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Analysis

Impact the impact of gut microbiota on gastric cancer via immune cells: a comprehensive Mendelian randomization study and mediation analysis

Chao Wang[1,2] · **Jia Wang**[2] · **Wenxian Guan**[3] · **Bojian Fei**[1,2]

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Abstract

Purpose Recent observational studies have highlighted the role of altered gut microbiota (GM) in the activation of the host immune system and the resulting of gastric cancer (GC). However, the exact causal relationship and mechanisms of action are still not fully understood.

Materials and methods Genetic data from published genome-wide association studies (GWASs) were employed to determine the causal effects of 207 taxa and 205 bacterial pathways on GC via two-sample Mendelian randomization (MR) and two-step mediation MR analysis. In this study, 731 immune cell traits served as potential mediators. An inverse variance-weighted (IVW) estimation, augmented by a range of alternative estimators, notably the Bayesian-weighted MR method, was employed as the primary methodological approach.

Results Four taxa and five bacterial pathways were found to be negatively correlated with GC, whereas one taxon and two bacterial pathways were a positively correlated with GC. Reverse causality was not found in the reverse MR analysis. Additional validation was performed using a sensitivity analysis. Mediation MR analyses revealed that the GM influences GC through various phenotypes of 16 immune cells that act as mediators. For example, *s_Alistipes_sp_AP11* was found to inhibit GC through NKT %T cell (total effect: -0.3234, mediation effect: 0.0212). This mediating effect further highlights the complex relationship among GMs, immune cell traits, and their combined effects on GC.

Conclusions Our findings highlight the genetic connection between specific GMs and GC, emphasizing the potential role of immune cells as mediators, and offering valuable perspectives on potential therapeutic strategies that manipulating the GM to address GC.

Keywords Gut microbiota · Gastric cancer · Immune cells · Mendelian randomization study · Single nucleotide polymorphisms

Chao Wang and Jia Wang contributed equally to this work and shared first authorship.

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✉ Wenxian Guan, wenxian_guan820@sina.com; ✉ Bojian Fei, jdfyfbj1971@163.com; Chao Wang, winnerwang0206@163.com; Jia Wang, wongj0916@163.com | [1]Department of Gastrointestinal Surgery, Affiliated Hospital of Jiangnan University, Wuxi 214062, Jiangsu, China. [2]Wuxi School of Medicine, Jiangnan University, Wuxi 214122, Jiangsu, China. [3]Division of Gastric Surgery, Department of General Surgery, Nanjing Drum Tower Hospital, Affiliated Hospital of Medical School, Nanjing University, Nanjing 210008, China.

Abbreviations

GM	Gut microbiota
GC	Gastric cancer
MR	Mendelian randomization
GWAS	Genome-Wide Association Studies
IVW	Inverse variance-weighted
SNP	Single nucleotide polymorphisms
IV	Instrumental variable
LD	Linkage disequilibrium
BWMR	Bayesian weighted MR
OR	Odds ratios
CI	Confidence intervals
H. pylori	Helicobacter pylori
ODN	Oligodeoxynucleotides
TNF	Tumor necrosis factor
Tregs	Regulatory T cells
TNFRSF	Tumor necrosis factor receptor family
TNFR	TNF receptor
ENTPD1	Triphosphate diphosphohydrolase 1
eIF5A	Eukaryotic initiation factor 5A

1 Introduction

Gastric cancer (GC) is a major healthcare issue worldwide, with approximately 1,089,103 new cases and 768,793 deaths recorded in 2020. As a result, GC is the fifth most common cancer and the fourth highest cause of cancer-related deaths globally [1], Early detection of GC is challenging as reliable biomarkers for accurate diagnosis and prognosis monitoring during the adjuvant stage are unavailable. As a result, GC is associated with a high mortality rate, with only 20% of patients surviving beyond five years after diagnosis [2, 3]. GC is influenced by genetics, environmental factors, and microbial factors, such as *Helicobacter pylori* infection [4, 5]. Various studies have indicated variations in stomach microbiota among individuals at various phases of gastric precancerous and malignant lesion progression, specifically a decline in total microbial variety and an increase in gut-friendly bacteria, particularly those with nitrosative capabilities [5]. The connection between the gut microbiota (GM) and GC may be due to the ongoing activation of the host's immune system by the GM, causing a breakdown in communication between host epithelial cells and microbes, ultimately leading to a condition of persistent inflammation [6]. However, the identification of non-*H. pylori* drivers associated with GC have not been identified. Therefore, more studies are needed to elucidate the exact mechanisms by which the GM affects the immune response during GC development and progression. Moreover, precise and easily accessible biomarkers are needed for the early detection of GC.

The human microbiome comprises a variety of microorganisms, such as viruses, fungi, and bacteria.

And *Firmicutes*, *Bacteroidetes*, *Actinobacteria* and *Proteobacteria* constitute the most abundant phyla in the human intestinal microbiota [7]. Dysbiosis, characterized by changes in the microbiota's composition and function of the microbiota, can be influenced by factors, such as antibiotic use, external microbial infections, and host genetics [8]. A well-regulated and consistent microbiome is essential to prevent cancer progression, whereas an imbalanced microbiome provides minimal defense and could potentially stimulates carcinogenesis. The gut microbiome is intricately linked to various mechanisms of carcinogenesis, such as inflammation, which facilitates tumor proliferation, modifications in the immune response, and generation of pro-carcinogenic metabolites [9]. Recent developments in metagenomics and high-throughput sequencing have revealed that the stomach, once thought to be devoid of microorganisms, harbors acid-resistant bacteria, including *H. pylori* [10]. The microbiota of the digestive system, such as *Lactobacillus, Streptococcus, Veillonella, Prevosia, Fusobacterium, Lachnospiraceae, Leptotrichia,* and *Clostridium*, are critical for the development of GC [5, 11]. Various immune-mediated inflammatory conditions have been found to alter GM composition and its metabolites. GM-derived metabolites have a wide range effects on various immune cell reactions, including T cells, B cells, dendritic cells, and macrophages [12]. Dysbiosis of the GM may lead to an atypical immune response, which can lead to the onset of GC.

Mendelian randomization (MR) analysis is a unique approach to uncovering causal relationships in observational data using genetic variants as instrumental variables (IVs) [13]. This approach is especially effective at elucidating the impact of the GM on disease pathogenesis via reducing confounding and reverse causation, which are inherent challenges in traditional observational studies. The immune system, with its various of cell types and signaling pathways, plays a crucial role in the development of GC. By analyzing 731 immune cell characteristics [14], specific immune pathways or cells that act as mediators connecting the gut microbiome to the onset or progression of GC can be identified.

This study aimed to explore the association between the GM, immune cells, and GC development. These findings improve our understanding of how the GM influences GC and shed light on the role of immune factors in disease progression.

2 Materials and methods

2.1 Study design

This study comprised two stages of analyses (Fig. 1**)**. In phase 1, a two-sample MR method was used to evaluate the links between 412 GM (207 taxa and 205 bacterial pathways) and GC using single nucleotide polymorphisms (SNPs) as instrumental variables (IVs) for each factor. In phase 2, mediators from 731 immune cell signatures were selected to explore the role of immune characteristics in connecting GM and GC. The mediating effects of these immune traits were calculated using a two-step MR mediation approach. For causal inference to be valid, IVs must meet three key assumptions [15]: (1) high correlation with the exposure variables; (2) no connection with confounding variables; and (3) no direct effect on the outcome, affecting the outcome solely through the exposure.

2.2 Sources of information on exposure, mediators, and outcome

The MR study used summary-level data from GWASs primarily conducted with individuals of European ancestry to gather information on exposure (207 species and 205 bacterial pathways), mediators (731 immune cell traits), and outcome (GC).

The complete GWAS information for 207 taxa and 205 bacterial pathways in 7,738 participants from the DMP cohort was downloaded directly from the NHGRI-EBI GWAS Catalog (https://www.ebi.ac.uk/gwas/) using the study access codes GCST90027446 to GCST90027857 (specific access codes for species and pathways are listed in Supplementary Table 1) or at https://dutchmicrobiomeproject.molgeniscloud.org [16].

GWAS data on immunity were used to examine the potential mediating functions of immune cells. Summary statistics for different immune characteristics were acquired from the GWAS Catalog, covering the accession numbers, GCST0001391 to GCST0002121 [14]. A total of 731 immunophenotypes were examined, including B cells, CDCs, mature T cells, monocytes, myeloid cells, TBNK (T, B cells, and natural killer cells), and Treg panels.

GC data were acquired from the https://storage.googleapis.com/finngen-public-data-r9/summary_stats/finngen_R9_C3_STOMACH_EXALLC.gz (737 cases and 287,137 controls).

All GWASs included in this study were authorized by the relevant institutional review boards and informed consent was obtained from all participants. Ethical approval was not required as summary-level data were employed in this study.

2.3 SNP selection

IVs were selected as SNPs linked to exposures showing a genome-wide significant association ($p < 5 \times 10^{-8}$) with the traits of interest. Subsequently, SNPs showing linkage disequilibrium (LD) were excluded from the study. To meet these requirements, the LD between the selected SNPs must have an r^2 value of less than 0.001 and be at least 10,000 kb apart [17, 18]. The explained variance (R^2) and F-statistic parameters were computed to assess the strength of the association between the identified IVs and exposure. Generally, SNPs with F-statistic values less than 10 are considered to be ineffective instruments. The genetic instruments for exposures were validated by calculating the F-statistic using the formula: $F = R^2 \times [(N - 1 - K)/K] \times (1 - R^2)$[19–21], where R^2 represents the cumulative variance accounted for by the selected SNPs, N denotes the size of the sample sizes, and K denotes the number of SNPs examined.

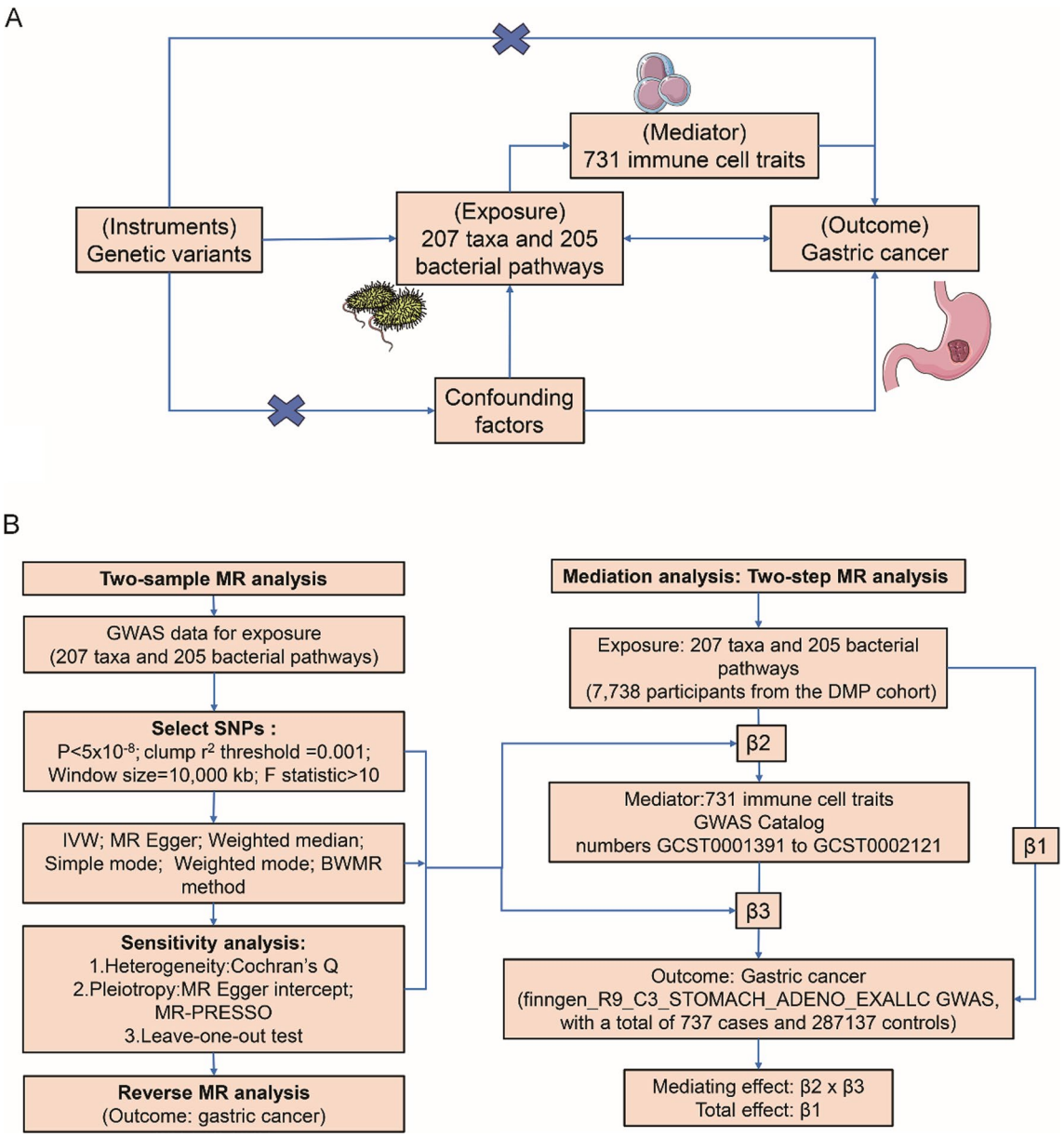

Fig.1 Assumptions and design of the relationship between gut microbiota and gastric cancer with immune cells as mediators in the bidirectional and mediation Mendelian randomization (MR) analyses. **a** A two-sample bidirectional Mendelian randomization (MR) approach was employed to examine the causal associations between gut microbiota (exposure) and gastric cancer (GC, outcome). A total of 731 immune cell traits were identified as potential mediators for further mediation analyses. Finally, a two-step MR analysis was conducted to determine the potential mediating role of immune cells. In step 1, the impact of gut microbiota on immune cells was determined while in step 2, the influence of immune cells on GC was examined. **b** Mendelian randomization analysis flow chart

2.4 MR analysis

Two-sample MR studies were conducted to explore the possible causal link between the makeup of GM and GC makeup. The inverse-variance-weighted (IVW) [22] and Bayesian weighted MR (BWMR) methods [23] were used to calculate impacts; beta (β) values with standard errors were reported for continuous results and odds ratios (OR) with 95% confidence intervals (CI) were reported for binary results. $P < 0.05$ was considered to indicate significance.

2.5 Mediation MR analysis

Two-step MR analysis was conducted to examine the involvement of immune cells as mediators. Initially, the causal associations between the GM and immune cells were individually examined using MR. Subsequently, the causal link between immune cells and GC was further investigated. To determine the role of immune cells in the connection between GM and GC, the total effect was represented as β1. The direct effect between GM and immune cells was designated as β2, and the direct effect between immune cells and GC was designated as β3. The product of β2 and β3 represented mediating effects.

2.6 Sensitivity analysis

Several MR methods, such as MR-Egger, weighted median, IVW, simple mode, weighted mode, BWMR, and MR-PRESSO approaches, were employed. However, the IVW method, which is a frequently used approach in MR research [24, 25], served as the main examination approach. Cochran's Q test was used to evaluate the heterogeneity of each SNP, and scatter plots were created to demonstrate the connections between SNPs, exposures, and outcomes in the MR analysis [25]. A leave-one-out analysis was conducted to assess the impact of each SNP on the results. Thereafter, MR-PRESSO [26] and MR-Egger regression [27] methods were applied to examine potential horizontal pleiotropy effects. MR-PRESSO was used to identify and address significant outliers, thereby correcting for horizontal pleiotropy. Statistical analyses were conducted using R software version 4.2.1 and the R-based package "TwoSampleMR" for MR analysis.

3 Results

3.1 Two sample MR analysis between GM and GC

To explore the causal effect of GM on GC, a two-sample MR analysis was performed using IVW and BWMR methods as the main analytical strategies. Supplementary Table 2 contains the precise information on the 4048 SNPs linked to 207 taxa and 205 bacterial pathway traits. As depicted in Fig. 2A, the IVW model identified 25 GMs associated with GC. The BWMR approach tackles the uncertainty surrounding the estimated minor effects and weak horizontal pleiotropic effects, and identifies outliers resulting from significant horizontal pleiotropic effects [23]. Therefore, the IVW results were validated using the BWMR method, which led to the identification of 18 GC-related GMs (Fig. 2B). Finally, a reverse MR study was performed using 18 sets of GM data that were statistically significant to mitigate the potential influence of reverse causation in the context of GC. Supplementary Table 3 shows the SNPs associated with GC. The results of the reverse MR analysis did not indicate of reverse causation ($P > 0.05$), as shown in Supplementary Table 4.

As displayed in Fig. 2, MR analysis using the IVW and BWMR methods indicated a genetic prediction of one taxon and three bacterial pathways linked to a higher risk of GC. The presence of *g_Haemophilus* (IVW OR = 1.4965, 95%CI = 1.0640–2.10480, P = 0.0205; BWMR OR = 1.5121, 95%CI = 1.0537–2.1698, P = 0.0248), "PWY.5659 GDP mannose biosynthesis" (IVW OR = 2.1118, 95%CI = 1.2293–3.6278, P = 0.0068; BWMR OR = 2.1384, 95%CI = 1.2067–3.7897, P = 0.0092), "PWY.724 superpathway of L lysine, L threonine and L methionine biosynthesis II" (IVW OR = 2.3932, 95%CI = 1.5945–1.0623, P = 0.0243; BWMR OR = 1.5768, 95%CI = 1.0472–2.3742, P = 0.0292), and "TRNA CHARGING PWY tRNA charging" (IVW OR = 1.5515, 95%CI = 1.0560–2.2796, P = 0.0253; BWMR OR = 1.5478, 95%CI = 1.0541–2.2729, P = 0.0258) were found to significantly elevate the risk of GC.

Eight taxa and six bacterial pathways were associated with a lower likelihood of developing GC. *g_Oxalobacter* (IVW OR = 0.7038, 95%CI = 0.5345–0.9269, P = 0.0124; BWMR OR = 0.6964, 95%CI = 0.5204–0.9320, P = 0.0150), *s_Alistipes_sp_AP11* (IVW OR = 0.7237, 95%CI = 0.5560–0.9420, P = 0.01620; BWMR OR = 0.7177, 95%CI = 0.5525–0.9322, P = 0.0129), *f_Oxalobacteraceae* (IVW OR = 0.7040, 95%CI = 0.5255–0.9430, P = 0.0186; BWMR OR = 0.6955, 95%CI = 0.5010–0.9485, P = 0.0218), *s_Oxalobacter_formigenes* (IVW OR = 0.7039, 95%CI = 0.5253–0.9433, P = 0.0187; BWMR OR = 0.6954, 95%CI = 0.5097–0.948, P = 0.0219), *o_Burkholderiales* (IVW OR = 0.6640, 95%CI = 0.4615–0.9553, P = 0.0273; BWMR OR = 0.6621, 95%CI = 0.4535–0.9666, P = 0.0327), *f_Bacteroidaceae* (IVW OR = 0.6483, 95%CI = 0.4380–0.9595, P = 0.0303; BWMR OR = 0.6436, 95%CI = 0.4260–0.9724, P = 0.0364), *s_Coprococcus_catus* (IVW OR = 0.5983, 95%CI = 0.3690–0.9701, P = 0.0372; BWMR OR = 0.5933, 95%CI = 0.3550–0.9914, P = 0.0463), *s_Escherichia_coli* (IVW OR = 0.7489,

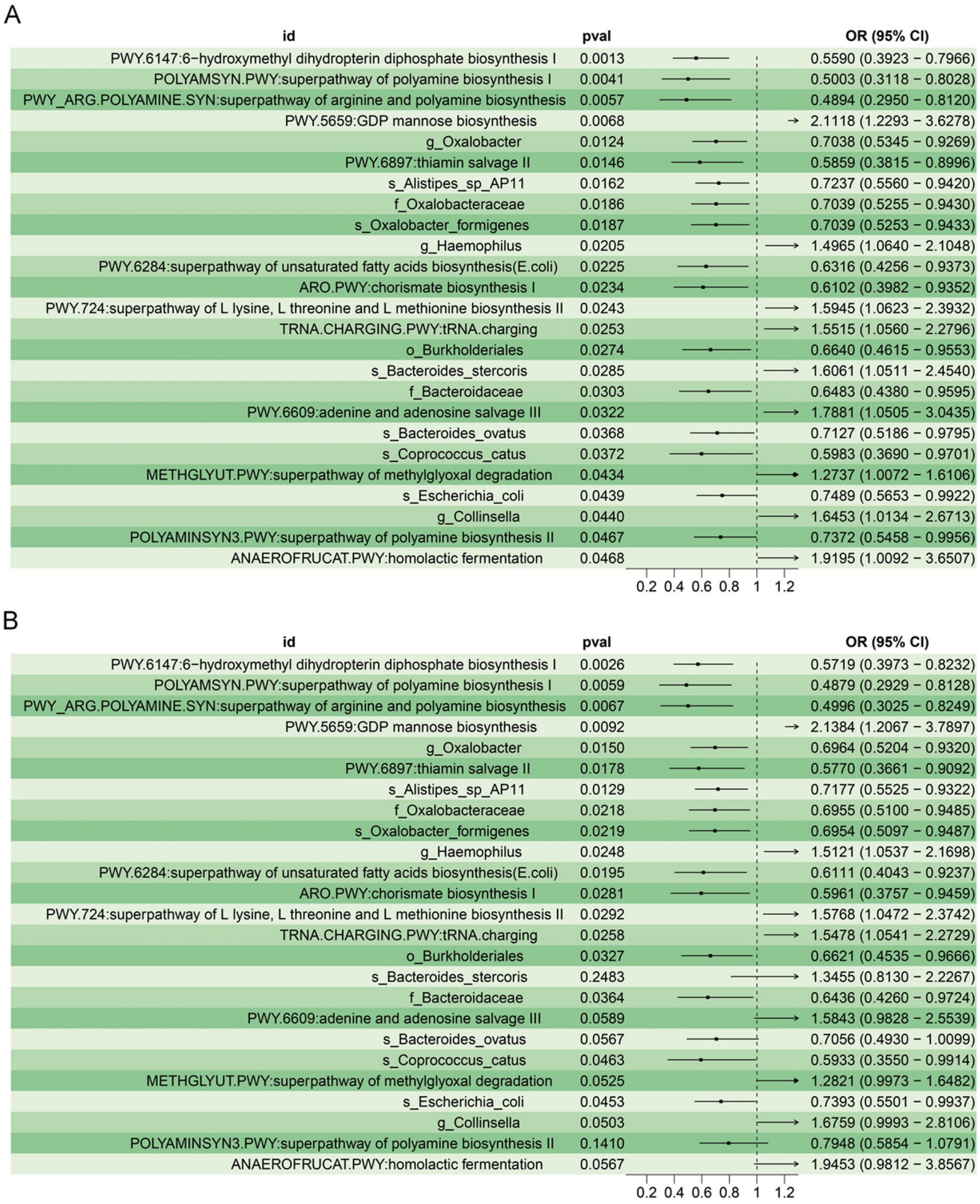

Fig.2 MR analyses highlight the causal effects between gut microbiota and gastric cancer. **a** Forest plot of the causal effect of gut microbiota on GC via the IVW method. **b** Forest plot of the causal effect of gut microbiota on GC via the BWMR method. *OR* odds ratio, *CI* confidence interval

95%CI = 0.5653–0.9922, P = 0.0439; BWMR OR = 0.7393, 95%CI = 0.5501–0.9937, P = 0.0453), "PWY.6147:6-hydroxymethyl dihydropterin diphosphate biosynthesis I" (IVW OR = 0.5590, 95%CI = 0.3923–0.7966, P = 0.0013; BWMR OR = 0.5719, 95%CI = 0.3973–0.8232, P = 0.0026), "POLYAMSYN.PWY: superpathway of polyamine biosynthesis I" (IVW OR = 0.5003, 95%CI = 0.3118–0.8028, P = 0.0041; BWMR OR = 0.4879, 95%CI = 0.29293 ~ 0.8128, P = 0.0059), "PWY_ARG.POLYAMINE.SYN:superpathway of arginine and polyamine biosynthesis" (IVW OR = 0.4894, 95%CI = 0.2950–0.8120, P = 0.0057; BWMR OR = 0.5000, 95%CI = 0.3025–0.8249, P = 0.0067), "PWY.6897:thiamin salvage II" (IVW OR = 0.5859, 95%CI = 0.3815–0.8996, P = 0.0146; BWMR OR = 0.5770, 95%CI = 0.3661–0.9092, P = 0.0178), "PWY.6284:superpathway of unsaturated fatty acids biosynthesis(E.coli)" (IVW OR = 0.6316, 95%CI = 0.4256–0.9373, P = 0.0225; BWMR OR = 0.6111, 95%CI = 0.4043–0.9237, P = 0.0195), and "ARO.PWY:chorismate biosynthesis I" (IVW OR = 0.6102, 95%CI = 0.3982–0.9352, P = 0.0234; BWMR OR = 0.5961, 95%CI = 0.3757–0.9459, P = 0.0281) were found to significantly decrease in the risk of GC (Fig. 2).

An additional investigation into the causal connection between the 18 GMs and GC is outlined in Fig. 3A–R. Sensitivity analysis provided detailed information confirming the strength of the observed causal relationships. The MR-Egger regression intercept method showed no bias from genetic pleiotropy in the results (Supplementary Table 5), and MR-PRESSO analysis confirmed the lack of horizontal pleiotropy in the MR study (P > 0.05, Supplementary Table 6). The Cochran's Q tests revealed no significant heterogeneity based on the results (P > 0.05; Supplementary Table 7). Furthermore, scatter plots (Fig. S1), forest plots (Fig. S2), and funnel plots (Fig. S3) further supported the stability of the results, and analyses using the "leave-one-out" method indicated that none of the individual variables had a significant impact on the causal inferences (Fig. S4).

3.2 Effect of 18 GMs on 731 immune cell traits

The crucial role of the immune system in GC is widely recognized. Thus, we proceeded to clarify how the GM affects immune cell characteristics and their potential influence on the risk of GC. Mediation MR analyses were performed with 731 immune cell characteristics as mediators to explore the relationships between the GM and GC. Significant relationships were found between the 18 bacterial traits and various immune cell traits. A total of 467 immune cell characteristics from a pool of 731 potential mediators met the screening criteria (P_IVW < 0.05) and were used in the mediation MR analyses (Supplementary Table 8).

3.3 Effect of each immune cell trait on GC

These results underscore the complex interplay between immune cell characteristics and the GM, laying the groundwork for further mediation analyses. Subsequent investigations revealed the potential mediating effects of exposure to these noteworthy mediators (467 immune cell traits from 18 gut microbiota) in GC. Based on IVW (**Fig.S5**), 12 GMs affected GC through 16 immune cell traits.

Following the elucidation of how immune cell features affect GC, we examined the direct influence of 12 GM on these crucial mediators (16 immune cell traits). Our analysis revealed several important discoveries (Fig. S6). The causal association between 12 GMs and 16 immune cell traits was investigated using a two-sample MR analysis. The MR-Egger regression intercept method revealed that genetic pleiotropy did not affect these findings (Supplementary Table 9). Moreover, Cochran's Q tests indicated no significant heterogeneity (P > 0.05, Supplementary Table 10), and MR-PRESSO analysis confirmed the absence of horizontal pleiotropy in the MR study (P > 0.05 Supplementary Table 11). The scatter plots in Fig. S7, forest plots in Fig. S8, funnel plots in Fig. S9, and "leave-one-out" analyses (Fig. S10) highlighted the consistency of the results.

MR analysis was conducted to determine the protective effects of the eight immunophenotypes on GC using the IVW method. These immunophenotypes include (6-hydroxymethyl dihydropterin diphosphate biosynthesis I) CD39 + CD4 + %CD4 +, *(f_Oxalobacteraceae)* CD4 Treg %T cell, (*f_Oxalobacteraceae*) CD8br and CD8dim %leukocyte, (s_Oxalobacter_formigenes) CD4 Treg %T cell, (GCST90027810) ebi-a-GCST90001614, (*g_Haemophilus*) HVEM on naive CD4 +, (chorismate biosynthesis I) CD28- CD8dim %CD8dim, and (tRNA.charging) CD39 + CD4 + %CD4 +. Comparable results were observed when four additional approaches were employed: MR eager, weighted median, simple mode, and weighted mode (Fig. S11).

The IVW approach was employed to examine the enhancing effect of four immunophenotypes on GC, specifically (superpathway of polyamine biosynthesis I) CD8br %T cell, (superpathway of arginine and polyamine biosynthesis) SSC-A on NKT, (thiamin salvage II) CD8br %T cell, (*s_Alistipes_sp_AP11*) NKT %T cell, (*g_Haemophilus*) CCR2 on CD14-CD16 + monocyte, (chorismate biosynthesis I) CD28- DN (CD4-CD8-) %T cell, (superpathway of L lysine, L threonine and

Fig.3 Forest plots display the causal associations between gut microbiota and GC using different methods. **a** Forest plot of the causal effect of the "6-hydroxymethyl dihydropterin diphosphate biosynthesis I" bacterial pathway on GC. **b** Forest plot of the causal effect of the "superpathway of polyamine biosynthesis I" bacterial pathway on GC. **c** Forest plot of the causal effect of the "superpathway of arginine and polyamine biosynthesis" bacterial pathway on GC. **d** Forest plot of the causal effect of the "GDP mannose biosynthesis" bacterial pathway on GC. **e** Forest plot of the causal effect of the *g_Oxalobacter* on GC. **f** Forest plot of the causal effect of the "thiamin salvage II" bacterial pathway on GC. **g** Forest plot of the causal effect of the *s_Alistipes_sp_AP11* on GC. **h** Forest plot of the causal effect of the *f_Oxalobacteraceae* on GC. **i** Forest plot of the causal effect of the *s_Oxalobacter_formigenes* on GC. **j** Forest plot of the causal effect of the *g_Haemophilus* on GC. **k** Forest plot of the causal effect of the "superpathway of unsaturated fatty acids biosynthesis (E. coli)" bacterial pathway on GC. **l** Forest plot of the causal effect of the "chorismate biosynthesis I" bacterial pathway on GC. **m** Forest plot of the causal effect of the "superpathway of L lysine, L threonine and L methionine biosynthesis II" bacterial pathway on GC. **n** Forest plot of the causal effect of the "TRNA.CHARGING.PWY:tRNA. charging" bacterial pathway on GC. **o** Forest plot of the causal effect of the *o_Burkholderiales* on GC. **p** Forest plot of the causal effect of the *f_Bacteroidaceae* on GC. **q** Forest plot of the causal effect of the *s_Coprococcus_catus* on GC. (r) Forest plot of the causal effect of the *s_Escherichia_coli* on GC. *IVW* inverse variance weighting, *OR* odds ratio, *CI* confidence interval

L methionine biosynthesis II) HLA DR on CD33br HLA DR + CD14dim, and (*s_Coprococcus_catus*) HLA DR on CD33br HLA DR + CD14dim. Four additional techniques, namely MR eager, weighted median, simple mode, and weighted mode, had similar outcomes (Fig. S11) to the above approaches.

The MR-Egger intercept (Supplementary Table 12) and MR-PRESSO analyses indicated no horizontal pleiotropy in the studied associations (Supplementary Table 13). Cochran's Q tests did not show any significant heterogeneity based on the results shown in Supplementary Table 14 ($P > 0.05$). The reliability of the findings was reinforced by scatter diagrams (Fig. S12), forest charts (Fig. S13), funnel charts (Fig. S14), and "leave-one-out" examinations (Fig. S15).

3.4 Mediation MR analysis

A two-step MR study revealed a causal relationship between the GM and GC, mediated by immune cell traits. The key factors affecting GC were determined, and the resulting impact of exposure on mediation was measured by calculating the mediation effect (indirect effect, Table 1).

Some data from Table 1 were briefly explained, particularly "PWY.6147:6-hydroxymethyl dihydropterin diphosphate biosynthesis I" hinders the advancement of GC by interacting with "CD39 + CD4 + %CD4 + " cells, resulting in a total effect size of -0.5815, of which the mediated effect size of "CD39 + CD4 + %CD4 + " was 0.0197. Moreover, "POLYAMSYN.PWY: superpathway of polyamine biosynthesis I" was demonstrated to impede the advancement of GC through mediation by "CD8br %T cell", with a total effect size of -0.6925; the mediated effect size of "CD8br %T cell" was 0.0418. The promotion of GC progression was facilitated by the interaction of "PWY.724: superpathway of L lysine, L threonine and L methionine biosynthesis II" and "HLA DR on CD33br HLA DR + CD14dim", resulting in a total effect size of 0.4665, with "HLA DR on CD33br HLA DR + CD14dim" cells contributing a mediated effect size of -0.0409. These findings emphasize the intricate relationship between distinct GM exposures, their mediators, and their collective influence on GC.

4 Discussion

The relationship between the GM and immune-related illnesses is a growing area of research. Imbalances in the GM can lead to immune system disorders; however, their impact on GC is complex and not fully understood. To our knowledge, this MR analysis is the first to elucidate the possible causal connection between immune cells in the GM and GC. We identified 12 GMs linked to GC with fluctuations in their abundance affecting immune cells and potentially increasing the risk of GC. We also identified 16 immune cells associated with these bacteria and GC, providing insights into the complex relationship between the GM and GC.

The GM coexists with the human host and has many advantages, such as regulation of immune homeostasis. An imbalance in the composition of the GM can lead to increased susceptibility to colonization of potentially harmful microorganisms and hinder the synthesis of key microbiota-derived metabolites necessary for immune cell maturation and maintenance. GM dysbiosis is associated with various diseases, including tumorigenesis and cancer progression. The treatment of many cancers directly affects gut bacteria. In fact, prophylactic antibiotics are needed to reduce the risk of infection. During radiotherapy and chemotherapy, gastrointestinal toxicity can occur in patients with cancer. Thus, treatment can subsequently alter the composition of the microbiota and may lead to persistent dysbiosis in cancer survivors. Exploring strategies to alleviate dysbiosis and restore microbiota balance plays a role in the management of inflammation-related neoplastic diseases. In the present study, GC was found to be positively

A

6-hydroxymethyl dihydropterin diphosphate biosynthesis I

method	nsnp	pval	OR (95% CI)
MR Egger	14	0.5027	0.5768 (0.1211 − 2.7471)
Weighted median	14	0.0159	0.5414 (0.3288 − 0.8916)
Inverse variance weighted	14	0.0013	0.5590 (0.3923 − 0.7966)
Simple mode	14	0.1374	0.5159 (0.2273 − 1.1706)
Weighted mode	14	0.1092	0.5159 (0.2426 − 1.0968)

B

superpathway of polyamine biosynthesis I

method	nsnp	pval	OR (95% CI)
MR Egger	8	0.4824	0.4961 (0.0792 − 3.1086)
Weighted median	8	0.0273	0.4915 (0.2615 − 0.9237)
Inverse variance weighted	8	0.0041	0.5003 (0.3118 − 0.8028)
Simple mode	8	0.0690	0.3513 (0.1351 − 0.9132)
Weighted mode	8	0.1125	0.4274 (0.1706 − 1.0707)

C

superpathway of arginine and polyamine biosynthesis

method	nsnp	pval	OR (95% CI)
MR Egger	6	0.9745	1.0347 (0.1452 − 7.3709)
Weighted median	6	0.0985	0.5861 (0.3110 − 1.1046)
Inverse variance weighted	6	0.0057	0.4894 (0.2950 − 0.8120)
Simple mode	6	0.3604	0.6249 (0.2501 − 1.5613)
Weighted mode	6	0.3577	0.6274 (0.2545 − 1.5466)

D

GDP mannose biosynthesis

method	nsnp	pval	OR (95% CI)
MR Egger	8	0.2410	5.3184 (0.4290 − 65.9335)
Weighted median	8	0.1059	1.7747 (0.8854 − 3.5573)
Inverse variance weighted	8	0.0068	2.1118 (1.2293 − 3.6278)
Simple mode	8	0.3518	1.7532 (0.5816 − 5.2845)
Weighted mode	8	0.4030	1.6623 (0.5428 − 5.0909)

E

g_Oxalobacter

method	nsnp	pval	OR (95% CI)
MR Egger	10	0.8730	1.1656 (0.1890 − 7.1872)
Weighted median	10	0.1343	0.7567 (0.5253 − 1.0899)
Inverse variance weighted	10	0.0124	0.7038 (0.5345 − 0.9269)
Simple mode	10	0.3452	0.7551 (0.4344 − 1.3124)
Weighted mode	10	0.3809	0.7686 (0.4391 − 1.3455)

F

thiamin salvage II

method	nsnp	pval	OR (95% CI)
MR Egger	10	0.5985	0.5480 (0.0639 − 4.7040)
Weighted median	10	0.0993	0.6349 (0.3699 − 1.0897)
Inverse variance weighted	10	0.0146	0.5859 (0.3815 − 0.8996)
Simple mode	10	0.3400	0.6433 (0.2727 − 1.5176)
Weighted mode	10	0.3089	0.6397 (0.2840 − 1.4408)

G

s_Alistipes_sp_AP11

method	nsnp	pval	OR (95% CI)
MR Egger	11	0.3509	0.5531 (0.1700 − 1.7997)
Weighted median	11	0.0979	0.7313 (0.5049 − 1.0593)
Inverse variance weighted	11	0.0162	0.7237 (0.5560 − 0.9420)
Simple mode	11	0.2002	0.6841 (0.3977 − 1.1770)
Weighted mode	11	0.2652	0.7298 (0.4326 − 1.2313)

H

f_Oxalobacteraceae

method	nsnp	pval	OR (95% CI)
MR Egger	9	0.8762	1.1617 (0.1886 − 7.1570)
Weighted median	9	0.2436	0.7819 (0.5171 − 1.1823)
Inverse variance weighted	9	0.0186	0.7039 (0.5255 − 0.9430)
Simple mode	9	0.4247	0.7691 (0.4171 − 1.4180)
Weighted mode	9	0.4242	0.7828 (0.4427 − 1.3841)

I

s_Oxalobacter_formigenes

method	nsnp	pval	OR (95% CI)
MR Egger	9	0.8662	1.1764 (0.1902 − 7.2759)
Weighted median	9	0.2314	0.7822 (0.5230 − 1.1696)
Inverse variance weighted	9	0.0187	0.7039 (0.5253 − 0.9433)
Simple mode	9	0.3966	0.7690 (0.4327 − 1.3664)
Weighted mode	9	0.4186	0.7827 (0.4457 − 1.3745)

J

g_Haemophilus

method	nsnp	pval	OR (95% CI)
MR Egger	5	0.5411	0.3809 (0.0243 − 5.9673)
Weighted median	5	0.0226	1.6538 (1.0731 − 2.5487)
Inverse variance weighted	5	0.0205	1.4965 (1.0640 − 2.1048)
Simple mode	5	0.1452	1.6568 (0.9579 − 2.8657)
Weighted mode	5	0.1405	1.6646 (0.9658 − 2.8689)

K

superpathway of unsaturated fatty acids biosynthesis(E.coli)

method	nsnp	pval	OR (95% CI)
MR Egger	10	0.6445	1.4312 (0.3304 − 6.1988)
Weighted median	10	0.4786	0.8303 (0.4965 − 1.3888)
Inverse variance weighted	10	0.0225	0.6316 (0.4256 − 0.9373)
Simple mode	10	0.8811	1.0778 (0.4151 − 2.7979)
Weighted mode	10	0.9225	1.0376 (0.5034 − 2.1388)

L

chorismate biosynthesis I

method	nsnp	pval	OR (95% CI)
MR Egger	12	0.4137	0.4836 (0.0911 − 2.5681)
Weighted median	12	0.1481	0.6565 (0.3712 − 1.1612)
Inverse variance weighted	12	0.0234	0.6102 (0.3982 − 0.9352)
Simple mode	12	0.7332	1.2123 (0.4119 − 3.5680)
Weighted mode	12	0.8441	1.1091 (0.4048 − 3.0388)

M

superpathway of L lysine, L threonine and L methionine biosynthesis II

method	nsnp	pval	OR (95% CI)
MR Egger	12	0.5347	1.7555 (0.3158 − 9.7604)
Weighted median	12	0.2740	1.3495 (0.7887 − 2.3089)
Inverse variance weighted	12	0.0243	1.5945 (1.0623 − 2.3932)
Simple mode	12	0.5953	1.2648 (0.5451 − 2.9349)
Weighted mode	12	0.5579	1.2814 (0.5733 − 2.8639)

N

TRNA.CHARGING.PWY:tRNA.charging

method	nsnp	pval	OR (95% CI)
MR Egger	12	0.5139	0.4481 (0.0438 − 4.5824)
Weighted median	12	0.0796	1.5624 (0.9487 − 2.5730)
Inverse variance weighted	12	0.0253	1.5515 (1.0560 − 2.2796)
Simple mode	12	0.3161	1.4842 (0.7104 − 3.1008)
Weighted mode	12	0.3483	1.4484 (0.6902 − 3.0397)

O

o_Burkholderiales

method	nsnp	pval	OR (95% CI)
MR Egger	14	0.2547	0.3975 (0.0877 − 1.8022)
Weighted median	14	0.1798	0.7163 (0.4398 − 1.1664)
Inverse variance weighted	14	0.0274	0.6640 (0.4615 − 0.9553)
Simple mode	14	0.4968	0.7476 (0.3308 − 1.6898)
Weighted mode	14	0.4141	0.7332 (0.3566 − 1.5077)

P

f_Bacteroidaceae

method	nsnp	pval	OR (95% CI)
MR Egger	13	0.0964	0.2260 (0.0455 − 1.1231)
Weighted median	13	0.1656	0.6944 (0.4146 − 1.1628)
Inverse variance weighted	13	0.0303	0.6483 (0.4380 − 0.9595)
Simple mode	13	0.1547	0.4717 (0.1789 − 1.2438)
Weighted mode	13	0.1273	0.4908 (0.2095 − 1.1499)

Q

s_Coprococcus_catus

method	nsnp	pval	OR (95% CI)
MR Egger	5	0.1860	0.1723 (0.0229 − 1.2948)
Weighted median	5	0.0396	0.5398 (0.3000 − 0.9712)
Inverse variance weighted	5	0.0372	0.5983 (0.3690 − 0.9701)
Simple mode	5	0.2260	0.5315 (0.2235 − 1.2641)
Weighted mode	5	0.1689	0.4986 (0.2210 − 1.1249)

R

s_Escherichia_coli

method	nsnp	pval	OR (95% CI)
MR Egger	12	0.7547	1.2077 (0.3818 − 3.8201)
Weighted median	12	0.1507	0.7644 (0.5298 − 1.1027)
Inverse variance weighted	12	0.0439	0.7489 (0.5653 − 0.9922)
Simple mode	12	0.6059	0.8481 (0.4616 − 1.5580)
Weighted mode	12	0.5276	0.8164 (0.4439 − 1.5016)

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Table 1 Mediation Mendelian randomization analyses of the causal effects among gut microbiota, immune cells and GC

Exposure	Mediator	Total effect	β1	β2	Indirect effect(β1*β2)
6-hydroxymethyl dihydropterin diphosphate biosynthesis I	CD39 + CD4 + %CD4 +	− 0.5815	− 0.1877	− 0.1051	0.0197
superpathway of polyamine biosynthesis I	CD8br %T cell	− 0.6925	0.3163	0.1323	0.0418
superpathway of arginine and polyamine biosynthesis	SSC-A on NKT	− 0.7145	− 0.3302	0.0783	− 0.0258
thiamin salvage II	CD8br %T cell	− 0.5347	0.2454	0.1323	0.0325
s_Alistipes_sp_AP11	NKT %T cell	− 0.3234	0.1378	0.1538	0.0212
f_Oxalobacteraceae	CD4 Treg %T cell	− 0.3511	− 0.1684	− 0.1782	0.0300
f_Oxalobacteraceae	CD8br and CD8dim %leukocyte	− 0.3511	0.1353	− 0.3758	− 0.0508
s_Oxalobacter_formigenes	CD4 Treg %T cell	− 0.3511	− 0.1685	− 0.1782	0.0300
s_Oxalobacter_formigenes	CD8br and CD8dim %leukocyte	− 0.3511	0.1354	− 0.3758	− 0.0509
g_Haemophilus	HVEM on naive CD4 +	0.4031	0.3610	− 0.1297	− 0.0468
g_Haemophilus	CCR2 on CD14− CD16 + monocyte	0.4031	0.1763	0.0908	0.0160
ARO.PWY:chorismate biosynthesis I	CD28− CD8dim %CD8dim	− 0.4940	0.2558	− 0.0621	− 0.0159
ARO.PWY:chorismate biosynthesis I	CD28− DN (CD4− CD8−) %T cell	− 0.4940	0.2642	0.1580	0.0418
superpathway of L lysine, L threonine and L methionine biosynthesis II	HLA DR on CD33br HLA DR + CD14dim	0.4665	− 0.3427	0.1193	− 0.0409
tRNA.charging	CD39 + CD4 + %CD4 +	0.4392	0.2298	− 0.1051	− 0.0241
s_Coprococcus_catus	HLA DR on CD33br HLA DR + CD14dim	− 0.5137	0.2997	0.1193	0.0357

correlated with *g_Haemophilus* and two bacterial pathways (Table 1). Such finding suggests that an increased presence of these specific types of taxonomic flora or alterations in these bacterial processes may lead to a higher likelihood of GC. Owing to its ability to reduce nitrates, the *Haemophilus* may play a role in the ongoing inflammatory process [28]. A negative relationship has also been found between the levels of *Haemophilus* and IL-1B mRNA [29]. Previous studies revealed a decrease in *Haemophilus* in the oral microbiome of individuals with GC and a reduction in the presence of *Haemophilus parainfluenzae* in GC [30]. Nevertheless, another study found indicated an increase in the prevalence of *Haemophilus* in GC [31], potentially linked to its connection with functional dyspepsia in the control group rather than intestinal metaplasia in the control group [29]. These results indicate that *Haemophilus* has different effects on GC at different stages. The present study shows that *Haemophilus* promotes effect on GC. Higher levels of tRNA and amino acid pairing typically lead to increased tumor formation [32]; however, specific metabolic processes can also contribute to the development of GC. The metabolic process of the "superpathway involving L lysine, L threonine, and L methionine biosynthesis II" remains largely unexplored in GC. Based on our findings, this metabolic pathway plays a role in promoting GC. Thus, how this pathway affects the GC progression through immune cells is further discussed.

The bacteria *s_Alistipes_sp_AP11*, *f_Oxalobacteraceae*, *s_Oxalobacter_formigenes*, and *s_Coprococcus_catus*, and the five bacterial pathways had an inverse relationship with GC. This finding suggests that these groups of organisms or bacterial processes could potentially provide defense mechanisms against the illnesses. *Alistipes*, a recently discovered group of bacteria, has mainly been found mainly in medical samples, although not as frequently as other bacteria in the Bacteroidetes phyla, which play significant roles in dysbiosis and disease. *Alistipes* can offer protection against certain illnesses, such as via cancer immunotherapy [33]. Similarly, we found that *s_Alistipes_sp_AP11* exerted a protective effect against occurrence of GC. Regarding *f_Oxalobacteraceae*, prior studies showed that following the successful elimination of *H._pylori* in patients with early-stage GC, the average of unidentified Oxalobacteraceae in the range of stomach mucosa-related microbiota was reduced in biopsy samples compared to that in patients without early-stage GC [34]. Reducing the levels of this bacterium may lower the risk of GC; however, further studies are needed to fully understand this connection after the successful elimination of *H. pylori*. *S_Oxalobacter_formigenes*, an exclusively anaerobic bacterium that depends entirely on oxalate for its development, plays a crucial role in the breakdown of oxalate in the digestive system of mammals [35]. Therefore, we speculate that reducing the abundance of this bacterium can regulate immune cells and thus reduce the risk of GC, which may be related to oxalate metabolism. Fermentation by various intestinal bacteria can result in the production of lactic acid, which can disturb the gut microbiome by reducing the pH of the lumen, leading to various negative health effects associated with its buildup. *Coprococcus catus* is a crucial species in the human gut and is essential for lactic acid metabolism. *C. catus* is important for the growth of lactic acid, sugar, or a combination of both, and influences how other intestinal bacterial species use lactic acid [36]. This role may explain the increased abundance of *C. catus*, which reduces the occurrence of GC.

The microbiota in the digestive system is important in the development of GC and may have an impact on the response to immunotherapy, enhancing the immune response to tumors in a various ways, such as by activating T cells in response to bacterial antigens that may also target tumor antigens and producing metabolites that have systemic effects on the host [37]. The current findings suggest that the gut microbiome increases the likelihood of GC by influencing 12 types of immune cells (Table 1). Furthermore, four GMs were found to decrease the likelihood of GC by controlling four immune cell characteristics (Table 1). CD39, also called ectonucleoside triphosphate diphosphohydrolase 1 (ENTPD1), is crucial for producing immunosuppressive adenosine and plays a significant role in cancer advancement [38]. In fact, high levels of CD39 in GC patients with GC post-surgery are linked to a negative outcome [39]. Our findings indicate that the "PWY.6147:6-hydroxymethyl dihydropterin diphosphate biosynthesis I" and "TRNA.CHARGING.PWY:tRNA.charging" are involved in promoting and inhibiting the risk of GC through "CD39 + CD4 + %CD4 + " cell, respectively. Currently, how these bacterial pathways interact with the "CD39 + CD4 + %CD4 + " cells remain unknown.

CD8 + T cells are the main cells in tumors that fight cancer; however, studies on their relationship with GC prognosis have yielded conflicting results, and their role in outcomes is debated [40]. Spermidine can boost fatty acid oxidation by activating mitochondrial trifunctional proteins, leading to increased mitochondrial activity and cytotoxic function in CD8 + T cells, ultimately improving anti-tumor immunity [41]. The breakdown of glutamine is a key feature of T cell activation and changes in metabolism. Studies using isotopic tracers on CD8 + T cells activated by antigens have indicated that glutamine is a primary source of carbon for the production of polyamines, such as putrescine, spermidine, and spermine. These polyamines are crucial for the proliferation of T cells triggered by activation and production of hypusine. Hypusine is a product of spermidine and is attached to the translational elongation factor, eukaryotic initiation factor 5A (eIF5A) [42]. Our findings indicate that "POLYAMSYN.PWY:superpathway of polyamine biosynthesis I" and "PWY.6897:thiamin

salvage I" are involved in mediating "CD8br %T" cell activity. However, the relationship between "PWY.6897: thiamin salvage II" and "CD8 + T" cells must to be further experimentally verified.

NK cells, also known as crucial lymphocytes in the innate immune system, are essential for inhibiting the onset, progression, and spread of GC. According to various clinical studies, enhancing NK cell quantity or NK cell anti-tumor function is a promising approach for patients with GC [43]. Arginase 2 (ARG2)-induced arginine depletion creates an immunosuppressive environment for traditional T cells. The "PWY_ARG.POLYAMINE.SYN: superpathway of arginine and polyamine biosynthesis", and s_Alistipes_sp_AP11 were discovered to reduce the likelihood of GC by affecting "SSC-A on NKT" cells. *Alistipes*, a genus in the phylum Bacteroidetes, is a symbiotic bacterium in the intestine that is not motile, produces indole, does not reduce nitrate, and does not hydrolyze arginine or urea. The bacterium has been demonstrated to play a positive role in cancer treatment by influencing the environment around the tumor, primarily by stimulating the production of tumor necrosis factor (TNF) by immune cells near the tumor and using a combination of intratumoral CpG-oligodeoxynucleotides (ODN) to activate TLR9 and inhibitory IL-10R antibodies, ultimately leading to tumor eradication [33]. Therefore, a possible connection exists between this taxon and GC involving the NKT cells.

Recent studies have shown that the recruitment of regulatory T cells (Tregs) to tumors is a strategy for immune evasion. Elevated levels of Tregs have been observed in the tumor mucosa of various types of cancers, such as GC, and these Tregs have been linked to unfavorable outcomes in GC [44]. The *f_Oxalobacteraceae* and *s_Oxalobacter_formigenes* are modulated via "CD4 Treg %T" cells, which is essential for immune system evasion. Therefore, the oxalate metabolism pathway may play a role in Tregs; however, further experiments are needed to verify this notion. Interestingly, *f_Oxalobacteraceae* and *s_Oxalobacter_formigenes* have been shown to reduce GC risk through "CD8br and CD8dim %leukocyte". Our findings also indicates that *g_Haemophilus* could potentially elevate the likelihood of GC by regulating of "HVEM on naive CD4 + " and "CCR2 on CD14- CD16 + monocyte". HVEM, a member of the tumor necrosis factor receptor family (TNFRSF14), is commonly altered in cancer and is believed to have a tumor suppressor function in certain cancer situations [45]. The activation of costimulatory signals through the binding of the TNF ligand to its corresponding TNF receptor (TNFR) superfamily is crucial for the proliferation, maturation, and viability of antigen-experienced CD4 + and CD8 + T cells, which play key roles in adaptive immunity and various diseases [46]. Monocytes travel through the bloodstream and move to areas of inflammation where they can have either negative or positive effects according to their characteristics [47]. Monocytes/macrophages that express CCR2, especially in the tumor microenvironments, can strongly suppress the immune system. Recently, studies have used CCR2 antagonism to attract immunosuppressive monocytes/macrophages to tumors to change the tumor environment and improve the anti-tumor immune response [48]. Currently, studies elucidating how g_Haemophilus interacts with the HVEM/CCR2 pathway are lacking.

CD28 plays key roles in activating T cells and regulating immune tolerance. Reduced CD28 expression in senescent T cells can lead to an increase in CD8 + CD28-senescent populations in tumors [49]. The "ARO.PWY:chorismate biosynthesis I" was found to be mediated through "CD28-CD8dim %CD8dim" and "CD28-DN (CD4-CD8-) %T" cells. However, further studies are required to elucidate regulation of immune cells through this metabolic pathway. HLA-DR, a part of the major histocompatibility complex II that exhibits abnormal expression in specific types of tumors reasons, might be a noteworthy indicator [50]. "PWY.724 superpathway of L lysine, L threonine and L methionine biosynthesis II" was discovered to elevate the likelihood of GC via "HLA DR on CD33br HLA DR + CD14dim" cells, whereas *s_Coprococcus_catus* was found to lower the likelihood of GC through "HLA DR on CD33br HLA DR + CD14dim" cells. These metabolites highlight the significant and diverse effects of microbial metabolism on the host immune response and overall well-being.

This study distinguishes itself from other related studies through its thorough methodology, incorporating several rigorous analyses to examine the connections between GM and GC. Our conclusions are reinforced by the uniformity of our findings using various analytical methods, including weighted median, MR-Egger, and primary IVW. We sought to thoroughly examine specific GM genera and their connections to GC, which is a significant aspect of our study. These findings offer compelling insights into potential biological mechanisms underlying GC development.

4.1 Limitations

This study used genetic data from the a GWAS database, which only included European populations; therefore, the findings may not apply to all ethnic groups and populations. Furthermore, our analysis primarily focused on adenocarcinoma as a singular category within the pathological classification of GC, neglecting other pathological classifications within GC. This omission may obscure the potential impact of the GM on the susceptibility to GC across various pathological classifications. In addition, owing to the intricate nature of the immune system, additional mediating factors may not have been considered. Therefore, caution should be exercised when interpreting these results in a clinical context,

particularly in relation to the individual health status of patients. The findings should be regarded as provisional and further verifications through subsequent investigations are warranted.

5 Conclusions

Our study emphasized the important role of the GM in affecting immune reactions and their possible effects on GC. The identified relationships and intermediary impacts established a basis for additional research, underscoring the importance of the gut-immune connection in well-being and illness.

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Data availability Data is provided within the manuscript or supplementary information files. Publicly available data were analyzed in this study, which can be found here: https://www.ebi.ac.uk/gwas/; https://storage.googleapis.com/finngen-public-data-r9/summary_stats/finngen_R9_C3_STOMACH_EXALLC.gz.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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