

RESEARCH

Open Access



Univariable and multivariable Mendelian randomization study identified the key role of gut microbiota in immunotherapeutic toxicity

Baike Liu^{1,2}, Zheran Liu³, Tianxiang Jiang^{1,2}, Xiangshuai Gu⁴, Xiaonan Yin^{1,2}, Zhaolun Cai^{1,2}, Xiaoqiao Zou^{1,2}, Lei Dai^{4*} and Bo Zhang^{1,2*}

Abstract

Background In cancer patients receiving immune checkpoint inhibitors (ICIs), there is emerging evidence suggesting a correlation between gut microbiota and immune-related adverse events (irAEs). However, the exact roles of gut microbiota and the causal associations are yet to be clarified.

Methods To investigate this, we first conducted a univariable bi-directional two-sample Mendelian randomization (MR) analysis. Instrumental variables (IVs) for gut microbiota were retrieved from the MiBioGen consortium (18,340 participants). GWAS summary data for irAEs were gathered from an ICIs-treated cohort with 1,751 cancer patients. Various MR analysis methods, including inverse variance weighted (IVW), MR PRESSO, maximum likelihood (ML), weighted median, weighted mode, and cML-MA-BIC, were used. Furthermore, multivariable MR (MVMR) analysis was performed to account for possible influencing instrumental variables.

Results Our analysis identified fourteen gut bacterial taxa that were causally associated with irAEs. Notably, *Lachnospiraceae* was strongly associated with an increased risk of both high-grade and all-grade irAEs, even after accounting for the effect of BMI in the MVMR analysis. *Akkermansia*, *Verrucomicrobiaceae*, and *Anaerostipes* were found to exert protective roles in high-grade irAEs. However, *Ruminiclostridium6*, *Coprococcus3*, *Collinsella*, and *Eubacterium (fissicatena group)* were associated with a higher risk of developing high-grade irAEs. *RuminococcaceaeUCG004*, and *DefluviitaleaceaeUCG011* were protective against all-grade irAEs, whereas *Porphyromonadaceae*, *Roseburia*, *Eubacterium (brachy group)*, and *Peptococcus* were associated with an increased risk of all-grade irAEs.

Conclusions Our analysis highlights a strong causal association between *Lachnospiraceae* and irAEs, along with some other gut microbial taxa. These findings provide potential modifiable targets for managing irAEs and warrant further investigation.

Keywords Gut microbiota, Immunotherapy toxicity, Immune-related adverse effects, irAEs, *Lachnospiraceae*, *Akkermansia*, *Ruminiclostridium6*, Mendelian randomization study, Multivariable Mendelian randomization study, MVMR

*Correspondence:

Lei Dai
daileiskb2012@163.com
Bo Zhang
zhangbo_scu@scu.edu.cn

Full list of author information is available at the end of the article



© The Author(s) 2024. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

Introduction

Applications of immune checkpoint inhibitors (ICIs), especially those targeting CTLA-4 (cytotoxic T-lymphocyte associated protein 4) and PD-1/PD-L1 (programmed cell death protein 1/programmed cell death ligand 1), have revolutionized the treatment of various aggressive cancers [1]. By blocking inhibitory signaling pathways and reinvigorating the natural anti-tumor immune response, these inhibitors have significantly prolonged the lives of numerous cancer patients [2–5]. However, due to the inhibition of the systemic brake of immune activation, ICIs can cause off-target effects resulting in immune-mediated toxicities to organs and non-malignant tissues. This newly recognized registry of iatrogenic effects, known as immune-related adverse events (irAEs), usually resemble autoimmune disorders, such as colitis, dermatitis, and thyroiditis [6]. Although the majority of irAEs manifest in a mild manner, still, up to 55% of patients develop serious irAEs in combined therapy (anti-CTLA-4 and anti-PD-1) [7]. Notably, serious irAEs pose significant risks to patients' well-being and may result in morbidity and mortality, not only due to the adverse event itself but also due to the need to suspend or terminate ICIs therapy and the potential impairment of the ICIs-induced immune response while using immunosuppressants (e.g., corticosteroids) [8–10]. Therefore, effective management of irAEs is critical to optimize the safety and efficacy of ICIs therapy.

The precise mechanisms underlying irAEs are not fully understood, but emerging evidence indicates that the gut microbiota, a complex and dynamic system of microorganisms colonizing the intestinal tract, may play a crucial role in the regulation of irAEs. Simpson et al. found a reduced alpha-diversity of intestinal microbiota in patients who developed severe irAEs [11]. Furthermore, antibiotics commonly prescribed prophylactically to hospitalized patients have been shown to increase the risk of ICIs therapy-related irAEs that are not limited to the gastrointestinal tract [12–14]. The gut microbiota closely interacts with the host immune system and has been implicated in the regulation of various autoimmune and inflammatory disorders [15, 16]. However, consensus on the core microbial drivers or protective microbes of irAEs is still lacking, due to inconsistent findings reported in previous studies [11, 17–19]. The discrepancies among previous studies may be attributed to limited sample sizes and susceptibility to confounding factors such as age, diet, and medication usage in observational designs [11, 20].

Mendelian randomization (MR), initially described by Katan in 1986 [21], is a novel method for inferring causal associations between modifiable risk factors and health outcomes using genetic variations as instrumental

variables (IVs) [22]. MR effectively addresses the limitations of confounding and measurement errors that often exist in observational studies, as the direction of causation is from the genetic polymorphism to the trait of interest, not vice versa [23]. Therefore, we aim to utilize MR, an increasingly popular method in drug discovery and epidemiology [24, 25], to investigate the potential association between the gut microbiota and irAEs, providing further evidence for the management of irAEs by targeting human gut microbiota.

Methods

Study design and data source

An overview of the study design was illustrated in Fig. 1. In general, we first performed univariable bi-directional two-sample MR analysis, which utilizes single-nucleotide polymorphisms (SNPs) from summary-level data as proxies for the risk factor under investigation. Then, several sensitivity analyses and multivariable MR analysis (MVMR) was conducted to further increase the robustness of our study. To ensure the validity of the MR results, three assumptions needed to be satisfied, as illustrated in Additional file 1: Fig. S1 [22]:

- (1) Relevance assumption: The genetic variants should demonstrate a strong association with the exposure;
- (2) Independence assumption: The genetic variants should not be associated with any confounders that could affect the relationship between the exposure and outcome;
- (3) Exclusion restriction assumption: The genetic variants should not have an independent effect on the outcome aside from their impact through the exposure.

This study is based on publicly available GWAS summary statistics and ethical approvals were acquired by the original studies.

Gut microbiota

Genetic variations associated with the composition of gut microbiota were derived from the most comprehensive genome-wide meta-analysis conducted to date by the MiBioGen consortium [26]. This study included a total of 18,340 individuals from 24 cohorts worldwide, mainly of European descent ($n=13,266$). Fecal DNA was extracted, and targeted sequencing of variable regions in the bacterial 16S rRNA gene (V4, V3–V4, and V1–V2 regions) was performed to profile the gut bacterial composition. To account for sequencing depth differences across cohorts, all cohorts were rarefied to 10,000 reads per sample. Taxonomic classification was performed using

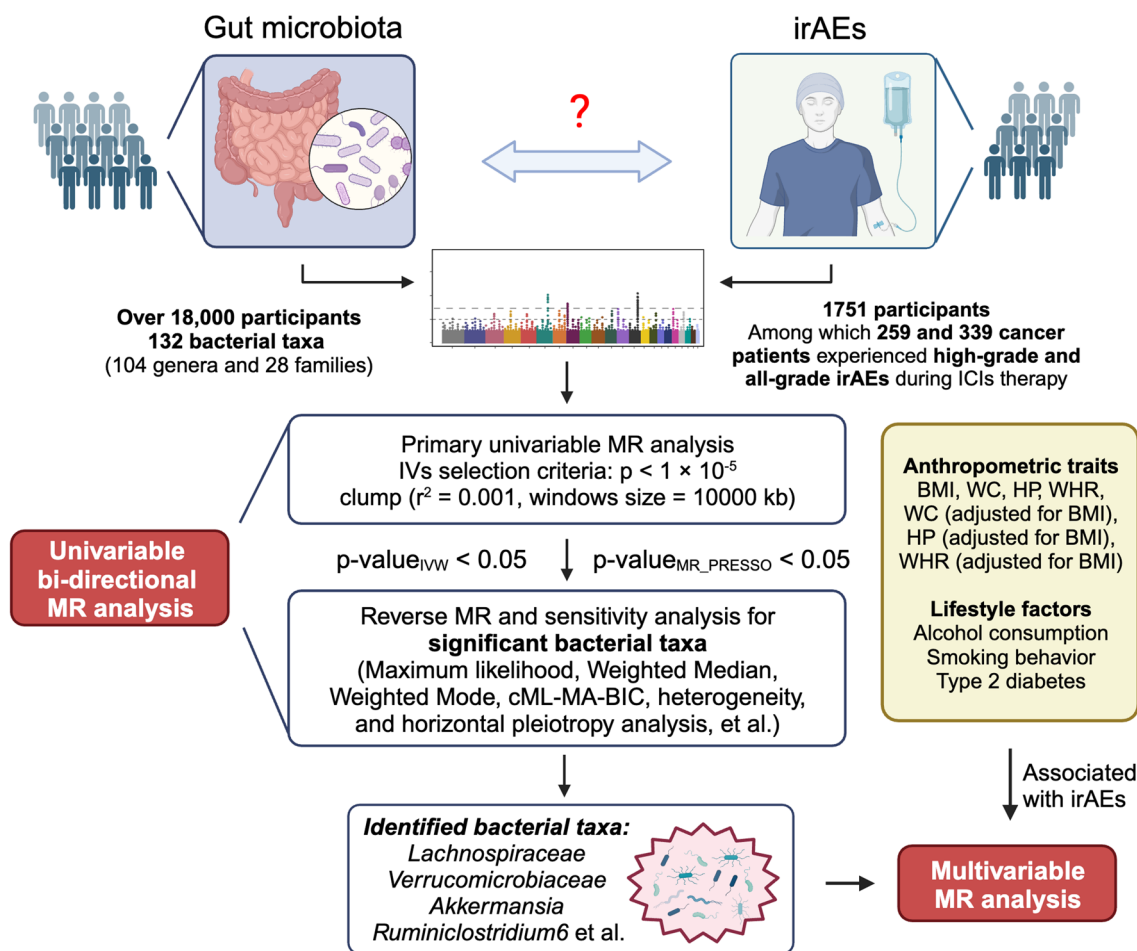


Fig. 1 Overview of the study design. Initially, IVs were selected from the summary GWAS data of the gut microbiota and irAEs. Subsequently, by employing thresholds of p value (IVW) < 0.05 and p value (MR PRESSO) < 0.05 , the identified gut microbiota that exhibited statistically significant associations were taken into further analysis. irAEs immune-related adverse events, ICIs immune checkpoint inhibitors, BMI body mass index, WC Waist circumference, HP Hip circumference; WHR waist-to-hip ratio. (Created with BioRender.com)

direct taxonomic binning method [26]. Following quality control, imputation, and post-imputation filtering procedures, gut bacterial taxa observed in over 10% of samples were included in the microbiota quantitative trait loci (mbQTL) mapping [26]. This allowed us to identify host genetic variants associated with the relative abundance of bacterial taxa. Further details on microbial data processing can be found in the original study. Genus-level and family-level taxa were included in our analysis, resulting in a total of 131 genus-level and 35 family-level taxa.

irAEs

Summary-level data of irAEs was obtained from a recent GWAS conducted in the Dana-Farber Cancer Institute (DFCI) cohort [27]. The study included 1,751 cancer patients of European ancestry who underwent ICIs treatments between 2013 and 2020. The majority of patients

(approximately 90%) received PD-1/PD-L1 inhibitors, while the remaining 10% received combined immunotherapy (CTLA4 and PD-1/PD-L1 inhibitors). Among the 1,751 cancer patients, 259 cases that experienced high-grade irAEs (grade 3–5 events) were manually curated according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events v.5 guidelines. In addition, algorithm-based autoimmune-like electronic health records were used to identify 339 patients who experienced any grade irAEs (referred to as all-grade irAEs). Most of these cases were grade 2 or higher events [27]. The tumor tissue of these patients was sequenced using the targeted OncoPanel sequencing platform. After quality control steps, germline SNPs were imputed by utilizing ultra-low-coverage off-target reads. Then, the GWAS was conducted in the DFCI cohort to investigate the association of all variants with the time

from the start of ICIs treatment to the occurrence of the two phenotypes of irAEs. For more detailed information, please refer to the original publication [27].

Selection of instrumental variables

Several steps were followed in the selection of IVs. First, for the gut microbiome, we selected SNPs associated with bacterial taxa with a *p* value less than 1×10^{-5} for further analysis [28, 29]. Second, potential SNPs were clumped for independence in the TwoSampleMR package in R software. We used the European 1000 Genomes Project

Phase 3 reference panel and set the linkage-disequilibrium threshold (r^2) at 0.001 within a 10 Mb window size. Third, we extracted SNPs from the outcome statistics and performed a harmonization procedure. SNPs that were not available in the outcome GWAS data were replaced with proxy SNPs ($r^2 > 0.8$), and palindromic SNPs were removed for further MR analysis. Furthermore, *F* statistics of selected IVs, which indicate instrument strength, was calculated as $[\text{Beta}/\text{SE}]^2$. Typically, *F* statistics > 10 suggest enough IVs strength to avoid weak instrument bias [30]. Finally, All SNPs with positive results were

High-grade irAEs

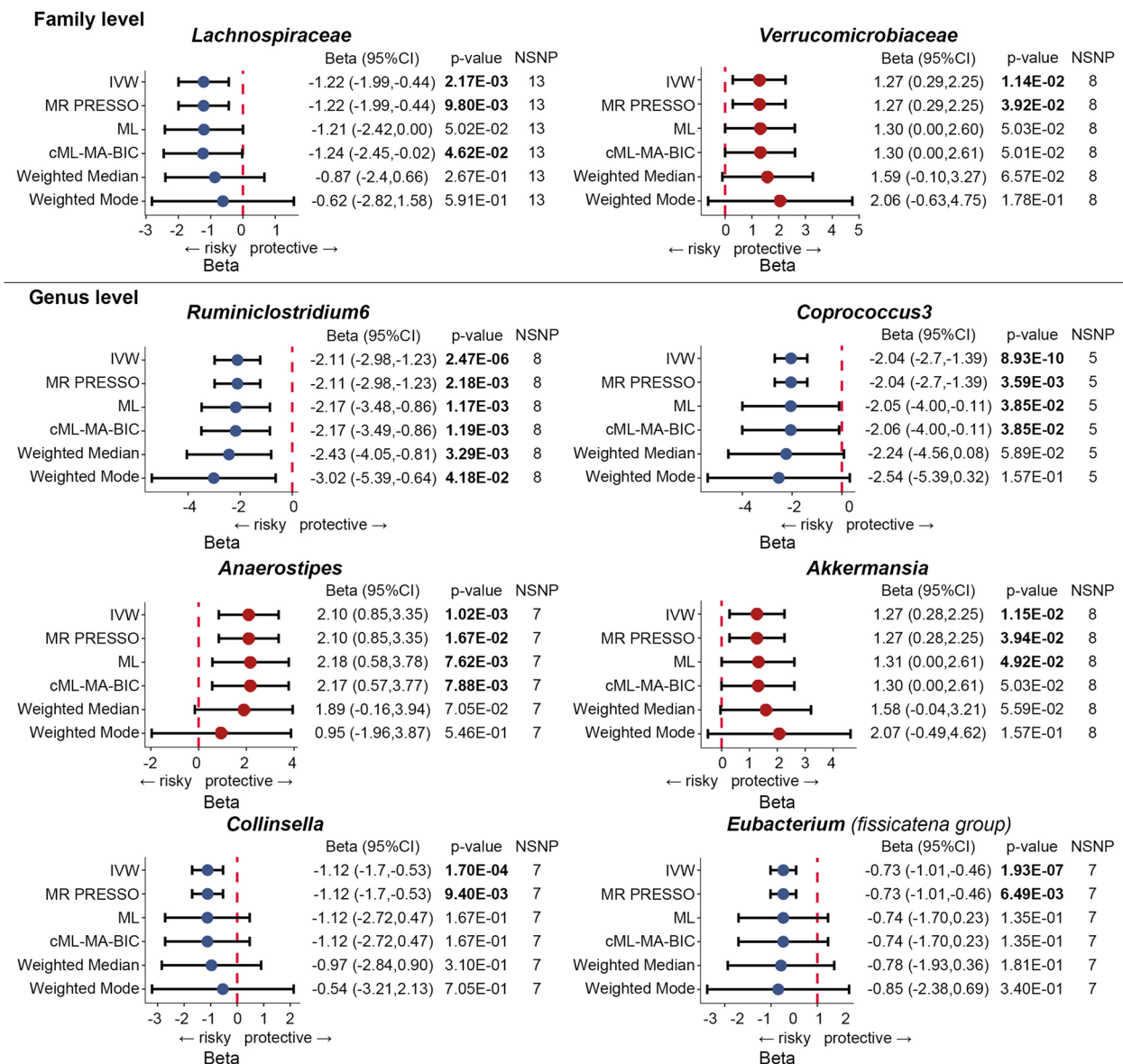


Fig. 2 Forest plots of MR results for the causal association between the identified eight gut microbial taxa and high-grade irAEs (grade 3–5 events). NSNP number of SNPs; Beta = effect size from the exposure to the outcome; CI = confidence interval

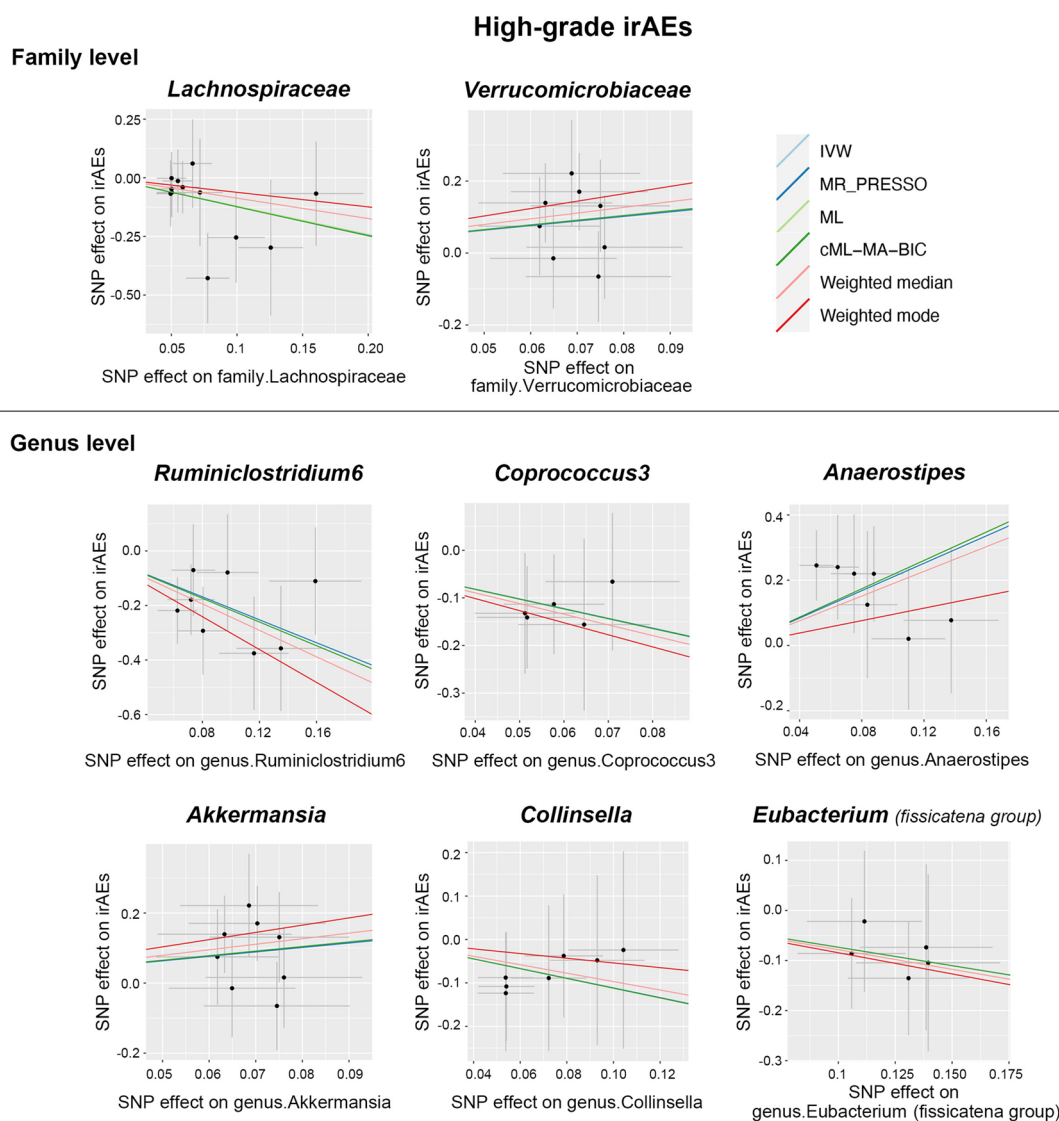


Fig. 3 Scatter plots of MR analysis between the gut microbial taxa and high-grade irAEs (grade 3–5 events)

re-examined using PhenoScanner package (version 1.0) in R software to investigate the presence of potential confounders. Bacterial taxa with less than 3 valid SNPs and unknown origin were excluded from the analysis to mitigate potential bias. Consequently, we included a total of 104 genus-level and 28 family-level bacterial taxa ($n = 132$) for further MR analysis.

Statistical analysis

In this study, we employed several MR analysis methods to explore the potential causal relationship between gut microbiota and irAEs. The methods used included IVW, MR PRESSO, ML, weighted median, weighted mode, and a constrained maximum likelihood and model

averaging-based method (cML-MA-BIC). IVW and MR PRESSO were used in the primary analysis. In general, IVW provides maximum statistical power when all instruments are valid [31], while MR PRESSO identifies and removes genetic variants that deviate significantly from the variant-specific causal estimates of other variants, thereby increasing statistical power and addressing potential outliers [32]. The ML method resembles the IVW approach which assumes the absence of both heterogeneity and horizontal pleiotropy. If these assumptions hold true, the ML method yields unbiased results with smaller standard errors compared to the IVW approach [33]. Considering the potential existence of IV pleiotropy, we also performed pleiotropy-robust methods including

weighted median, weighted mode, and cML–MA–BIC in the sensitivity analysis. These methods relax the instrumental variable assumptions. Weighted median were introduced when the exclusion restriction assumption was violated (uncorrelated pleiotropy), which typically assume fewer than 50% of genetic variants are invalid [34]. The weighted-mode method clusters genetic variants based on their similarity in causal effect and estimates the overall causal effect based on the cluster with the most number of IVs [35]. The cML–MA–BIC method is a novel approach developed for MR analysis, specifically addressing the issue of invalid IVs exhibiting both uncorrelated and correlated pleiotropy (violation of the independence assumption) [36]. By being robust to such violations, cML–MA–BIC improves the accuracy of MR analysis, reduces Type I error, and increases statistical power [36].

Next, heterogeneity and directional pleiotropy were assessed using Cochra's Q statistics and MR Egger intercept. Leave-one-out (LOO) analysis was conducted to identify possible reliance on a specific variant, which involved excluding one SNP at a time for all valid SNPs in the IVW analysis. In addition, reverse MR analysis between irAEs and the identified significant gut bacterial taxa was performed. Moreover, MVMR analysis, which could help us better understand the intricate interplay between the risk factors was further conducted [37]. We considered a Bonferroni-corrected p value of 3.89×10^{-4} (0.05/132) as the significance threshold for gut microbiota. Two-tailed p value < 0.05 was considered suggestive of significance. All analyses were conducted using R packages "TwoSampleMR" (version 0.5.6), "MRPRESSO" (version 1.0), and "MRcML" (version 0.0.0.9) in R software (version 4.2.2).

Results

Genetic instruments and primary MR analysis

A total of 870 single-nucleotide polymorphisms (SNPs) were selected as IVs for the 132 gut bacterial taxa (Additional file 2: Table S1). The F statistics for each SNP ranged from 16.91 to 36.57, with a median value of 21.66. Using the IVW and MR-PRESSO methods, eight gut bacterial taxa associated with high-grade irAEs were identified with p values < 0.05 . These taxa include *Lachnospiraceae*, *Verrucomicrobiaceae*, *Ruminiclostridium6*, *Coprococcus3*, *Anaerostipes*, *Akkermansia*, *Collinsella*, and *Eubacterium (fissicatena group)*. For all-grade irAEs, seven gut bacterial taxa, including *Lachnospiraceae*, *Porphyromonadaceae*, *Roseburia*, *RuminococcaceaeUCG004*, *DeFluviitaleaceaeUCG011*, *Eubacterium (brachy group)*, and *Peptococcus*, were identified. Given previous studies suggest that pre-existing autoimmune conditions, such as inflammatory

bowel disease, psoriasis, and rheumatoid arthritis, may predispose individuals to irAEs susceptibility [38, 39]. We further examined the SNPs associated with the significant bacterial taxa using PhenoScanner. Only one SNP (rs11597285) for the *Collinsella* genus was found to be associated with allergic disease (e.g., allergic rhinitis and eczema) (refer to Additional file 2: Table S9). However, the results of *Collinsella* remained uninfluenced after removing rs11597285 in the LOO analysis (described below). The complete results of the primary MR analysis can be found in Additional file 2: Tables S2 and S3.

Main MR results and sensitivity analysis for high-grade irAEs

As shown in Fig. 2, the IVW estimate suggested the abundance of *Lachnospiraceae* family was associated with a shortened time to high-grade irAEs (Beta = -1.22, 95% CI -1.99 to -0.44, $p = 2.17 \times 10^{-3}$), indicating that *Lachnospiraceae* serves as a risk factor for the development of high-grade irAEs. The deleterious effect remained significant in pleiotropy-robust cML–MA–BIC estimation (Beta = -1.24, 95% CI -2.45 to -0.02, $p = 4.62 \times 10^{-2}$). Surprisingly, *Ruminiclostridium6* genus was significantly associated with an increased risk of high-grade irAEs in all MR approaches, including IVW (Beta = -2.11, 95% CI -2.98 to -1.23, $p = 2.47 \times 10^{-6}$), cML–MA–BIC (Beta = -2.17, 95% CI -3.49 to -0.86, $p = 1.19 \times 10^{-3}$), Weighted median (Beta = -2.43, 95% CI -4.05 to -0.81, $p = 3.29 \times 10^{-3}$), and other methods. In addition, the IVW estimate indicated a protective effect of the *Akkermansia* genus on high-grade irAEs (Beta = 1.27, 95% CI 0.28–2.25, $p = 0.01$), and this finding was confirmed by the IVW estimate of its paternal taxon *Verrucomicrobiaceae* (Beta = 1.27, 95% CI 0.29–2.25, $p = 0.01$). The IVW estimate of *Anaerostipes* genus also indicated a suggestive protective effect against high-grade irAEs (Beta = 2.1, 95% CI 0.85–3.35, $p = 1.02 \times 10^{-3}$), as well as cML–MA–BIC (Beta = 2.17, 95% CI 0.57–3.77, $p = 7.88 \times 10^{-3}$). Moreover, significant effects of the *Coprococcus3* (Beta = -2.04, 95% CI -2.7 to -1.39, $p = 8.93 \times 10^{-10}$), *Collinsella* (Beta = -1.12, 95% CI -1.7 to -0.53, $p = 1.7 \times 10^{-4}$), and *Eubacterium (fissicatena group)* genus (Beta = -0.73, 95% CI -1.01 to -0.46, $p = 1.93 \times 10^{-7}$) were all revealed by IVW estimate, indicating an increased risk of high-grade irAEs. Scatter plots reflecting the effect size of IVs on both bacterial taxa and high-grade irAEs are shown in Fig. 3.

In the subsequent analysis of heterogeneity and horizontal pleiotropy, Cochra's Q statistics revealed no significant heterogeneity (p value > 0.05) among the IVs for the gut bacterial taxa in high-grade irAEs analysis (see Table 1). No significant evidence for directional

Table 1 Results of heterogeneity and horizontal pleiotropy analysis

Bacterial taxa (exposure)	irAEs (outcome)	Heterogeneity test			Horizontal pleiotropy results		
		Cochran's Q	df	p value	Egger intercept	SE	p value
<i>Lachnospiraceae</i>	High-grade irAEs	5.18	12	0.95	0.04	0.11	0.73
<i>Verrucomicrobiaceae</i>	High-grade irAEs	4.18	7	0.76	0.36	0.61	0.57
<i>Eubacterium (fissicatena group)</i>	High-grade irAEs	0.33	4	0.99	0.07	0.53	0.9
<i>Akkermansia</i>	High-grade irAEs	4.18	7	0.76	0.37	0.61	0.56
<i>Anaerostipes</i>	High-grade irAEs	4.13	6	0.66	0.38	0.2	0.11
<i>Collinsella</i>	High-grade irAEs	0.82	6	0.99	-0.19	0.23	0.43
<i>Coprococcus3</i>	High-grade irAEs	0.47	4	0.98	-0.28	0.46	0.59
<i>Ruminiclostridium6</i>	High-grade irAEs	3.54	7	0.83	-0.17	0.18	0.38
<i>Lachnospiraceae</i>	All-grade irAEs	17.3	12	0.14	0.09	0.11	0.43
<i>Porphyromonadaceae</i>	All-grade irAEs	0.66	4	0.96	0.05	0.28	0.87
<i>Eubacterium (brachy group)</i>	All-grade irAEs	1.41	5	0.92	-0.11	0.29	0.73
<i>DefluviitaleaceaeUCG011</i>	All-grade irAEs	0.48	4	0.98	-0.03	0.25	0.92
<i>Peptococcus</i>	All-grade irAEs	1.87	5	0.87	-0.12	0.24	0.66
<i>Roseburia</i>	All-grade irAEs	3.75	7	0.81	-0.31	0.22	0.21
<i>RuminococcaceaeUCG004</i>	All-grade irAEs	2.73	7	0.91	-0.23	0.3	0.47

df, degree of freedom; SE, standard error; High-grade irAEs, grade 3–5 irAEs

horizontal pleiotropy was found in the MR–Egger regression intercept analysis and MR PRESSO global test (Table 1 and Additional file 2: Table S4). In addition, the LOO analysis identified no predominant SNP that influenced the results (Fig. 4). We further performed reverse MR analysis and demonstrated no reverse causation exists between high-grade irAEs and the abundance of gut bacterial taxa (Additional file 2: Table S6).

Main MR results and sensitivity analysis for all-grade irAEs

Figure 5 reveals the association between bacterial taxa and all-grade irAEs. It is noteworthy that the deleterious impact of the *Lachnospiraceae* family was also detected in all-grade irAEs, as revealed by IVW (Beta = -2.05, 95% CI -3.27 to -0.82, $p = 1.06 \times 10^{-3}$), cML–MA–BIC (Beta = -2.21, 95% CI -3.32 to -1.1, $p = 9.16 \times 10^{-5}$), and Weighted median estimates (Beta = -1.72, 95% CI -3.2 to -0.24, $p = 2.25 \times 10^{-2}$). The IVW estimate of *Roseburia* genus also showed an increased risk of all-grade irAEs (Beta = -2.07, 95% CI -3.21 to -0.93, $p = 3.76 \times 10^{-4}$). Consistent results were observed in ML (Beta = -2.07, 95% CI -3.67 to -0.47, $p = 1.14 \times 10^{-2}$) and cML–MA–BIC (Beta = -2.08, 95% CI -3.71 to -0.45, $p = 1.23 \times 10^{-2}$) estimates. On the contrary, *RuminococcaceaeUCG004* (Beta = 1.07, 95% CI 0.43–1.72, $p = 1.05 \times 10^{-3}$) and *DefluviitaleaceaeUCG011* (Beta = 0.82, 95% CI 0.38–1.25, $p = 2.15 \times 10^{-4}$) were identified to decrease the risk of all-grade irAEs according to the IVW approach. Subsequently, the results for *RuminococcaceaeUCG004* were consistent with the cML–MA–BIC (Beta = 1.1, 95% CI

0.04–2.15, $p = 4.12 \times 10^{-2}$) and ML method (Beta = 1.09, 95% CI 0.04–2.14, $p = 4.16 \times 10^{-2}$). Moreover, the IVW estimates suggested that *Porphyromonadaceae* (Beta = -1.11, 95% CI -1.83 to -0.39, $p = 2.46 \times 10^{-3}$), *Eubacterium (brachy group)* (Beta = -0.71, 95% CI -1.14 to -0.27, $p = 1.38 \times 10^{-3}$), and *Peptococcus* (Beta = -0.71, 95% CI -1.24 to -0.18, $p = 8.82 \times 10^{-3}$) may increase the risk of all-grade irAEs. Scatter plots reflecting the effect size of each IV on both bacterial taxa and all-grade irAEs are shown in Fig. 6.

Similarly, Cochran's Q statistics indicated an absence of notable heterogeneity in the IVs of gut bacterial taxa (refer to Table 1). In addition, the results from the MR–Egger regression intercept analysis and the MR PRESSO global test demonstrated no significant evidence of directional horizontal pleiotropy (Table 1 and Additional file 2: Table S4). Next, no significant single SNP was identified in the LOO analysis that influenced the results (Fig. 7). Overall, these results provide evidence for the association between specific gut bacterial taxa and the development of high-grade and all-grade irAEs, and highlight the potential role of the gut microbiota in irAEs.

Multivariable MR analysis

Given the strong correlation between body mass index (BMI) and gut microbiota [40, 41], we incorporated BMI and weight-associated anthropometric traits, as well as several lifestyle risk factors associated with irAEs [42], into the univariable MR analysis. Detailed GWAS data sets information of these anthropometric traits and

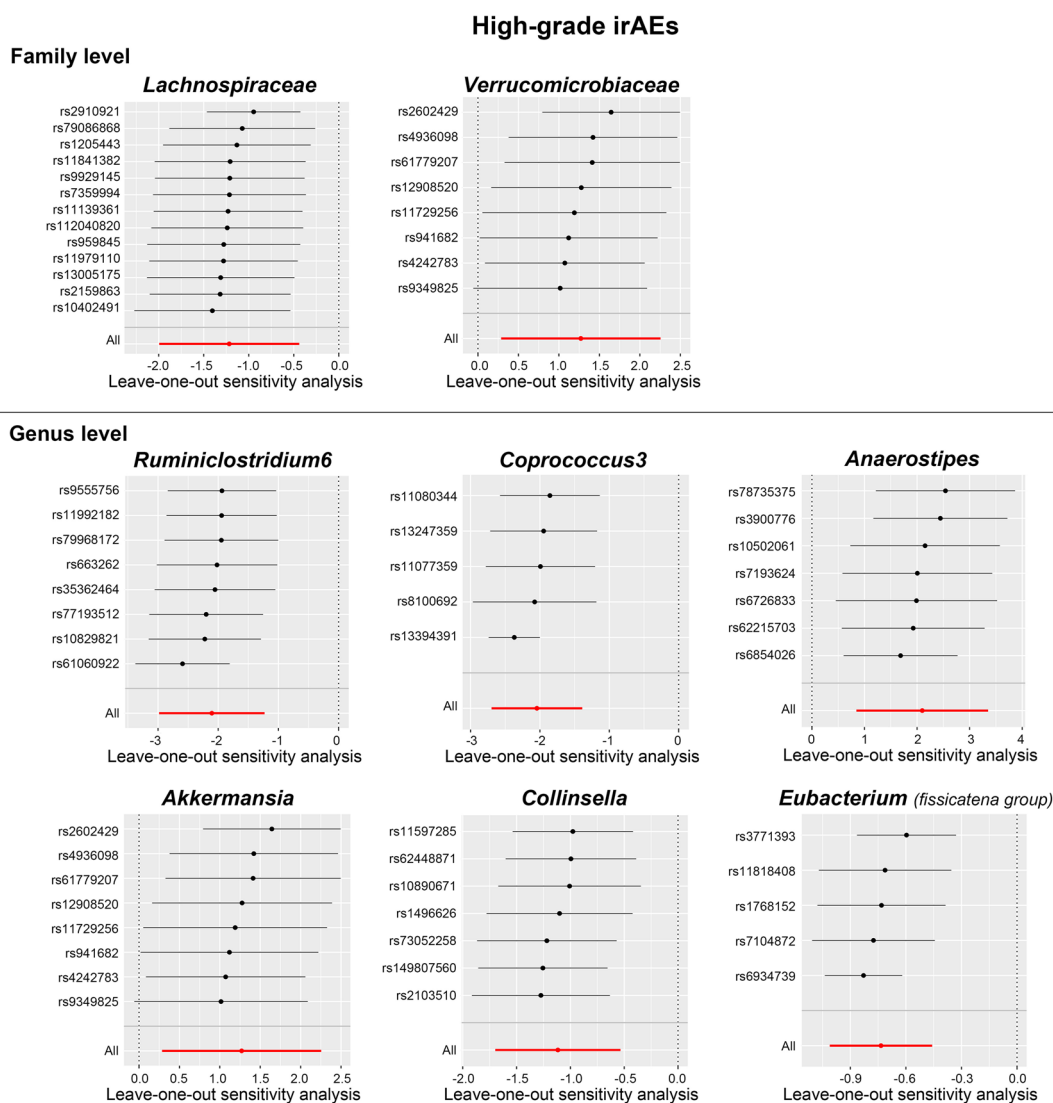


Fig. 4 Leave-one-out plots of MR analysis between the gut microbial taxa and high-grade irAEs (grade 3–5 events)

lifestyle risk factors can be found in Additional file 2: Table S10. The univariable MR analysis suggested a correlation between BMI and an increased risk of all-grade irAEs (Table 2). Thus, we performed subsequent MVMR analysis by including BMI as an additional exposure alongside previously identified gut microbial taxa (Additional file 2: Table S11). Notably, after accounting for the effect of BMI in the MVMR analysis, *Lachnospiraceae* still remained significantly associated with the risk of developing all-grade irAEs (Beta = -1.07, 95% CI -1.96 to -0.18, $p = 0.02$), and high-grade irAEs (Beta = -0.91, 95% CI -1.84 to 0.01, $p = 0.05$) with a marginal p value (Table 3).

Discussion

In this study, univariable bi-directional MR and MVMR analyses were conducted to infer the causal association between gut microbiota and irAEs. We identified fourteen gut bacterial taxa that were causally associated with high-grade and all-grade irAEs. Surprisingly, *Lachnospiraceae* strongly associated with an increased risk of both irAEs phenotypes, even after accounting for the effect of BMI in the MVMR analysis. In addition, we found robust evidence indicating that *Ruminiclostridium6* predisposes ICI recipients to developing high-grade irAEs. *Coprococcus3*, *Collinsella*, and *Eubacterium (fissicatena group)* were also associated with an increased risk of high-grade irAEs, while *Akkermansia*, *Verrucomicrobiaceae*, and *Anaerostipes* exhibited protective roles

All-grade irAEs

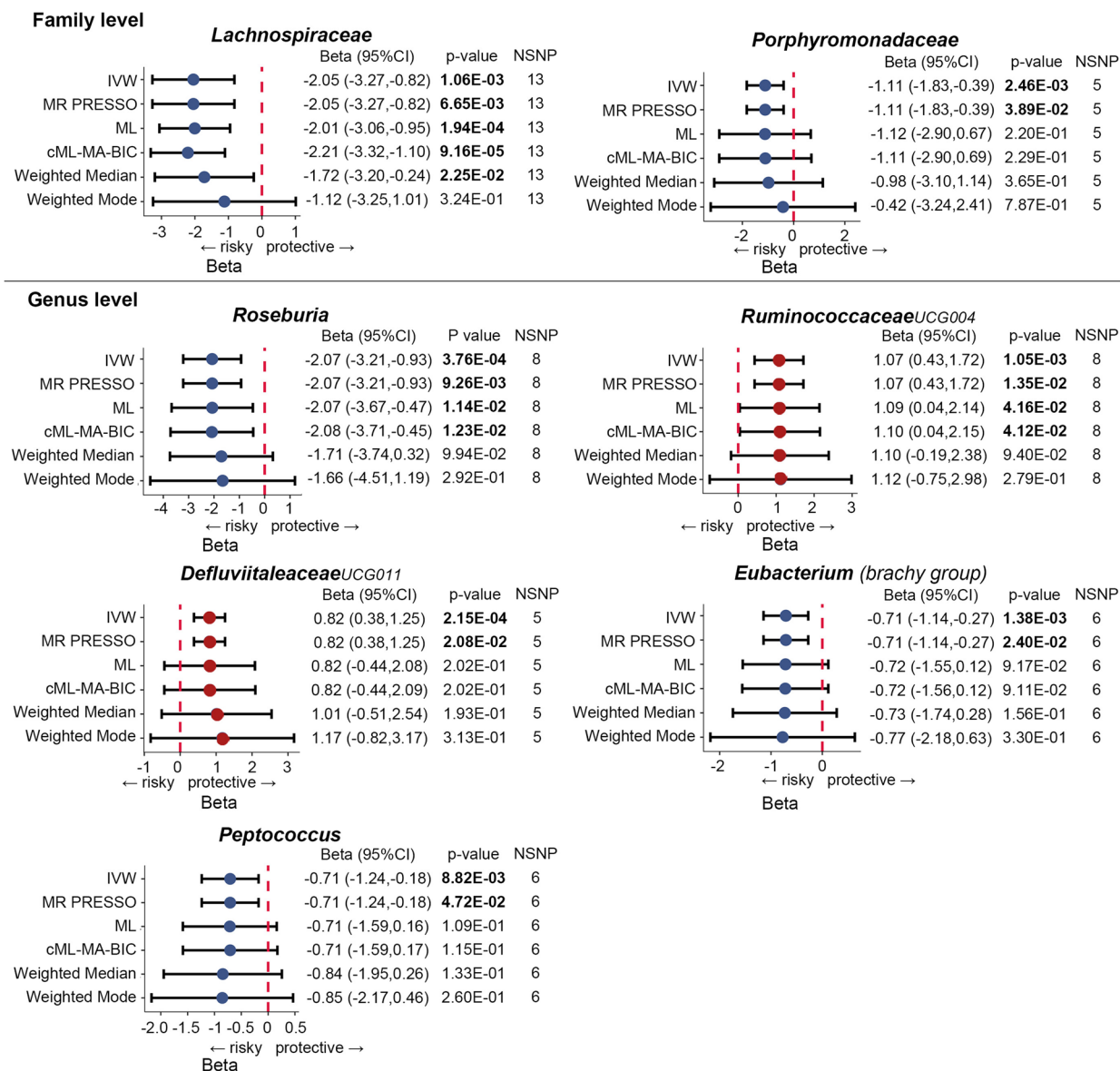


Fig. 5 Forest plots of MR results for the causal association between the identified seven gut microbial taxa and all-grade irAEs (grade 1–5 events). NSNP number of SNPs; Beta = effect size from the exposure to the outcome; CI = confidence interval

in high-grade irAEs. For all-grade irAEs, *Porphyromonadaceae*, *Roseburia*, *Eubacterium (brachy group)*, and *Peptococcus* were associated with an elevated risk, while *Ruminococcaceae*UCG004 and *Defluviitaleaceae*UCG011 were associated with a reduced risk.

Several observational studies have demonstrated associations between gut microbiota and irAEs [11, 17–19, 43, 44]. *Lachnospiraceae* species (such as *Coprococcus* and *Roseburia*), which are obligately anaerobic, variably spore-forming bacteria, were found to

correlate with increased risk of various types of irAEs [17, 18, 45]. In a more recent study, two species of the *Lachnospiraceae* family were specifically enriched in irAEs that occur in endocrine organs [46]. Consistent with these findings, we also identified the *Lachnospiraceae* family, and its two genera (i.e., *Coprococcus*3 and *Roseburia*) associated with an increased risk of irAEs. Importantly, the harmful influence of the *Lachnospiraceae* family was observed in high-grade irAEs and all-grade irAEs, even after adjusting the effect of

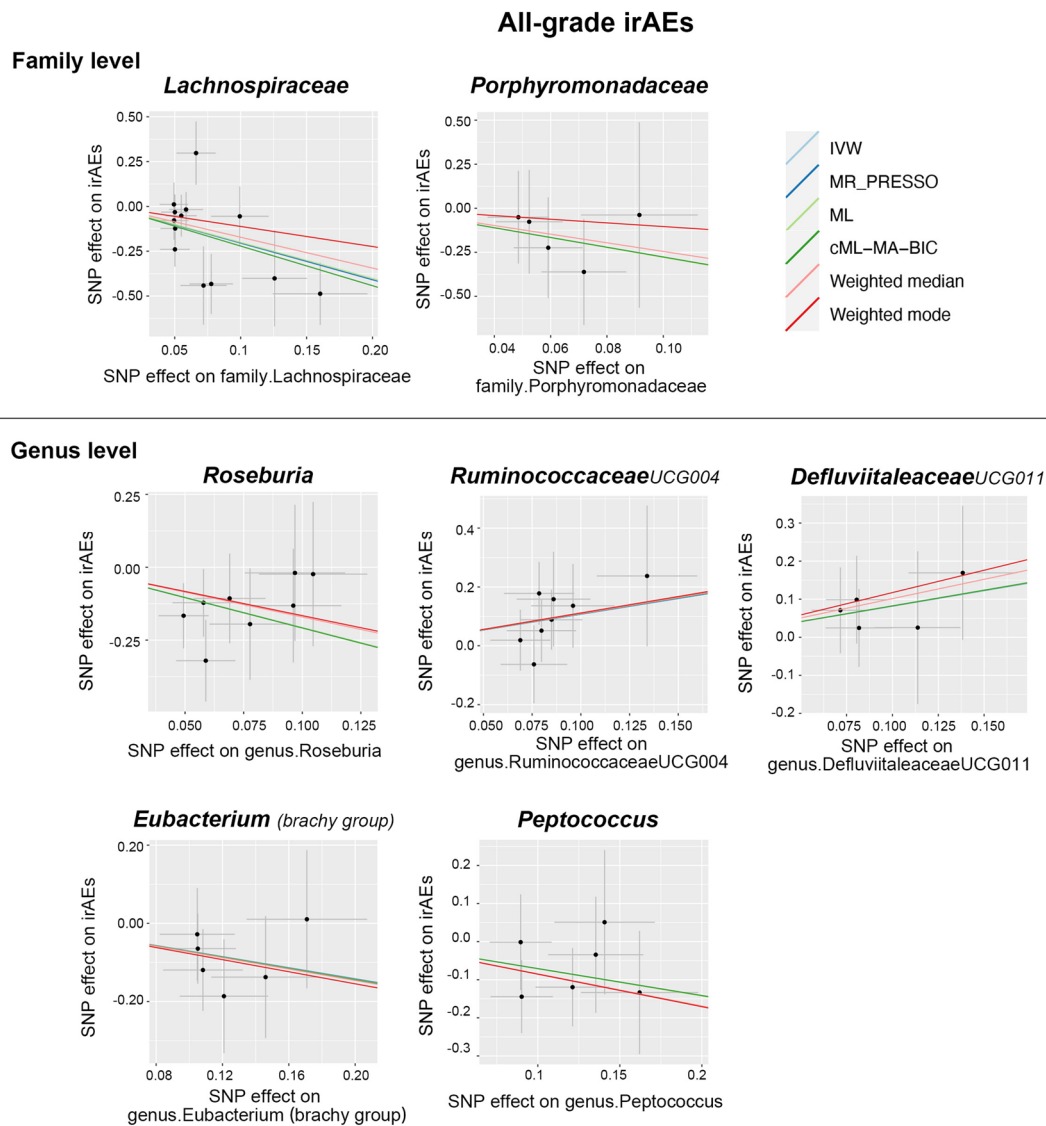


Fig. 6 Scatter plots of MR analysis between the gut microbial taxa and all-grade irAEs (grade 1–5 events)

BMI, which provides further validity and robustness to our study. *Ruminiclostridium6* has been less studied in irAEs, but some studies have observed its accumulation in a mouse model of DSS-induced colitis. While treated with phloretin (a dihydrochalcone flavonoid) or sodium butyrate (one of the short chain fatty acids [SCFAs]), both of which alleviates DSS-induced colitis, the abundance of *Ruminiclostridium6* was reduced [47, 48]. In addition, the *Ruminiclostridium* genus has also been associated with autoimmune-related diseases, such as the experimental multiple sclerosis model and Alzheimer’s disease [49, 50]. Based on our strong association of *Ruminiclostridium6* with increased risk of high-grade irAEs, it is suggested that *Ruminiclostridium6*

may play a pivotal role in the development of autoimmune conditions and could be a potential target for relieving irAEs symptoms, although more evidence is needed.

Akkermansia muciniphila, an anaerobic gram-negative species that belongs to *Akkermansia* genus, and *Verrucomicrobiaceae* family, has gained much attention in immunotherapy due to their association with a favorable response in ICIs therapy [51–53]. *Akkermansia muciniphila* has also been shown to exhibit a protective role in ICIs-associated colitis [54]. Mechanically, Wang et al. demonstrated that *Akkermansia muciniphila* and its purified membrane protein mitigated colitis by regulating macrophages and CD8+T cells in the colon

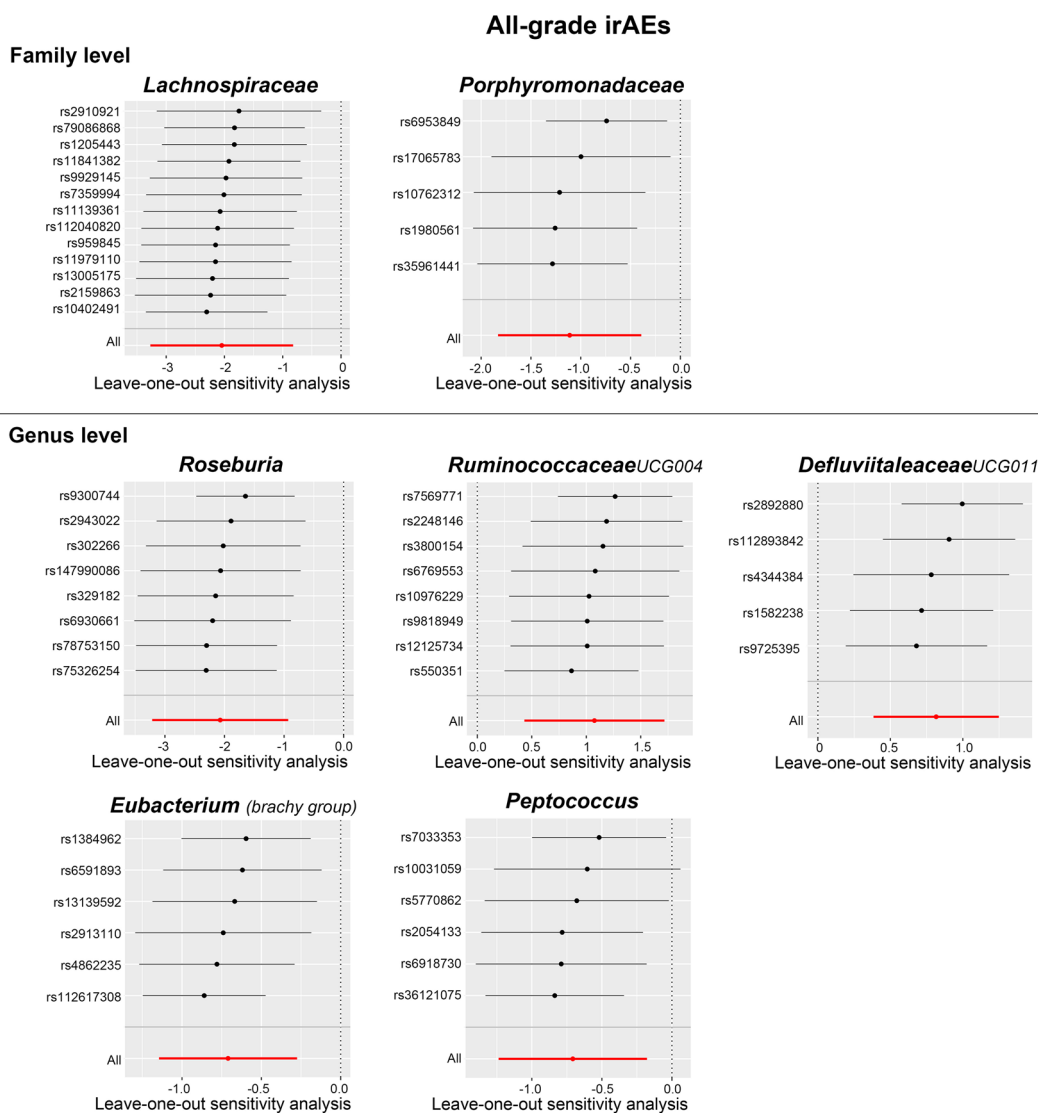


Fig. 7 Leave-one-out plots of MR analysis between the gut microbial taxa and all-grade irAEs (grade 1–5 events)

tissue [55]. Our study further supported the protective role of *Akkermansia* in high-grade irAEs. *Ruminococcaceae*, a key family of bacteria producing short-chain fatty acids (SCFAs), has been observed to be enriched in ICIs responders without severe irAEs [11]. Previous studies have found that *Faecalibacterium prausnitzii*, a species belonging to the *Ruminococcaceae* family, was decreased in non-responders and those experiencing severe irAEs [11, 17, 45]. These findings suggest a potential role of *Ruminococcaceae* as protective bacteria, possibly through the facilitation of SCFAs accumulation, in the mitigation of irAEs. *Collinsella* and *Anaerostipes* have limited evidence in irAEs, but the *Collinsella* genus has been reported to increase the production of IL-17A and enhance rheumatoid arthritis severity [56]. In contrast,

Anaerostipes, which belongs to the *Lachnospiraceae* family, was conversely associated with the risk of high-grade irAEs in our study [11, 18]. These discrepancies observed in previous clinical studies might be attributed to several factors, including limited sample sizes in previous observational studies, heterogeneity among the samples, and inadequate exploration of the taxonomic classification at the genus level of the gut microbiota. Therefore, a more detailed taxonomy for gut microbiota is crucial in dissecting the underlying mechanisms.

Gut microbiota plays a pivotal role in modulating human immune homeostasis, and an imbalance in gut microbial composition, known as gut dysbiosis, has been implicated in several autoimmune diseases [15, 16, 57]. IrAEs resemble autoimmune diseases in many aspects

Table 2 Univariate MR estimates between common risk factors and irAEs

Exposures	Method	NSNP	Outcomes			
			High-grade irAEs		All-grade irAEs	
			Beta (95%CI)	p value	Beta (95%CI)	p value
Anthropometric factors						
BMI	IVW	314	-0.60 (-1.34, 0.14)	0.11	-0.74 (-1.41, -0.06)	0.03
Waist circumference	IVW	26	0.61 (-1.01, 2.23)	0.46	0.05 (-1.15, 1.24)	0.94
Waist circumference (adjusted for BMI)	IVW	46	0.00 (-1.37, 1.38)	1.00	-0.39 (-1.5, 0.73)	0.50
Hip circumference	IVW	38	0.09 (-0.92, 1.10)	0.86	-0.63 (-1.7, 0.44)	0.25
Hip circumference (adjusted for BMI)	IVW	52	0.65 (-0.38, 1.69)	0.21	0.47 (-0.52, 1.45)	0.35
Waist-to-hip ratio	IVW	20	-0.28 (-2.21, 1.64)	0.77	-1.69 (-3.56, 0.18)	0.08
Waist-to-hip ratio (adjusted for BMI)	IVW	25	0.84 (-0.88, 2.55)	0.34	-0.54 (-1.94, 0.85)	0.44
Lifestyle factors						
Alcoholic drinks per week	IVW	14	-0.44 (-4.21, 3.33)	0.82	-0.06 (-3.24, 3.12)	0.97
Cigarettes smoked per day	IVW	13	0.11 (-0.93, 1.16)	0.83	0.67 (-0.29, 1.63)	0.17
Smoking initiation	IVW	54	0.08 (-1.21, 1.37)	0.90	-0.10 (-1.23, 1.04)	0.87
Type 2 diabetes	IVW	79	-0.16 (-0.48, 0.17)	0.34	0.01 (-0.33, 0.34)	0.97

BMI body mass index, IVW Inverse variance weighted method, NSNP number of SNPs, irAEs immune-related adverse events, 95% CI 95% confidential interval, High-grade irAEs, grade 3–5 irAEs

Table 3 Multivariable MR estimates between *Lachnospiraceae*, BMI and irAEs

Exposures in MVMR	Outcomes			
	High-grade irAEs		All-grade irAEs	
	Beta (95% CI)	p value	Beta (95% CI)	p value
<i>Lachnospiraceae</i>	-0.91 (-1.84, 0.01)	0.05	-1.07 (-1.96, -0.18)	0.02
BMI	-0.43 (-1.2, 0.34)	0.28	0.01 (-0.33, 0.34)	0.34

MVMR multivariable MR analysis, BMI body mass index, irAEs immune-related adverse events, 95% CI 95% confidential interval, High-grade irAEs, grade 3–5 irAEs

[38, 58, 59]. Thus, despite the underlying mechanisms by which gut microbiota manipulates the development of irAEs remain poorly understood, we hypothesize that there might be some shared etiology of microbiota in autoimmune diseases and irAEs. These mechanisms include: (1) “Molecular mimicry”: Evidence has shown that exposure to homologous amino acid sequences or epitopes of microbiota and aberrant activation of autoreactive B or T cells leads to multiple autoimmune diseases, such as multiple sclerosis [60], Guillain–Barré syndrome [61], Type 1 diabetes [62], Rheumatoid arthritis [63], and primary biliary cholangitis [64], which is referred to as “molecular mimicry” [65]. It is believed that the systematic activation of the immune system during ICIs treatment triggers irAEs by bypassing self-tolerance in normal organs. One intriguing fact is that most irAEs occur in barrier organs (e.g., the intestinal tract, skin, and lungs) [58, 66]. This implies that the activated immune response might target the commensal microbiome

as antigenic targets, although this hypothesis has not been fully demonstrated. (2) Decreased accumulation of SCFAs: SCFAs, including acetate, propionate, and butyrate, are a group of organic compounds primarily produced by the gut microbiota during the fermentation of dietary fibers. These metabolites were shown to improve the anti-cancer function of effector T cells, but they also seem to exhibit anti-inflammatory characteristics [67, 68]. Butyrate, one of the well-studied SCFAs, was shown to inhibit the activation of NF- κ B and its downstream pathway [69], thereby reducing the production of pro-inflammatory cytokines, such as IL-8 [70], while increasing the levels of anti-inflammatory factors, such as IL-10 [71]. Moreover, SCFAs serve as a key energy source for colonocytes and maintain intestinal barrier integrity [72]. Thus, the reduced abundance of SCFAs-producing bacteria along with its metabolites may participate in the development of irAEs. [3] Other mechanisms: stimulation of the immune response by microbial-associated

molecular patterns (e.g., include lipopolysaccharides (LPS), lipoproteins, flagellin and bacterial DNA) [73] and compromised vitamin B and polyamine metabolism that associated with gut dysbiosis [19] may also contribute to irAEs.

Taken together, the gut microbiota and the human immune system maintain a delicate balance under normal physiological conditions. Once the balance has been disturbed (e.g., ICIs treatment), the dysregulated microbiota might lead to the development of undesirable irAEs. The primary management strategy for irAEs (>grade 2) involves the suspension of ICIs and/or utilizing immunosuppressive therapy [74]. Nevertheless, one concern is that discontinuing ICIs or using immunosuppressants may compromise treatment efficacy. Ideally, approaches to boost ICIs efficacy while reducing the accompanied irAEs are to be expected in the future. Fecal Microbiota Transplantation (FMT), an approach to modulate gut microbiota, has been shown to increase ICIs efficacy in melanoma patients [75, 76], and emerging evidence has demonstrated the mitigation of ICIs-related colitis through FMT in clinical practice [77]. Interestingly, while irAEs and ICIs efficacy are often considered two sides of the same coin, certain gut bacteria, such as *Akkermansia muciniphila* and *Faecalibacterium prausnitzii*, have been shown to ameliorate irAEs and reinforce ICIs efficacy at the same time [78]. This suggests that targeting gut microbiota could be an ideal approach to relieve irAEs symptoms and maintain ICIs efficacy, but further real-world evidence is needed to support this hypothesis.

Our study possesses several strengths. First, we applied the MR approach to infer the causal associations between gut microbiota and irAEs, which effectively mitigates the influence of confounding factors and provides robust causal inference. Second, we conducted reverse MR analyses, confirming the absence of reverse causation, thereby enhancing the validity of our study. Third, we applied MVMR in our analysis which further strengthened the validity and robustness of our study. Moreover, we incorporated several pleiotropy-robust methods such as MR PRESSO and cML-MA-BIC, further strengthening the robustness of our study. However, there are also certain inherent limitations in our study that should be considered when interpreting the findings. First, our analysis is based on European-derived GWAS summary statistics, which might confine the generalization of the findings to other populations. Second, due to the utilization of summary statistics instead of raw data in the analysis, subgroup analyses based on ICIs regimes (e.g., PD-1/PD-L1 group, CTLA-4 group, and combined therapy group) or different cancer subtypes (e.g., gastrointestinal cancer and genitourinary cancer) could not be performed. Third, the gut microbiota is shaped by multiple environmental

factors, which confines the number of the identified significant gene loci in the GWAS [26]. Thus, we relaxed the significant threshold of IVs to 1×10^{-5} [29] and employed Bonferroni correction to mitigate potential false positive results. Dietary patterns may influence the composition of gut microbiota and the changes of gut microbial taxa may also contribute to the development of irAEs. However, due to the limitations of summary statistics, detailed analysis among diet, gut microbiota and irAEs was not available. Further study is needed in the future. In addition, future studies may focus on the role of different microbial patterns residing on different barrier interfaces of human organs in the development of irAEs.

Conclusion

Our univariable and multivariable MR analysis identified a strong causal association between *Lachnospiraceae* and irAEs, along with several other gut microbial taxa, such as *Akkermansia* and *Ruminiclostridium6* etc.. However, whether the FMT or probiotics could be used as interventional approaches to mitigate irAEs while reserving ICIs efficacy, additional randomized clinical trials (RCTs) are warranted. Furthermore, in-depth investigations are needed to elucidate the precise mechanisms through which the gut microbiota influences the development of irAEs.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s40001-024-01741-7>.

Additional file 1: Figure S1. Illustration of MR assumptions

Additional file 2: Table S1. All Instrumental variables used for gut microbiota in the MR analysis. **Table S2.** Full results of primary MR analysis between bacterial taxa and all-grade irAEs. **Table S3.** Full results of primary MR analysis between bacterial taxa and high-grade irAEs. **Table S4.** Results of MR PRESS Global test. **Table S5.** Instrumental variables for irAEs in the reverse MR analysis. **Table S6.** Results of reverse MR analysis between all-grade irAEs and the identified significant gut bacterial taxa. **Table S7.** Results of reverse MR analysis between high-grade irAEs and the identified significant gut bacterial taxa. **Table S8.** Results of heterogeneity and horizontal pleiotropy analysis in reverse MR analysis. **Table S9.** PhenoScanner results of instrumental variables used for significant bacterial taxa in the MR analysis. **Table S10.** Description of data sets used for weight-associated anthropometric and lifestyle factors in univariable MR analysis. **Table S11.** Multivariate MR estimates between gut microbiota (adjusted for BMI) and irAEs.

Acknowledgements

We acknowledge all the investigators who have made their GWAS data available.

Author contributions

BL conceptualized this study. BL, ZL, and TJ were involved in the analyses and manuscript drafting of this study. XG and XZ were involved in the data curation. BL, TJ, XY, ZC, and LD were involved in the interpretation of the methodology and results. BL and ZL were involved in the visualization of the results. BZ was involved in obtaining funding and critical revision of the manuscript.

Funding

This study was supported by the National Nature Science Foundations of China (Grant number 82203108), China Postdoctoral Science Foundation (Grant number 2022M722275), and the Key R&D Program of Sichuan Province, China (Grant number 2023YFS0278).

Data availability

This study was conducted using public-available data. The GWAS summary statistics for gut microbiota are available at www.mibiogen.org, and GWAS summary statistics for irAEs are available at <https://zenodo.org/record/6800429>. The summary statistics for irAEs were downloaded from <https://zenodo.org/record/6800429>.

Declarations

Ethics approval and consent to participate

Not applicable.

Competing interests

All authors declare no conflict of interest.

Author details

¹Department of General Surgery, West China Hospital, Sichuan University, Chengdu 610041, Sichuan, People's Republic of China. ²Gastric Cancer Center, West China Hospital, Sichuan University, Chengdu 610041, Sichuan, People's Republic of China. ³Department of Biotherapy and National Clinical Research Center for Geriatrics, Cancer Center, West China Hospital, Sichuan University, Chengdu, Sichuan, People's Republic of China. ⁴State Key Laboratory of Biotherapy and Cancer Center, West China Hospital, Sichuan University and Collaborative Innovation Center for Biotherapy, Chengdu 610041, Sichuan, People's Republic of China.

Received: 25 September 2023 Accepted: 22 February 2024

Published online: 12 March 2024

References

- Ribas A, Wolchok JD. Cancer immunotherapy using checkpoint blockade. *Science*. 2018;359(6382):1350–5.
- Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, et al. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med*. 2010;363(8):711–23.
- Motzer RJ, Escudier B, McDermott DF, George S, Hammers HJ, Srinivas S, et al. Nivolumab versus everolimus in advanced renal-cell carcinoma. *N Engl J Med*. 2015;373(19):1803–13.
- Overman MJ, McDermott R, Leach JL, Lonardi S, Lenz HJ, Morse MA, et al. Nivolumab in patients with metastatic DNA mismatch repair-deficient or microsatellite instability-high colorectal cancer (CheckMate 142): an open-label, multicentre, phase 2 study. *Lancet Oncol*. 2017. [https://doi.org/10.1016/S1470-2045\(17\)30422-9](https://doi.org/10.1016/S1470-2045(17)30422-9).
- Gandhi L, Rodríguez-Abreu D, Gadgeel S, Esteban E, Felip E, De Angelis F, et al. Pembrolizumab plus chemotherapy in metastatic non-small-cell lung cancer. *N Engl J Med*. 2018;378(22):2078–92.
- Robert C. A decade of immune-checkpoint inhibitors in cancer therapy. *Nat Commun*. 2020;11(1):3801.
- Postow MA, Chesney J, Pavlick AC, Robert C, Grossmann K, McDermott D, et al. Nivolumab and Ipilimumab versus Ipilimumab in Untreated Melanoma. *N Engl J Med*. 2015;372(21):2006–17.
- Suresh K, Naidoo J. Lower survival in patients who develop pneumonitis following immunotherapy for lung cancer. *Clin Lung Cancer*. 2020;21(3):e169–70.
- Schadendorf D, Wolchok JD, Hodi FS, Chiarion-Sileni V, Gonzalez R, Rutkowski P, et al. Efficacy and safety outcomes in patients with advanced melanoma who discontinued treatment with nivolumab and ipilimumab because of adverse events: a pooled analysis of randomized phase ii and iii trials. *J Clin Oncol Off J Am Soc Clin Oncol*. 2017;35(34):3807–14.
- Brahmer JR, Lacchetti C, Schneider BJ, Atkins MB, Brassil KJ, Caterino JM, et al. Management of immune-related adverse events in patients treated with immune checkpoint inhibitor therapy: American society of clinical oncology clinical practice guideline. *J Clin Oncol Off J Am Soc Clin Oncol*. 2018;36(17):1714–68.
- Simpson RC, Shanahan ER, Batten M, Reijers ILM, Read M, Silva IP, et al. Diet-driven microbial ecology underpins associations between cancer immunotherapy outcomes and the gut microbiome. *Nat Med*. 2022;28(11):2344–52.
- Jing Y, Chen X, Li K, Liu Y, Zhang Z, Chen Y, et al. Association of antibiotic treatment with immune-related adverse events in patients with cancer receiving immunotherapy. *J Immunother Cancer*. 2022;10(1):e003779.
- Abu-Sbeih H, Herrera LN, Tang T, Altan M, Chaftari AP, Okhuysen PC, et al. Impact of antibiotic therapy on the development and response to treatment of immune checkpoint inhibitor-mediated diarrhea and colitis. *J Immunother Cancer*. 2019;7(1):242.
- Mohiuddin JJ, Chu B, Facciabene A, Poirier K, Wang X, Doucette A, et al. Association of antibiotic exposure with survival and toxicity in patients with melanoma receiving immunotherapy. *J Natl Cancer Inst*. 2021;113(2):162–70.
- Yoo JY, Groer M, Dutra SVO, Sarkar A, McSkimming DI. Gut microbiota and immune system interactions. *Microorganisms*. 2020;8(10):1587.
- Belkaid Y, Hand T. Role of the microbiota in immunity and inflammation. *Cell*. 2014;157(1):121–41.
- Chaput N, Lepage P, Coutzac C, Soularue E, Le Roux K, Monot C, et al. Baseline gut microbiota predicts clinical response and colitis in metastatic melanoma patients treated with ipilimumab. *Ann Oncol Off J Eur Soc Med Oncol*. 2017;28(6):1368–79.
- McCulloch JA, Davar D, Rodrigues RR, Badger JH, Fang JR, Cole AM, et al. Intestinal microbiota signatures of clinical response and immune-related adverse events in melanoma patients treated with anti-PD-1. *Nat Med*. 2022;28(3):545–56.
- Dubin K, Callahan MK, Ren B, Khanin R, Viale A, Ling L, et al. Intestinal microbiome analyses identify melanoma patients at risk for checkpoint-blockade-induced colitis. *Nat Commun*. 2016;2(7):10391.
- Bajinka O, Tan Y, Abdelhalim KA, Özdemir G, Qiu X. Extrinsic factors influencing gut microbes, the immediate consequences and restoring eubiosis. *AMB Express*. 2020;10(1):130.
- Katan MB. Apolipoprotein E isoforms, serum cholesterol, and cancer. *Lancet Lond Engl*. 1986;1(8479):507–8.
- Davies NM, Holmes MV, Davey SG. Reading Mendelian randomisation studies: a guide, glossary, and checklist for clinicians. *BMJ*. 2018;12(362):k601.
- Davey Smith G, Hemani G. Mendelian randomization: genetic anchors for causal inference in epidemiological studies. *Hum Mol Genet*. 2014;23(11):R89–98.
- Chen L, Peters JE, Prins B, Persyn E, Traylor M, Surendran P, et al. Systematic Mendelian randomization using the human plasma proteome to discover potential therapeutic targets for stroke. *Nat Commun*. 2022;17(13):6143.
- Zhang X, Theodoratou E, Li X, Farrington SM, Law PJ, Broderick P, et al. Genetically predicted physical activity levels are associated with lower colorectal cancer risk: a Mendelian randomisation study. *Br J Cancer*. 2021;124(7):1330–8.
- Kurilshikov A, Medina-Gomez C, Bacigalupe R, Radjabzadeh D, Wang J, Demirkan A, et al. Large-scale association analyses identify host factors influencing human gut microbiome composition. *Nat Genet*. 2021;53(2):156–65.
- Groha S, Alaiwi SA, Xu W, Naranbhai V, Nassar AH, Bakouny Z, et al. Germ-line variants associated with toxicity to immune checkpoint blockade. *Nat Med*. 2022;28(12):2584–91.
- Liu X, Tong X, Zou Y, Lin X, Zhao H, Tian L, et al. Mendelian randomization analyses support causal relationships between blood metabolites and the gut microbiome. *Nat Genet*. 2022;54(1):52–61.
- Sanna S, van Zuydam NR, Mahajan A, Kurilshikov A, Vich Vila A, Vösa U, et al. Causal relationships among the gut microbiome, short-chain fatty acids and metabolic diseases. *Nat Genet*. 2019;51(4):600–5.
- Zheng J, Baird D, Borges MC, Bowden J, Hemani G, Haycock P, et al. Recent Developments in Mendelian Randomization Studies. *Curr Epidemiol Rep*. 2017;4(4):330–45.
- Burgess S, Davey Smith G, Davies NM, Dudbridge F, Gill D, Glymour MM, et al. Guidelines for performing Mendelian randomization investigations. *Wellcome Open Res*. 2019;4:186.

32. Verbanck M, Chen CY, Neale B, Do R. Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. *Nat Genet.* 2018;50(5):693–8.
33. Burgess S, Dudbridge F, Thompson SG. Combining information on multiple instrumental variables in Mendelian randomization: comparison of allele score and summarized data methods. *Stat Med.* 2016;35(11):1880–906.
34. Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent estimation in mendelian randomization with some invalid instruments using a weighted median estimator. *Genet Epidemiol.* 2016;40(4):304–14.
35. Hartwig FP, Davey Smith G, Bowden J. Robust inference in summary data Mendelian randomization via the zero modal pleiotropy assumption. *Int J Epidemiol.* 2017;46(6):1985–98.
36. Xue H, Shen X, Pan W. Constrained maximum likelihood-based Mendelian randomization robust to both correlated and uncorrelated pleiotropic effects. *Am J Hum Genet.* 2021;108(7):1251–69.
37. Sanderson E, Spiller W, Bowden J. Testing and correcting for weak and pleiotropic instruments in two-sample multivariable Mendelian randomization. *Stat Med.* 2021;40(25):5434–52.
38. Champiat S, Lambotte O, Barreau E, Belkhir R, Berdelou A, Carbonnel F, et al. Management of immune checkpoint blockade dysimmune toxicities: a collaborative position paper. *Ann Oncol Off J Eur Soc Med Oncol.* 2016;27(4):559–74.
39. Plaçais L, Dalle S, Dereure O, Trabelsi S, Dalac S, Legoupil D, et al. Risk of irAEs in patients with autoimmune diseases treated by immune checkpoint inhibitors for stage III or IV melanoma: results from a matched case–control study. *Ann Rheum Dis.* 2022;81(10):1445–52.
40. Stanislowski MA, Dabelea D, Lange LA, Wagner BD, Lozupone CA. Gut microbiota phenotypes of obesity. *NPJ Biofilms Microbiomes.* 2019;5(1):18.
41. Deng K, Shuai M, Zhang Z, Jiang Z, Fu Y, Shen L, et al. Temporal relationship among adiposity, gut microbiota, and insulin resistance in a longitudinal human cohort. *BMC Med.* 2022;20(1):171.
42. Chennamadhavuni A, Abushahin L, Jin N, Presley CJ, Manne A. Risk factors and biomarkers for immune-related adverse events: a practical guide to identifying high-risk patients and rechallenging immune checkpoint inhibitors. *Front Immunol.* 2022. <https://doi.org/10.3389/fimmu.2022.779691>.
43. Liu T, Xiong Q, Li L, Hu Y. Intestinal microbiota predicts lung cancer patients at risk of immune-related diarrhea. *Immunotherapy.* 2019;11(5):385–96.
44. Chau J, Yadav M, Liu B, Furqan M, Dai Q, Shahi S, et al. Prospective correlation between the patient microbiome with response to and development of immune-mediated adverse effects to immunotherapy in lung cancer. *BMC Cancer.* 2021;21(1):808.
45. Liu W, Ma F, Sun B, Liu Y, Tang H, Luo J, et al. Intestinal microbiome associated with immune-related adverse events for patients treated with anti-PD-1 inhibitors, a real-world study. *Front Immunol.* 2021;16(12):756872.
46. Zhang Y, Cheng S, Zou H, Han Z, Xie T, Zhang B, et al. Correlation of the gut microbiome and immune-related adverse events in gastrointestinal cancer patients treated with immune checkpoint inhibitors. *Front Cell Infect Microbiol.* 2023. <https://doi.org/10.3389/fcimb.2023.1099063>.
47. Dou X, Gao N, Yan D, Shan A. Sodium butyrate alleviates mouse colitis by regulating gut microbiota dysbiosis. *Anim Open Access J MDPI.* 2020;10(7):1154.
48. Wu M, Li P, An Y, Ren J, Yan D, Cui J, et al. Phloretin ameliorates dextran sulfate sodium-induced ulcerative colitis in mice by regulating the gut microbiota. *Pharmacol Res.* 2019;150:104489.
49. Johanson DM, Goertz JE, Marin IA, Costello J, Overall CC, Gaultier A. Experimental autoimmune encephalomyelitis is associated with changes of the microbiota composition in the gastrointestinal tract. *Sci Rep.* 2020;10(1):15183.
50. Ning J, Huang SY, Chen SD, Zhang YR, Huang YY, Yu JT. Investigating casual associations among gut microbiota, metabolites, and neurodegenerative diseases: a mendelian randomization study. *J Alzheimers Dis JAD.* 2022;87(1):211–22.
51. Routy B, Le Chatelier E, Derosa L, Duong CPM, Alou MT, Daillère R, et al. Gut microbiome influences efficacy of PD-1–based immunotherapy against epithelial tumors. *Science.* 2018;359(6371):91–7.
52. Lee KA, Thomas AM, Bolte LA, Björk JR, de Ruijter LK, Armanini F, et al. Cross-cohort gut microbiome associations with immune checkpoint inhibitor response in advanced melanoma. *Nat Med.* 2022;28(3):535–44.
53. Derosa L, Routy B, Thomas AM, Iebba V, Zalcman G, Friard S, et al. Intestinal *Akkermansia muciniphila* predicts clinical response to PD-1 blockade in patients with advanced non-small-cell lung cancer. *Nat Med.* 2022;28(2):315–24.
54. Wang Y, Wiesnoski DH, Helmink BA, Gopalakrishnan V, Choi K, DuPont HL, et al. Fecal microbiota transplantation for refractory immune checkpoint inhibitor-associated colitis. *Nat Med.* 2018;24(12):1804–8.
55. Wang L, Tang L, Feng Y, Zhao S, Han M, Zhang C, et al. A purified membrane protein from *Akkermansia muciniphila* or the pasteurised bacterium blunts colitis associated tumorigenesis by modulation of CD8+ T cells in mice. *Gut.* 2020;69(11):1988–97.
56. Chen J, Wright K, Davis JM, Jeraldo P, Marietta EV, Murray J, et al. An expansion of rare lineage intestinal microbes characterizes rheumatoid arthritis. *Genome Med.* 2016;8(1):43.
57. Miyauchi E, Shimokawa C, Steimle A, Desai MS, Ohno H. The impact of the gut microbiome on extra-intestinal autoimmune diseases. *Nat Rev Immunol.* 2023;23(1):9–23.
58. Martins F, Sofiya L, Sykiotis GP, Lamine F, Maillard M, Fraga M, et al. Adverse effects of immune-checkpoint inhibitors: epidemiology, management and surveillance. *Nat Rev Clin Oncol.* 2019;16(9):563–80.
59. Postow MA, Sidlow R, Hellmann MD. Immune-related adverse events associated with immune checkpoint blockade. *N Engl J Med.* 2018;378(2):158–68.
60. Du C, Yao SY, Ljunggren-Rose Å, Sriram S. Chlamydia pneumoniae infection of the central nervous system worsens experimental allergic encephalitis. *J Exp Med.* 2002;196(12):1639–44.
61. Rodríguez Y, Rojas M, Pacheco Y, Acosta-Ampudia Y, Ramírez-Santana C, Monsalve DM, et al. Guillain-Barré syndrome, transverse myelitis and infectious diseases. *Cell Mol Immunol.* 2018;15(6):547–62.
62. Gulden E, Wong FS, Wen L. The gut microbiota and type 1 diabetes. *Clin Immunol.* 2015;159(2):143–53.
63. Lundberg K, Kinloch A, Fisher BA, Wegner N, Wait R, Charles P, et al. Antibodies to citrullinated α -enolase peptide 1 are specific for rheumatoid arthritis and cross-react with bacterial enolase. *Arthritis Rheum.* 2008;58(10):3009–19.
64. Tanaka A, Leung PSC, Gershwin ME. Pathogen infections and primary biliary cholangitis. *Clin Exp Immunol.* 2019;195(1):25–34.
65. Rojas M, Restrepo-Jiménez P, Monsalve DM, Pacheco Y, Acosta-Ampudia Y, Ramírez-Santana C, et al. Molecular mimicry and autoimmunity. *J Autoimmun.* 2018;95:100–23.
66. Wang SJ, Dougan SK, Dougan M. Immune mechanisms of toxicity from checkpoint inhibitors. *Trends Cancer.* 2023;9(7):543–53.
67. Park J, Kim M, Kang SG, Jannasch AH, Cooper B, Patterson J, et al. Short-chain fatty acids induce both effector and regulatory T cells by suppression of histone deacetylases and regulation of the mTOR–S6K pathway. *Mucosal Immunol.* 2015;8(1):80–93.
68. Luu M, Riestler Z, Baldrich A, Reichardt N, Yuille S, Busetto A, et al. Microbial short-chain fatty acids modulate CD8+ T cell responses and improve adoptive immunotherapy for cancer. *Nat Commun.* 2021;12(1):4077.
69. Zhou G, Zhang N, Meng K, Pan F. Interaction between gut microbiota and immune checkpoint inhibitor-related colitis. *Front Immunol.* 2022;27(13):1001623.
70. Böcker U, Nebe T, Herweck F, Holt L, Panja A, Jobin C, et al. Butyrate modulates intestinal epithelial cell-mediated neutrophil migration. *Clin Exp Immunol.* 2003;131(1):53–60.
71. Lee C, Kim BG, Kim JH, Chun J, Im JP, Kim JS. Sodium butyrate inhibits the NF- κ B signaling pathway and histone deacetylation, and attenuates experimental colitis in an IL-10 independent manner. *Int Immunopharmacol.* 2017;51:47–56.
72. den Besten G, van Eunen K, Groen AK, Venema K, Reijngoud DJ, Bakker BM. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *J Lipid Res.* 2013;54(9):2325–40.
73. Wang Y, Jenq RR, Wargo JA, Watowich SS. Microbiome influencers of checkpoint blockade-associated toxicity. *J Exp Med.* 2023;220(3):e20220948.
74. Schneider BJ, Naidoo J, Santomaso BD, Lacchetti C, Adkins S, Anadkat M, et al. Management of immune-related adverse events in patients treated

with immune checkpoint inhibitor therapy: ASCO guideline update. *J Clin Oncol.* 2021;39(36):4073–126.

75. Davar D, Dzutsev AK, McCulloch JA, Rodrigues RR, Chauvin JM, Morrison RM, et al. Fecal microbiota transplant overcomes resistance to anti-PD-1 therapy in melanoma patients. *Science.* 2021;371(6529):595–602.
76. Baruch EN, Youngster I, Ben-Betzalel G, Ortenberg R, Lahat A, Katz L, et al. Fecal microbiota transplant promotes response in immunotherapy-refractory melanoma patients. *Science.* 2021;371(6529):602–9.
77. Chen M, Liu M, Li C, Peng S, Li Y, Xu X, et al. Fecal microbiota transplantation effectively cures a patient with severe bleeding immune checkpoint inhibitor-associated colitis and a short review. *Front Oncol.* 2022;10(12):913217.
78. Soularue E, Lepage P, Colombel JF, Coutzac C, Faleck D, Marthey L, et al. Enterocolitis due to immune checkpoint inhibitors: a systematic review. *Gut.* 2018;67(11):2056–67.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.