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ORIGINAL INVESTIGATION

Mendelian randomization analysis reveals causal relationships between gut microbiome and optic neuritis

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Abstract

Background It is unclear whether gut microbiota (GM) affects the risk of optic neuritis (ON) through the "gut-brain" axis and the "gut-retina" axis. To examine the causal relationship between GM and ON, we conducted Mendelian randomization (MR) study.

Methods Up to 18,340 samples of 24 population-based cohorts were included in genome-wide association study (GWAS) of 196 GM taxa. ON outcomes were selected from the FinnGen GWAS (951 ON cases and 307,092 controls). In addition, the GWAS based on UK Biobank (UKB) (105 ON cases and 456,243 controls) was used for further exploration. Inverse variance weighted (IVW) was carried out to estimate their effects on ON risk and the MR assumptions were evaluated in sensitivity analyses.

Results Among the 196 GM taxa, the IVW results confirmed that Family -*Peptococcaceae* ($P = 2.17 \times 10^{-3}$), Genus- *Hungatella* ($P = 4.57 \times 10^{-3}$) and genus-*Eubacterium_rectale_group* ($P = 0.02$) were correlated with the risk of ON based on Finngen GWAS. Based on data from UKB, Genus- *Eubacterium_hallii_group* ($P = 1.50 \times 10^{-3}$) and Genus- *Ruminococcaceae_UCG_002* ($P = 0.02$) were correlated with the risk of ON. At the phylum, class and order levels, no GM taxa were causally related to ON ($P > 0.05$). Heterogeneity ($P > 0.05$) and pleiotropy ($P > 0.05$) analysis confirmed the robustness of the MR results.

Conclusion Our MR findings support the causal effect of specific GM taxa on ON. GM may affect the risk of ON through the "gut-brain" axis and the "gut-retina" axis. However, further research is needed to confirm the relevant mechanism of the relationship between GM and ON.

Introduction

Optic neuritis (ON) is a neuroinflammatory disease involving axial injury and demyelination (Lee et al. 2020; Toosy et al. 2014) and has a global incidence of up to 2.18 per 100,000/year (Toosy et al. 2014). The symptoms of vision loss and eye pain may deteriorate rapidly, but recovery may span over a year or more. Therefore, effective prevention and treatment in clinical practice are particularly important.

ON is usually related to immune diseases such as neuromyelitis optica spectrum disorders (NMOSD) and multiple sclerosis (MS). However, the specific mechanism of ON remains unclear. Interestingly, many studies have confirmed that the gut microbiome (GM) influences immune diseases such as ON (Ghezzi et al. 2021; Zamvil et al. 2018). Cui et al. (2020) found that inflammation caused by GM ecological imbalance leads to NMOSD, resulting in spinal cord and optic nerve injury. Furthermore, autoantibody aquaporin-4

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is related to NMOSD and also has extensive homology with GM (Varrin-Doyer et al. 2012). Similarly, Ghezzi et al. (2021) described the "gut-brain" axis as a bidirectional communication between the nervous system and GM, which allows targeted GM therapy to exert a certain effect on MS. Rowan et al. (2017) also found an association between the GM and the retina and proposed the concept of the "gut-retina" axis. However, optic nerve involvement has not been investigated in "gut-retina" axis studies. As the optic nerve is an extension of the brain to the eye, GM may possibly play a role in optic neuritis or retinal damage. In terms of anatomical location, the optic nerve is distant from the gastrointestinal tract, and many factors may interfere with the connection between the two. Accurate conclusions cannot be reasonably drawn from randomized controlled studies (RCTs), and no studies have successfully explored this issue.

As opposed to RCT results that may be impacted by confounding factors, Mendelian randomization (MR) studies based on genetic variation offer an alternative method for exploring the effects of GM on distal tissues (Thanassoulis and O'Donnell 2009). Due to the random principle of meiosis, MR analysis is not susceptible to reverse causality and confusion. Zhuang et al. (2020) found a direct causal association between GM and neuropsychiatric disorders through the MR framework, confirming the association of the "gut-brain" axis. Xu et al. (2021) also confirmed the impact of GM on the risk of autoimmune diseases through MR studies. Therefore, this study assesses the possibility of a causal association between each GM taxa and ON through MR studies, which may strengthen the theoretical basis for the "gut-brain" axis and the "gut-retina axis" and provide new ideas for the prevention and treatment of optic neuritis.

Methods

Study overview

To evaluate the causal relationships among GM taxa and ON, a two-sample MR was conducted using instrumental variables (IVs) curated from the largest study to date to investigate the genetics of GM. To avoid sample overlap, summary-level data of ON were obtained from the FinnGen Project (https://r7.finngen.fi/).

Ethics statement

The summary-level data of the study used for analysis consisted of de-identified public data and were obtained from published studies. All studies were performed in accordance with the declaration of Helsinki and were approved by the ethics committee of the corresponding institution. Moreover, our results were reported in line with the MR-STROBE guidance (Davey Smith and Hemani 2014).

Exposure sources of GM taxa

The summary statistics for GM taxa were provided by the GWAS of the MiBioGen consortium (Kurilshikov et al. 2021). As the largest GM genetics research of human microbiome at present, a total of 18,340 samples of 16S rRNA gene sequencing data were obtained from 24 population-based cohorts (11 countries of Asian, African, European, Middle Eastern and Hispanic ancestries). The data of GM taxa were obtained by 7 fecal DNA extraction methods. The composition of GM taxa was analyzed for three 16S rRNA regions. After rarefying to 10,000 reads for rarefaction reproducibility, GM was classified by level (phylum, class, order, family, genus) using direct taxonomic binning. Following adjustment for age, principal genetic components, technical covariates, sex and 5 (monoethnic cohorts) or 10 (multiethnic cohorts) principal components (PCs), 122,110 genetic loci were obtained from 211 taxa. More details of the GM data were described elsewhere (Kurilshikov et al. 2021). Among the 211 GM taxa, the unknown GM taxa were removed, and the 196 known GM taxa were analyzed.

Considering that the data source of ON was European, the GWAS from the TwinsUK Registry (Goodrich et al. 2016) was used as the exposure data source for exploration. Goodrich et al. collected 3,261 fecal samples from 1126 twin pairs and analyzed the 16S rRNA sequencing data. These twin pairs were all European. After adjusting for age, gender, shipment date, collection method and first 3 PCs, 163 GM taxa were obtained. More details on the method of GWAS can be found in the original articles (Goodrich et al. 2016).

Outcome sources of ON

ON data were obtained from the FinnGen consortium (release R7, 2022) (FinnGen 2022). A total of 16,962,023 variables were obtained from Finnish biobanks, and the digital health record data of 309,154 subjects were obtained from Finnish health registries. The diagnosis of ON was based on the definition by the International Classification of Diseases 10th version (ICD-10). After adjusting for age, sex, genetic relatedness, genotyping batch, and first 10 PCs, 951 ON cases and 307,092 controls of Finnish ancestry were used for the analysis of ON.

In addition, the GWAS of Jiang et al. (2021) was used for verification. Jiang et al. analyzed the data of 456,348 individuals (11,842,647 variables) in the UK Biobank (UKB). In total, 2989 binary traits were analyzed, including ON. The 2989 traits were generated from the ICD-10 records from the UKB. After adjusting for age, sex, and the top 20 PCs provided by the UKB as covariates, 105 ON cases and

456,243 European ancestry controls were included in the analysis of ON.

Quality control of IVs

To obtain reliable results, MR analysis needs to satisfy the following 3 assumptions (Davey Smith and Hemani 2014) (Fig. 1): (1) The IVs eventually comprised independent single nucleotide polymorphisms (SNPs) associated with each GM taxa; (2) All IVs that passed quality control should not be associated with confounding factors; (3) The effects of the IVs on the risk of each GM taxa are only mediated by ON.

Quality control was performed on SNPs to meet the above assumptions for MR analysis. Similar to most current MR studies, the genome-wide significance threshold ($P<5\times10^{-8}$) was selected to screen SNPs. In addition, considering the small number of IVs obtained, the locus-wide significance ($P<1\times10^{-5}$) was selected as the threshold for obtaining IVs by referring to the current MR research on GM. Based on the European-based 1000 Genome Projects, a linkage disequilibrium (LD) analysis ($R^2<0.001$, clumping distance = 10,000 kb) was carried out to assure statistical independence. Subsequently, the palindromic SNPs were removed to ensure that the effects of the SNPs on each GM taxa corresponded to the same allele as the effects on ON. The F statistic was then used to exclude weak IVs ($F<10$). The specific formula of the F statistic was $\frac{R^2(n-k-1)}{k(1-R^2)}$ (R^2: GM taxa variance explained by SNPs; n: sample size; k: the number of included IVs). MR Steiger filtering (Hemani et al. 2017) was used to test the causal direction of each IV on the GM taxa and ON. To minimize the correlation between SNPs and confounders, PhenoScanner (http://www.phenoscanner.medschl.cam.ac.uk/) was used to check all included IVs and the SNPs associated with confounders were removed.

MR analysis

Based on the presence or absence of heterogeneity, the inverse variance weighted (IVW) test in the fixed/random-effects model was used as the principal analysis for causal associations to obtain unbiased estimates. Four additional methods were employed to validate the results. (1) The weighted median (WM) method (Bowden et al. 2016a): If more than 50% of weights were derived from valid SNPs, the weighted median (WM) method could provide an effective estimate of causality between GM taxa and ON; (2) MR Egger (Bowden et al. 2016b): for pleiotropic results

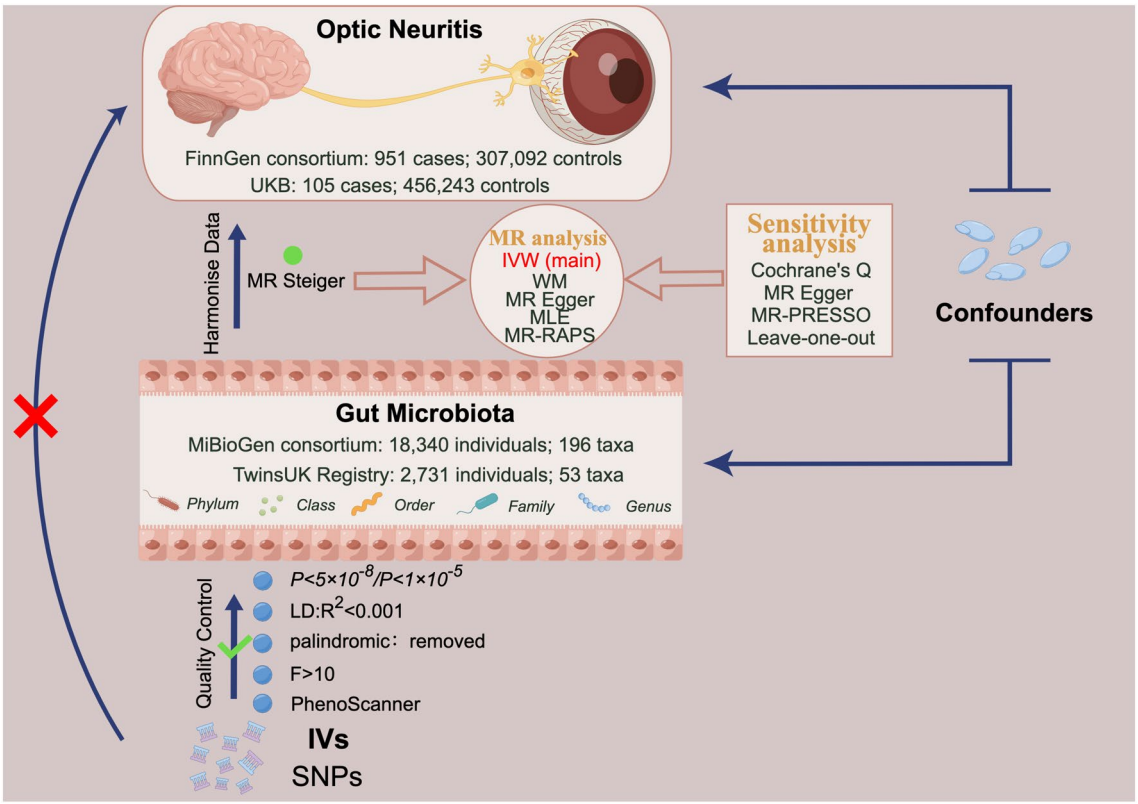

Fig. 1 Overview of the analysis process of the causal relationship between the gut microbiome and optic neuritis through MR analyses

(the results of MR Egger are still valid even if all IVs are invalid), the MR Egger results should be considered in combination. Notably, this method had the lowest power among the 5 methods; (3) Maximum likelihood estimator (MLE) (Burgess et al. 2015): the results assume the linear correlation of ON and each GM taxa with normal distribution and allow for uncertainty in both gene–GM taxa and gene–ON associations; (4) MR robust adjusted profile score (MR-RAPS) (Zhao et al. 2019): if many weak IVs are included, MR-RAPS can provide higher statistical power. Therefore, after sensitivity analysis, comprehensive consideration of the results of the above 5 methods strengthens the evidence of causality. In addition, for each GM taxa, causal associations were assessed using the Wald ratio (WR) method if only a single SNP passed quality control.

Sensitivity analyses

Cochrane's Q method was used to test the heterogeneity of the MR analysis ($P<0.05$ was considered as possible heterogeneity in IVs). The pleiotropic analysis was preliminarily judged by the intercept of MR Egger regression ($P<0.05$ was considered as possible pleiotropy in IVs). For the causality between GM taxa and ON based on MR results, the pleiotropy was further assessed with MR-PRESSO ("MR-PRESSO" package), and the possible outliers were removed. To ensure the robustness of MR results, the leave-one-out method (Xiang et al. 2021) was employed to rule out the possibility of the influence of a single SNP on the causality between GM taxa and ON.

Statistical analysis

All analyses were conducted using R software (Version 4.1.1). MR analysis of the causal associations between GM taxa and ON was performed using the R package "TwoSampleMR". The odds ratio (OR) revealed the influence of GM taxa on ON. $P<0.05$ was considered suggestive of evidence for a potential causal effect (Waters and Ley 2019; Xiang et al. 2021). At each GM level, the false discovery rate (FDR) was used to adjust the results of multiple comparisons (Benjamini and Yekutieli 2001). In addition, the website (http://cnsgenomics.com/shiny/mRnd/) was used to calculate the power (Hartwig et al. 2017).

Result

Selection of IVs related to each GM taxa (MiBioGen consortium)

Through LD analysis, deletion of palindromic SNPs and PhenoScanner, 1872 SNPs ($P<1\times10^{-5}$) were finally obtained from 196 bacterial taxa (excluding the unknown taxa) based on FinnGen consortium (Supplementary Table 1). These SNPs ($P<1\times10^{-5}$) were used as IVs to analyze the causal relationship with ON. The 196 bacterial taxa comprised 9 phyla, 16 classes, 20 orders, 32 families and 119 genera. All 1872 SNPs were not weak IVs (the minimum value of F was 14.589). At the same time, MR Steiger was used to ensure the directional accuracy of the causal relationship for each IV. Under the threshold of genome-wide statistical significance ($P<5\times10^{-8}$), only 15 SNPs passed the quality control and were used as IVs, which suggested sufficient validity ($F>10$). These IVs were associated with 2 orders, 3 families and 10 genera (Supplementary Table 1).

Based on the GWAS of Jiang et al. (2022) from UKB data, 1900 SNPs ($P<1\times10^{-5}$) related to 196 GM taxa were used as IVs (Supplementary Table 1). All 1900 SNPs were not weak IVs ($F>10$) and MR Steiger ensured the directional accuracy of the causality. Under the threshold of genome-wide statistical significance ($P<5\times10^{-8}$), only 11 SNPs (related to 1 order, 2 family and 8 genera, respectively) were used as IVs (Supplementary Table 1),

Results of MR Analysis (locus-wide significance, $P<1\times10^{-5}$)

Based on data from Finngen consortium

Figure 2A and Supplementary Table 2 show the impact of changes in 196 bacterial taxa abundance on ON risk based on data from the Finngen consortium. At the family level, there is suggestive evidence that an increase in *Peptococcaceae* abundance resulted in increased ON risk (IVW: $P=2.17\times10^{-3}$; WM: $P=4.10\times10^{-3}$; MLE: $P=2.21\times10^{-3}$; MR-RAPS: $P=3.30\times10^{-3}$) (Fig. 2A, Table 1). The IVW result of *Peptococcaceae* tends to pass FDR correction (FDR = 0.0695) (Supplementary Table 2). The power (more than 80%) is enough to test causal effects of *Peptococcaceae* on ON (Supplementary Table 3). At the genus level, the IVW results indicated that *Hungatella* ($P=4.57\times10^{-3}$) and *Eubacterium_rectale_group (E. rectale)* ($P=0.02$) were protective factors against ON (Fig. 2A, Table 1). Furthermore, this conclusion was confirmed by the results of WM ($P_{Hungatella}=8.06\times10^{-3}$, $P_{E.\ rectale}=0.02$), MLE ($P_{Hungatella}=5.88\times10^{-3}$, $P_{E.\ rectale}=0.02$), and MR- RAPS ($P_{Hungatella}=8.82\times10^{-3}$, $P_{E.\ rectale}=0.03$) (Fig. 2A, Table 1). The MR results of *Hungatella* and *E. rectale* failed to pass FDR correction (FDR > 0.05). There is limited power (less than 80%) to test the causality of 2 genera on ON (Supplementary Table 3). For all the 3 bacterial taxa mentioned above, the MR Egger results showed no association with ON ($P>0.05$) (Fig. 2A, Table 1).

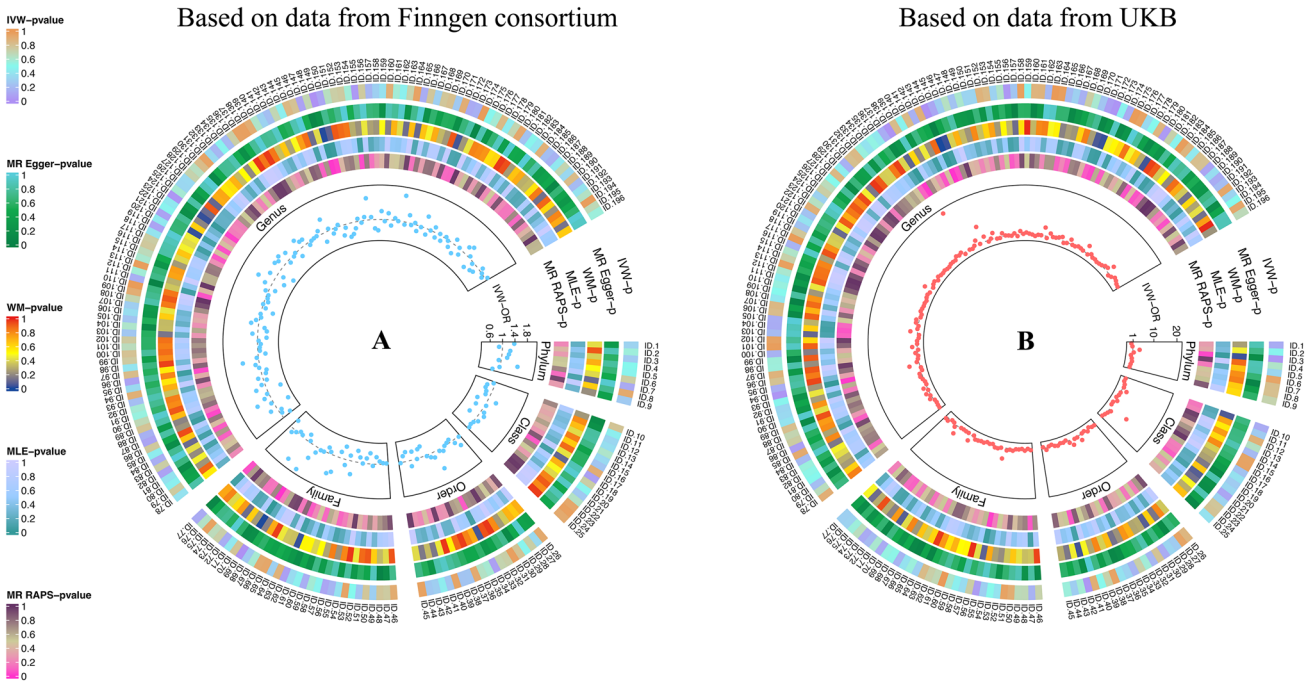

Fig. 2 Causal analysis of gut microbiome taxa and optic neuritis based on MR analyses (locus-wide significance, $P < 1 \times 10^{-5}$). From outside to inside, the P values of IVW, MR Egger, WM, MLE, and MR- RAPS are represented, respectively. The GM taxa name represented by each ID can be found in Supplementary Table 2. **A**. MR results based on Finngen consortium. **B**. MR results based on UKB

Table 1 The MR results between GM taxa and ON based on Finngen consortium

Bacterial taxa	Method	OR	95%CI	*P*
Family -Peptococcaceae	IVW	1.96	1.28–3.02	2.17×10^{-3}
Family -Peptococcaceae	WM	2.33	1.31–4.14	4.10×10^{-3}
Family -Peptococcaceae	MR Egger	2.67	0.81–8.73	0.15
Family -Peptococcaceae	MLE	2.01	1.60–2.52	2.21×10^{-3}
Family -Peptococcaceae	MR-RAPS	2.00	1.58–2.53	3.30×10^{-3}
Genus-Hungatella	IVW	0.56	0.38–0.84	4.57×10^{-3}
Genus-Hungatella	WM	0.49	0.29–0.83	8.06×10^{-3}
Genus-Hungatella	MR Egger	0.11	0.01–1.25	0.17
Genus-Hungatella	MLE	0.55	0.45–0.69	5.88×10^{-3}
Genus-Hungatella	MR-RAPS	0.55	0.44–0.69	8.82×10^{-3}
Genus-E. rectale	IVW	0.47	0.24–0.90	0.02
Genus-E. rectale	WM	0.37	0.15–0.88	0.02
Genus-E. rectale	MR Egger	0.22	0.02–2.35	0.26
Genus-E. rectale	MLE	0.46	0.32–0.64	0.02
Genus-E. rectale	MR-RAPS	0.46	0.32–0.65	0.03

MR mendelian randomization, *IVW* inverse variance weighted, *WM* Weighted median, *MLE* maximum likelihood estimator, *MR-RAPS* MR robust adjusted profile score, *E. rectale Eubacterium_rectale_group*

Based on data from UKB

Figure 2B and Supplementary Table 4 show the impact of changes in 196 bacterial taxa abundance on ON risk based on data from UKB. At the genus level, *Eubacterium_hallii_group* (*E. hallii*) (IVW: $P = 1.50 \times 10^{-3}$; WM: $P = 1.35 \times 10^{-3}$; MR Egger: 0.03; MLE: $P = 7.46 \times 10^{-4}$; MR-RAPS: $P = 1.12 \times 10^{-3}$) and *Ruminococcaceae_UCG_002* (IVW: $P = 0.02$; WM: $P = 0.03$; MLE: $P = 0.04$; MR-RAPS: $P = 0.03$) indicated a causal relationship with ON (Fig. 2B, Table 2). The power (more than 80%) is enough to test the causality of *Ruminococcaceae_UCG_002* on ON and the power is limited (less than 80%)to test the causality of *E. hallii* (Supplementary Table 3). For all the 2 bacterial taxa mentioned above, the MR results did not pass FDR correction ($P > 0.05$).

Sensitivity analyses

Based on data from the Finngen consortium

Heterogeneity and pleiotropy analyses of MR results were performed for 196 GM taxa based on data from the Finngen consortium (Supplementary Table 2). The results of the heterogeneity and pleiotropy analyses further validated the accuracy of the MR results of 1 family and 2 genera on ON.

Table 2 The MR results between GM taxa and ON based on UKB

Bacterial taxa	Method	OR	95%CI	*P*
Genus- E. hallii	IVW	8.35	2.25–30.95	1.50×10^{-3}
Genus- E. hallii	WM	18.03	3.07–105.71	1.35×10^{-3}
Genus- E. hallii	MR Egger	29.01	1.96–428.84	0.03
Genus- E. hallii	MLE	9.07	4.72–17.44	7.46×10^{-4}
Genus- E. hallii	MR-RAPS	9.03	4.59–17.73	1.12×10^{-3}
Genus- Ruminococcaceae_UCG_002	IVW	0.24	0.07–0.80	0.02
Genus- Ruminococcaceae_UCG_002	WM	0.14	0.03–0.79	0.03
Genus- Ruminococcaceae_UCG_002	MR Egger	0.13	0.01–3.40	0.24
Genus- Ruminococcaceae_UCG_002	MLE	0.31	0.17–0.55	0.04
Genus- Ruminococcaceae_UCG_002	MR-RAPS	0.28	0.16–0.51	0.03

MR Mendelian randomization, *IVW* Inverse variance weighted, *WM* Weighted median, *MLE* maximum likelihood estimator, *MR-RAPS* MR robust adjusted profile score, *E. hallii Eubacterium_ hallii _group*

At the family level, no heterogeneity (IVW: $P=0.56$; MR Egger: $P=0.48$) and no pleiotropy was found in *Peptococcaceae* (MR Egger: $P=0.60$; MR-PRESSO: $P=0.606$) (Table 3). In addition, the leave-one-out analysis revealed no significant difference in causal estimations of *Peptococcaceae* on ON (Supplementary Fig. 1A). At the genus level, no heterogeneity was observed in the 2 GM taxa (IVW: $P_{Hungatella}=0.68$, $P_{E.\ rectale}=0.42$; MR Egger: $P_{Hungatella}=0.91$, $P_{E.\ rectale}=0.36$) (Table 3). The MR Egger regression showed no pleiotropy in *Hungatella* ($P=0.28$) and *E. rectale* ($P=0.53$) for ON (Table 3). Moreover, MR-PRESSO analysis of *Hungatella* ($P=0.718$) and *E. rectale* ($P=0.465$) showed no outliers (Table 3). On the other hand, the leave-one-out results revealed that no single IV drives the causal associations between identified bacterial taxa and ON (Supplementary Fig. 1B and 1C).

Based on data from the UKB

Heterogeneity and pleiotropy analyses of MR results were performed for 196 GM taxa based on data from the UKB (Supplementary Table 2). Cochrane's Q revealed no heterogeneity in *Genus- E. hallii* (IVW: $P=0.32$; MR Egger: $P=0.33$) *and Ruminococcaceae_UCG_002* (IVW: $P=0.44$; MR Egger: $P=0.38$) (Table 3). Meanwhile, no pleiotropy was shown in *Genus- E. hallii (*MR Egger regression: $P=0.32$; MR PRESSO: $P=0.384$) *and Genus- Ruminococcaceae_UCG_002* (MR Egger regression: $P=0.69$; MR PRESSO: $P=0.439$) (Table 3). The leave-one-out results revealed that no single IV drives the causal associations between identified bacterial taxa and ON (Supplementary Fig. 2A and 2B).

Results of MR Analysis (genome-wide statistical significance, $P<5\times10^{-8}$)

Based on data from the Finngen consortium, WR results of each GM taxa showed that only *Genus-Oxalobacter* reduced the risk of ON (OR = 0.37, 95%CI, 0.17–0.79, $P=1.04\times10^{-2}$) (Fig. 3). The MR results of other GM taxa did not show a causal association with ON ($P>0.05$) (Fig. 3). Based on data from UKB, there was no significant causal association between each GM taxa and the risk of

Table 3 The sensitivity analyses of causality between GM taxa and ON based on MR results

Bacterial taxa	*Q-P* (IVW)	*Q-P* (MR Egger)	Pleiotropy- *P*	MR-PRESSO
Based on data from Finngen consortium				
Family -Peptococcaceae	0.56	0.48	0.60	0.606
Genus-Hungatella	0.68	0.91	0.28	0.718
Genus-E. rectale	0.42	0.36	0.53	0.465
Based on data from UKB				
Phylum- Verrucomicrobia	0.75	0.73	0.44	0.751
Genus- E. hallii	0.32	0.33	0.32	0.384
Genus- Ruminococcaceae_UCG_002	0.44	0.38	0.69	0.439

MR Mendelian randomization, *IVW* Inverse variance weighted, *WM* Weighted median, *MLE* maximum likelihood estimator, *MR-RAPS* MR robust adjusted profile score, *E. rectale, Eubacterium_rectale_group*; *E. hallii, Eubacterium_ hallii _group*

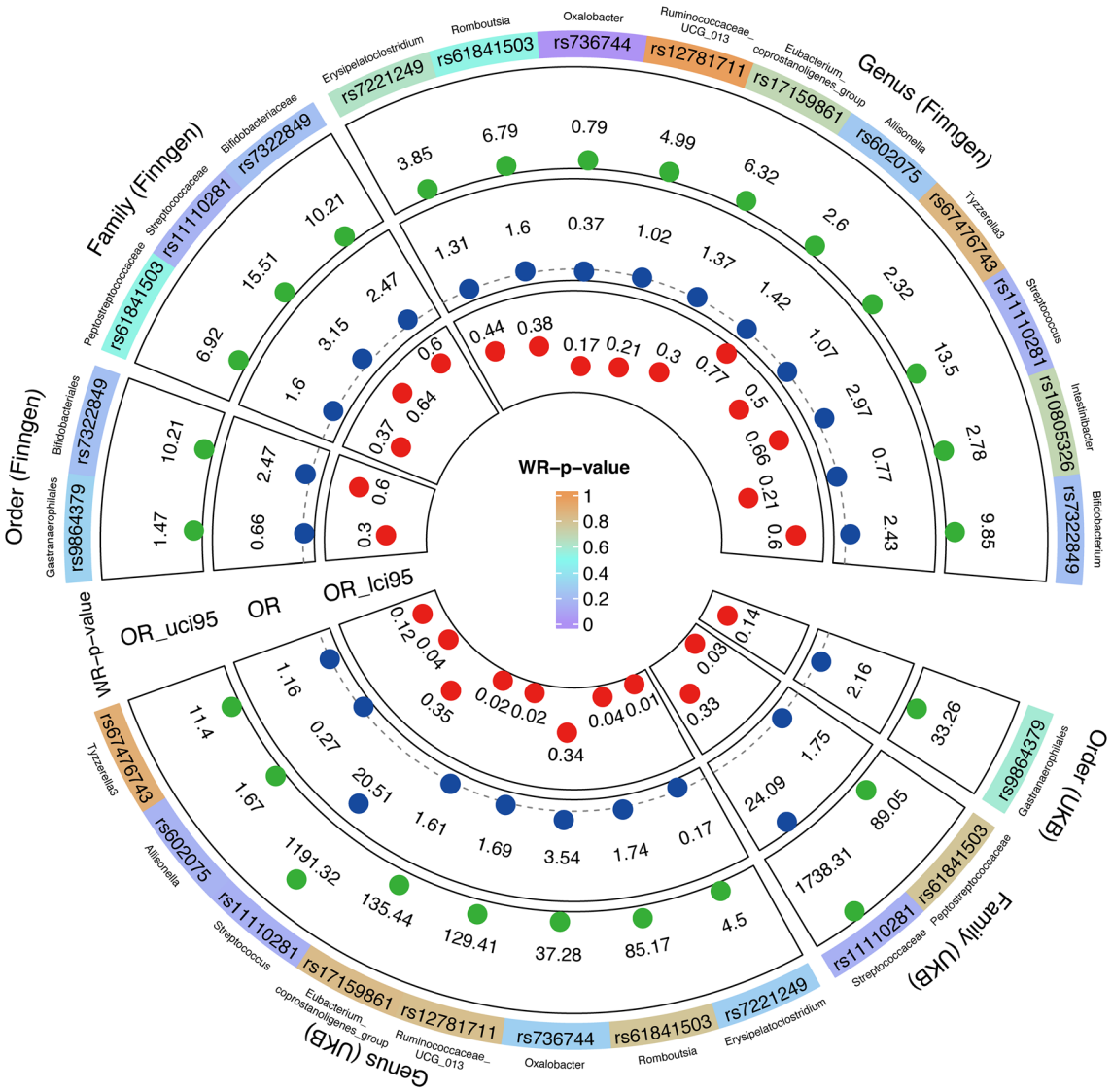

Fig. 3 The results of Wald ratio (genome-wide statistical significance, $P<5\times10^{-8}$) between gut microbiome taxa and optic neuritis based on Finngen consortium and UKB

optic neuritis ($P>0.05$) (Fig. 3). Furthermore, the number of IVs was too small to conduct a sensitivity analysis.

Results of MR and sensitivity analysis based on twinsUK registry

Based on the TwinsUK Registry, only the 608 SNPs that have passed the quality control were used as IVs (Supplementary Table 3). These IVs (F > 10) were related to 5 phyla, 8 classes, 9 orders, 15 families and 16 genera, respectively (Supplementary Table 4).

The 5 methods of MR analysis demonstrated no effect of GM taxa on the risk of ON (all P >0.05) (Supplementary Table 4). The sensitivity analysis results supported the robustness of the MR analysis (Supplementary Table 5).

Discussion

This study analyzed for the first time the causal relationship between GM taxa and ON through multiple data sets, including the largest general population-based cohort. Concerning the GWAS of Finngen consortium, our study supports *Peptococcaceae* as a risk factor for ON at the family level. At the genus level, increases in the abundance of *Hungatella* and *E. rectale* may lead to a reduced risk of ON. These results were not validated in the GWAS based on UKB. However, the GWAS based on UKB found two other GM taxa (*E. hallii and Ruminococcaceae_UCG_002)* were related to the risk of ON at the genus level. It is worth noting that both *E. rectale* and *E. hallii* belong to *Eubacterium*. In addition, due to the limited sample size and the difference in detection

accuracy, the above GM taxa yielded no IVs in the TwinsUK Registry and could not be evaluated. On higher taxonomic levels than families, no relationship was observed between GM taxa and ON, which is in accordance with the results based on the TwinsUK Registry. Therefore, the abundance of these specific GM taxa at the family or genus level are likely new biomarkers for ON. Targeted regulation of the abundance of these GM taxa may be applied in the treatment and prevention of ON.

Immune diseases often lead to ON. Although no study has reported the effect of specific GM taxa on ON, the association between specific GM taxa and immune diseases has been previously explored (Hu et al. 2020). Studies speculate that it may be due to changes in the abundance of GM taxa that make the immune system respond to the body (Donaldson et al. 2018; Hu et al. 2020). Bosák et al. (2021) found a significant enrichment of *Hungatella* in immunodeficient patients and believed that *Hungatella* could interfere with the immune system by regulating IgA. In addition, Navarro et al. (Navarro-López et al. 2022) also found *Hungatella* to cause dysbiosis in patients with MS. Similarly, Pröbstel et al. (2020) also reported the relationship between the changes in GM and MS, and found that GM-specific IgA, as the mediator of MS, plays a key role during neuroinflammation. Interestingly, the detection of cerebrospinal fluid in patients with ON revealed that IgA was significantly negatively correlated with the biomarker myelin basic protein. Thus, *Hungatella* may also control IgA and influence the development of ON. Zhao et al. (2018) found that an increased abundance of *E. hallii* in Hashimoto's thyroiditis patients. This study reflected the results of some RCTs demonstrating the association between *Hungatella* and *E. hallii* and might provide further evidence for ON treatment and prevention.

At present, many studies have confirmed that GM taxa have an impact on the brain and neurological diseases and put forward the concept of the "gut-brain" axis. The optic nerve is an extension of the brain, and our investigations into ON have further verified the mechanism of the "gut-brain" axis. In the study of Cattaneo et al. (2017), anti-inflammatory *E. rectale* affected the levels of pro-inflammatory cytokines IL-1β, NLRP3, and CXCL2. These are possibly associated with a peripheral inflammatory state in patients with Alzheimer's disease. Hence, *E. rectale* and *E. hallii* may also affect the disease status of ON through these inflammatory markers, thereby affecting the risk of ON. Our results also confirm this view. Through the evaluation of patients with concussions, Soriano et al. (2022) found a lower abundance of *E. rectale*, suggesting that targeted treatment may improve brain injury, confirming the connection between *E. rectale* and the brain. Lu et al. (2019) found a significant increase in the abundance of *E. rectale* in patients with bipolar depression after treatment. The same study also found a significant correlation between GM taxa and T cells. Notably, a significant increase in activated T cells was observed during the onset of ON, indicating that T cells play a role in the pathogenesis of ON (Matsuya et al. 2011). Thus, *E. rectale* can affect ON by activating T cells. Gong et al. found a difference in the abundance of *E. hallii* in patients with encephalitis, indicating that *E. hallii* may affect the pathogenesis of encephalitis through the metabolic pathway of the host and influence the production of inflammatory factors. In addition, Jiang et al. (2022) found that *Ruminococcaceae_UCG_002* is the main genus mediating the positive association between chronic insomnia and cardiometabolic diseases. Our results also found that *Ruminococcaceae_UCG_002* had a positive impact. Although the sample size of the above studies is not as large as the data source we used (the MiBioGen consortium), they all confirm the impact of *E. rectale*, *E. hallii* and *Ruminococcaceae_UCG_002* on the brain and nerves. Considering the association of the "gut-brain" axis, we believe that targeted regulation of specific GM taxa (like *E. rectale*) can also exert a similar effect on the improvement of ON.

The occurrence of ON leads to retinal damage. Therefore, our results also support the connection of the "gut-retina" axis. Rowan et al. (2017) found for the first time that regulating GM abundance could improve retinal damage and described the "gut-retina" axis connection. Changes in GM taxa lead to corresponding changes in the gut barrier, affecting the homeostasis of the immune system and regulating retina-specific immune cells (Andriessen et al. 2016; Fernandes et al. 2019). The microbiome analysis of Beli et al. (2018) showed that phylum *Firmicutes* might provide a neuroprotective effect in the retina of mice. Our previous study also found that *E. rectale* was associated with diabetes retinopathy (Liu et al. 2022). Notably, *Hungatella*, *E. rectale*, *E. hallii* and *Ruminococcaceae_UCG_002* all belong to the phylum *Firmicutes,* which indicates that some taxa of *Firmicutes* are involved in the "gut-retina" axis. However, no significant causality was found at the phylum level, which may be due to the interference caused by the interaction of lower taxonomic levels. Our study found a significant correlation at a lower taxonomic level, which raises the possibility of targeted treatment for improving retinal damage caused by optic neuritis in the future.

The current study also has certain limitations. The genetic risk score approach can also find the difference between the GWAS of GM taxa and the GWAS of ON. However, to protect the privacy and genetic information of the subjects, we cannot calculate without obtaining individual-level data. When using the GWAS based on UKB (105 ON cases) for verification, the results were not consistent with those of Finngen (951 cases). This may be due to the difference in the number of cases of ON and race. Meanwhile, the failure of our results to pass FDR correction may also be related to the sample size. Since the sample size of the TwinsUK Registry

is only 2731, few IVs were obtained. Although using the TwinsUK Registry reduced the interference of ethnic factors as much as possible, no significant causal relationship was found. Therefore, in future research, a larger sample of the same race is required to further confirm the impact of each GM taxa. Also, a more comprehensive GWAS will hopefully yield more IVs with consistent effects and generalize the findings across multi-ancestry studies.

On the whole, our work is based on the largest genetic study of human microbiology, exploring the possible mechanisms of the "gut-brain" axis and the "gut-retina" axis. We confirmed a potential causal effect between some GM taxa (*Peptococcaceae*, *Hungatella*, *E. rectale*, *E. hallii* and *Ruminococcaceae_UCG_002*) and ON. Further research is required to replicate the novel associations between GM taxa and ON and to clarify the mechanisms between them.

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Author contributions KL and ZY designed the study. KL, PW, JZ and ZY analyzed the data and drew the figures. All authors critically revised the manuscript. All authors read and approved the final manuscript.

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Data availability Data can be obtained by a reasonable request to the corresponding author.

Declarations

Conflict of interest All authors declare no conflict of interests.

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