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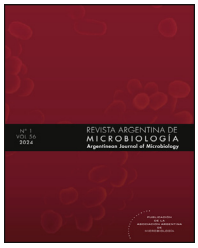

ORIGINAL ARTICLE

Causal relationships between *Burkholderiales*, *Eubacteriaceae* and colorectal cancer mediated by plasma metabolites

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Circulating metabolites;
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Abstract While *Burkholderiales* and *Eubacteriaceae* are associated with colorectal cancer, a causal relationship has not yet been fully established. This study aimed to assess their causal effects and associated plasma metabolites on colorectal cancer. Summary statistics from genome-wide association studies were analyzed for gut microbiota (n = 7738), 1400 plasma metabolites (n = 8299), and colorectal cancer (n = 321 040). Genetic correlations were estimated using linkage disequilibrium score regression. Causal relationships were assessed using Mendelian randomization. Furthermore, a two-step mediation analysis was performed to estimate the proportion of the effect of *Burkholderiales* and *Eubacteriaceae* on colorectal cancer mediated by plasma metabolites. The inverse variance weighted method was primarily used to estimate the effect. Our results suggested the causal effects of 13 *Burkholderiales* and eight *Eubacteriaceae* taxa on colorectal cancer. The positive effect of *Oxalobacteraceae*, *Oxalobacter* and *Oxalobacter formigenes* was potentially mediated by dihomo-linolenoylcarnitine, while the protective effect of *Eubacterium siraeum* may involve 1-linoleoyl-GPE. Sensitivity analyses indicated no significant heterogeneity or pleiotropy. This study suggests the potential causal effects of the *Burkholderiales* and *Eubacteriaceae* taxa on colorectal cancer, with effects potentially mediated by the metabolites dihomo-linolenoylcarnitine and 1-linoleoyl-GPE, respectively. It should be emphasized that these findings are inherently observational in nature and do not establish definitive causality, which is a limitation of the study due to the relaxed statistical threshold for instrumental variable selection and the restriction of the analysis to populations of European ancestry. Further experimental and clinical studies are required to elucidate these mechanistic relationships.

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PALABRAS CLAVE
Microbiota intestinal;
Metabolitos circulantes;
Cáncer colorrectal;
Regresión por puntuación de desequilibrio de ligamiento;
Aleatorización mendeliana

Relaciones causales entre *Burkholderiales*, *Eubacteriaceae* y cáncer colorrectal mediadas por metabolitos plasmáticos

Resumen Aunque Burkholderiales y Eubacteriaceae están asociados con el cáncer colorrectal (CCR), su relación causal no ha sido completamente dilucidada. Este estudio tuvo como objetivo evaluar sus efectos causales, junto con los metabolitos plasmáticos asociados, sobre el CCR. Se analizaron las estadísticas resumidas de los estudios de asociación de genoma completo de la microbiota intestinal (N = 7738), 1400 metabolitos plasmáticos (N = 8299) y CCR (N = 321.040). Las correlaciones genéticas se estimaron mediante regresión de puntuación de desequilibrio de ligamiento. Las relaciones causales se evaluaron mediante aleatorización mendeliana. Además, se realizó un análisis de mediación en dos pasos para estimar la proporción del efecto de Burkholderiales y Eubacteriaceae sobre el CCR mediado por metabolitos plasmáticos. Se utilizó principalmente el método de la ponderación de la varianza inversa para evaluar la estimación del efecto. Nuestros resultados sugirieron efectos causales de 13 taxones de Burkholderiales y de 8 taxones de Eubacteriaceae sobre el CCR. El efecto positivo de Oxalobacteraceae, Oxalobacter y Oxalobacter formigenes estuvo potencialmente mediado por la dihomo-linolenoilcarnitina, mientras que el efecto protector de Eubacterium siraeum podría involucrar a la 1-linoleoil-glicero-3-fosfoetanolamina (1-linoleoil-GPE). Los análisis de sensibilidad no indicaron heterogeneidad o pleiotropía significativas. Este estudio sugiere posibles efectos causales de taxones de Burkholderiales y Eubacteriaceae sobre el CCR, con efectos potencialmente mediados por los metabolitos dihomo-linolenoilcarnitina y 1-linoleoil-GPE, respectivamente. Cabe enfatizar que estos hallazgos son inherentemente observacionales y no establecen una causalidad definitiva, una limitación subrayada por el umbral estadístico relajado para la selección de variables instrumentales y la restricción del análisis a poblaciones de ascendencia europea. Se requieren más estudios experimentales y clínicos para investigar estas relaciones mecanísticas.

Introduction

Colorectal cancer (CRC) ranks as the third most common malignancy worldwide, with recent data indicating a continued rise in both its incidence and mortality[5]. According to 2023 colorectal cancer statistics, there is a noticeable trend toward diagnosis at a younger age and more advanced stages[31]. This is reflected in the increased mortality observed among individuals under 50 years of age. Additionally, approximately 25–35% of CRC patients will eventually develop liver metastases[2]. As reported by the American Cancer Society, patients with metastatic disease have a low five-year survival rate of less than 12%[30]. Currently, the diagnosis of CRC primarily relies on magnetic resonance imaging, endoscopic biopsy, and tumor marker testing. However, these methods are associated with considerable limitations, including the high heterogeneity of imaging findings, restricted sampling depth during colonoscopy, and the relatively low specificity of available biomarkers[15]. Therefore, identifying more accurate diagnostic biomarkers is crucial for improving the clinical diagnosis and management of CRC.

The human gut microbiota is a diverse ecosystem harboring over 10^{14} microorganisms, which complements a wide range of host physiological functions, biochemical processes, and metabolic activities[33]. Accumulating evidence has linked gut microbiota dysbiosis to the development of colorectal cancer. This is primarily characterized by an increased abundance of pathogenic bacteria, such as *Fusobacterium nucleatum*, *Escherichia coli*, and *Bacteroides fragilis*, coupled with a depletion of protective bacteria that produce short-chain fatty acids (SCFAs)[38]. Furthermore, emerging bacterial species such as *Parvimonas micra* and *Solobacterium* have also been increasingly implicated in CRC[46]. Although modern techniques can adequately depict the enormous diversity of the gut microbiome, the specific role of most bacterial species in colorectal cancer remains largely unknown. Among these, the order *Burkholderiales* has been implicated in multiple host cellular functions, including nutrient acquisition, the production of intra- and extracellular metabolites, and the regulation of metabolic homeostasis. Notably, *Burkholderia glumae*, a member of this order, is recognized as a pathogen that perturbs nutrient uptake and nucleotide metabolism[1]. While *Burkholderiales* has been reported as a potential biomarker for stages II–III CRC in a Chinese cohort, its association with CRC development in other regions is unclear[49]. *Eubacteriaceae* produce medium-chain fatty acids that contribute to energy metabolism, immune modulation, and gut barrier integrity[29]. Nonetheless, their specific mechanisms in CRC progression remain unclear. Establishing a causal link between the gut microbiota and CRC is particularly challenging due to the complexity of confounding factors such as diet and lifestyle, coupled with the technical limitations

of current intervention methods such as fecal microbiota transplantation[44].

Alterations in the metabolic state are more readily detectable than genomic or proteomic changes, making them promising non-invasive biomarkers for cancer diagnosis, prognosis, and treatment[39]. Furthermore, metabolites produced by the gut microbiota can enter systemic circulation and exert regulatory functions at various target sites within the host[10,41]. Multiple studies have identified serum metabolites from host and microbiota as potential biomarkers for detecting colorectal cancer[48]. Nishiumi et al. developed a CRC prediction model incorporating 2-hydroxybutyrate, kynurenine, aspartic acid, and cystamine, with an AUC of 0.9097[27]. Reduced circulating levels of amino acids such as tryptophan, methionine, alanine, and glutamine are commonly observed in CRC patients and correlate with shorter disease-free survival[3,50]. However, the interactive mechanisms by which specific gut microbes and metabolites collectively promote CRC development remain unclear. Moreover, patients with early-onset colorectal cancer typically have a poorer prognosis and lower survival rates than those with late-onset cancer[37]. Thus, elucidating the complex interactions between the gut microbiome, metabolites, and CRC is essential for achieving earlier detection and intervention. Harnessing the potential of identified metabolites may improve clinical approaches to early diagnosis and management.

To assess causality, we applied linkage disequilibrium score regression (LDSC) and Mendelian randomization (MR) – methods robust to confounding and reverse causation[7,21,43]. This study specifically examines the causal effects of the gut microbiome (*Burkholderiales* and *Eubacteriaceae*) and plasma metabolites on colorectal cancer risk. The findings are expected to clarify pathogenic pathways, thereby advancing the understanding of CRC etiology.

Materials and methods

Study design

We applied LDSC and a two-step Mendelian randomization framework to estimate the genetic correlation and potential causal relationships between genetically predicted gut microbiome features and colorectal cancer. The diagram of the study design, the causal interpretation of Mendelian randomization, and three necessary assumptions are illustrated in Figure 1. Single nucleotide polymorphisms (SNPs) should strongly predict the exposures; then, there should be no confounders of the instrument and the outcome. Finally, the SNPs are associated with the outcome only through the exposures[19,42]. This study was conducted in accordance with the STROBE-MR reporting guidelines[32] (Table S1).

Data sources

The characteristics of the corresponding GWAS (genome wide association studies) data sources are described in Figures 1 and 2. Summary data of gut microbes were obtained from a study conducted by Lopera-Maya et al.[23], who reported 207 taxa and 205 pathways involving 7738 participants. Various plasma metabolites were derived from Chen et al.'s study, involving the cohort of 8299 individuals of European ancestry from the Canadian Longitudinal Study on Aging (CLSA)[11]. A total of 15.4 million SNPs were retained for the GWAS following stringent pre-GWAS genotype quality control. Summary statistics were deposited in the GWAS Catalog (https://www.ebi.ac.uk/gwas/). Accession numbers for the European GWAS datasets are as follows: GCST90199621-90201020. Among them, there were 1091 blood metabolites (850 known substances and 241 unknown entities) and 309 metabolite ratios in the genome-wide association analyses (Table S2). To better understand broader biological processes, investigation of genetic determinants of substrate-product ratios is essential given that several metabolites serve as substrates and products of enzymatic reactions[45]. A colorectal cancer risk-related dataset was obtained from FinnGen R10, which included 6847 CRC patients and 314 193 controls. The original data utilized in this GWAS was approved by the ethical committee, and all participants have duly signed their informed consent forms.

Selection of genetic instrumental variants

Instrumental variables were selected through the following steps. First, genetic variants associated with microbiota traits were identified at a genome-wide significance threshold set at $p < 1 \times 10^{-5}$. Next, we excluded the SNPs with linkage disequilibrium (LD) given the parameters of $r^2 < 0.001$ and distance >10 000 kb[26,34]. Finally, we included the SNPs whose effect allele frequency was >0.01 and *F*-statistic was >10 to minimize weak instrumental bias[9]. The SNPs used as instruments for each exposure are listed in Table S3.

Genetic analyses to elucidate causality using LDSC

We assessed the genetic correlation between the gut microbiota and colorectal cancer using the recommended settings in the software package LDSC[6]. LDSC examines the relationship between test statistics and linkage disequilibrium to quantify the contribution of inflation from a true polygenic signal or bias. It can estimate genetic correlation from GWAS summary statistics and is not biased by sample overlap[6,7]. The LDSC method estimates the genetic correlation between two traits by regressing the LD score of each SNP against the effect sizes of the two traits[42,43].

Genetic analyses to elucidate causality using MR

Five methods were involved, including weighted median, MR-Egger, weighted mode, simple mode and inverse variance weighted (IVW). The IVW method is regarded as the most precise and robust approach for estimating causal effects, provided that all selected SNPs serve as valid instrumental variables (IVs)[8]. Results are reported as beta (β) value with standard error for the continuous outcome and odds ratio (OR) with a 95% confidence interval (CI). A threshold of $p < 0.05$ was considered nominally significant. For

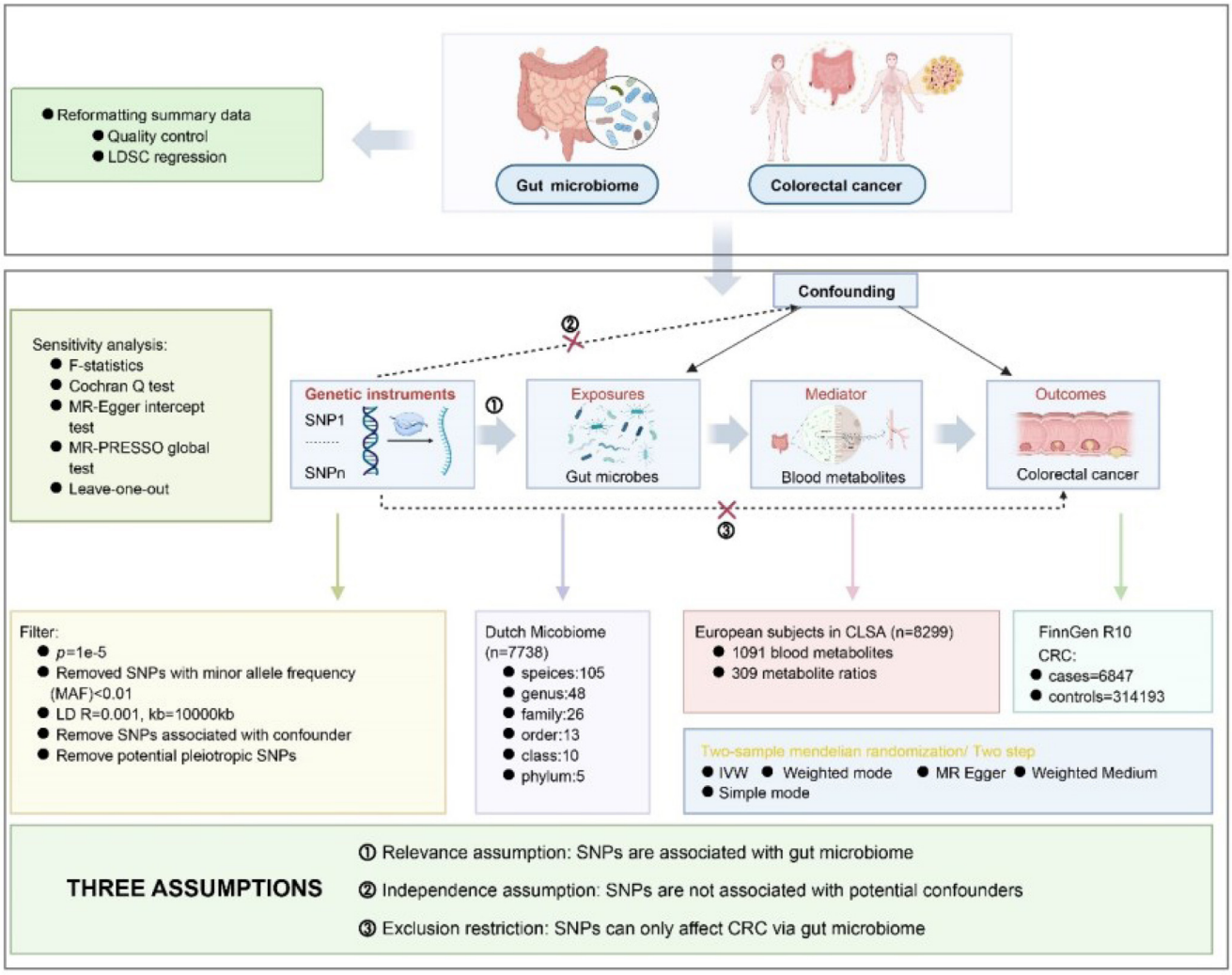

Figure 1 Assumptions and design of the bidirectional mediation Mendelian randomization (MR) analyses. First, a two-sample bidirectional MR was performed to investigate the causal relationships between gut microbiota (exposure) and colorectal cancer (outcome). Second, 1400 blood metabolites (mediators) were selected for subsequent mediation analyses. Finally, a two-step MR analysis was conducted to detect potential mediating metabolites (Step 1, the effect of the gut microbiota on metabolites; Step 2, the effect of metabolites on colorectal cancer). LDSC: linkage disequilibrium score regression; CLSA: Canadian Longitudinal Study on Aging; IVW: inverse variance weighted.

bacterial taxa containing only one IV, we used the Wald ratio for MR analysis.

Mediation analyses link ''intestinal microbiota-blood metabolites-colorectal cancer''

We used summary statistics of blood metabolites from 8299 individuals of European ancestry from the CLSA, including 1091 blood metabolites and 309 metabolite ratios from the GWAS catalog. To discover potential novel metabolites as mediators between the two species and colorectal cancer, we adopted a two-step Mendelian randomization (TSMR) to decompose the direct and indirect effects of the gut microbiome and blood metabolites on colorectal cancer. Two estimates were calculated: the causal effect of the exposure on the mediator and the causal effect of the mediator on colorectal cancer.

Heterogeneity, pleiotropy, and sensitivity assessment

Through the radial MR-Egger intercept and MR-PRESSO global tests, horizontal pleiotropy was not found in our study, as shown in Table S5[4]. Heterogeneity was assessed using Cochran's *Q* test, with a *p*-value >0.05 indicating no significant heterogeneity (Table S4). In addition, a leave-one-out analysis was used to detect SNP outliers. All analyses were performed in R (version 4.3.2) using the following R packages: ''LdlinkR'', ''TwoSampleMR''[18], ''tidyverse'', and ''MR-PRESSO''[40].

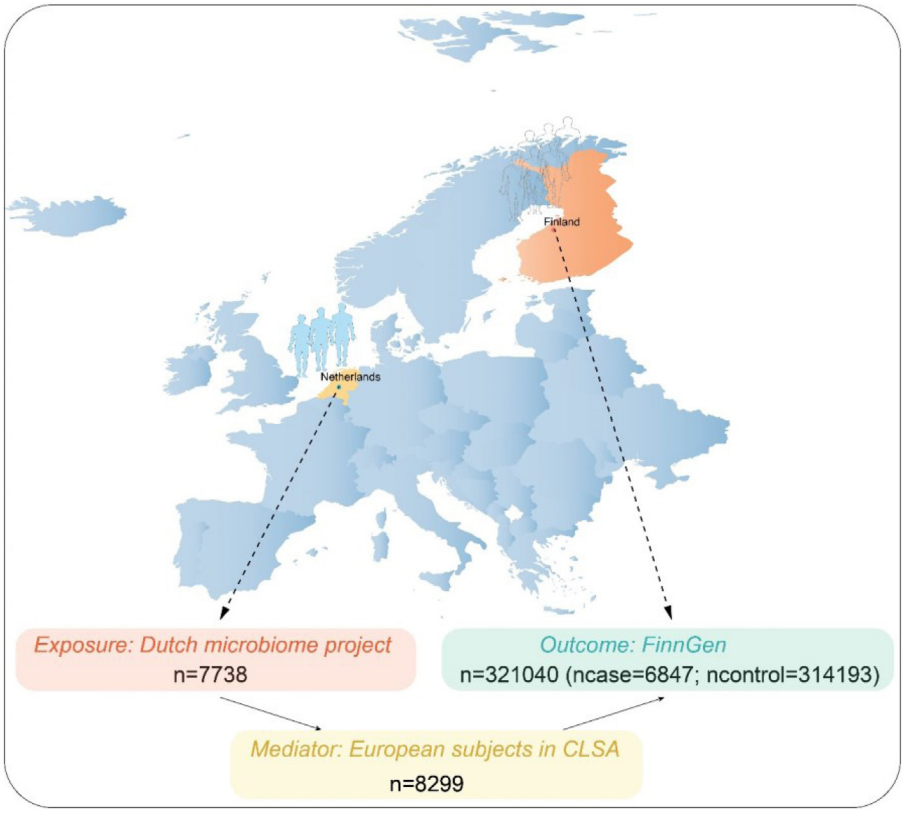

Figure 2 The geographical locations of exposure, mediators and outcome; summarization of the sample sizes of the MR analyses.

Results

Instrumental variable selection

The number of SNPs used as instrumental variables ranged from four to fourteen (median, ten) for *Burkholderiales*, which comprised thirteen taxa from a pool of 7738 Dutch participants (Table S6). The number of SNPs for eight microbes belonging to *Eubacteriaceae* ranged from six to fourteen (median, nine) (Table S7). We extracted the effect allele, the other allele, the corresponding beta-coefficient (β), standard error (SE), and *p* values of these SNPs for analysis. The *F*-statistic for each instrumental variable exceeded 10, which is a standard threshold for minimizing weak instrument bias.

Genetic causality and correlation between the gut microbiota and colorectal cancer

To assess the genetic correlation between 412 species-level gut microbiota and colorectal cancer, we conducted an LDSC regression analysis. Some species could not be used for the above analysis owing to limitations such as low heritability and small sample size[12]. Finally, we obtained the estimations of genetic correlation between 113 species and colorectal cancer. LDSC showed a suggestive correlation between *Burkholderiales* and colorectal cancer as shown in Figure 3 and Table S8 ($r_g = -9.208$, $p = 0.033$). Through our LDSC analysis, *Burkholderiales* was identified as being associated with CRC. Separately, a strong association between *Eubacteriaceae* and CRC was supported by existing literature[16]. Then we conducted bidirectional MR analyses to explore the causal relationship between the gut microbiome and colorectal cancer, including 13 taxa belonging to *Burkholderiales* and eight microbes within *Eubacteriaceae* (Fig. 4A). The significant taxa were *Oxalobacteraceae* (OR: 1.115; 95%CI: 1.011–1.231; $p = 0.030$), *Oxalobacter* (OR: 1.119; 95%CI: 1.020–1.228; $p = 0.017$) and *Oxalobacter_formigenes* (OR: 1.115; 95%CI: 1.011–1.231; $p = 0.030$) (Fig. 4C). They were all positively associated with colorectal cancer. In addition, among the eight bacterial taxa belonging to *Eubacteriaceae*, only one was found to be positively associated with colorectal cancer (Fig. 4B), while *Eubacterium_siraeum* was negatively correlated. *Eubacterium_siraeum* was demonstrated to exert a potent protective effect against colorectal cancer (OR: 0.846; 95%CI: 0.759–0.943; $p = 0.003$) (Fig. 4C). The *Q*-statistics of the IVW test and MR-Egger indicated no notable heterogeneity (*p*-values between 0.676 and 0.963) (Table S4). The *p*-values of the MR-Egger intercepts were between 0.462 and 0.777, suggesting no horizontal pleiotropy in the MR study (Table S5). The results of the ''leave-one-out'' analysis supported the reliability of the MR analysis (Figs. S1–S4). The overall effect of the gut microbiota on colorectal cancer was illustrated by the scatter plots (Figs. S5–S8). Moreover, the forest plots indicated the causal associations between the intestinal microbiome and colorectal cancer (Figs. S9–S12).

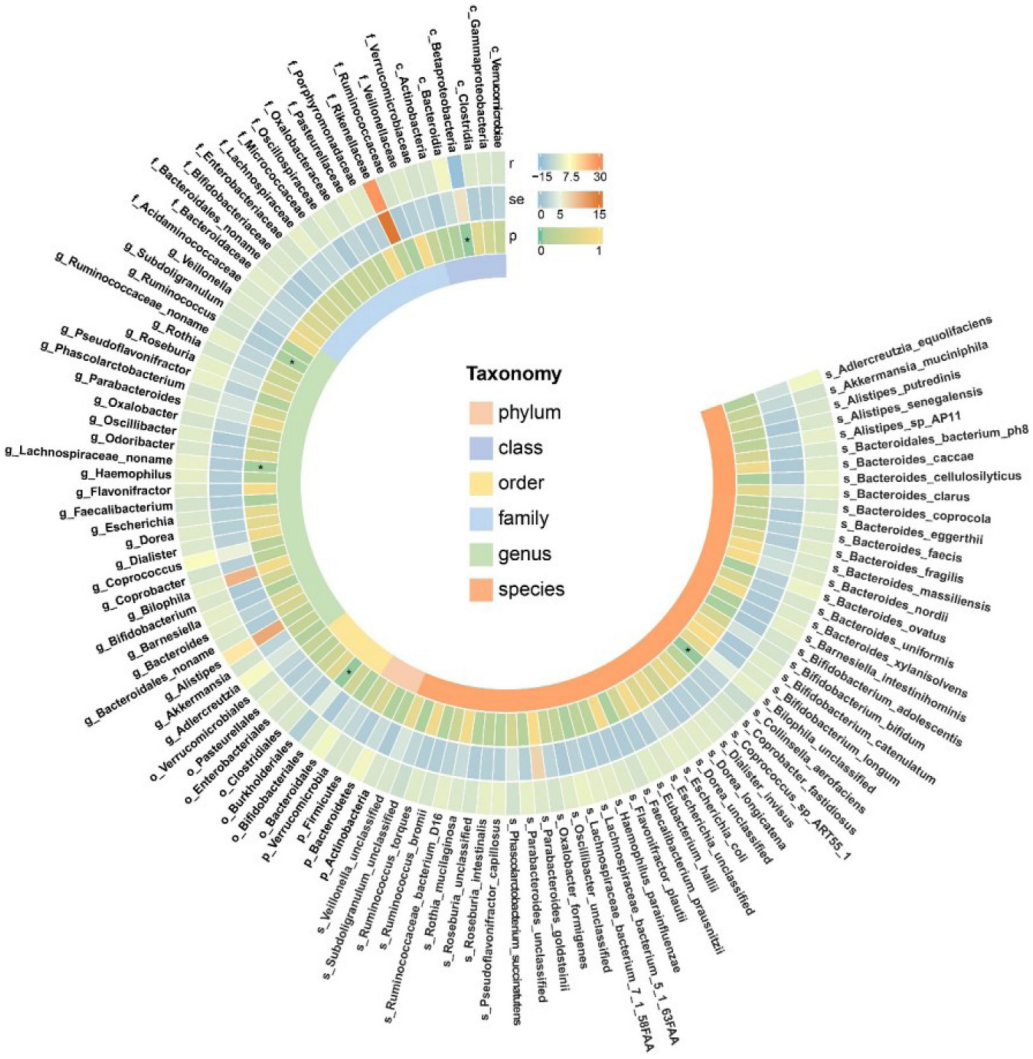

Figure 3 Circular heatmap of suggestive genetic correlation between gut microbes and colorectal cancer.

Mediation analyses of potential blood metabolites

In this study, blood metabolites were investigated as potential mediators in the causal pathway from the gut microbiota to colorectal cancer. First, we tested for a causal association between four gut microbiota and 1400 metabolites by two-sample MR analysis (Tables S9–S12). Subsequently, we tested for a causal association between the metabolites identified in step 1 (those associated with the gut microbiota) and CRC using a two-sample MR (Tables S13–S16). Among the four taxa causally associated with colorectal cancer, four were significantly associated with the two metabolites based on IVW methods (Table 1). Specifically, *Eubacterium_siraeum*, as a protective taxon against colorectal cancer, increased 1-linoleoyl-GPE (OR: 1.114; 95%CI: 1.005–1.236; $p = 0.041$). *Oxalobacteraceae*, *Oxalobacter* and *Oxalobacter_formigenes* all exerted detrimental effects on colorectal cancer by decreasing the levels of dihomo-linolenoylcarnitine. The odds ratios for both the *Oxalobacteraceae* family and the *Oxalobacter_formigenes* species were 0.910, indicating that these metabolites act as mediators between the gut microbiota and colorectal cancer.

Discussion

To the best of our knowledge, our research represents the first application of MR to explore the causal link between the gut microbiota at the species level and colorectal cancer, while also investigating the potential mediating role of 1400 circulating metabolites in this relationship. Furthermore, we utilized LDSC to estimate the genetic correlation between the gut microbiota and colorectal cancer. Notably, our findings indicated that *Oxalobacter*, *Oxalobacteraceae* and *Oxalobacter formigenes* affiliated with *Burkholderiales* showed a positive association with CRC risk, while *Eubacterium_siraeum* appeared to restrict the progression of CRC, conferring a protective effect on the host.

In an MR study to evaluate the causal association between 119 gut microbes based on 16S rRNA sequencing and colorectal cancer, Xiang et al.[44] found that family

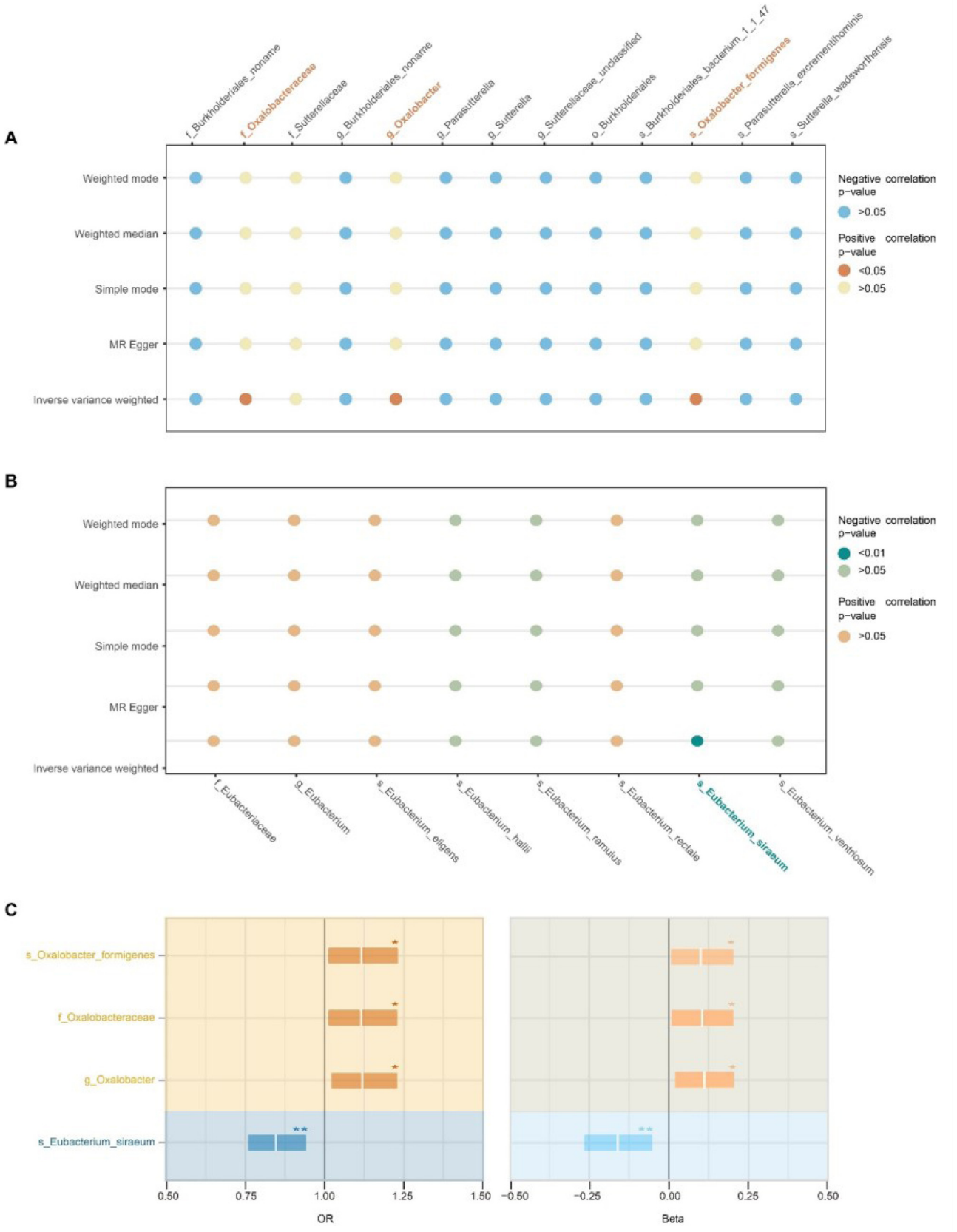

Figure 4 Suggestive causal effects of gut microbes on colorectal cancer. (A) MR results of causal association between gut microbes belonging to *Burkholderiales* and colorectal cancer. (B) MR results of causal association between gut microbes belonging to *Eubacteriaceae* and colorectal cancer. (C) Significant causal estimates from genetically predicted gut microbes to colorectal cancer. MR: Mendelian randomization; OR: odds ratio.

Table 1 Metabolites as intermediates in the causal effects of the gut microbiota on colorectal cancer.

Exposur	$\beta_{e\text{-}i}$	$OR_{e\text{-}i}$	$P_{e\text{-}i}$	Intermediate	$\beta_{i\text{-}o}$	$OR_{i\text{-}o}$	$P_{i\text{-}o}$	Outcome	$\beta_{e\text{-}o}$	$OR_{e\text{-}o}$
f_Oxalobacteraceae	−0.095	0.910	0.046	Dihomo-linolenoylcarnitine	−0.067	0.936	0.040	CRC	0.109	1.115
g_Oxalobacter	−0.092	0.912	0.031	Dihomo-linolenoylcarnitine	−0.067	0.936	0.040	CRC	0.113	1.119
s_Oxalobacter_formigenes	−0.095	0.910	0.046	Dihomo-linolenoylcarnitine	−0.067	0.936	0.040	CRC	0.109	1.115
s_Eubacterium_siraeum	0.108	1.114	0.041	1-Linoleoyl-GPE	−0.198	0.821	0.011	CRC	−0.167	0.846

Porphyromonadaceae, genus *Slackia*, genus *Anaerotruncus*, and genus *Intestinibacter* exhibited a significant causal association with CRC risk. Furthermore, *Eubacterium coprostanoligenes* was positively associated with CRC risk[44]. In our study, however, *Eubacterium siraeum* was negatively correlated with the risk of CRC. Although they all belong to the Firmicutes phylum, the discrepancy may originate from differences in databases and study populations. The LDSC analysis, identified the order *Burkholderiales* as correlated with colorectal cancer. Among the 13 taxa within *Burkholderiales*, three anaerobic bacteria – *Oxalobacteraceae*, *Oxalobacter* and *Oxalobacter formigenes* – were found to exert detrimental effects on colorectal cancer. As specialized symbionts relying solely on oxalic acid[47], these bacteria were found to increase CRC risk. *Oxalobacteraceae* and *Oxalobacter* were also reported to participate in the unfavorable link between Crohn's disease and esophageal cancer[13,14,28], both of which are diseases of the digestive system.

In our mediation analyses, *Oxalobacteraceae*, *Oxalobacter*, *Oxalobacter_formigenes* were all associated with dihomo-linolenoylcarnitine, suggesting that it may be an important element in mediation. Dihomo-linolenoylcarnitine (C20:3n3 or 6) is a long-chain acylcarnitine, i.e., compounds associated with the transport of fatty acids across the mitochondrial membrane for energy utilization.'' should be revised into ''which are compounds associated with the transport of fatty acids across the mitochondrial membrane for energy utilization. Moreover, it is essential for the β-oxidation of long-chain fatty acids and energy production in mammals[22]. In our study, we found that *Oxalobacter_formigenes* was negatively associated with dihomo-linolenoylcarnitine. We hypothesized that an increase in the abundance of *Oxalobacter_formigenes* decreased dihomo-linolenoylcarnitine, causing the dysfunction of fatty acids transport and oxidation, which may in turn promote carcinogenesis. However, the underlying mechanism requires further investigation.

1-Linoleoyl-GPE, classified as a lysophospholipid, was reported to be associated with better outcomes in mild traumatic brain injury, as it may improve lipid membrane repair and subsequently enhance functional outcomes[17]. Tamura et al. hypothesized that the depletion of lipid storage beyond supply may lead to a decrease in lipids serving as energy sources, which may predict a poor prognosis in renal cell carcinoma[35,36]. Therefore, we deduced that the enrichment of *Eubacterium_siraeum* may increase 1-linoleoyl-GPE, which may upregulate energy resources to reduce the risk of colorectal cancer and improve survival.

Previous studies were mainly focused on the observational phenomenon, which cannot be used as evidence to support a direct causal association between gut microbes and CRC due to confounding factors such as diet, age and environment[24]. The significant advantage of our research is the selection of genetic variants strongly associated with the gut microbes as IVs, which is neither influenced by other factors nor directly correlated to CRC. Additionally, our dataset was based on metagenomic sequencing and species-level microbes, which better revealed the interplay between gut microbes and CRC. We targeted *Burkholderiales* based on the results of the LDSC analysis, which was not biased by sample overlap and derived from a true polygenic signal[6,7]. Moreover, our study provided genetic evidence supporting a potential mechanism through which gut microbes may impact colorectal cancer by modulating metabolites, which could have important implications for future clinical applications. Nonetheless, our study has several limitations and perspectives for future research. First, the selection of instrumental variables based on a relaxed significance threshold ($p < 1 \times 10^{-5}$) may influence the accuracy of the results. However, the *F*-statistics of IVs were all greater than ten, suggesting a decreased likelihood of weak instrument bias[25]. Second, our analysis was restricted to individuals of European ancestry; therefore, the generalizability of the findings to other populations is limited. Third, due to the polygenic nature of the traits, it is challenging to completely rule out the presence of polymorphisms. The biological implications of the selected SNPs have not been comprehensively explored. Finally, and most importantly, the MR analysis is inherently a hypothesis-generating method. Thus, experimental and clinical studies are essential to investigate the causal relationship between the gut microbiome and colorectal cancer[20].

Conclusion

Our mediation analysis using MR indicated a causal pathway involving the gut microbiota, plasma metabolites, and colorectal cancer. Specifically, the regulatory effects of three taxa within the order *Burkholderiales* on CRC were mediated through a metabolic pathway involving dihomo-linolenoyl carnitine. Furthermore, we suggested that the protective effect of *Eubacterium siraeum* against CRC may be achieved through 1-linoleoyl-GPE. It is crucial to acknowledge that these findings are derived from a genetic instrumental variable analysis and remain subject to important limitations. Key among these limitations are the use of a less stringent genome-wide significance threshold for instrumental variable selection and the restriction of the analysis to individuals of European ancestry, which may affect the generalizability of the results. Future research should prioritize experimental studies to validate the underlying biological mechanisms, along with clinical investigations to explore the translational potential of modulating these microbiota-metabolite axes for CRC prevention or intervention.

Authors' contributions

Study conception: Y.G. Study design: X.Z., L.Z., Y.S. Data analysis: J.M., J.Z. Manuscript drafting: Y.G. and J.M. All of the coauthors have approved the submitted final version and agreed to the publication.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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Conflict of interest

The authors declare that they have no conflict of interest.

Data availability

We thank all the GWAS for making the summary data publicly available, and we are grateful for all the investigators and participants who contributed to those studies. We appreciate the BioRender (https://www.biorender.com) for the help of the figures production during the preparation of this manuscript.

No datasets were generated or analyzed during the current study.

Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version available at https://doi.org/10.1016/j.ram.2026.100719.

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