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Exploring the Causal Relationship between Gut Microbiome and Programmed Cell Death Protein-1/Programmed Cell Death Ligand-1: Mediating Effects of Serum Lipid and Amino Acid Metabolic Biomarkers in a Two-step Mendelian Randomization Study

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ABSTRACT

Programmed cell death protein-1/programmed cell death ligand-1 (PD-1/PD-L1) plays a pivotal role in tumor immune evasion. The efficacy of these treatments is limited by variable patient responses and adverse effects. It is necessary for a deeper understanding of the underlying biological mechanisms.

This study used a 2-step Mendelian randomization (MR) approach to investigate causal relationships among gut microbiota, lipid and amino acid metabolic traits, and PD-1/PD-L1. The summary statistics for 412 traits of the gut microbiome (N=7738), 249 traits of serum metabolites (N=115 078), and 2 traits of PD-1/PD-L1 (N=3301) were derived from publicly genome-wide association studies. The primary method employed for MR was inverse-variance weighted regression. We conducted a series of sensitivity analyses to evaluate the reliability of the causal estimates. Subsequently, mediation analysis was undertaken to elucidate the pathway from gut microbiome to PD-L1, mediated by serum metabolic markers.

Our analyses identified 28 gut microbial traits significantly affecting PD-L1 and 14 affecting PD-1, 8 of which remained consistently linked to PD-L1 after sensitivity analysis. Furthermore, 13 serum lipid and amino acid metabolic traits exhibited significant causal effects on PD-L1, with 6 remaining robust post analysis. Notably, *Bacteroides dorei* demonstrated a causal effect on PD-L1, mediated 9.6% by the metabolic biomarker phenylalanine.

These findings highlight the intricate interplay among gut microbiome, metabolic biomarkers, and immune regulation. They suggest novel therapeutic targets for cancer treatment that emphasize the value of microbiome and metabolic biomarkers in improving immunotherapy outcomes and promoting personalized medicine.

Keywords: Amino acid metabolism; Gastrointestinal microbiome; Lipid metabolism; Mendelian randomization analysis; Programmed death-ligand 1

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INTRODUCTION

The prevalence and mortality rates of cancer pose significant challenges to global health, leading to considerable socioeconomic burdens, including increased healthcare costs and loss of workforce productivity. Cancer accounts for 16.8% of deaths and 22.8% of deaths from noncommunicable diseases worldwide.[1] Immune checkpoint inhibitors (ICIs) have become a significant breakthrough in cancer research, offering a potent way to enhance anticancer effects against various cancers.[2] ICIs are monoclonal antibodies targeting programmed cell death protein 1 (PD-1) and its ligand PD-L1, blocking immune regulatory interactions.[3] This leads to enhanced T-cell activation and a robust antitumor immune response. However, despite the success of ICIs, only a subset of patients respond, and resistance limits the number achieving durable responses. Additionally, immune-related adverse events complicate treatment.[4,5] Notably, elevated PD-L1 expression has been correlated with the effectiveness of ICIs,[6] underscoring the need for innovative strategies to improve treatment outcomes and reduce adverse effects.

Emerging research has highlighted the pivotal role of gut microbiome in modulating immune responses and influencing ICIs efficacy by regulating PD-1/PD-L1 signaling pathways. Several studies have reported associations between gut microbiome composition and patient responses to ICIs, suggesting that specific microbial profiles may predict treatment outcomes.[7–9] These insights have emphasized the potential of gut microbiome as a therapeutic target and biomarker for enhancing immunotherapy strategies against cancer, particularly in relation to PD-1/PD-L1 interactions. Additionally, research has found that gut microbiome may regulate the efficacy of ICIs through amino acid and lipid metabolism.[10] However, the interaction among gut microbiome, serum lipids, and amino acid metabolic biomarkers in PD-1/PD-L1 signaling pathways has not been thoroughly investigated. Addressing these knowledge gaps is critical for developing novel therapeutic approaches. These approaches could enhance immunotherapy efficacy and reduce side effects.

To explore these relationships, our study takes a 2-sample Mendelian randomization (MR) approach. It leverages genetic variants as instrumental variables to determine the causal effects of gut microbiome on PD-1/PD-L1 signaling. This methodology reduces confounding commonly seen in observational studies, thereby enhancing the reliability of causal inferences. The primary objective of this research was to elucidate how gut microbiome may influence PD-1/PD-L1 signaling via pathways mediated by serum lipids and amino acid metabolic biomarkers. By identifying these mediating effects, we aim to provide foundational insights, which will inform future clinical interventions and personalized treatment strategies for patients with malignancies.

In summary, our study aimed to address current research gaps by systematically investigating the complex interactions among gut microbiome, PD-1/PD-L1, and related metabolic biomarkers. The outcomes are expected to significantly advance our understanding of immune modulation in cancer therapy. This knowledge will pave the way for innovative microbiome-targeted therapies that improve the efficacy of current immunotherapies.

MATERIALS AND METHODS

Study Design

This study used a 2-stage analytical design. In the first stage, we applied univariable Mendelian randomization (UVMR) to examine causal links between gut microbiome (exposures) and PD-1/PD-L1 expression (outcomes). In the second stage, we selected lipid and amino acid metabolic biomarkers as biologically plausible mediators of the gut microbiome to PD-1/PD-L1 axis for mediation analysis. We used single-nucleotide polymorphisms (SNPs) as instrumental variables for each exposure, mediator, and outcome. Finally, we applied a 2-step MR approach to test whether metabolic biomarkers mediate the causal effect of the gut microbiome on PD-1/PD-L1 expression.

This study was conducted in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology Using Mendelian Randomization (STROBE-MR) Statement.[11] The study also adhered to the prescribed checklist. The flowchart of the study is shown in Figure 1.

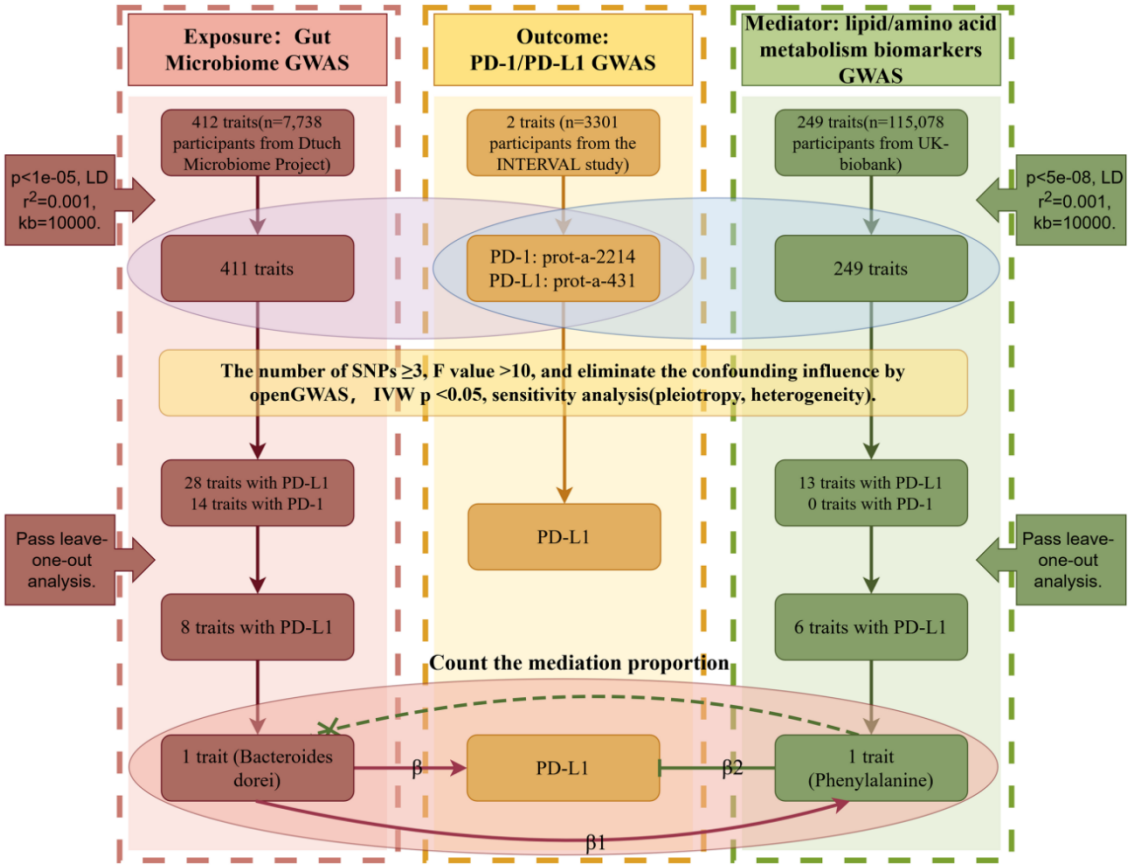

Figure 1. The flow chart of Mendelian randomization in this study. GWAS: genome-wide association studies; SNPs: single-nucleotide polymorphisms; LD: linkage disequilibrium; IVW: inverse-variance weighted; PD-1: programmed death protein 1; PD-L1: programmed death ligand 1.

Data Sources of Exposure

The Dutch Microbiome Project (DMP) conducted a large-scale genome-wide association study (GWAS) and produced a species-level dataset on the gut microbiome.[12] This analysis encompassed 7738 participants of European ancestry, which delivered the most comprehensive genomic characterization of gut microbiome to date. The researchers analyzed stool samples using shotgun metagenomic sequencing, successfully identifying 207 taxonomic classifications (including 105 species, 48 genera, 26 families, 13 orders, 10 classes, and 5 phyla) and 205 metabolic pathways connected to gut microbiome functions. The GWAS summary statistics in DMP represent inverse-rank normalized values, and the effect sizes (β) are expressed in units of standard deviation (SD). Data were obtained from the OpenGWAS database (https://gwas.mrcieu.ac.uk/, accessed on 10 May 2025, ebi-a-GCST90027446 to ebi-a-GCST90027857), with full GWAS dataset IDs detailed in Supplementary Table 1. SNPs with $p<1\times10^{-5}$ and clumped at a linkage disequilibrium threshold of $r^2<0.001$ (with a clumping distance of 10 000 kb) were used as instrumental variables for gut microbiome analyses. All F-statistic estimates exceeded 10, minimizing the risk of weak instrument bias. To reduce potential confounding, we screened instrumental variables with the OpenGWAS database (https://gwas.mrcieu.ac.uk/) for secondary phenotypes beyond the target exposures and outcomes. No SNPs exhibited associations with confounding phenotypes. Therefore, no exclusions were required in the subsequent analyses.

Data Sources of Mediators

In this study, we extracted genetic variations associated with 249 serum lipidomic and amino acid traits from the UK-Biobank dataset, which includes 115 078 individuals of European descent. The effect

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sizes (β) represent change in standardized, covariate-adjusted metabolite concentration (in SD units) per effect allele, based on inverse-rank normalized residuals of log-transformed values. We sourced the data from the OpenGWAS database (https://gwas.mrcieu.ac.uk/, accessed on 10 May 2025), with full GWAS dataset IDs detailed in Supplementary Table 2. We identified SNPs reaching genome-wide significance ($p<5\times10^{-8}$). Then, we performed clumping using an LD threshold of $r^2<0.001$ within 10 000-kb windows to ensure SNP independence. Crucially, we assessed bidirectionality using inverse-variance weighted MR. This verified that the causal effect flows solely from gut microbiome to metabolites, thereby eliminating reverse causation bias in mediation analysis.[13]

Data Sources of Outcomes

We identified genetic predictors of PD-1/PD-L1 protein abundance using summary statistics from the INTERVAL study.[14] GWAS dataset IDs detailed in Supplementary Table 3. This study recruited 3301 healthy participants of European ancestry, with a mean age of 44 years and 48.9% female. The GWAS summary statistics represent rank-based inverse normal transformed residuals of natural log-transformed relative protein abundances. Additionally, to satisfy the MR assumption requiring genetic variants to affect outcomes solely through exposure, we systematically verified that all SNPs showed no significant associations in the outcome ($p>0.05$ or at least more than that in the exposure).

Mendelian Randomization Analyses

We conducted a 2-sample UVMR to assess the overall impact of exposures and mediators on PD-1/PD-L1. All MR analyses adhere to 3 fundamental assumptions: (1) genetic variants must be strongly associated with the exposure; (2) genetic variants must not be associated with confounders that influence the exposure-outcome relationship; (3) genetic variants affect PD-1/PD-L1 only through the exposure. We used inverse-variance weighting (IVW) as the primary method for MR. Additionally, 5 complementary methods—MR Egger, simple mode, weighted median, unweighted mode regression, and the robust adjusted profile score—were used to strengthen the accuracy of our findings. We aimed to observe consistent trends in odds ratios (OR) across these 5 methods that matched those from IVW, or at minimum, similar trends in β values. Additionally, false discovery rate (FDR) correction was applied to account for multiple hypothesis testing. Colocalization analysis was performed to evaluate whether the genetic variants associated with the exposure, mediator, and outcome shared common causal loci, thereby reinforcing the robustness of the inferred causal pathways.

Sensitivity Analyses

Sensitivity analyses were conducted to evaluate the robustness of the results. We assessed heterogeneity among SNPs using Cochran Q test. Fixed-effects IVW models were applied when no substantial heterogeneity was evident ($p\geq0.05$), while random-effects IVW models were used when significant heterogeneity existed ($p<0.05$). Concurrently, we evaluated horizontal pleiotropy using MR Egger regression, where a statistically significant intercept term ($p<0.05$) indicated potential bias from multieffect variants. An intercept approaching zero with $p>0.05$ suggested negligible pleiotropic bias. Furthermore, to comprehensively assess horizontal pleiotropy, we performed the MR-PRESSO global test based on 1000 simulations. A significance threshold of $p<0.05$ was applied to indicate the presence of detectable horizontal pleiotropy.

Count the Mediation Proportion

To quantify mediation effects in the gut microbiome → metabolic biomarker → PD-1/PD-L1 pathway, we employed a 2-step MR approach. This was necessary because the absence of overlapping SNPs between gut microbiome and metabolic biomarkers prevented multivariable MR analysis. First, UVMR estimated the causal effect of gut microbiome composition on metabolic biomarkers (β_1). Second, independent UVMR analyses were used to quantify the effect of metabolic biomarkers on PD-1/PD-L1 expression (β_2). The total causal effect of gut microbiome on PD-1/PD-L1 (β) was estimated using the primary UVMR analysis. Mediation effects were calculated as $\beta_1\times\beta_2$, with the mediation proportion calculated as $(\beta_1\times\beta_2) / \beta$.

Statistical Analysis

All statistical analyses were performed using R software (v4.4.3) with the `TwoSampleMR` package for MR modeling and mediation calculations. Visualizations including forest plots, funnel plots, scatter plots, and leave-one-out sensitivity plots were generated using `ggplot2` within the R environment.

RESULTS

Causal Effects of Gut Microbiome on PD-1/PD-L1

We conducted MR analyses on 412 gut microbial traits, and after SNP quality control, retained 411 traits for causal assessment on PD-1/PD-L1. Subsequent analyses identified 28 traits that exhibited significant causal effects on PD-L1 and 14 traits on PD-1, with all associations robust in sensitivity analyses. Given the lack of findings in mediation analyses involving PD-1–associated traits, we therefore focused exclusively on PD-L1–related microbiome in subsequent analyses. Among the 28 traits causally linked to PD-L1, leave-one-out sensitivity analyses identified influential outlier SNPs in 20 traits. After removing these pleiotropic variants, the causal associations for these 20 traits became statistically nonsignificant. Finally, this refinement identified 8 robust gut microbial traits exhibiting consistent causal relationships with PD-L1 expression, and only 1 (*Bacteroides dorei*, GWAS ID: ebi-a-GCST90027824) of which was subsequently incorporated into the formal mediation analysis (Table S4, S7, Figures S1A–C and 2A). We further investigated the causal relationship between *B dorei* and PD-L1. Following FDR correction for multiple testing, the association between *B dorei* and PD-L1 was attenuated and did not retain formal statistical significance (p_{FDR}=0.066). However, the effect estimate remained consistent in direction with the initial finding. We assessed potential horizontal pleiotropy for *B dorei* using the MR-PRESSO global test. The results indicated no evidence of significant pleiotropy (p=0.761), suggesting that the causal estimate from the main IVW analysis is robust. Additionally, colocalization analysis revealed weak evidence for shared causal variants between *B dorei* and PD-L1 loci (PP.H4=0.069), suggesting distinct genetic mechanisms underlying this association.

Causal Effect of Lipid/Amino Acid Metabolic Biomarkers on PD-1/PD-L1

MR analyses were performed on 249 serum lipid and amino acid metabolic biomarker traits. After applying standard SNP quality control, we retained all 249 traits for subsequent analysis. We did not identify any significant causal associations between these traits and PD-1 expression. In contrast, 13 metabolic traits showed significant causal effects on PD-L1 expression. All these traits passed sensitivity analyses for heterogeneity and horizontal pleiotropy. Leave-one-out sensitivity analyses detected influential outlier SNPs in 7 of the 13 traits. After removing these pleiotropic variants, the causal associations for those 7 traits became statistically nonsignificant. Therefore, 6 metabolic biomarker traits remained strongly associated with PD-L1 expression (Table S5 and Figure S2A–C), with only 1 (phenylalanine, GWAS ID: met-d-Phe) subsequently incorporated into the formal mediation analysis (Table S8 and Figure 2C). Following FDR correction for multiple testing, the association between phenylalanine and PD-L1 keep robust (p_{FDR}=0.019). Furthermore, we assessed potential horizontal pleiotropy for phenylalanine using the MR-PRESSO global test (p=0.395). Colocalization analysis between phenylalanine and PD-L1 yielded a PP.H4 value of 0.143, indicating insufficient evidence for shared genetic causal variants.

Causal Links between Gut Microbiome and Lipid/Amino Acid Metabolic Biomarkers

We selected 8 gut microbiome traits as exposures and 6 lipid and amino acid metabolic markers as outcomes, and performed MR analysis, which identified only *B dorei* (ebi-a-GCST90027824) retained a robust causal effect on phenylalanine (met-d-Phe), as shown in Table S6 and Figure 2B. Moreover, the causal relationship between the 2 variables remained stable after FDR correction (IVW, β=0.035; OR, 1.036; 95% CI, 1.007–1.065; p=0.013; p_{FDR}=0.038), and passed sensitivity analysis and horizontal multiple effect testing (Table S6). To eliminate potential reverse causation in mediation analysis, we conducted bidirectional MR (Table S6 and Figure 2D). By reversing the roles of exposure and outcome for these validated biomarkers (phenylalanine as exposure and *B dorei* as outcome), no causal effect was detected (IVW, β=0.018; OR, 1.019; 95% CI, 0.812–1.277; p=0.874). This confirms unidirectional causality exclusively from gut microbiome to the metabolic mediator, and meets the mediation criteria required for subsequent analysis.

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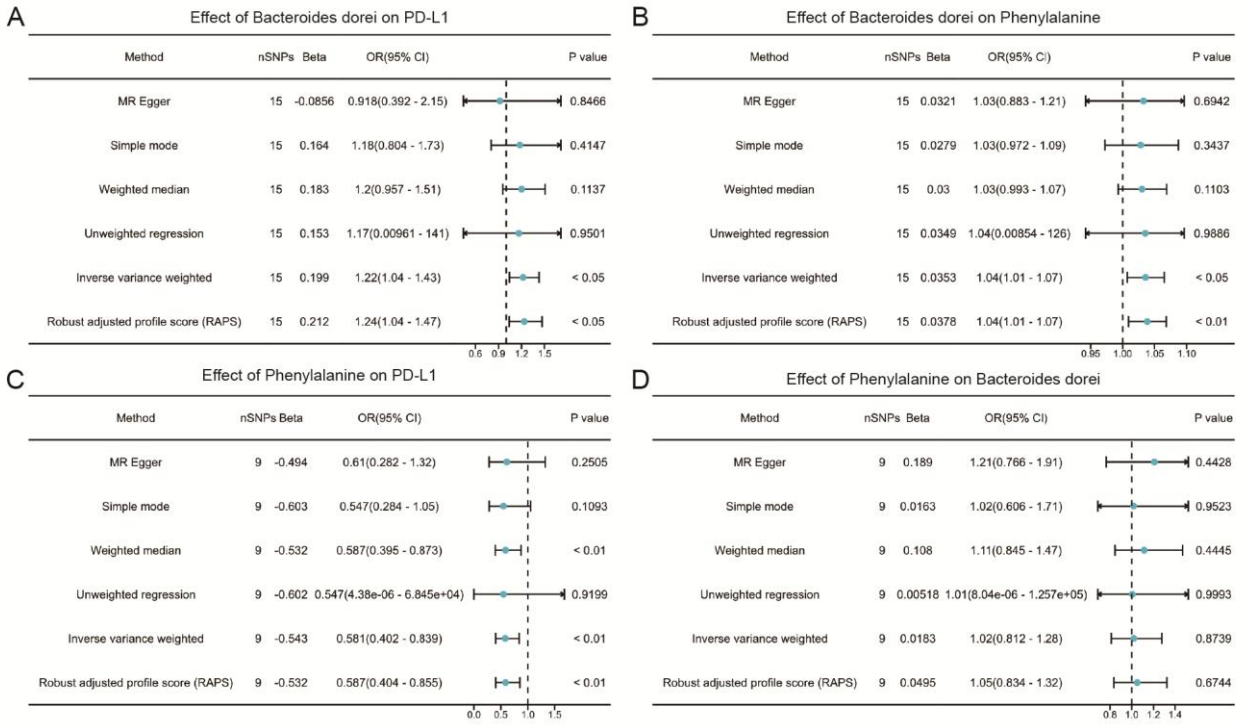

Figure 2. Forest plots of results from Mendelian randomization (MR) analysis in this study. A. Forest plot showing the causal effects of *Bacteroides dorei* on PD-L1 estimated by 6 MR methods; B. Forest plot showing the causal effects of *B dorei* on Phenylalanine estimated by 6 MR methods; C. Forest plot showing the causal effects of Phenylalanine on PD-L1 estimated by 6 MR methods; D. Forest plot showing the causal effects of Phenylalanine on *B dorei* estimated by 6 MR methods. OR: odds ratio.

Mediating Role of Metabolic Biomarkers in Microbiome–PD-L1 Pathway

This study performed a 2-step MR analysis because no overlapping SNPs were found between these instruments. UVMR analyses identified that *B dorei* increases PD-L1 protein expression (IVW, β=0.199; OR, 1.221 [95% CI, 1.043–1.429]; *p*=0.013; Figure 3A), whereas phenylalanine decreases the risk (IVW, β=−0.543; OR, 0.581 [95% CI, 0.402–0.839]; *p*=0.004; Figure 3C). We further performed reverse MR analyses to assess potential reverse causality. The results demonstrated no significant causal effects of PD-L1 expression levels on the abundance of *B dorei* (IVW, β=−0.040; OR, 0.961 [95% CI, 0.867–1.065]; *p*=0.447) or on phenylalanine levels (IVW, β=−0.002; OR, 0.998 [95% CI, 0.984–1.013]; *p*=0.821), supporting the unidirectional causal directions identified in our primary analyses (Table S6). Both associations were robust in sensitivity analyses (Table S6, Figure 3D–I). *B dorei* causally increased phenylalanine levels (IVW, β=0.035; OR, 1.036 [95% CI, 1.007–1.065]; *p*=0.013; Figure 3B) without evidence of reverse causation (IVW, β=0.018; OR, 1.019 [95% CI, 0.812–1.277]; *p*=0.874; Table S6). Mediation analysis indicated that phenylalanine accounts for 9.6% (95% CI, 1.38% to 46.54%) of *B dorei*'s effect on PD-L1 expression, indicating partial mediation where phenylalanine attenuates the overall causal pathway.

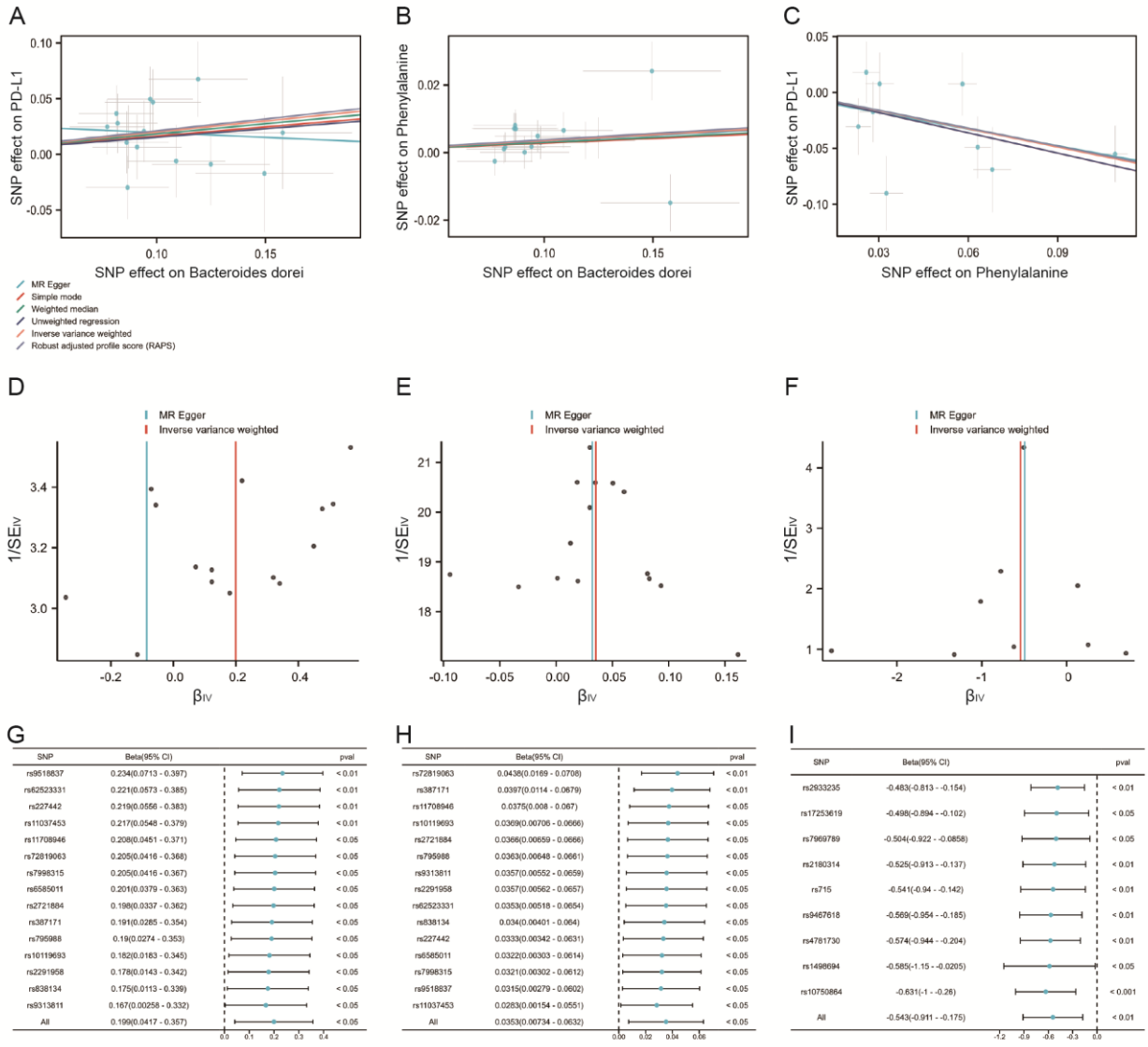

Figure3. Mendelian randomization sensitivity analysis. A. Scatter plot, D. Funnel plot, and G. Leave-one-out plot for Bacteroides dorei on PD-L1; B. Scatter plot, E. Funnel plot, and H. Leave-one-out plot for *Bacteroides dorei* on Phenylalanine; C. Scatter plot, F. Funnel plot, and I. Leave-one-out plot for Phenylalanine on PD-L1.

DISCUSSION

This study employed a 2-stage MR approach to investigate the causal relationships between gut microbiome and PD-1/PD-L1 expression. It particularly focused on the mediating roles of lipid and amino acid metabolic biomarkers. By using genetic instrumental variables, this methodology allowed for a robust inference of causality while minimizing confounding factors, thereby enhancing the reliability of our findings. The primary objective was to elucidate how specific gut microbial traits and metabolic pathways interact to influence immune regulation, especially in cancer progression and treatment. The integration of microbiome analysis and metabolic profiling highlights the intricate connections between these biological systems and paves the way for future research to develop targeted therapeutic interventions that improve patient outcomes in cancer immunotherapy.

Our study provides evidence that gut microbiome

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traits causally influence PD-1/PD-L1 expression, offering a novel perspective on immune regulation in cancer. The identification of 8 gut microbial traits with significant causal effects on PD-L1 underscores the potential of these microorganisms as modulators of immune checkpoint pathways. The strong association between certain gut microbiome and PD-L1 expression suggests these microbes may modulate immune responses through metabolic pathways, potentially influencing immunotherapy efficacy. For example, *B dorei* influences PD-L1 expression, highlighting the gut microbiome's role in enhancing immune checkpoint activity. These results are consistent with previous studies highlighting the role of gut microbiome in shaping immune responses, which in turn influence cancer progression and treatment outcomes.[15-17]

Moreover, our analysis of lipid and amino acid metabolic biomarkers revealed that 6 traits significantly influenced PD-L1 expression after sensitivity analyses. This finding highlights the intricate relationship between metabolism and immune regulation, suggesting that metabolic pathways mediate the influence of gut microbiome on immune regulation. The identification of specific lipid and amino acid metabolic traits that influence PD-L1 expression may offer new biomarkers for predicting immunotherapy efficacy. And it implies metabolic interventions could serve as novel therapeutic strategies in cancer treatment that could enhance the effectiveness of existing immunotherapies.[18,19] This suggests that tailoring therapeutic strategies to an individual's metabolic and microbiome profile could improve treatment outcomes and reduce adverse effects from immune checkpoint blockade therapies.[20]

The identified causal pathway linking *B dorei*, phenylalanine, and PD-L1 underscores the pivotal function of the gut microbiome in modulating both metabolic homeostasis and immune checkpoint-related treatment efficacy. *B dorei* is a Gram-negative, anaerobic, non-spore-forming rod-shaped bacterium isolated from healthy human feces and formally identified and named in 2006.[21] *B dorei* exhibits remarkable immunomodulatory properties. It can reduce key proinflammatory factors (IL-1β, IL-6, TNF-α), decrease microbial products with proinflammatory and potential tumor-promoting effects, such as lipopolysaccharides, and produce secondary bile acids like ursodeoxycholic acid, which possess anti-inflammatory and cell-protective effects.[22–24] Therefore, maintaining an appropriate abundance of *B dorei* in the gut may help create an anti-inflammatory intestinal microenvironment, thereby indirectly reducing the risk of certain inflammation-driven cancers. A clinical study[25] showed that patients with phenylketonuria have a significantly reduced relative abundance of *Bacteroides* in the gut. These bacteria may indirectly affect phenylalanine metabolism by degrading complex carbohydrates, thus influencing the availability of metabolic substrates. Additionally, research[26] suggests that modulating the relative abundance of *Bacteroides* can change phenylalanine metabolic pathways, which may help alleviate symptoms of rheumatoid arthritis. Research[27] showed metal ion-chelated L-phenylalanine nanostructures can reshape the tumor immune-suppressive microenvironment by activating dendritic cells via the NF-κB pathway, potentially affecting PD-L1 expression. Clinical studies reveal small cell lung cancer patients responding well to immune checkpoint blockade therapies (progression-free survival >6 months) have reduced serum phenylalanine levels.[28] In patients with non-small-cell lung cancer receiving combination immunotherapy, phenylalanine is a significant prognostic marker. The proportion of patients with decreased phenylalanine levels after 2 treatment cycles compared to baseline correlates with improved progression-free survival (p<0.0001) and overall survival (p<0.005).[29] Consequently, integrating *B dorei* abundance and phenylalanine levels as biomarkers could enhance patient stratification for immunotherapy, inform therapeutic decisions, and guide microbiome-based combination strategies. Taken together, the modulation of the gut microbiome could be a viable strategy to improve metabolic profiles and consequently enhance immunotherapy responses. The mediating role of phenylalanine in the microbiome/PD-L1 pathway further illustrates the complexity of these interactions and the potential for developing targeted interventions leveraging metabolic pathways to enhance immune responses in cancer therapy. Identifying the connections between gut microbiome, metabolic biomarkers, and PD-L1 underscores the need for future research on the underlying mechanisms. Additionally, dietary interventions aimed at modifying gut microbiome composition may serve as adjunctive therapies to enhance the efficacy of existing cancer treatments.[30,31] This integrative approach could facilitate the development of innovative strategies that harness the gut microbiome to improve clinical outcomes in cancer patients.[32,33]

There are several limitations in the careful interpretation of our findings. Firstly, although we found links between gut microbiome and PD-L1 expression, the complex pathways may involve other mediators beyond lipid and amino acid biomarkers, such as inflammatory cytokines or epigenetic regulators. Therefore, future multiomics mediation analyses should incorporate these factors. Secondly, only using European GWAS datasets restricts the generalizability of our conclusions, as gut microbiome and PD-L1 pathways may vary by ethnicity due to diet and environment, requiring validation in diverse cohorts. Moreover, our research data were mainly obtained from the healthy population, while there may be differences in the microbiome and the metabolic biomarkers of cancer patients. However, several studies have suggested that the dysregulation of the gut microbiota and metabolites can contribute to cancer development, and this hypothesis has also been supported in animal and clinical experiments.[34–37] Finally, this exploratory study provides initial evidence showing modest effect estimates for the microbiome and limited colocalization with PD-L1. However, it establishes a valuable framework for future research. Future cohort studies employing nonlinear analyses are necessary to confirm potential threshold effects in the gut microbiome–PD-L1 axis and to elucidate its intricate immune dynamics.

In conclusion, our findings highlight the intricate interactions among gut microbiome composition, metabolic biomarkers, and PD-L1 expression. These insights could lead to reshaping therapeutic strategies in cancer treatment. The robust causal relationships we identified not only highlight the potential of gut microbiome as a modulator of immune checkpoint regulation but also suggest that metabolic intermediates serve as critical mediators in this pathway. These results advocate shifting to integrate microbiome profiling and metabolic assessments into personalized medicine, thereby enhancing immunotherapy efficacy. Ultimately, this work lays the foundation for future studies to harness gut microbiome and metabolic pathways to improve cancer treatment outcomes.

STATEMENT OF ETHICS

Not applicable, as the data used in this manuscript are from previously published studies that have undergone ethical review by relevant institutions and obtained informed consent from patients.

FUNDING

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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DATA AVAILABILITY

The datasets used in this research are accessible through an online database. The names of these collections and their accession numbers can be found in the main article or the Supplementary Material.

AI ASSISTANCE DISCLOSURE

This study did not use AI assistance.

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