohorts covering various ancestries, such as Asian, African, European, Middle Eastern, and Hispanic. This heterogeneity in the GWAS source data introduces the risk of population structure bias, as it may not align with the ancestry composition of the dAMD outcome data, which was sourced solely from the FinnGen project, comprising individuals of European descent. To address this issue, we utilized principal components (PCs) as covariates in our analysis to account for population stratification. However, even with PCs, some underlying differences in genetic architecture across ancestry groups may persist, potentially impacting the validity of our causal estimates. To further minimize this risk, we conducted sensitivity analysis focusing only on IVs derived from cohorts with European ancestry. This approach ensures that the population structure for IV construction closely resembles the structure of the outcome data. Additionally, we implemented the MR-PRESSO approach to detect and correct for outliers that might arise from population stratification or other forms of bias. The MR-PRESSO analysis helped identify and remove outliers, ensuring the robustness of our causal estimates. Furthermore, the causal relationship between GM taxa and dAMD cannot be directly established due to the complex nature of the gut-retina axis and the multifactorial etiology of dAMD. Finally, the selection of IVs was restricted by a limited number, and expanding the sample size in future studies would provide more comprehensive insights into the connection between GM taxa and dAMD.

Our study provides compelling evidence for the role of GM in dAMD, highlighting the influence of *Faecalibacterium* and *Bilophila* on the risk of this condition. The gut-retina axis emerges as a key area for further exploration, with the potential for therapeutic interventions targeting the GM to prevent or manage dAMD. These findings not only contribute to the existing body of research but also offer new directions for clinical practice and future research.

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Authors' contributions: Huang HY: Conceptualization, Data analysis, Writing. Wang J: Writing–review & editing, Investigation, Visualization. Qin B: Analysis, Writing. Tan Y: Resources, Reviewing, Project administration. All authors have directly accessed and verified the underlying data reported in the manuscript.

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