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Original Article

Causal Relationship Between Gut Microbiota and Liver Cirrhosis: 16S rRNA Sequencing and Mendelian Randomization Analyses

Mengqin Yuan[1#], Xue Hu[1#], Lichao Yao[1], Ping Chen[1], Zheng Wang[1], Pingji Liu[1], Zhiyu Xiong[1], Yingan Jiang[1*] and Lanjuan Li[1,2*]

[1]Department of Infectious Diseases, Renmin Hospital of Wuhan University, Wuhan, Hubei, China; [2]State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, National Clinical Research Centre for Infectious Diseases, Collaborative Innovation Centre for Diagnosis and Treatment of Infectious Diseases, The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, Zhejiang, China

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Abstract

Background and Aims: Accumulating evidence highlights the association between the gut microbiota and liver cirrhosis. However, the role of the gut microbiota in liver cirrhosis remains unclear. ***Methods:*** We first assessed the differences in the composition of the bacterial community between CCl4-induced liver cirrhosis and control mice using 16S rRNA sequencing. We then performed a two-sample Mendelian randomization (MR) analysis to reveal the underlying causal relationship between the gut microbiota and liver cirrhosis. Causal relationships were analyzed using primary inverse variance weighting (IVW) and other supplemental MR methods. Furthermore, fecal samples from liver cirrhosis patients and healthy controls were collected to validate the results of the MR analysis. ***Results:*** Analysis of 16S rRNA sequencing indicated significant differences in gut microbiota composition between the cirrhosis and control groups. IVW analyses suggested that Alphaproteobacteria, Bacillales, NB1n, Rhodospirillales, *Dorea*, *Lachnospiraceae*, and *Rhodospirillaceae* were positively correlated with the risk of liver cirrhosis, whereas *Butyricicoccus*, *Hungatella*, *Marvinbryantia*, and *Lactobacillaceae* displayed the opposite effects. However, the weighted median and MR-PRESSO estimates further showed that only *Butyricicoccus* and *Marvinbryantia* presented stable negative associations with liver cirrhosis. No significant heterogeneity or horizontal pleiotropy was observed in the sensitivity analysis. Furthermore, the result of 16S rRNA sequencing also showed that healthy controls had a higher relative abundance of *Butyricicoccus* and *Marvinbryantia* than liver cirrhosis patients. ***Conclusions:*** Our study provides new causal evidence for the link between gut microbiota and liver cirrhosis, which may contribute to the discovery of novel strategies to prevent liver cirrhosis.

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Introduction

Liver cirrhosis is a chronic liver disease that results from acute or chronic liver injury, such as hepatitis virus infection, alcoholism, and obesity.[1] As an advanced liver disease, liver cirrhosis leads to a range of serious complications, including liver cancer and liver failure.[2,3] Approximately one million people worldwide die annually from liver cirrhosis.[4] Despite this, effective and specific antifibrotic strategies are still lacking.[5] Therefore, it is essential to determine the risk factors for liver cirrhosis and to identify novel strategies for its prevention.

The liver and gut communicate closely via the porta hepatis and biliary systems, and the gut microbiota affects liver metabolism through the gut–liver axis. Ample evidence links enteric dysbiosis to the progression of liver cirrhosis.[6,7] Qin *et al*.[8] observed an obvious change in the composition of the bacterial community in liver cirrhosis patients. Chen *et al*.[9] studied the bacterial community composition in cirrhotic and healthy patients and found that the cirrhosis group was highly enriched in *Enterobacteriaceae* and *Streptococcaceae*. However, the abundance of *Lachnospiraceae* declined. Nev-

Keywords: 16S rRNA gene sequencing; Liver cirrhosis; Gut microbiota; Causality; Two-sample Mendelian randomization study.

Abbreviations: BMI, body mass index; CCL4, carbon tetrachloride; CI, confidence interval; GWAS, genome-wide association study; HE, hematoxylin-eosin; ICD, International Classification of Diseases; IVW, inverse variance weighting; KEGG, Kyoto Encyclopedia of Genes and Genomes; LD, linkage disequilibrium; MR, Mendelian randomization; MR PRESSO, MR pleiotropy residual sum and outlier; OR, odds ratio; OTU, operational taxonomic unit; PICRUSt, Phylogenetic Investigation of Communities by Reconstruction of Unobserved States; SCFA, short-chain fatty acid; SE, standard error; SNP, single nucleotide polymorphism.

[#]Contributed equally to this work.

[*]**Correspondence to:** Lanjuan Li, State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, National Clinical Research Centre for Infectious Diseases, Collaborative Innovation Centre for Diagnosis and Treatment of Infectious Diseases, The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, Zhejiang 310003, China; Department of Infectious Diseases, Renmin Hospital of Wuhan University, Wuhan, Hubei 430060, China. ORCID: https://orcid.org/0000-0001-6945-0593. Tel/Fax: +86-571-87236459, E-mail: ljli@zju.edu.cn; Yingan Jiang, Department of Infectious Diseases, Renmin Hospital of Wuhan University, Wuhan, Hubei 430060, China. ORCID: https://orcid.org/0000-0003-4912-7494. Tel/Fax: +86-27-88041911, E-mail: jiangya_cn@aliyun.com.

ertheless, adverse alterations in the gut microbiota can partly be attributed to type 2 diabetes, obesity, alcohol abuse, and inflammation, all of which are important factors that induce the onset and progression of cirrhosis.[10,11] It is imperative to acknowledge that conventional observational studies remain susceptible to inherent confounders and the challenges posed by reverse causality. Consequently, it becomes crucial to investigate whether a causal link exists between gut microbiota and liver cirrhosis.

Mendelian randomization (MR) is an innovative analytical approach that leverages genetic variants as instrumental variables (IVs) to ascertain the causal association between exposure and outcome.[12] According to the law of independent assortment, wherein the progeny randomly inherits parental alleles, MR analysis circumvents reverse causality and ameliorates residual confounding.[13] Recently, MR methodologies have been used to evaluate causal relationships between the gut microbiota and disease progression.[14,15] An example is the work of Xiang *et al*.,[16] in which MR was employed to delineate several gut microbiota taxa, potentially mitigating the risk of systemic lupus erythematosus. This pioneering approach provides an unprecedented avenue for gauging the causal association between gut microbiota and liver cirrhosis. To the best of our knowledge, no previous study has comprehensively elucidated the causal links between gut microbiota and liver cirrhosis.

In this study, we employed 16S rRNA gene sequencing to discern variances in gut microbiota composition between cohorts afflicted with liver cirrhosis and control groups. Subsequently, we harnessed the two-sample MR methodology to explore the causal effect of gut microbiota on liver cirrhosis. Finally, we validated the relationship by sequencing data from fecal samples from patients with liver cirrhosis and healthy control individuals. We aimed to investigate the potential causality between gut microbiota and liver cirrhosis. By doing so, we sought to enhance the foundational understanding of the etiology of liver cirrhosis to prevent the occurrence of liver cirrhosis.

Methods

Study design

First, we sequenced the 16S rRNA gene and compared the gut bacterial communities between a mouse model of carbon tetrachloride (CCl4)-induced cirrhosis and a control group. Subsequently, we used a two-sample MR analysis to further evaluate the causal effects of the gut microbiota on liver cirrhosis. To screen genetic variants that can be used to estimate causal effects, three key assumptions should be met: (1) IVs are strongly correlated with the gut microbiome, (2) IVs are independent of other confounding factors, and (3) IVs are independent of cirrhosis, except for the gut microbiota.[17] Finally, fecal samples were collected from both liver cirrhosis patients and healthy controls to authenticate the outcomes of the MR analysis.

Construction of CCl4-induced cirrhosis mouse model

C57BL/6J mice (7–8 weeks; 20±2 g) were obtained from Beijing Vital River Laboratory Animal Technology (Beijing, China). One week after adaptive nutrition, mice were randomized into the CCl4 model and the control groups. Mice were injected intraperitoneally with CCl4 (1 mL/kg, twice a week, for 12 weeks) to induce liver cirrhosis.[18] Control mice were injected with the same volume and frequency of PBS. After the model was established, blood, liver, and fecal specimens were collected from all mice.

Histological examination

Liver specimens fixed with 4% paraformaldehyde and embedded with paraffin were stained with hematoxylin-eosin (HE) and Masson trichrome.[19] The percentages of fibrotic areas were calculated using the ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Measurement of liver function

Blood was collected using the eyeball method, coagulated at room temperature for 6 h, and then centrifuged to extract the serum. The levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) in the serum were assayed using commercial kits (Shanghai Enzyme Link Biotechnology Co., Ltd., Shanghai, China).[20]

Fecal sample storage and 16s rRNA sequencing analysis

To validate the results of the MR analysis, we also collected additional fecal samples from 13 patients diagnosed with liver cirrhosis and seven healthy controls at the Renmin Hospital of Wuhan University. All participants gave their informed consent. We collected middle fecal samples from all participants to reduce the variability arising from the collection of samples from different fecal locations. Following collection, the fecal samples were promptly frozen and preserved within a dedicated −80°C refrigerator to ensure their stability and integrity.

Genomic DNA was extracted from microbiome samples.[21] Primers were designed based on the conserved region of the sequence, with the specific barcode sequence of the sample incorporated. The 16S rRNA gene was then amplified using PCR. The PCR-amplified products were purified and recycled using clean VAHTSTM DNA beads (Vazyme Biotech Co, Nanjing, China). Subsequently, the recovered products were subjected to a fluorescence assay using a Quant-iT PicoGreen dsDNA Assay Kit and a microplate reader (FLx800; BioTek, Winooski, VT, USA).

Amplified DNA was sequenced using the MiSeq platform. DADA2 and VSearch were used to denoise or cluster the sequences. The DADA2 method is primarily used for primer removal, quality filtering, denoising, splicing, and chimeric removal. In the context of DADA2, each instance of repetition in its variants is referred to as an amplicon sequence variant or characteristic variant corresponding to an operational taxonomic unit (OTU) variant. The variants found in higher abundance within the samples are specifically labeled as the characteristic variants (corresponding to the OTU variant). VSearch is the default method used in functional genetic engineering analysis. Bioinformatic analysis was performed using QIIME2 (version 1.9.1).[22] Alpha diversity analysis was conducted by calculating Chao 1, Faith's phylogenetic diversity (PD), Good's coverage, observed species, Pielou's evenness, and Shannon and Simpson indices. Beta diversity was assessed through principal coordinate analysis at the amplicon sequence variant/OTU level, based on the Bray–Curtis distance. Community phylogenetic studies were performed to predict microbial functions by simulating unobserved states (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States; PICRUSt2) in the Kyoto Encyclopedia of Genes and Genome (KEGG) and MetaCyc databases.[23]

Data sources and instruments of MR analysis

A flowchart outlining the MR analysis is shown in Fig. 1. First, the single nucleotide polymorphisms (SNPs) associated with 211 bacterial traits were obtained from the most

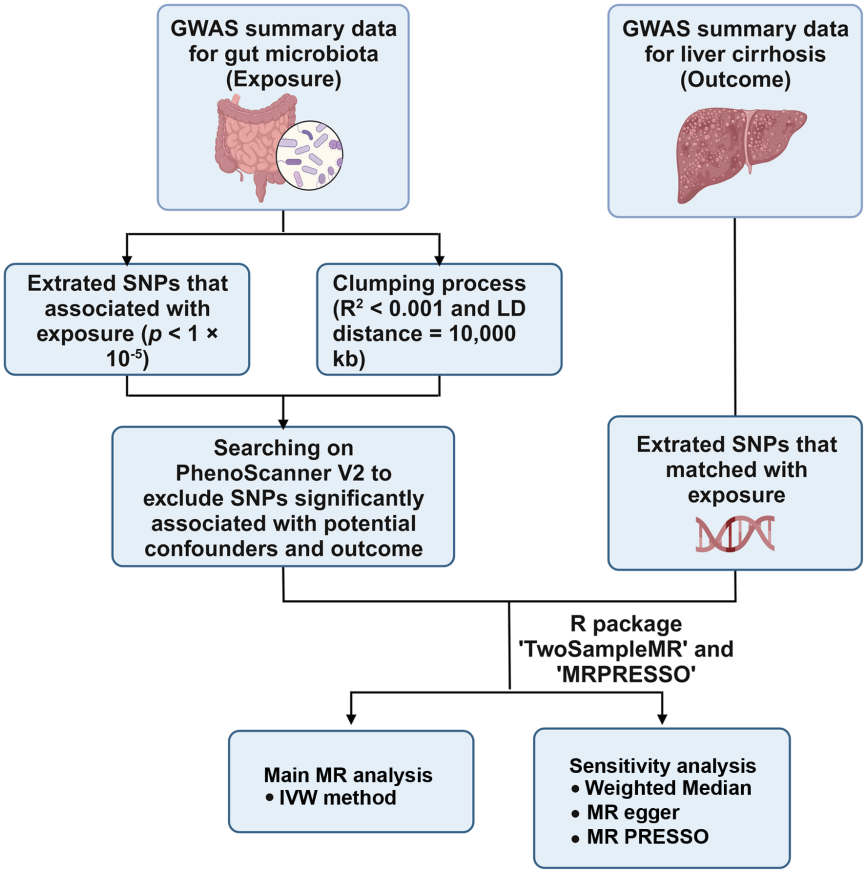

Fig. 1. Study design and workflow of MR analysis. GWAS, genome-wide association study; IVW, inverse variance weighting; LD, linkage disequilibrium; MR, Mendelian randomization; MR PRESSO, MR pleiotropy residual sum and outlier; SNP, single nucleotide polymorphism.

extensive and up-to-date genome-wide association study (GWAS) meta-analysis conducted by the MiBioGen consortium.[24] To elucidate the influence of human genetics on the gut microbiota, we harmonized the 16S rRNA gene sequencing profiles and genome-wide genotypes of 18,340 individuals from 24 distinct cohorts. We excluded 15 bacterial taxa lacking specific species names (unidentified families or genera). Consequently, the present study included 196 bacterial taxa (comprising 119 genera, 32 families, 20 orders, 16 classes, and 9 phyla) for the subsequent analysis. Second, to obtain more comprehensive results, we set the threshold for genome-wide level of significance at $p<1\times10^{-5}$, as suggested by Sanna *et al*.[25] Third, we excluded SNPs with a linkage disequilibrium (LD) R^2 value of <0.001, and an LD distance of 10,000 kb.[26] Fourth, we SNPs linked to potential confounding factors and other characteristics associated with liver cirrhosis (such as hepatitis virus infection, BMI, type 2 diabetes, dyslipidemia, alcohol consumption, and smoking) by cross-referencing each SNP against the PhenoScanner V2 database.[27]

Genetic variants of liver cirrhosis were obtained from the FinnGen research project, which included 811 cases and 213,592 controls with a total of 16,380,458 SNPs. Liver cirrhosis patients were defined according to the International Classification of Diseases (ICD)-10, ICD-9, or ICD-8 code (majorly the ICD-10 code). Led by the University of Helsinki, the FinnGen research project aims to combine genomic and health information to investigate disease mechanisms and develop new treatments.[28] The project involved almost all biobanks in Finland and their respective institutions. We downloaded GWAS data from the Integrative Epidemiology Unit OpenGWAS database. Detailed information on data acquisition and screening is presented in Table 1. Furthermore, we replicated the causal association between exposure and outcome among 361,194 participants of European descent using summary statistics of British biobanks provided by the Neale Lab (http://www.nealelab.is/uk-biobank/) (Supplementary Table 1). As our analysis employed published studies and publicly available aggregated statistics, there was no requirement for ethical approval or patient consent for the MR study.

MR analyses

To evaluate the causal impact of the gut microbiota on liver cirrhosis, the primary MR method employed was inverse variance weighting (IVW) analysis.[29] The MR-Egger regression technique was used to estimate the degree of pleiotropy.[30] The IVW analysis yielded unbiased estimates in cases where horizontal pleiotropy was balanced. The Cochran Q statistic was utilized to measure heterogeneity.[31] When no significant heterogeneity was observed, the IVW fixed-effects model was adopted; otherwise, the random-effects IVW model was adopted. The weighted median method was used to obtain valid estimates when a substantial portion (up to 50%) of the analytical weight was derived from weak instrumental variables.[31] We further applied the MR pleiotropy residual sum and outlier (MR-PRESSO) alongside the weighted median approach to detect and correct for multi-effect outliers at any

Table 1. Characteristics of GWAS used in the MR study

Exposures	GWAS ID	Consortium	Sample size	Total No. of strongly Related SNPs	Total No. of enrolled SNPs	Adjustment	Population
Gut microbiota	ebi-a-GCST90016908-ebi-a-GCST90017118	MiBioGen	14,306	2,786	2,179	Hepatitis virus infection, BMI, type 2 diabetes mellitus, dyslipidemia, drinking and smoking	European

Outcomes	GWAS ID	Consortium	Cases	Control	Population
Liver cirrhosis	finn-b-K11_FIBROCHIRLIV	FinnGen	811	213,592	European

BMI, body mass index; GWAS, genome-wide association study; MR, Mendelian randomization; SNP, single nucleotide polymorphism.

level, as MR-Egger regression has limited statistical power.[32] These gut microbiota were considered risk or protective factors for liver cirrhosis when all MR analysis results reached nominal significance. All analyses were conducted using the TwoSampleMR and MR-PRESSO packages of the R software (version 4.1.2).

Results

Establishment of the animal model of liver cirrhosis

To evaluate the association between liver cirrhosis and the gut microbiota, we first established a CCl4-induced cirrhosis model (Fig. 2A). The CCl4-induced cirrhotic mice had remarkably lower body weights than the control mice (Fig. 2B). Conversely, the ratio of liver weight to body weight was higher in the experimental group than in the control group (Fig. 2C). Histological analysis using HE staining revealed extensive hepatocellular vacuoles and hepatocyte damage (Fig. 2D). Masson's staining also indicated larger fibrotic areas in the CCl4-induced liver cirrhosis model (Fig. 2D–E). In addition, the CCl4-induced liver cirrhosis model exhibited significantly elevated serum levels of ALT, AST, and ALP (Fig. 2F). These results demonstrated the successful construction of a CCl4-induced cirrhosis model.

Diversity of gut microbiota in liver cirrhosis mice

We evaluated the diversity of gut microbiota using high-throughput 16S rRNA gene sequencing. We employed a range of indices to evaluate microbial alpha diversity in mice with liver cirrhosis, including Shannon, Pielou's evenness, Good's coverage, observed species, Simpson, Chao1, and Faith's PD. The results showed that the microbial alpha diversity indices did not differ between the cirrhosis and control groups (Fig. 3A). We further compared beta diversity to reflect differences in species diversity among the groups. As shown in Figure 3B, a notable separation trend in beta diversity was observed between the control and liver cirrhosis groups. Heat maps were generated at the genus level to further display differences in microbial composition. Figure 3C illustrates the top 50 genera with the highest average abundances. These results strongly suggested a marked difference between the gut microbiota communities of the cirrhosis and the control groups.

Functional assessment of gut microbiota

To evaluate the functional and metabolic changes in the gut microbiota, PICRUSt2 was used to analyze all OTUs. PICRUSt2 analysis revealed that metabolic pathways were the most enriched KEGG pathways in the primary function analysis (Fig. 4A–B). Among secondary metabolic pathways, carbohydrate and cofactor metabolism emerged as the two secondary metabolic pathways with the highest relative abundance, followed by amino acid, terpenoid, and polyketide metabolism. We further analyzed the metabolism-related functions of the gut microbiota using PICRUSt2 in the MetaCyc database. As shown in Figure 4C–D, the biosynthesis-related pathways were the most enriched primary functions. Furthermore, among the secondary functional pathways, amino acid, nucleoside, nucleotide, and vitamin biosynthesis, along with cofactors, electron carriers, and prosthetic groups, exhibited the highest relative abundances.

IV selection

A total of 2786 SNPs (statistical threshold of genome-wide significance set at $p<1\times10^{-5}$) for 196 bacterial genera were collected as potential IVs. According to the screening criteria, 2197 SNPs linked to 196 bacterial traits were selected as IVs for subsequent MR analyses.

Two-sample MR analysis

The IVW method indicated a statistically significant association between 11 bacterial genera and liver cirrhosis risk, suggesting that these bacterial genera may influence the development of liver cirrhosis (Table 2). Specifically, *Alphaproteobacteria* [odds ratio (OR) 1.72 [95% confidence interval (CI) 1.00–2.94], p=0.049), *Bacillales* (OR 1.40 [95% CI 1.02–1.93], p=0.035), *NB1n* (OR 1.51 [95% CI 1.12–2.03], p=0.007), *Rhodospirillales* (OR 1.46 [95% CI 1.02–2.09], p=0.038), *Dorea* (OR 1.97 [95% CI 1.05–3.70], p=0.034), *Lachnospiraceae* (OR 1.86 [95% CI 1.07–3.23], p=0.027), and *Rhodospirillaceae* (OR 1.47 [95% CI 1.01–2.15], p=0.045) were positively associated with the risk of liver cirrhosis. In contrast, *Butyricicoccus* (OR 0.41 [95% CI 0.23–0.76], p=0.004), *Hungatella* (OR 0.57 [95% CI 0.35–0.92], p=0.021), *Marvinbryantia* (OR 0.48 [95% CI 0.28–0.82], p=0.007), and *Lactobacillaceae* (OR 0.66 [95% CI 0.44–1.00], p=0.048) exhibited a protective effect on liver cirrhosis.

However, the results remained consistent for only two microbial genera using the other two approaches (Fig. 5A–B). As shown in Figure 5C–D, the weighted median and MR-PRESSO tests corroborated the primary analysis results, underscoring that *Butyricicoccus* (weighted median: OR 0.42 [95% CI 0.18–0.97], p=0.041; MR-PRESSO: OR 0.43 [95% CI 0.28–0.68], p=0.007) and *Marvinbryantia* (weighted median: OR 0.46 [95% CI 0.22–0.96], p=0.038; MR-PRESSO: OR 0.51 [95% CI 0.32–0.82], p=0.018) showed protective effects on liver cirrhosis. Additionally, no evidence of horizontal pleiotropy was found between IVs and liver cirrhosis, and no SNP outlines were detected through MR-Egger regression (p=0.365 for *Butyricicoccus* and p=0.074 for *Marvinbryantia*) or MR-PRESSO tests (p=0.818 for *Butyricicoccus*;

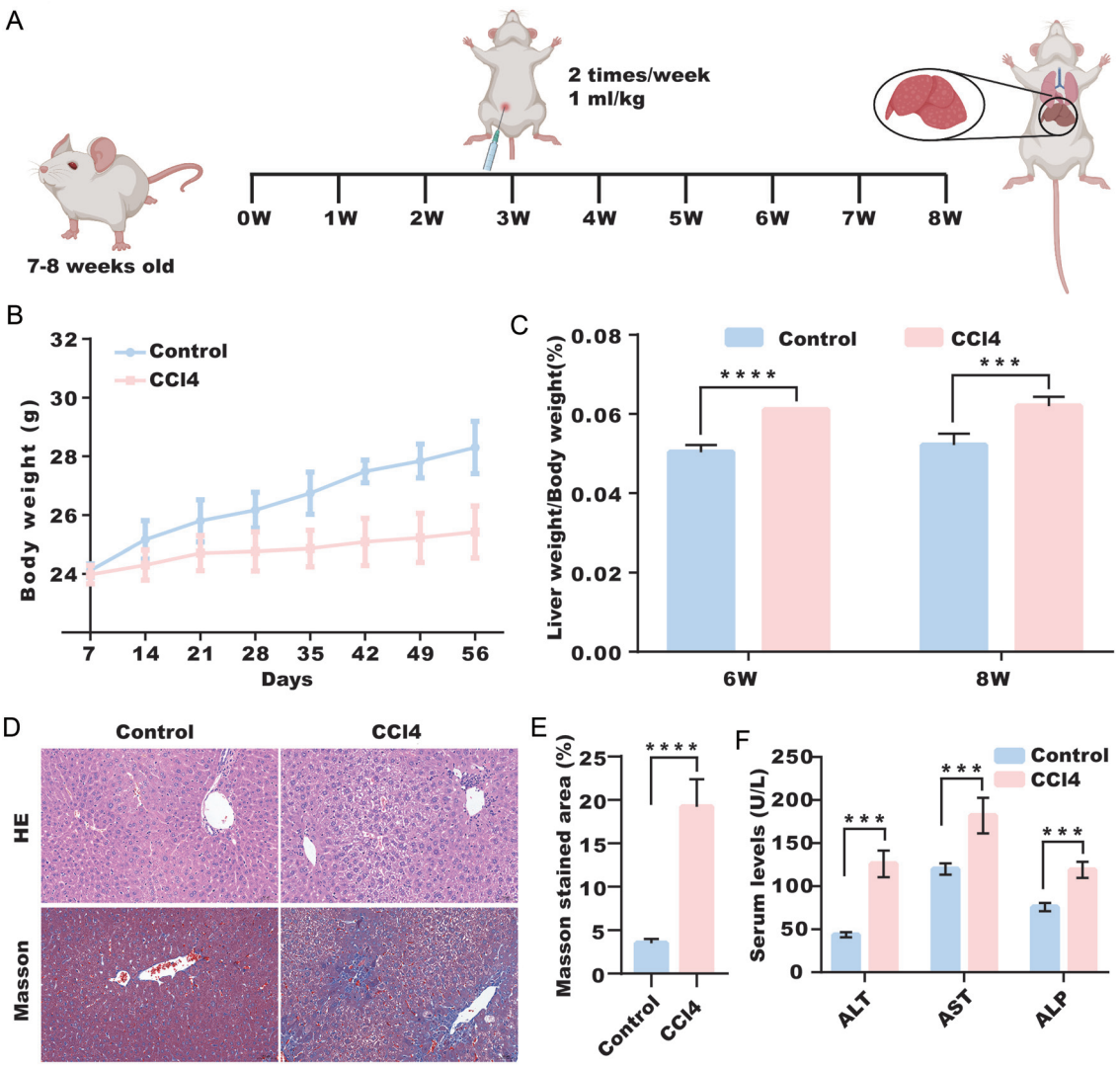

Fig. 2. Construction of CCl4-induced liver cirrhosis mice model. (A) The experimental design diagram presentation of CCl4-induced liver fibrosis. (B) Body weight changes of the two groups. (C) Liver weight/Body weight of the two groups at the 6th and 8th week after intraperitoneal injection of CCl4. (D–E) Representative images of HE and Masson staining of liver sections. (F) Serum ALT, AST and ALP level. ***$p<0.001$, ****$p<0.0001$. CCl4, carbon tetrachloride; HE, hematoxylin-eosin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

$p=0.600$ for *Marvinbryantia*). The SNPs selected as genetic instruments for *Butyricicoccus* and *Marvinbryantia* are listed in Supplementary Table 2. The leave-one-out approach further confirmed the causality between *Butyricicoccus* and *Marvinbryantia*; the removal of any single SNP did not significantly alter liver fibrosis (Supplementary Fig. 1).

When adopting a more stringent genome-wide statistical significance threshold of $p<5\times10^{-6}$ as the second threshold to determine stricter causal associations, we found that *Marvinbryantia* was also causally associated with liver cirrhosis (Supplementary Table 3). However, we could not establish a more stringent causal link between *Butyricicoccus* and liver cirrhosis as only two SNPs were identified when the genome-wide statistical significance threshold was set at $p<5\times10^{-6}$. Furthermore, the validation set replicated the causal association with *Marvinbryantia*, with the direction of the effect aligning with that in the discovery set. This alignment of outcomes further strengthened our confidence in the authentic causal connection (Supplementary Table 4).

Validation of MR

Following the confirmation of the causal direction through MR analysis, we validated our conclusions by leveraging data derived from the 16S rRNA sequencing analyses of liver cirrhosis patients. We extracted the relative abundances of the genera *Butyricicoccus* and *Marvinbryantia*, which were then visually represented using bar plots. Notably, the relative abundances of both *Butyricicoccus* and *Marvinbryantia* genera were significantly higher in the healthy control group when compared to liver cirrhosis patients (Fig. 6A–B). This congruence between the results of MR and sequencing analyses further supports the notion that these specific microbiota genera potentially exhibit protective attributes in the context of liver cirrhosis development.

Discussion

In this study, we employed an integrative approach, encom-

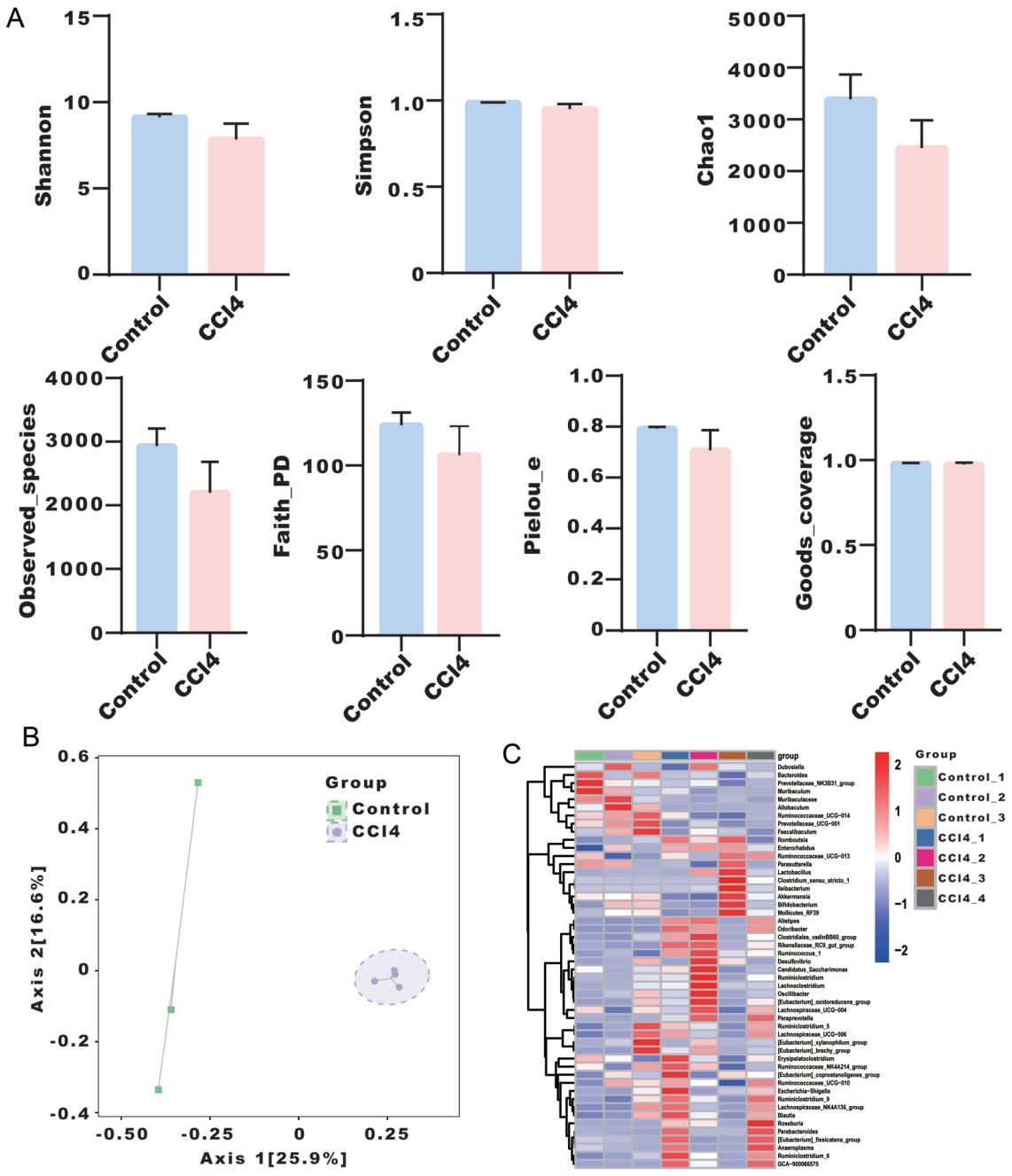

Fig. 3. Comparisons of alpha and beta diversity between control and CCl4-induced liver cirrhosis mice. (A) Alpha diversity of two groups using Shannon, Simpson, Chao1, Observed species, Faith's PD, Pielou's evenness, and Good's coverage indices. (B) Beta diversity shown by principal coordinate analysis. (C) Comparative analysis of the gut microbiota between control and CCl4-induced liver cirrhosis by Heat map analysis. CCl4, carbon tetrachloride.

passing 16S rRNA gene sequencing technology and a two-sample MR method, to comprehensively investigate the intricate relationship between the gut microbiota and the risk of liver fibrosis. To the best of our knowledge, this two-sample MR study is the first to investigate the causal interconnections between gut microbiota and liver cirrhosis, leveraging publicly accessible genetic databases. Our findings underscore the discernible variation in bacterial composition within the gut microbiota of mice with liver cirrhosis compared to the control group. MR analyses consistently corroborated the protective roles of *Butyricicoccus* and *Marvinbryantia* against the risk of liver fibrosis. Furthermore, we confirmed the discrepancy in the relative abundances of the gut microbiota between healthy controls and individuals with liver cirrhosis. Consequently, our research not only unveils a causal relationship between liver fibrosis and the gut microbiota, but also sheds profound light on the pivotal role of the gut microbiota in the progression of liver cirrhosis.

The human gastrointestinal tract is the most densely colonized organ, with a staggering count of up to 100 trillion

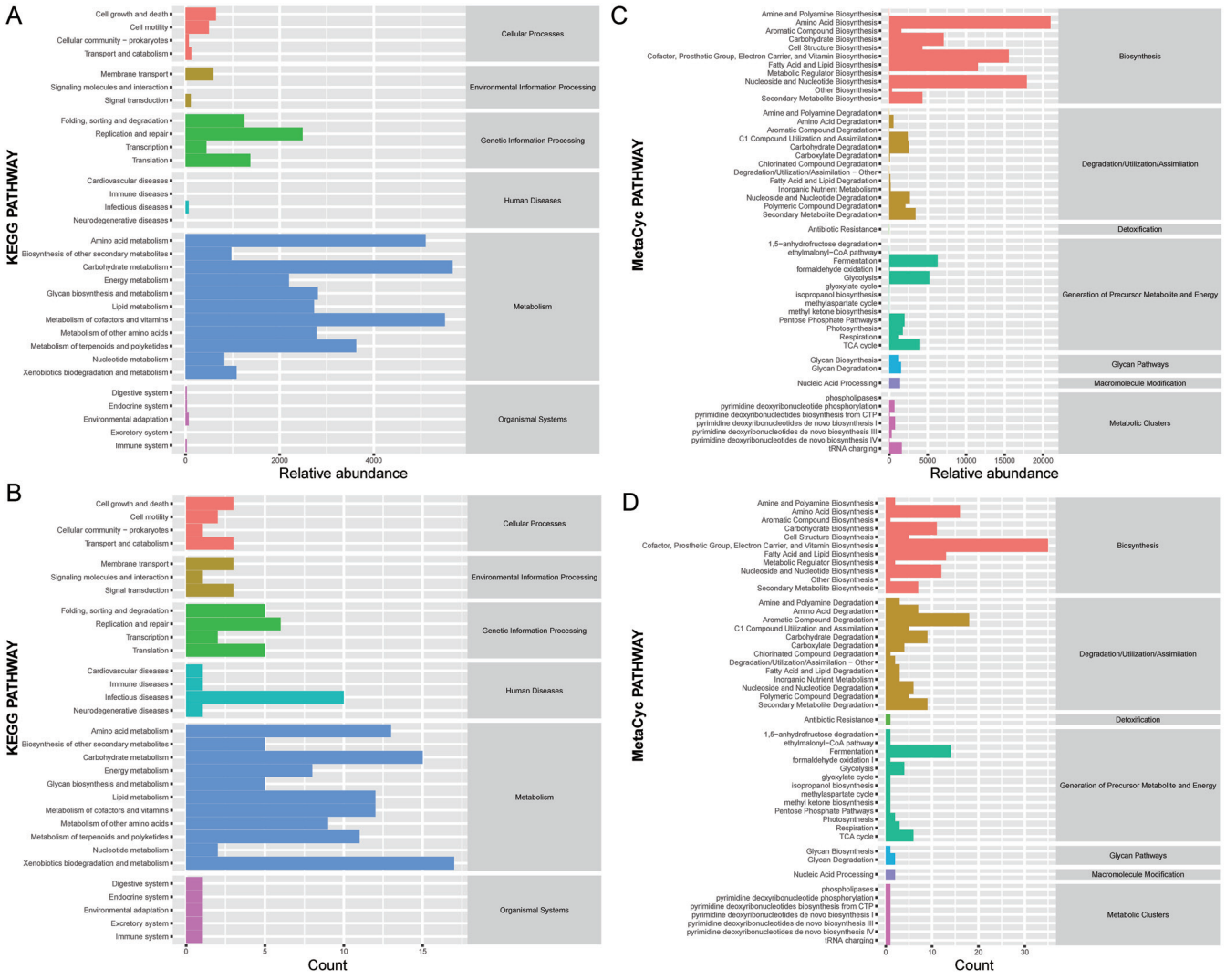

Fig. 4. Metabolic pathway difference analysis. (A–B) Functional prediction based on the KEGG database. (C–D) Metabolic pathway difference analysis based on the MetaCyc database.

microorganisms, nearly tenfold greater than the number of human cells.[33] The portal vein, which functions as the principal channel connecting the gastrointestinal tract and the liver, plays a pivotal role in facilitating the direct and expeditious transport of substances from the intestinal milieu to the liver. Remarkably, this intricate interlinkage establishes a reciprocal conduit for communication extending from the liver to the intestine. This dynamic interaction is facilitated by mechanisms such as bile excretion and antibody release.[34] This intricate mutual interaction is commonly referred to as the gut–liver axis. Studies have consistently underscored the pronounced correlation between cirrhosis and discernible alterations in the composition of the gut microbiota.[35–38] A study by Qin *et al*.[8] revealed that individuals with liver cirrhosis exhibited a notable transformation in their gut microbiota compared to healthy controls. Importantly, the correlation between the abundance of these invading microbial species and disease severity indicated their potential active involvement in the underlying pathological mechanisms. This intriguing relationship suggests that interventions to rectify these substantial dysbiotic imbalances within the gut microbiota may offer innovative avenues for treating liver cirrhosis.

In this study, an inverse correlation was identified between *Butyricicoccus*, *Marvinbryantia* and the risk of liver cirrhosis. Notably, both these microbial genera, as documented by existing research, possess the ability to generate short-chain fatty acids (SCFAs).[39,40] These SCFAs are synthesized primarily through glycolytic fermentation of dietary carbohydrates and play a pivotal role in sustaining both metabolic and immune equilibrium.[41,42] A pertinent observation was the decline in the prevalence of SCFA-associated species, corresponding to an escalation in the severity of liver disease.[43] Jin *et al*.[44] reported a compromised ability of the gut microbiota in liver cirrhosis patients to effectively ferment carbohydrates into SCFAs, resulting in a notable decrease in butyrate in these patients compared to healthy controls. Notably, the genus *Butyricicoccus* can degrade polysaccharides via autocrine multi-enzyme complexes, culminating in the production of SCFAs, particularly butyrate. Butyrate, a recognized substrate for maintaining intestinal integrity, notably enhances intestinal barrier function by upregulating the expression of

Table 2. Summary of the causal relationship between the gut microbiota and the risk of liver cirrhosis based on the IVW method

Classification		No. of SNP	β	SE	OR (95% CI)	*p* value	Horizontal pleiotropy			Heterogeneity		*p* for MR PRESSO Global Test
							Egger intercept	SE	*p* value	Cochran's Q	*p* value	
Class	Alphaproteobacteria	7	0.54	0.28	1.72 (1.00, 2.94)	0.049	0.05	0.05	0.365	4.48	0.723	0.818
Order	Bacillales	7	0.34	0.16	1.40 (1.02, 1.93)	0.035	−0.05	0.11	0.684	5.35	0.500	0.632
	NB1n	12	0.41	0.15	1.51 (1.12, 2.03)	0.007	0.08	0.08	0.346	10.00	0.530	0.307
	Rhodospirillales	14	0.38	0.18	1.46 (1.02, 2.09)	0.038	0.09	0.07	0.223	13.65	0.399	0.529
Family	*Lachnospiraceae*	14	0.62	0.28	1.86 (1.07, 3.23)	0.027	−0.02	0.06	0.769	8.43	0.814	0.875
	Lactobacillaceae	7	−0.42	0.21	0.66 (0.44, 1.00)	0.048	0.08	0.08	0.366	3.03	0.804	0.943
	Rhodospirillaceae	15	0.39	0.19	1.47 (1.01, 2.15)	0.045	0.12	0.08	0.145	17.18	0.247	0.401
Genus	*Butyricicoccus*	8	−0.88	0.31	0.41 (0.23, 0.76)	0.004	0.05	0.05	0.365	4.48	0.723	0.818
	Dorea	10	0.68	0.32	1.97 (1.05, 3.70)	0.034	0.07	0.06	0.269	6.43	0.697	0.125
	Hungatella	5	−0.57	0.25	0.57 (0.35, 0.92)	0.021	−0.12	0.21	0.617	4.94	0.294	0.36
	Marvinbryantia	10	−0.73	0.27	0.48 (0.28, 0.82)	0.007	−0.19	0.09	0.074	8.26	0.508	0.600

CI, confidence interval; OR, odds ratio; MR PRESSO, MR pleiotropy residual sum and outlier; SE, standard error; SNP, single nucleotide polymorphism.

essential tight junction proteins such as claudin-1 and Zonula Occludens-1.[45] Zheng *et al*. and Quan *et al*.[46,47] provided additional support for the utility of butyrate, demonstrating its potential to enhance hepatic lipid metabolism, mitigate hepatic steatosis, and ameliorate nonalcoholic fatty liver disease by influencing the composition of the intestinal microbiome. Conversely, *Marvinbryantia* is a beneficial intestinal bacterium known for its ability to uphold both the diversity and operational efficacy of the gut microbiota, consequently yielding potential benefits to human health. Notably, its multifaceted positive effects include safeguarding the integrity of intestinal epithelial cells against degenerative processes and exhibiting notable anti-inflammatory attributes.[48] However, investigations on the correlation between *Marvinbryantia* and liver cirrhosis are currently limited. Promisingly, a comparative study involving the analysis of the gut microbiota in 24 liver cirrhosis patients and 20 healthy individuals indicated a marked predominance of *Marvinbryantia* within the latter group. This substantial disparity alludes to the plausible role of *Marvinbryantia* in mitigating the risk of liver cirrhosis.[49] These studies revealed their beneficial roles in human diseases and support our findings.

It is also imperative to acknowledge that dietary factors considerably influence gut microbiota composition. Specifically, evidence suggests an association between a high-fat diet and alterations in the gut microbiota, including the augmentation of *Marvinbryantia.*[50] Therefore, while our findings point towards a potential relationship between *Marvinbryantia* and liver cirrhosis, further exploration is imperative to elucidate this connection. Overall, our findings contribute to a better understanding of the plausible causal relationships between various gut microbiota genera and the onset of liver cirrhosis, thereby enhancing our insight into the intricate mechanisms underlying this pathological condition.

The paramount strength of our study lies in the adoption of the two-sample MR method to investigate the relationship between the gut microbiota and the risk of liver cirrhosis. This approach effectively minimized the potential impact of confounding variables and the reverse causality bias. Furthermore, the IVs of the gut microbiota were identified from the largest published GWAS meta-analysis, thus ensuring the robustness of the MR analysis. Therefore, this study may be more compelling than other observational studies.

However, this study had some limitations. First, we were unable to further explore the causal effects of the gut microbiota on liver cirrhosis at the species level because the lowest taxonomic level explored in this study was the genus. Additionally, the SNPs listed in our analysis, while instrumental in our study, did not meet the conventional threshold for nominal genome-wide significance ($p<5\times10^{-8}$), indicating the need to encompass a broader array of genetic variations as instrumental variables. Furthermore, the absence of basic demographic information and clinical manifestations precluded us from conducting a thorough subgroup analysis. Finally, the exclusive inclusion of individuals of European descent in our participant cohort warrants prudence in generalizing our observations to other racial or ethnic groups. This necessitates future investigations for validation across diverse populations, although we validated participants from Asian populations.

Conclusions

Collectively, our study marks a pivotal advancement in unraveling the intricate interplay between the gut microbiota and the progression of liver cirrhosis. By illuminating the

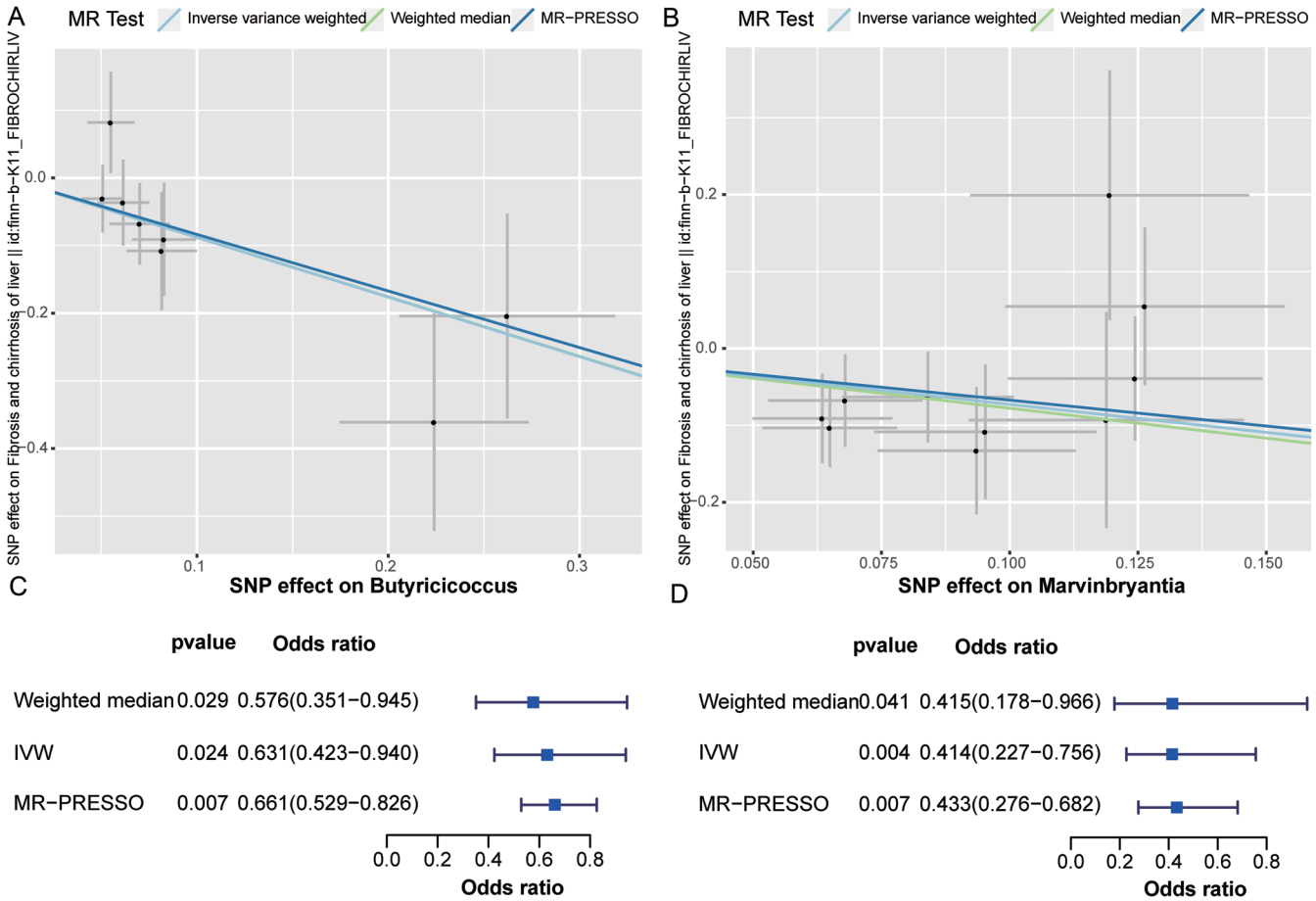

Fig. 5. Causal associations between two gut microbiota with the risks of liver fibrosis. (A–D) Forest plots of (A) *Butyricicoccus* and (B) *Marvinbryantia*. (C–D) Scatter plots of (C) *Butyricicoccus* and (D) *Marvinbryantia*. IVW, inverse variance weighting; MR, Mendelian randomization; MR PRESSO, MR pleiotropy residual sum and outlier.

causal relationships of specific microbial genera, namely *Butyricicoccus* and *Marvinbryantia*, in influencing liver fibrosis risk, we not only contribute to a deeper comprehension of the disease's mechanisms but also unveil potential targets for innovative therapeutic strategies. These findings highlight the crucial role of the gut microbiota in liver health and warrant further exploration, promising novel avenues for research and intervention in the realm of liver cirrhosis.

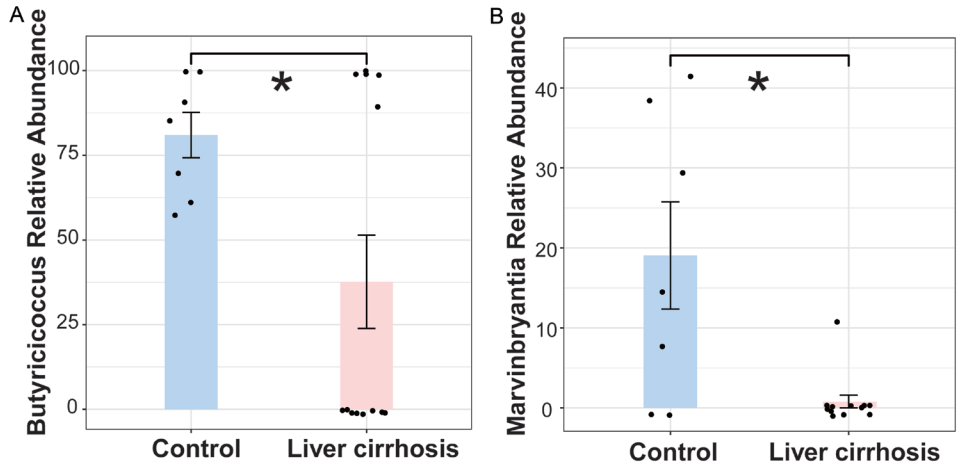

Fig. 6. Bar plots to validate MR. (A–B) Comparative relative abundance of genes_*Butyricicoccus* (A) and genes_*Marvinbryantia* (B) in healthy controls and liver cirrhosis patients. *$p<0.05$.

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Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Study concept and design (LL, YJ), acquisition of data (MY, XH), analysis and interpretation of data (MY, XH, LY, PC), drafting of the manuscript (MY, XH), critical revision of the manuscript for important intellectual content (ZW, PL, ZX), administrative, technical, or material support (YJ), and study supervision (LL). All authors have contributed significantly to this study and approved the final manuscript.

Ethical statement

The animal study was conducted following the Declaration of Helsinki, and the protocol was approved by the Animal Care and Use Committee of Renmin Hospital of Wuhan University (no. 20211204). Furthermore, the human experiments also complied with the Declaration of Helsinki and were approved by the Institutional Review Board of Renmin Hospital of Wuhan University [protocol: 2018Q-C026 (Y01)]. The individual consent for this retrospective analysis was waived.

Data sharing statement

The datasets generated for this study are available from the corresponding author upon reasonable request.

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