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Causal Links between Gut Microbiota, Blood Metabolites, Immune Cells, Inflammatory Proteins, and Myopia: A Mendelian Randomization Study

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Purpose: This study aimed to investigate causal relationships between gut microbiota, blood metabolites, immune cell traits, circulating inflammatory proteins, and myopia through Mendelian randomization (MR) analysis.

Design: Mendelian randomization study.

Subjects: Genome-wide association study (GWAS) data of 412 gut microbiota, 1400 blood metabolites/metabolite ratios, 731 immune cell traits, and 91 circulating inflammatory proteins from the public GWAS database. Genome-wide association study data of myopia from the public GWAS database and FinnGen consortium.

Methods: Two-sample MR analysis and meta-analysis were employed using 4 methods, with inverse-variance weighted as the primary approach, to investigate potential causal links. Metabolic pathway analysis was conducted to explore metabolic pathways. The Cochran Q-test, MR-Egger intercept test, and MR-PRESSO were used for sensitivity analyses. Mediation and reverse MR analyses were also carried out to identify potential mediation relationships and modification effects of myopia.

Main Outcome Measures: Causal relationships between gut microbiota, blood metabolites, immune cell traits, circulating inflammatory proteins, and myopia.

Results: We identified causal effects of 34 and 22 gut microbiota/bacterial pathways, 131 and 98 blood metabolites/metabolite ratios, 60 and 37 immune cell traits, and 5 and 2 circulating inflammatory proteins on myopia (ukb-b-6353 and R10_H7_MYOPIA, respectively). Overlapping causal relationships were found for 1 gut bacterial pathway, 10 blood metabolites/metabolite ratios, and 2 immune cell traits across both outcomes; however, none of these overlaps reached significance after meta-analysis. The Small Molecule Pathway Database and Kyoto Encyclopedia of Genes and Genomes database enriched 14 significant pathways. Flavin adenine dinucleotide was involved in 8 pathways in both databases. Furthermore, the causal effect of glycochenodeoxycholate glucuronide on myopia was mediated by acetyl-CoA fermentation to butanoate II, with mediation proportion of 19.03% (ukb-b-6353) and 19.48% (R10_H7_MYOPIA). Reverse MR analysis identified modification effects of myopia (ukb-b-6353) on gut microbiota, blood metabolites, and circulating inflammatory proteins.

Conclusions: These findings demonstrated significant causal relationships between gut microbiota, blood metabolites, immune cell traits, circulating inflammatory proteins, and myopia. Gut microbiota pathway may mediate the causal effects of blood metabolite on myopia. This may provide researchers with a new perspective in exploring the biological mechanisms of myopia and may lead to the exploration of earlier treatment strategies.

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Myopia, acknowledged as a leading cause of distance vision impairment, represents a significant and escalating global public health challenge, characterized by a dramatic rise in prevalence and associated morbidity. Epidemiological evidence reveals that the condition affects 10% to 30% of adults across diverse countries and an alarming 80% to 90% of young adults in specific East and Southeast Asian territories.[1–3] Projections suggest that by 2050, 4.758 billion people will suffer from myopia, with 938 million having high myopia.[4] The etiology of myopia is multifaceted, involving a complex interplay between traditional genetic and environmental factors, culminating in excessive elongation of the eye's axial length and altered refractive status. Notably, hereditary factors predominate, accounting for nearly 94% of risk determinants and driving the extension of ocular axial length.[5] Over 438 genetic markers have been implicated in

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myopia,[6,7] yet recent investigations highlight the role of dysregulated cytokines and blood metabolites, influenced by aberrant gut microbiota composition, in various ocular pathologies, including keratitis, glaucoma, uveitis, diabetic retinopathy, age-related macular degeneration, and Sjögren's syndrome–associated dry eye.[8–16]

Of particular interest, Yu et al reported positive correlations between aqueous humor concentrations of interleukin-6, matrix metalloproteinase-2, and malondialdehyde and ocular axial length, whereas total antioxidant capacity showed a negative correlation. These findings suggest a potential association between mild intraocular inflammation and oxidative stress imbalance and myopia.[17] However, the precise causal connections linking gut microbiota, blood metabolites, circulating immune factors, and myopia have not been fully elucidated.

Observational studies, confounded by multiple factors, struggle to directly assess causal effects. Yet, the high heritability of the human genome presents a viable approach to uncover the intricate pathogenetic mechanisms underlying myopia and investigate its possible associations with the gut microbiome, blood metabolomics, and immunological factors. Genome-wide association studies (GWAS) and related research over the past 2 decades have identified more than 400 gene loci associated with myopia and refractive errors.[18] Whether causal links exist between gut microbiota, blood metabolites, immune cell traits, circulating inflammatory proteins, and myopia remains uncertain.

Mendelian randomization (MR) analysis provides insights into causal relationships by utilizing genetic variants strongly associated with exposures as instrumental variables (IVs).[19–25] This method has been applied to explore causal associations in ocular diseases such as cataract, glaucoma, and age-related macular degeneration.[26–33] When observational studies are limited, MR findings are particularly compelling if they align with observational data and demonstrate consistent associations. Additionally, mediation effects through intermediaries and metabolic pathways can be elucidated through MR analysis and metabolic pathway analysis.[34,35]

Our study employed 2-sample MR analysis, meta-analysis, and bioinformatic metabolic pathway analysis to assess the causal relationship between gut microbiota, blood metabolites, immune cell traits, circulating inflammatory proteins, and myopia. Furthermore, the potential mediation effect was investigated through a 2-step MR analysis and a multivariable MR (MVMR) analysis. We also employed a reverse MR analysis to explore the modification effect of myopia. The assessment of exposures and outcomes may demonstrate the genetic influence of gut microbiota, blood metabolites, and immune factors on myopia and the potential biological processes, providing evidence for further studies.

Methods

Study Design and Ethics Statement

This study employed a 2-sample MR analysis approach to explore causal associations using GWAS data pertaining to gut microbiota, blood metabolites, immune cell characteristics, circulating inflammatory proteins, and 2 independent myopia GWAS consortia, which were retrieved from publicly available databases. To elucidate the potential mediating effects, we conducted both 2-step MR and MVMR analyses. In addition, a reverse MR analysis was undertaken to assess the modifying impact of myopia on these factors. A graphical overview of the study design is provided in Figure 1.

Considering that all GWAS datasets used in this study were sourced from publicly accessible repositories, no additional ethical approval was required for this secondary data analysis. This research adhered to the tenets of the Declaration of Helsinki. Because this study did not involve primary data collection or patient interaction, individual patient consent was not required.

Data Source

The myopia GWAS data were obtained from 2 sources: the Medical Research Council Integrative Epidemiology Unit's Open GWAS Database (available at https://gwas.mrcieu.ac.uk/), specifically under identifier ukb-b-6353, and the FinnGen consortium (https://www.finngen.fi/en), identified as dataset R10_H7_MYOPIA. The ukb-b-6353 dataset ("phenotype: reason for glasses/contact lenses: for short-sightedness, i.e., primarily or exclusively for distance viewing, such as driving or watching movies, referred to as 'myopia'") encompassed 460 536 Europeans, comprising 37 362 cases of myopia and 423 174 controls. Meanwhile, the R10_H7_MYOPIA dataset, defined as "a refractive error where light rays entering the eye parallel to the optic axis are focused in front of the retina when ocular accommodation is relaxed, resulting from either an excessively curved cornea or an elongated anteroposterior length of the eyeball, commonly known as nearsightedness," included data from 429 209 individuals with 4106 identified cases.

For gut microbiota, GWAS data encompassing 207 taxa and 205 pathways from 7738 participants were retrieved from the NHGRI-EBI GWAS Catalog (accessions GCST90027446-GCST90027857; Table S1, available at www.ophthalmologyscience.org).[36] Genome-wide association study data for 1400 blood metabolites/metabolite ratios (1091 blood metabolites and 309 metabolite ratios) from 8299 individuals in the Canadian Longitudinal Study on Aging cohort were retrieved from the GWAS Catalog (accessions GCST90201021-90204063; Table S2, available at www.ophthalmologyscience.org).[37] Genome-wide association study data covering 731 immune cell traits from 3757 Sardinians were also publicly available in the GWAS Catalog (accession GCST0001391-GCST0002121; Table S3, available at www.ophthalmologyscience.org).[38] Lastly, GWAS data for 91 circulating inflammatory proteins measured in 14 824 European participants using the Olink Target Inflammation panel came from 11 cohorts (accessions GCST90274758-GCST90274848; Table S4, available at www.ophthalmologyscience.org).[39]

Instrumental Variable Selection and Quality Control

The MR analysis conducted in this study hinged upon 3 pivotal assumptions: (1) IVs demonstrate a strong association with the exposure variable, (2) IVs are independent of any confounding factors, and (3) IVs affect the outcome exclusively through the exposure pathway. To ensure adherence to these foundational principles, genetic factors were meticulously selected according to stringent criteria encompassing statistical significance thresholds, minimal linkage disequilibrium (LD), and physical distance.[40]

The selection of genetic variants related to gut microbiota was based on a stringent association threshold of $P < 1 \times 10^{-5}$, with the LD assessed at $r^2 < 0.001$ within a clumping window of 10 000 KB, utilizing the European 1000 Genomes Project reference panel.

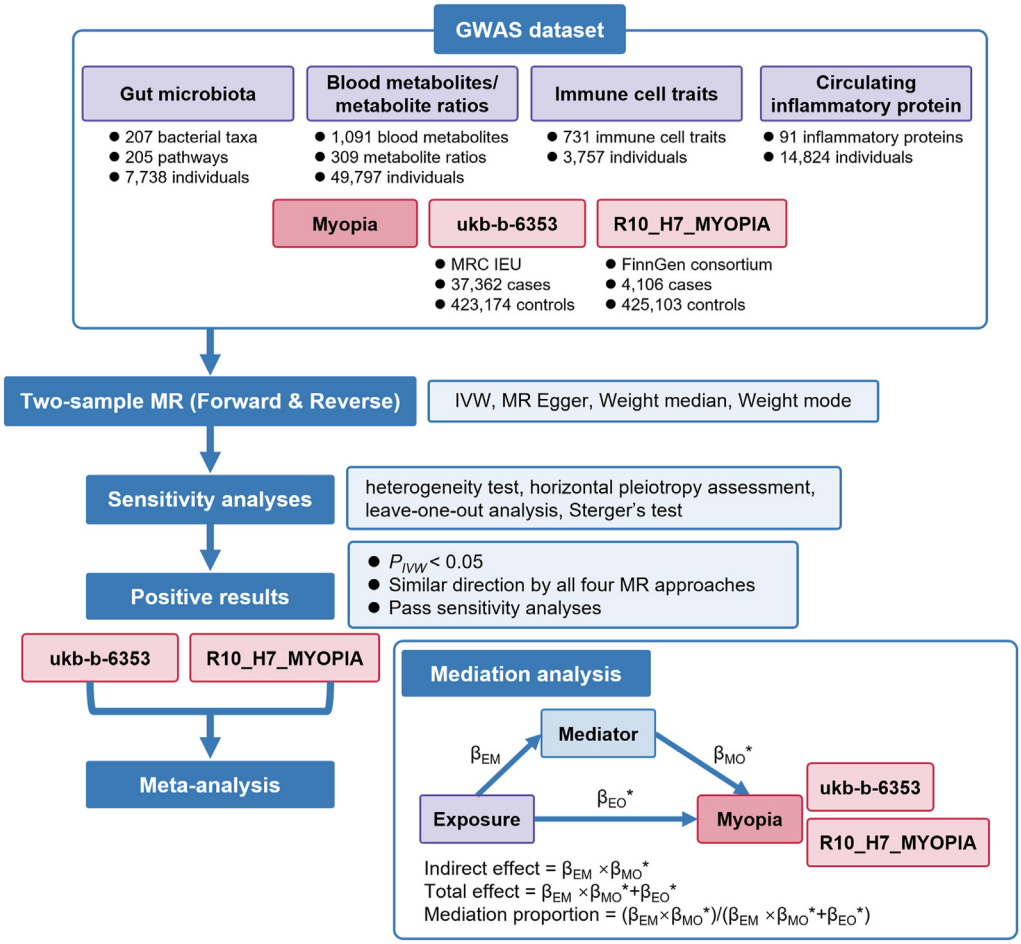

Figure 1. Summary of the study design. GWAS = genome-wide association studies; IVW = inverse variance weighted; MR = Mendelian randomization; MR-PRESSO = Mendelian randomization pleiotropy residual sum and outlier.

For blood metabolites, the genetic variants were extracted with association thresholds of $P < 1 \times 10^{-5}$ and LD $r^2 < 0.1$ within a clumping window of 500 KB. For immune cell traits, the association threshold was set at $P < 1 \times 10^{-5}$, and the LD was determined at $r^2 < 0.1$ within a 500 KB. In the case of circulating inflammatory proteins, an association threshold of $P < 5 \times 10^{-6}$ was applied, coupled with an LD criterion of $r^2 < 0.001$ within a clumping window of 10 000 KB.

Moreover, the criteria for selecting genetic variants associated with myopia included (1) a myopic trait association threshold of $P < 5 \times 10^{-8}$, (2) an LD of $r^2 < 0.001$, and (3) an LD distance >10 000 KB. To eliminate the potential bias from weak instruments, the explained variance (R^2) and F statistic parameters were calculated. The single-nucleotide polymorphisms with $F < 10$ were excluded from further analysis.[41]

Two-Sample MR Analysis

To evaluate the potential causal role of gut microbiota, blood metabolites, immune cells, and inflammatory proteins in the onset of myopia, a 2-sample MR analysis was performed. Myopia data from 2 databases (ukb-b-6353 and R10_H7_MYOPIA) served as outcomes, while the aforementioned biological factors were treated as exposures. The analysis employed a suite of methods including inverse variance weighted (IVW), MR-Egger, weighted median, weighted mode, and simple mode, with IVW designated as the principal method.[42–45] Statistical significance was determined by a corrected P value (P_{IVW}) for multiple testing, employing false discovery rate (FDR) correction (Benjamini–Hochberg) <0.1. The causal relationships with a $P_{IVW} < 0.05$ and $P_{FDR} < 0.1$ were considered as significant associations. Moreover, the causal relationships with a $P_{IVW} < 0.05$ but $P_{FDR} \geq 0.1$ were considered as potential causal relationships. Moreover, the beta coefficients from the IVW, MR-Egger, weighted median, and weighted mode methods had to exhibit consistent directionality, either uniformly positive or negative, to confirm the reliability of the findings. A meta-analysis with a random-effects model was applied to combine MR results across both myopia outcomes, calculating an overall effect size for each exposure. Results were expressed as odds ratios (ORs) accompanied by 95% confidence intervals (CIs).

MR-Egger sensitivity analysis was conducted to screen for directional horizontal pleiotropy, with $P < 0.05$ indicating potential bias.[46] The MR-PRESSO test was utilized to detect and adjust for pleiotropy by identifying and removing outliers.[47] Leave-one-out analysis ensured the robustness of the results by assessing the impact of individual single-nucleotide polymorphisms on pleiotropy, excluding results with a global test $P \leq 0.05$. The Cochran Q test was applied to check for significant heterogeneity,

with $P < 0.05$ indicating significant heterogeneity. The Steiger test was employed to confirm that the IVs influenced myopia exclusively through the specified exposures.

Furthermore, a reverse MR analysis was also undertaken to explore whether myopia might modify gut microbiota, blood metabolites, immune cell profiles, and inflammatory protein levels. The same analytical methods were employed, maintaining IVW as the primary approach.

Metabolic Pathway Analysis

Metabolic pathway analysis was performed using the MetaboAnalyst 6.0 (https://new.metaboanalyst.ca/) to investigate the relationship between metabolic pathways and myopia.[48] Enriched metabolic pathways were selected from the Small Molecule Pathway Database (SMPDB) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database, which included 99 and 80 metabolite sets based on human metabolic pathways.

Mediation Analysis

Two-sample MR and MVMR analyses were conducted to identify potential mediators.[49] Initially, a 2-sample MR analysis was executed to pinpoint significant causal links between exposures and putative mediators (β_{EM}). Subsequently, an MVMR analysis was performed to assess the mediating role of these mediators on the outcomes.[49]

The effect of each mediator on myopia adjusting for the genetic effect of exposure (β_{MO}*) and the effect of exposure on myopia adjusting for each mediator (β_{EO}*) were calculated. The indirect effect of exposures on myopia via each mediator was calculated by multiplying β_{EM} and β_{MO*}, whereas the direct effect of exposures on myopia was β_{EO}*. The total effect was defined as the sum of direct and indirect effect ($\beta_{EM} \times \beta_{MO}* + \beta_{EO}*$). Additionally, the proportion of mediation was calculated as "indirect effect/total effect" ($[\beta_{EM} \times \beta_{MO}*]/[\beta_{EM} \times \beta_{MO}* + \beta_{EO}*]$). It is notable that the direction of the indirect effect, direct effect, and total effect must be consistent. Any mediation relationships displaying discordant directions of effect should be excluded from further consideration.

Analysis Software and Packages

All the statistical analyses and data visualizations were performed using the R packages "TwoSampleMR," "Mendelian randomization," "MRPRESSO," "MVMR" (version 4.3.1), and "meta," strictly following the STrengthening the Reporting of Observational studies in Epidemiology-MR guidelines.[50]

Results

Causal Effects of Gut Microbiota on Myopia

Utilizing the ukb-b-6353 myopia GWAS dataset as the outcome and adhering to the predefined selection criteria, we identified 19 gut microbiota species and 15 gut bacterial pathways significantly associated with myopia (Fig 2A and Table S5, available at www.ophthalmologyscience.org). Among these, 21 factors were linked to an elevated risk of myopia (OR >1) and 13 factors correlated with a decreased risk (OR <1). However, given that the P_{FDR} >0.1, these relationships were classified as potential causal associations.

In parallel, when using the R10_H7_MYOPIA myopia dataset, which served as the outcome, the MR analysis

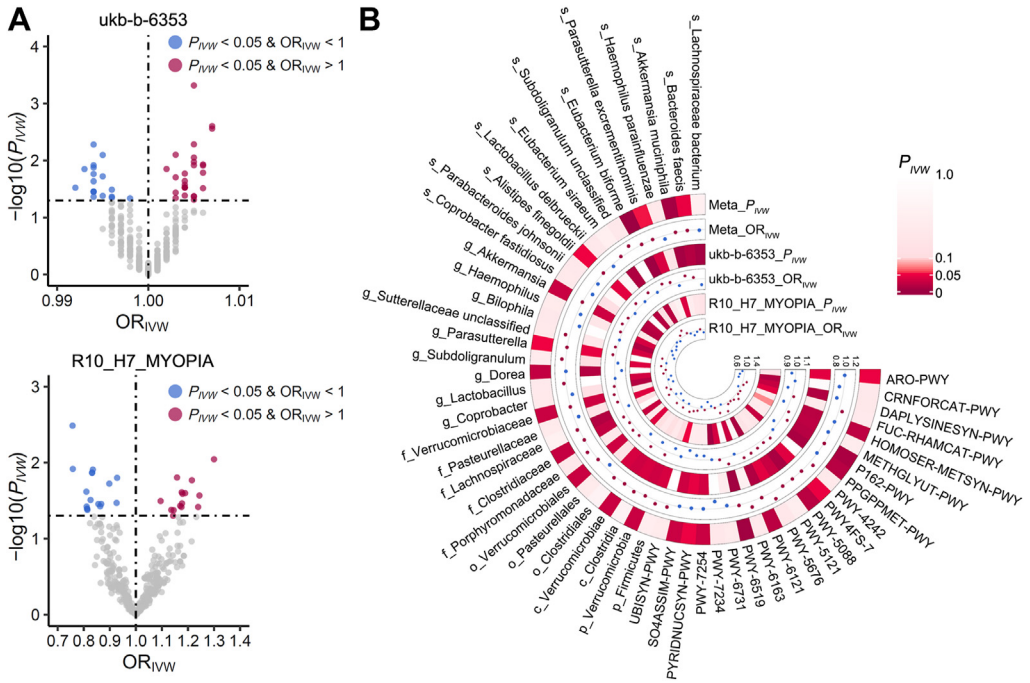

Figure 2. The causal impact of gut microbiota on myopia. **A**, Volcano plots of the causal impact of gut microbiota on myopia from the inverse variance weighted (IVW) method. The X-axis represents the logarithmic odds ratio (OR), and the Y-axis represents the −log10(P_{IVW}). The exposure with P_{IVW} < 0.05 and OR >1 is indicated in red, while the exposure with P_{IVW} <0.05 and OR <1 is indicated in blue. **B,** Circular heatmap of the significant gut microbiota identified in meta-analysis.

revealed 14 gut microbiota species and 8 gut microbiota pathways significantly associated with myopia ($P_{IVW} < 0.05$, Fig 2A and Table S5). Within these findings, 10 factors were associated with an increased risk of myopia (OR >1) and 12 factors with a decreased risk (OR <1). These associations, similarly, were categorized as potential causal relationships.

The meta-analysis revealed 22 significant correlations between gut microbiota and myopia. Among these, 15 were associated with an increased risk (OR > 1, $P_{IVW\text{-}meta} < 0.05$) and 7 were associated with a reduced risk of myopia (OR < 1, $P_{IVW\text{-}meta} < 0.05$; Fig 2B and Table S6, available at www.ophthalmologyscience.org). It is noteworthy that the "Acetyl-CoA fermentation to butanoate II" pathway (PWY-5676) was the sole gut bacterial pathway demonstrating a significant association with 2 myopia outcomes ($P_{IVW} < 0.05$). However, this association did not reach significance post-meta-analysis (OR = 1.121, 95% CI = 0.874 to 1.438, $P_{IVW\text{-}meta} = 0.3696$), indicative of substantial heterogeneity ($I^2 = 84.8\%$).

Causal Effects of Blood Metabolites and Metabolite Ratios on Myopia

In the ukb-b-6353 myopia dataset, we identified 131 blood metabolites/metabolite ratios, which were categorized into 10 significant categories (Fig 3A and Table S7, available at www.ophthalmologyscience.org). Specifically, these comprised 42 lipids, 32 metabolite ratios, 20 amino acids, 15 uncharacterized compounds, 13 xenobiotics, 2 cofactors and vitamins, 2 carbohydrates, 2 nucleotides, 2 partially characterized molecules, and 1 peptide. Using the FDR method, we identified 47 significant associations, comprising 18 risk factors (OR >1, $P_{FDR} < 0.1$) and 29 protective factors (OR <1, $P_{FDR} < 0.1$).

Considering myopia (R10_H7_MYOPIA) as the outcome, the IVW methods revealed 98 significant cause–effect relationship between blood metabolites/metabolite ratios and myopia ($P_{IVW} < 0.05$, Fig 3A and Table S7), including 44 risk factors (5 in the amino acid group, 1 in the carbohydrate group, 8 in the lipid group,

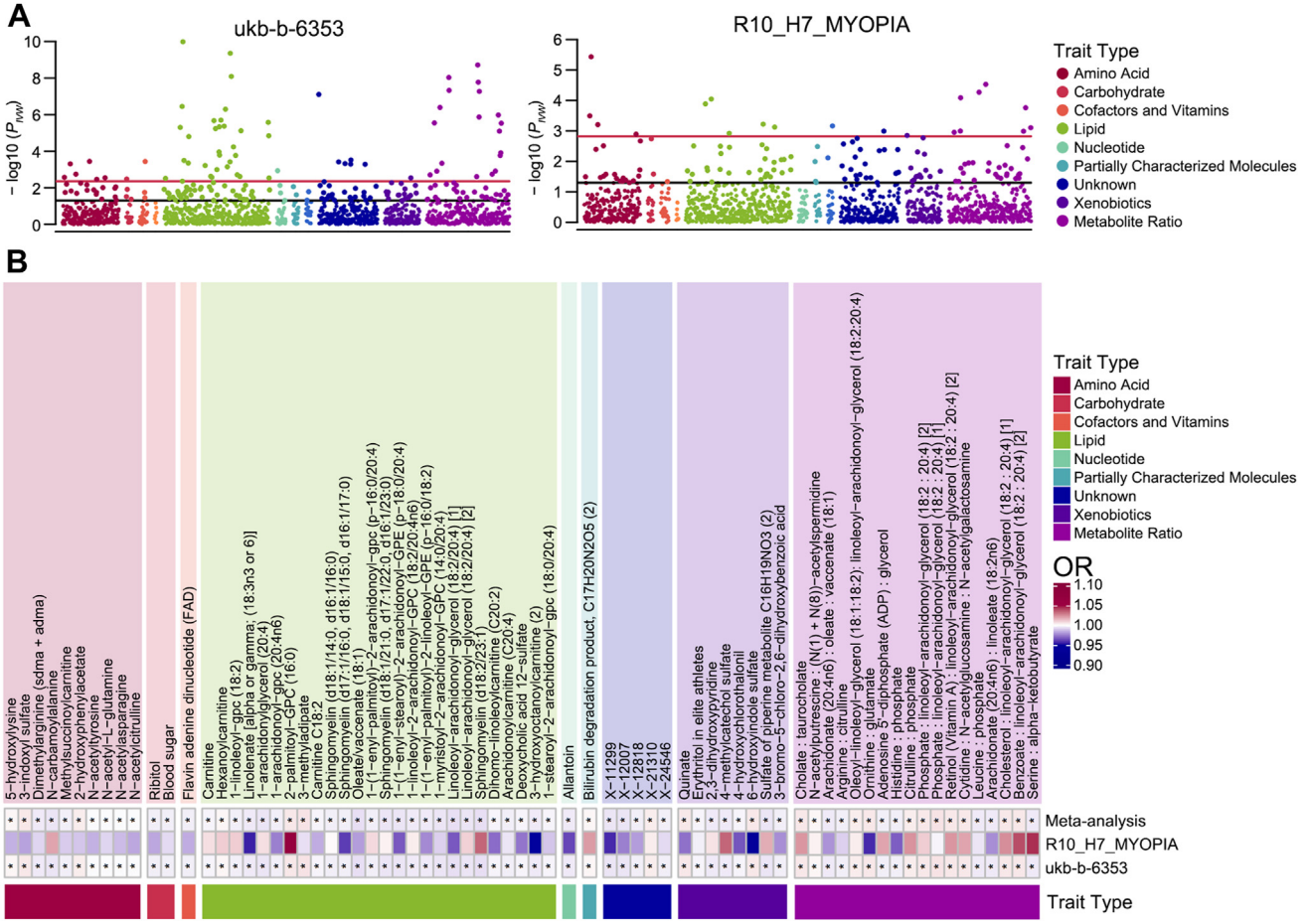

Figure 3. The causal impact of blood metabolites and metabolite ratios on myopia. **A**, Manhattan plot of MR results for all metabolites on myopia. The horizontal black and red lines indicate a significant association at $P_{IVW} < 0.05$ and $P_{FDR} < 0.1$, respectively. **B,** Heatmap of the significant blood metabolites and metabolite ratios identified in meta-analysis. The asterisk (*) indicates the suggestive evidence for a potential causal association ($P_{IVW} < 0.05$ or $P_{IVW\text{-}meta} < 0.05$). FDR = false discovery rate; IVW = inverse variance weighted; MR = Mendelian randomization.

13 in the metabolite ratio group, 2 in the partially characterized molecules group, 7 in the unknown group, and 8 in the xenobiotics group) and 54 protective factors (11 in the amino acid group, 1 in the carbohydrate group, 18 in the lipid group, 10 in the metabolite ratio group, 1 in the peptide group, 11 in the unknown group, and 2 in the xenobiotics group). There were 18 significant relationships identified by FDR methods (P_{FDR} <0.1).

As shown in Figure 3B and Table S8 (available at www.ophthalmologyscience.org), the meta-analysis yielded 72 significant exposures, including 21 risk factors (OR >1, $P_{IVW\text{-}meta}$ <0.05) and 51 protective factors (OR <1, $P_{IVW\text{-}meta}$ <0.05). Although 10 significant factors overlapped in the 2 outcomes with consistent effects (ukb-b-6353 and R10_H7_MYOPIA), their heterogeneity (I^2) was too high with $P_{IVW\text{-}meta}$ >0.05.

For the 6 metabolites with significant causal effects common to both outcomes, no significantly enriched pathways were identified in the SMPDB and KEGG databases. Of the 49 metabolites with significant causal effects identified in the meta-analysis, the SMPDB database highlighted 10 significant pathways, whereas the KEGG database pinpointed 4 pathways, encompassing a total of 6 metabolites (Fig 4). Specifically, flavin adenine dinucleotide was implicated in 8 pathways across both databases, while carnitine and D-glucose were each associated with 4 pathways.

Causal Effects of Immune Cell Traits on Myopia

Utilizing the ukb-b-6353 dataset as the outcome, we identified a total of 60 suggestive associations between immune cell traits and myopia (Fig 5A and Table S9, available at www.ophthalmologyscience.org). These associations spanned various cell types: 12 related to B cells, 6 to dendritic cells, 8 to T-cell maturation stages (MST), 4 to monocytes, 1 to myeloid cells, 8 to TBNK (T, B, NK) cells, and 21 to Treg (regulatory T) cells. Among these, 22 traits were linked to an increased risk (OR >1, P_{IVW} <0.05) and 38 were associated with a reduced risk of myopia (OR <1, P_{IVW} <0.05). After FDR testing, 5 immune cell traits were found to have a significant association with myopia risk (P_{FDR} <0.1), specifically $CD39^+$ activated Treg %activated Treg (OR = 0.996, 95% CI = 0.994−0.998), CD38 on transitional B cell (OR = 1.002, 95% CI = 1.001−1.003), CD3 on $CD28^+$ CD45RA-CD8br (OR = 0.998, 95% CI = 0.997−0.999), CD3 on activated Treg (OR = 0.999, 95% CI = 0.998−0.999), and FSC-A on CD8br (OR = 0.996, 95% CI = 0.993−0.998).

When using R10_H7_MYOPIA as the outcome, 37 immune cell traits were significantly associated with myopia, including 16 B-cell panels, 1 within dendritic cell panel, 4 MST panels, 3 monocyte panels, 3 TBNK panels, and 10 Treg panels (Fig 5A and Table S9). Of these, 21 were risk factors (OR >1, P_{IVW} <0.05) and 16 were protective factors (OR <1, P_{IVW} <0.05). However, upon further adjustment for multiple testing (using P_{FDR} <0.1 as the threshold), no trait retained statistical significance.

Notably, the only immune cell trait that was consistently associated with an increased risk across both outcomes was the CM CD8br %CD8br (ukb-b-6353: OR = 1.002, 95% CI = 1.000−1.004; R10_H7_MYOPIA: OR = 1.091, 95% CI = 1.023−1.164). Conversely, the only trait associated with a protective effect across both outcomes was CD3 on $CD28^+$ DN ($CD4^-CD8^-$) (ukb-b-6353: OR = 0.998, 95% CI = 0.997−1.000; R10_H7_MYOPIA: OR = 0.945, 95% CI = 0.900−0.993).

Following meta-analysis, the association between 24 immune cell traits and myopia revealed statistical significance ($P_{IVW\text{-}meta}$ <0.05) (Fig 5B and Table S10, available at www.ophthalmologyscience.org). Nevertheless, the 2

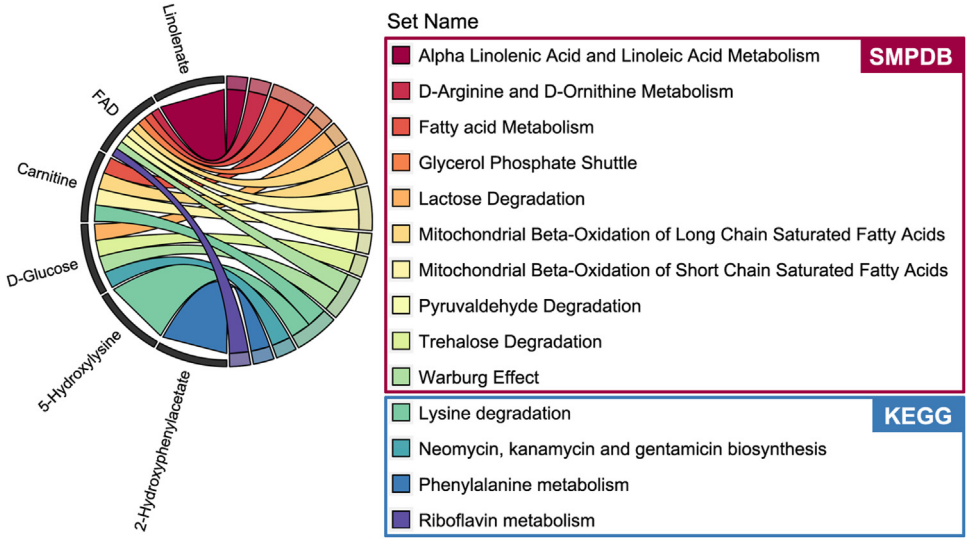

Figure 4. The network of significant metabolic pathways and metabolites identified in KEGG and SMPDB pathway databases. FAD = flavin adenine dinucleotide; KEGG = Kyoto Encyclopedia of Genes and Genomes; SMPDB = Small Molecule Pathway Database.

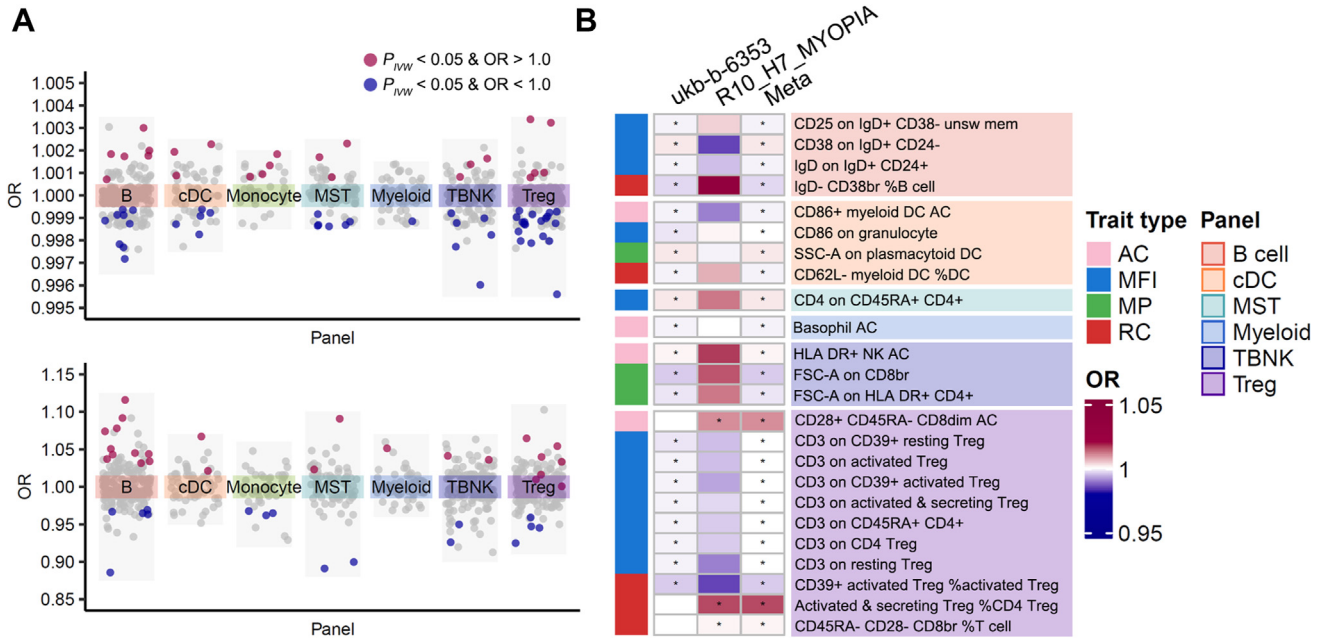

Figure 5. The causal impact of immune cell traits on myopia. **A**, Scatter plots of the causal impact of immune cell traits across 7 panels on myopia from the inverse variance weighted (IVW) method. The X-axis represents the panels, and the Y-axis represents the logarithmic odds ratio (OR). The exposure with P_{IVW} <0.05 and OR >1 is indicated in red, while the exposure with P_{IVW} <0.05 and OR <1 is indicated in blue. **B**, Heatmap of the significant immune cell traits identified in meta-analysis. The asterisk (*) indicates the suggestive evidence for a potential causal association (P_{IVW} <0.05 or $P_{IVW\text{-}meta}$ <0.05).

immune cell traits that exhibited significant relationships across both outcomes showed substantial heterogeneity and did not demonstrate a significant causal effect post-meta-analysis (CM CD8br %CD8br: I^2 = 85.0%, $P_{IVW\text{-}meta}$ = 0.3641; CD3 on $CD28^+$ DN ($CD4^-CD8^-$): I^2 = 78.8%, $P_{IVW\text{-}meta}$ = 0.3777).

Causal Effects of Circulating Inflammatory Proteins on Myopia

Using the IVW method and comprehensive screening criteria, 5 circulating inflammatory proteins were found to have significant relationships with ukb-b-6353 myopia outcome (Fig 6A and Table S11, available at www.ophthalmologyscience.org). Elevated levels of glial cell line–derived neurotrophic factor (OR = 1.004, 95% CI = 1.000–1.007), interleukin-18 (OR = 1.003, 95% CI = 1.000–1.007), and sulfotransferase 1A1 (OR = 1.003, 95% CI = 1.000–1.006) were associated with an increased risk of myopia. Conversely, lower levels of interleukin-17C (OR = 0.996, 95% CI = 0.992–1.000) and monocyte chemoattractant protein 2 (OR = 0.998, 95% CI = 0.997–1.000) were associated with a reduced risk of myopia. However, none of these associations maintained statistical significance following FDR correction (P_{FDR} >0.1).

Regarding the R10_H7_MYOPIA myopia outcome, reduced levels of interleukin-33 (OR = 0.818, 95% CI = 0.677–0.989) and adenosine deaminase (OR = 0.891, 95% CI = 0.815–0.975) were associated with a decreased risk of myopia. Unfortunately, these findings did not withstand statistical significance after FDR adjustment (Fig 6A and Table S11).

Notably, despite the absence of significant overlapping exposures between the 2 outcome datasets, our meta-analysis revealed potential causal relationships (Fig 6B and Table S12, available at www.ophthalmologys

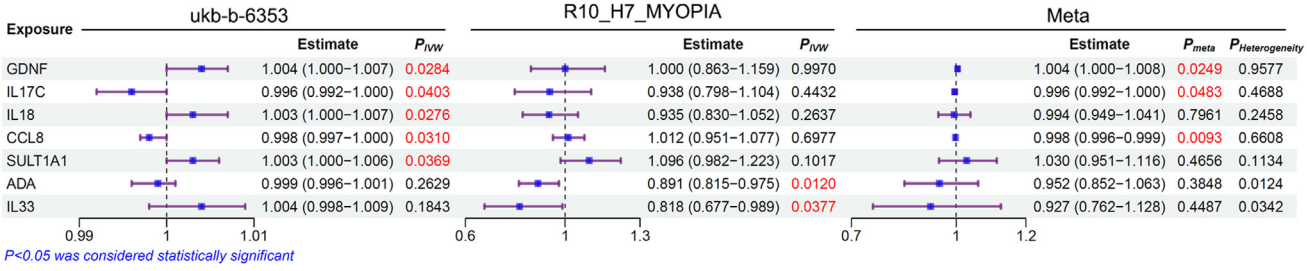

Figure 6. Forest plot for the causal effect of the significant circulating inflammatory proteins identified in 2 myopia databases and meta-analysis.

cience.org). Glial cell line–derived neurotrophic factor, IL17C, and MCP-2/CCL8 emerged as factors exerting significant causal effects on myopia.

Mediation Analysis

In accordance with the foundational principles of a 2-step MR analysis for mediation analysis, we selected 1 gut microbiota, 10 blood metabolites/metabolite ratios, and 2 immune cell traits that displayed statistically significant causal relationships with both myopia outcomes (ukb-b-6353 and R10_H7_MYOPIA) for further investigation. From the preliminary 2-sample MR analysis, 4 significant causal effects were identified: 1 significant causal effect of blood metabolites on gut microbiota composition, 1 significant causal effect of blood metabolites on an immune cell trait, and 2 significant causal effects of gut microbiota on blood metabolite levels (Table S13, available at www.ophthalmologyscience.org).

Furthermore, to ascertain the independent effects of candidate mediators and exposures on the 2 myopia outcomes, we employed the MVMR approach (Table S14, available at www.ophthalmologyscience.org). The MVMR analysis pinpointed glycochenodeoxycholate glucuronide as a protective effector against myopia through PWY-5676. Acetyl-CoA fermentation to butanoate II, with a mediated proportion of 19.03% and 19.48% of the total effect in ukb-b-6353 and R10_H7_MYOPIA datasets, respectively (Fig 7).

Reverse MR Analysis

The reverse MR analysis was undertaken to investigate the potential influence of myopia on the gut microbiota, blood metabolites/metabolite ratios, immune cell traits, and circulating inflammatory proteins (Fig 8 and Table S15, available at www.ophthalmologyscience.org).

With the ukb-b-6353 myopia dataset serving as the exposure variable, we identified significant associations between myopia and 1 gut microbiota species (*Eubacterium hallii*, OR = 0.100, 95% CI = 0.012–0.799), 1 circulating inflammatory protein (fibroblast growth factor 23, OR = 4.644, 95% CI = 1.724–12.512), and 15 blood metabolites/metabolite ratios (6 lipids, 1 peptide, 2 unknown compounds, 1 xenobiotic, and 5 metabolite ratios).

When utilizing the R10_H7_MYOPIA dataset as the exposure, no significant causal relationships survived the Steiger's test criterion.

Discussion

Recent studies have highlighted the potential causal connections between gut microbiota, blood metabolites, immune cell traits, circulating inflammatory proteins, and ocular diseases, including myopia.[51–54] Omar et al[55] reported higher abundances of *Bifidobacterium, Bacteroides, Megamonas, Faecalibacterium, Coprococcus, Dorea, Roseburia,* and *Blautia* in myopic individuals compared to emmetropes. Li et al[56] observed an increase in *Actinobacteria phylum* and a decrease in *Firmicutes phylum* in myopic mice, accompanied by lower concentrations of L-Glutamate and L-Glutamine. However, the underlying causal mechanisms linking these elaborate factors to myopia remained unexplored. Our study uniquely investigates these causal mechanisms for the first time, employing 6 GWAS datasets encompassing gut microbiota, blood metabolites, immune cell traits, circulating inflammatory proteins, and 2 myopia datasets.

Through 2-sample MR analyses, we identified 1 gut microbiota pathway (acetyl CoA fermentation to butanoate II), 10 blood metabolites/metabolite ratios, and 2 immune cell traits with causal effects on myopia (ukb-b-6353 and R10_H7_MYOPIA). Meta-analysis further revealed significant relationships involving 22 gut microbiotas, 72 blood metabolites/metabolite ratios, 24 immune cell traits, and 3 circulating inflammatory proteins in relation to myopia. Despite the absence of significant overlap across the 2 myopia outcomes after meta-analysis, our comprehensive approach ensures a robust investigation of causality.

Our findings corroborate prior hypotheses that suggest gut microbiota dysbiosis and alterations in blood metabolites

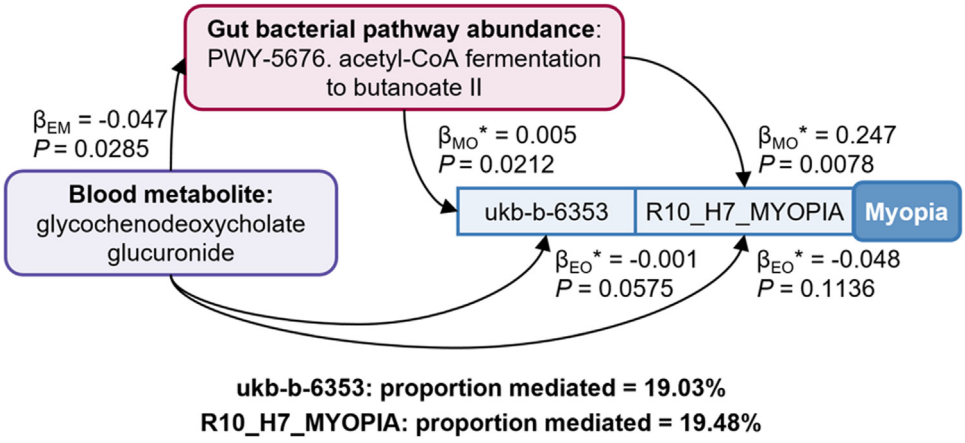

Figure 7. The mediation effects of gut bacterial pathway abundance PWY-5676. Acetyl-CoA fermentation to butanoate II on the association between blood metabolite glycochenodeoxycholate glucuronide and myopia.

Exposure	Outcome	Type	NSNP	Estimate	P_{IVW}
ukb-b-6353	s_Eubacterium_hallii	gut microbiota	28	0.100 (0.012-0.799)	0.0299
ukb-b-6353	1-palmitoyl-GPC (16:0)	blood metabolites	29	0.246 (0.064-0.945)	0.0412
ukb-b-6353	Gamma-glutamylglycine	blood metabolites	29	0.241 (0.065-0.895)	0.0335
ukb-b-6353	3,4-dihydroxybutyrate	blood metabolites	29	0.228 (0.064-0.820)	0.0236
ukb-b-6353	5alpha-androstan-3beta,17beta-diol disulfate	blood metabolites	29	5.055 (1.603-15.938)	0.0057
ukb-b-6353	Androstenediol (3beta,17beta) monosulfate (2)	blood metabolites	29	4.334 (1.163-16.155)	0.0289
ukb-b-6353	Docosahexaenoylcarnitine (C22:6)	blood metabolites	29	4.710 (1.039-21.348)	0.0444
ukb-b-6353	Methyl vanillate sulfate	blood metabolites	29	0.130 (0.020-0.857)	0.0340
ukb-b-6353	Cholic acid glucuronide	blood metabolites	29	0.127 (0.020-0.792)	0.0271
ukb-b-6353	X-11787	blood metabolites	29	0.238 (0.064-0.880)	0.0314
ukb-b-6353	X-12100	blood metabolites	29	0.256 (0.075-0.877)	0.0301
ukb-b-6353	Pyruvate : 3-methyl-2-oxobutyrate	metabolite ratios	29	0.222 (0.057-0.869)	0.0307
ukb-b-6353	Cysteinylglycine : taurine	metabolite ratios	29	4.251 (1.146-15.761)	0.0304
ukb-b-6353	Aspartate : N-acetylglucosamine : N-acetylgalactosamine	metabolite ratios	29	4.375 (1.198-15.972)	0.0255
ukb-b-6353	Mannose : N-acetylglucosamine : N-acetylgalactosamine	metabolite ratios	29	4.613 (1.184-17.975)	0.0276
ukb-b-6353	Histidine : asparagine	metabolite ratios	29	4.974 (1.287-19.227)	0.0200
ukb-b-6353	FGF23	circulating inflammatory proteins	30	4.644 (1.724-12.512)	0.0024

P_{IVW}<0.05 was considered statistically significant

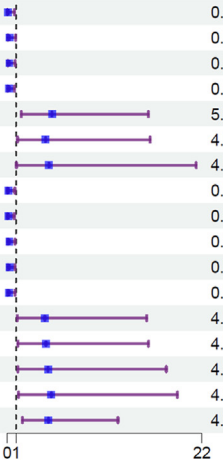

Figure 8. Forest plot for the causal effect of myopia on gut microbiota, blood metabolites and metabolite ratios, and circulating inflammatory proteins.

might contribute to myopia. Notably, though no specific gut microbiota exhibited a causal effect on both myopia outcomes, our study highlights a mediating effect of the acetyl-CoA fermentation to butanoate II pathway (PWY-5676) on glycochenodeoxycholate glucuronide in the pathophysiology of myopia. Increased levels of glycochenodeoxycholate glucuronide appear to mitigate the risk-enhancing effect of the acetyl-CoA fermentation to butanoate II pathway on myopia progression. Considering butyrate, a product of this pathway, is crucial for gut health and immune regulation.[57,58] The rise in glycochenodeoxycholate glucuronide levels may impair butyrate's anti-inflammatory properties, potentially leading to chronic inflammation and immune dysregulation. Such conditions could affect ocular tissue remodeling and axial length, thereby contributing to myopia.[17,59] This mediating relationship provides insights into myopia's pathogenesis and points to novel therapeutic targets. Further mechanistic studies are necessary to elucidate the underlying processes.

Significant associations were observed between numerous exposures and the 2 myopia outcomes (ukb-b-6353 and R10_H7_MYOPIA) in our study, with consistent OR directions. However, meta-analysis yielded fewer significant results with considerable heterogeneity, likely due to differences in population demographics and sample sizes between the UK Biobank and FinnGen databases. The UK Biobank's larger, primarily European cohort offers high statistical power and genetic homogeneity, whereas FinnGen's smaller, Finnish population captures unique genetic variants. These disparities contribute to heterogeneity and potential biases in population-based genetic studies. Future investigations should employ stratified analyses or meta-regression to address population-specific differences and identify sources of heterogeneity, enhancing the validity of genetic research findings pertinent to myopia.

While MR is a valuable tool for identifying potential causal relationships by minimizing confounding and reverse causation, it cannot fully eliminate the influence of behavioral or environmental factors. Evidence accumulated over the last 2 decades suggests that most school myopia is primarily driven by environmental factors, with over 400 genes explaining only 10% to 20% of the phenotypic variance.[60] Axial length growth, while central to myopia development, is influenced by diverse mechanisms.[61,62] For instance, men typically exhibit longer axial lengths than women due to flatter corneas and less powerful lenses, irrespective of refractive error. Similar trends are observed among taller individuals compared to shorter ones, driven by mechanisms such as retinal defocus detection during early development.[63] Robust processes like defocus and contrast detection in the parafoveal region govern axial growth and can be modulated by treatments like orthokeratology and peripheral defocus spectacles. Moreover, environmental factors, including light exposure, play a critical role in myopia progression.[64] Outdoor activity has been shown to reduce myopia risk, whereas prolonged exposure to low-light indoor environments and reading with low contrast black letters can accelerate ocular growth. These findings underscore that visual and light cues predominantly regulate eye growth, contrasting with growth modulation in other tissues by inflammation, nutrition, or infections. While inflammation may influence ocular growth mechanisms, its role in directly modifying refractive error remains uncertain. Thus, any suggestion of inflammation as a causal factor in myopia development must be approached cautiously.

Still, there are some limitations in our study. First, the diverse and limited sample sizes across populations may compromise statistical power and mask subtle effects. Myopia prevalence is particularly high in Asia, and the genetic architecture and environmental exposures in these populations may differ substantially. Second, although MR inherently assumes that genetic variation is not influenced by behavioral or environmental factors, such as diet and living habits, we agree that the lack of behavioral and environmental data may limit the interpretation of myopia as an exposure.[65] Future studies are needed to validate our

findings in a more diverse population influenced by various behavioral and environmental factors. Third, the GWAS data sourced from public databases may lack standardization, affecting accuracy and reliability. Fourth, our study lacks experimental validation of the predicted causal relationships and study on the magnitude of the effect, which may help address correctly the clinical significance of the associations found. Despite these constraints, our comprehensive MR analyses establish credible causal links, providing a foundation for future studies to probe deeper into the mechanisms underlying myopia.

In summary, our study has undertaken a comprehensive and systematic examination of the causal associations between gut microbiota, blood metabolites/metabolite ratios, immune cell traits, circulating inflammatory proteins, and myopia, employing MR analysis. Notably, our mediation analysis shed light on the potential mediating role of the acetyl-CoA fermentation to butanoate II pathway (PWY-5676) in gut microbiota on glycochenodeoxycholate glucuronide (a blood metabolite) in the etiology of myopia. This may provide researchers with a new perspective in exploring the biological mechanisms of myopia and may lead to the exploration of earlier treatment strategies.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Footnotes and Disclosures

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No animal subjects were used in this study.

Author Contributions:

Conception and design: Lv, Wang, Huang, Yu, Li, Song

Data collection: Lv, Wang, Huang, Yu

Analysis and interpretation: Wang, Huang

Obtained funding: Li

Overall responsibility: Wang, Huang, Li, Song

Abbreviations and Acronyms:
CI = confidence interval; **FDR** = false discovery rate; **GWAS** = genome-wide association study; **IV** = instrumental variable; **IVW** = inverse variance weighted; **KEGG** = Kyoto Encyclopedia of Genes and Genomes; **LD** = linkage disequilibrium; **MR** = Mendelian randomization; **MVMR** = multivariable Mendelian randomization; **OR** = odds ratio.

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