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Investigating causal associations among gut microbiota, metabolites, and liver diseases: a Mendelian randomization study

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Objective: There is some evidence for an association between gut microbiota and nonalcoholic fatty liver disease (NAFLD), alcoholic liver disease (ALD), and viral hepatitis, but no studies have explored their causal relationship.

Methods: Instrumental variables of the gut microbiota (N = 13266) and gut microbiota-derived metabolites (N = 7824) were acquired, and a Mendelian randomization study was performed to explore their influence on NAFLD (1483 European cases and 17,781 European controls), ALD (2513 European cases and 332,951 European controls), and viral hepatitis risk (1971 European cases and 340,528 European controls). The main method for examining causality is inverse variance weighting (IVW).

Results: IVW results confirmed that *Anaerotruncus* (p = 0.0249), *Intestinimonas* (p = 0.0237), *Lachnoclostridium* (p = 0.0245), *Lachnospiraceae NC2004 group* (p = 0.0083), *Olsenella* (p = 0.0163), and *Peptococcus* (p = 0.0472) were protective factors for NAFLD, and *Ruminococcus 1* (p = 0.0120) was detrimental for NAFLD. The higher abundance of three genera, *Lachnospira* (p = 0.0388), *Desulfovibrio* (p = 0.0252), and *Ruminococcus torques group* (p = 0.0364), was correlated with a lower risk of ALD, while *Ruminococcaceae UCG 002* level was associated with a higher risk of ALD (p = 0.0371). The *Alistipes* (p = 0.0069) and *Ruminococcaceae NK4A214 group* (p = 0.0195) were related to a higher risk of viral hepatitis. Besides, alanine (p = 0.0076) and phenyllactate (p = 0.0100) were found to be negatively correlated with NAFLD, while stachydrine (Op = 0.0244) was found to be positively associated with NAFLD. The phenylacetate (p = 0.0353) and ursodeoxycholate (p = 0.0144) had a protective effect on ALD, while the threonate (p = 0.0370) exerted a detrimental influence on ALD. The IVW estimates of alanine (p = 0.0408) and cholate (p = 0.0293) showed their

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suggestive harmful effects against viral hepatitis, while threonate (p = 0.0401) displayed its suggestive protective effect against viral hepatitis.

Conclusion: In conclusion, our research supported causal links between the gut microbiome and its metabolites and NAFLD, ALD, and viral hepatitis.

KEYWORDS

alcoholic liver disease, nonalcoholic fatty liver disease, viral hepatitis, gut microbiota, gut microbiota-derived metabolites, mendelian randomization analysis

1 Introduction

Nonalcoholic fatty liver disease (NAFLD) is a prevailing form of chronic liver disease that is marked by the accumulation of hepatic fat in patients who do not have a history of heavy alcohol intake (1). It comprises a wide range of gradually deteriorating pathological disorders, ranging from a straightforward case of nonalcoholic fatty liver to a more serious case of nonalcoholic steatohepatitis (NASH), the latter of which has a higher risk of developing cirrhosis, organ failure, and hepatocellular carcinoma (2–4). Alcoholic liver disease (ALD) is a disease due to chronic and excessive alcohol intake. The accumulation of fat in the liver cells is one of the early responses to excessive alcohol use. When alcohol abuse persists, steatosis may develop into steatohepatitis, fibrosis, cirrhosis, and ultimately hepatocellular cancer (5). As an inflammation of the liver, hepatitis can either go away on its own or develop into a serious condition that results in cirrhosis or hepatocellular cancer. Globally, the main cause of hepatitis is viral, with hepatitis B and C virus infections usually developing into chronic hepatitis (6). There is an urgent need to identify potential causal risk factors for NAFLD, ALD, and viral hepatitis since they pose a significant health burden globally.

The gut microbiota, as the "forgotten organ", is a dynamic and intricate community of ecological bacteria (7). The liver is the first organ crossed by the portal vein of the intestine. The phrase "gut-liver axis" was coined to describe the close connection between the intestinal flora, the immune system, and the intestinal barrier that occurs in the gut and liver (8). Through the portal vein, the liver gets 75% of its blood from the gut. By secreting bile and other mediators, it also gives the intestines feedback (9). Thus, various gut factors, such as gut microbiota, bacterial composition, and gut microbiota-derived metabolites, are deeply involved in the homeostasis of the liver.

Recently, there has been growing evidence that intestinal flora is closely related to human health and is involved in the etiology of various complex diseases, including liver diseases (9, 10). However, there is controversy among these studies. For example, Zhu et al. revealed a higher relative abundance of *Prevotella* and no distinct alternation in *Bacteroides* in NAFLD patients than the control (11). However, Boursier et al. found that, compared to healthy controls, patients with NASH had higher levels of *Bacteroides* and lower levels of *Prevotella* (12). Besides, when compared to controls, several studies have demonstrated an increase in the *Firmicutes* to *Bacteroidetes* ratio in NAFLD and NASH (13, 14), while others have shown a decrease in this ratio (11, 15, 16). Confounding or reverse causation in observational studies could be to blame for the contradictory results in gut microbial dysbiosis in NAFLD.

As we know, confounding factors and reverse causation may both affect the findings of current observational epidemiological research, making causal inference difficult. The Mendelian randomization (MR) method using genetic variants as instrumental variables (IVs) in the epidemiological investigation has been generally accepted to estimate the causal influence of exposure on diseases (17). Based on the Mendelian inheritance rule, parental genetic alleles are randomly dispersed to their offspring during the meiotic process, which is regarded as a randomized controlled study (RCT). This method was chosen because it was practical, economical, and less likely to be confounded by covariables (18). Also, since genetic variants are already set at the time of conception, MR is less susceptible to the influence of reverse causation. Previous genetic research has shown that host genetic variants can affect the intestinal flora, allowing us to explore the relationship between gut microbiota and liver diseases using the MR approach.

Thus, in this study, the summary data from genome-wide association studies (GWASs) was used to explore the causal association of gut microbiota and metabolites with NAFLD, ALD, and viral hepatitis using the two-sample MR analysis.

2 Materials and methods

2.1 Study design

MR analysis is a genetic method that infers the causal effects of exposure on outcomes by using the random allocation of genetic variants at conception. The SNPs employed as IVs need to meet the following basic assumptions. First, there has to be a solid association between the SNPs and the exposure; second, the SNPs should not be related to the outcome *via* confounders; and third, the SNPs should not impact the outcome directly. Earlier research detailed further particulars of this method (19). The STROBE-MR guidelines were used to design this research (20). Figure 1 shows the flowchart of the MR study between gut microbiota and metabolites with liver diseases.

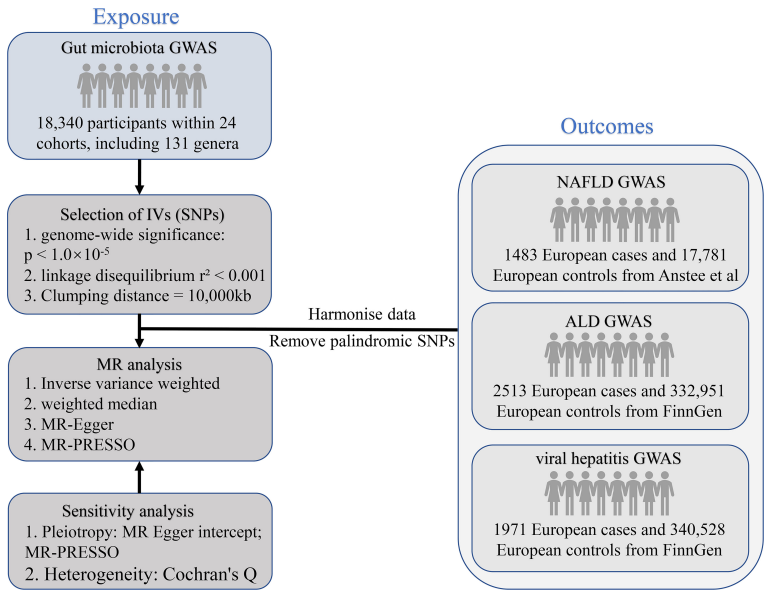

FIGURE 1
The study design of the present MR study of the associations of gut microbiota and metabolites and liver diseases. MR, Mendelian randomization; SNP, single nucleotide polymorphism; NAFLD, non-alcoholic fatty liver disease; ALD, alcoholic liver disease; GWAS, genome-wide association study; IV, instrumental variables.

2.2 Exposure sources

Genetic instruments of intestinal microbiome were acquired from the largest genome-wide meta-analysis published by the MiBioGen consortium (21). The study contained 24 cohorts with 18,340 individuals, most of whom were of European ancestry (16 cohorts, N = 13,266). The study targeted variable regions V4, V3–V4, and V1–V2 of the 16S rRNA gene to profile the microbial composition and to conduct taxonomic classification using direct taxonomic binning. For each cohort, microbiota quantitative trait loci (mbQTL) mapping analysis included only the taxa presented in > 10% of the samples (21). The lowest taxonomic level in this study was genus, and 131 genera with a mean abundance > 1% were found, including 12 unknown genera (21). Thus, 119 genus-level taxa were obtained in our study for MR analysis. The included cohorts all made adjustments for sex and age as covariates in their calculations (21).

We also used summary-level data from the human metabolome GWAS performed among subjects of European descent (TwinsUK and KORA, N = 7824) in light of the significant roles gut metabolites play in microbiota-host interaction (22). Then we utilized HMDB (23) to acquire a list of 12 gut microbiota-derived metabolite traits from all the measured metabolites in the GWAS, such as betaine, carnitine, cholate, choline, alanine, phenylacetate, phenyllactate, stachydrine, threonate, and ursodeoxycholate.

2.3 Outcome sources

The genetic association with NAFLD was extracted from the newly published GWAS summary statistics by Anstee et al., consisting of 1483 European cases and 17,781 European controls (24). The top 5 genetic principal components and genotyping batch were corrected during the analysis (24). GWAS summary-level data for ALD (2513 European cases and 332,951 European controls) and viral hepatitis (1971 European cases and 340,528 European controls) were downloaded from FinnGen consortium R8 release data (25). During the analysis, age, sex, the first 10 principal components, and the genotyping batch were corrected (25).

2.4 Genetic instrument selection

To satisfy the above MR assumption, we selected IVs with linkage disequilibrium $r^2 < 0.001$ and distance > 10,000 kb and attaining genome-wide significance ($p < 1.0\times10^{-5}$) (26). The linkage disequilibrium reference panel was established utilizing the 1000 Genomes Project European sample (27). Each IV's strength was determined utilizing the F statistics = $beta^2/se^2$ (28). For adequate strength to be determined, the F-statistics had to be >10.

2.5 Statistical analysis

The primary statistical analysis method was the inverse variance weighted (IVW) method under random effects. This method was supplemented with weighted median analysis (29), MR-Egger regression (30), and MR-PRESSO methods (31). IVW assumes that all genetic variation SNPs are valid IVs with an overall bias of zero. As for the weighted median analysis, this estimate is consistent even if up to half of the weights are from invalid

instruments. Besides, MR-Egger analysis can identify horizontal pleiotropy through the intercept ($p < 0.05$ for the intercept indicates pleiotropy) (30). The MR-PRESSO method can detect possible outliers and generate causal estimates after the removal of outlying IVs (31). To measure the degree of heterogeneity, the Q-value from Cochrane was applied. The causal relationship is considered significant if: 1) the *p*-value of the IVW method is less than < 0.05; 2) the estimations obtained using the MR-Egger, weighted median, and IVW methods all have the same direction; and 3) neither the MR-Egger intercept test nor the MR-PRESSO global test has statistical significance ($p > 0.05$) (32). Furthermore, in addition to meeting the 3 conditions mentioned above, for the connection between gut microbiota or metabolites and liver diseases, a Bonferroni-adjusted IVW *p* (*p*FDR) value of 4.2×10^{-5} ($p = 0.05/119$) or 5×10^{-4} (P = 0.05/10) was employed as the cut-off for statistical significance. $p < 0.05$ but more than the Bonferroni corrected significance level was seen as suggestive of evidence for a potential association (33, 34). Each test was two-sided and conducted utilizing the TwoSampleMR and MR-PRESSO packages in the R software (version 4.2.1) (31, 35).

3 Results

3.1 Causal effect of gut microbiota on NAFLD

The results of IVW analyses demonstrated that *Anaerotruncus* (OR = 0.595, 95% CI: 0.378-0.937, $p = 0.0249$), *Intestinimonas* (OR = 0.726, 95% CI: 0.550-0.958, $p = 0.0237$), *Lachnoclostridium* (OR = 0.523, 95% CI: 0.297-0.920, $p = 0.0245$), *Lachnospiraceae NC2004 group* (OR = 0.676, 95% CI: 0.505-0.904, $p = 0.0083$), *Olsenella* (OR = 0.770, 95% CI: 0.623-0.953, $p = 0.0163$), and *Peptococcus* (OR = 0.817, 95% CI: 0.669-0.998, $p = 0.0472$) were negatively associated with NAFLD (Table 1 and Figure 2), indicating a protective impact of the above genera on NAFLD (Table 1). The results of IVW analyses revealed that *Ruminococcus 1* (OR = 1.833, 95% CI: 1.142-2.940, $p = 0.0120$) was positively related to NAFLD, suggesting a detrimental effect on NAFLD (Table 1 and Figure 2). These associations were also supported by the MR-PRESSO method, as shown in Table 1. Besides, the MR estimates of the weighted median analysis showed similar results in *Anaerotruncus* (OR = 0.519, 95%

TABLE 1 Association of genetically predicted gut microbiota with non-alcoholic fatty liver disease.

Methods	IVs	OR	95% CI	*p* value	Egger intercept, *p* value	Heterogeneity (Q, *p* value)	MR-PRESSO (Global test *p* value)
Anaerotruncus							
IVW	13	0.595	0.378-0.937	**0.0249**	-0.003, 0.952	11.377, 0.497	0.494
Weighted median	13	0.519	0.273-0.985	**0.0449**			
MR-Egger	13	0.625	0.119-3.301	0.5914			
MR-PRESSO	13	0.595	0.382-0.926	**0.0400**			
Intestinimonas							
IVW	17	0.726	0.550-0.958	**0.0237**	0.010, 0.761	18.312, 0.306	0.337
Weighted median	17	0.787	0.544-1.140	0.2049			
MR-Egger	17	0.651	0.308-1.376	0.2785			
MR-PRESSO	17	0.726	0.550-0.958	**0.0380**			
Lachnoclostridium							
IVW	13	0.523	0.297-0.920	**0.0245**	-0.087, 0.234	18.982, 0.089	0.107
Weighted median	13	0.429	0.225-0.816	**0.0099**			
MR-Egger	13	1.893	0.237-15.130	0.5593			
MR-PRESSO	13	0.523	0.297-0.920	**0.0441**			

(Continued)

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TABLE 1 Continued

Methods	IVs	OR	95% CI	*p* value	Egger intercept, *p* value	Heterogeneity (Q, *p* value)	MR-PRESSO (Global test *p* value)
Lachnospiraceae NC2004 group							
IVW	9	0.676	0.505-0.904	**0.0083**	-0.046, 0.542	7.121, 0.524	0.464
Weighted median	9	0.694	0.455-1.058	0.0891			
MR-Egger	9	0.998	0.290-3.466	0.9967			
MR-PRESSO	9	0.676	0.514-0.889	**0.0233**			
Olsenella							
IVW	11	0.770	0.623-0.953	**0.0163**	-0.060, 0.405	4.987, 0.892	0.897
Weighted median	11	0.743	0.562-0.982	**0.0370**			
MR-Egger	11	1.257	0.410-3.854	0.6979			
MR-PRESSO	11	0.770	0.663-0.895	**0.0067**			
Peptococcus							
IVW	12	0.817	0.669-0.998	**0.0472**	0.023, 0.690	14.159, 0.224	0.265
Weighted median	12	0.941	0.736-1.203	0.6284			
MR-Egger	12	0.683	0.283-1.646	0.4156			
MR-PRESSO	12	0.817	0.669-0.998	**0.0472**			
Ruminococcus 1							
IVW	10	1.833	1.142-2.940	**0.0120**	-0.023, 0.670	7.555, 0.580	0.897
Weighted median	10	1.800	0.920-3.523	0.0862			
MR-Egger	10	2.435	0.635-9.332	0.2305			
MR-PRESSO	10	1.833	1.188-2.826	**0.0228**			

IV, instrumental variables; OR, Odd Ratio; IVW, inverse variance weighted; Bold represents $p < 0.05$.

CI: 0.273-0.985, $p = 0.0449$), *Lachnoclostridium* (OR = 0.429, 95% CI: 0.225-0.816, $p = 0.0099$), and *Olsenella* (OR = 0.743, 95% CI: 0.562-0.982, $p = 0.0370$) (Table 1 and Figure 2). Whereas, for *Lachnoclostridium* and *Olsenella*, MR-Egger analysis results were in the opposite direction to IVW and weighted median analysis results (Table 1 and Figure 2). Detailed statistics for the remaining genera are shown in Table S1. We do not find significant heterogeneity across these results using Cochrane Q statistics (Table 1). The estimation of the intercept that was generated from the MR-Egger regression was centered around 0 and did not offer definitive evidence of horizontal pleiotropy (Table 1). No outliers were found by MR-PRESSO. The average F-statistic was 21.386, ranging from 17.045 to 28.784, revealing that there was no weak IV bias (Table S2).

3.2 Causal effect of gut microbiota on ALD

In the IVW method, we found that the genetically predicted higher relative abundance of three genera, *Lachnospira* (OR = 0.568, 95% CI: 0.332-0.971, $p = 0.0388$), *Desulfovibrio* (OR = 0.744, 95% CI: 0.574-0.964, $p = 0.0252$), and *Ruminococcus torques group* (OR

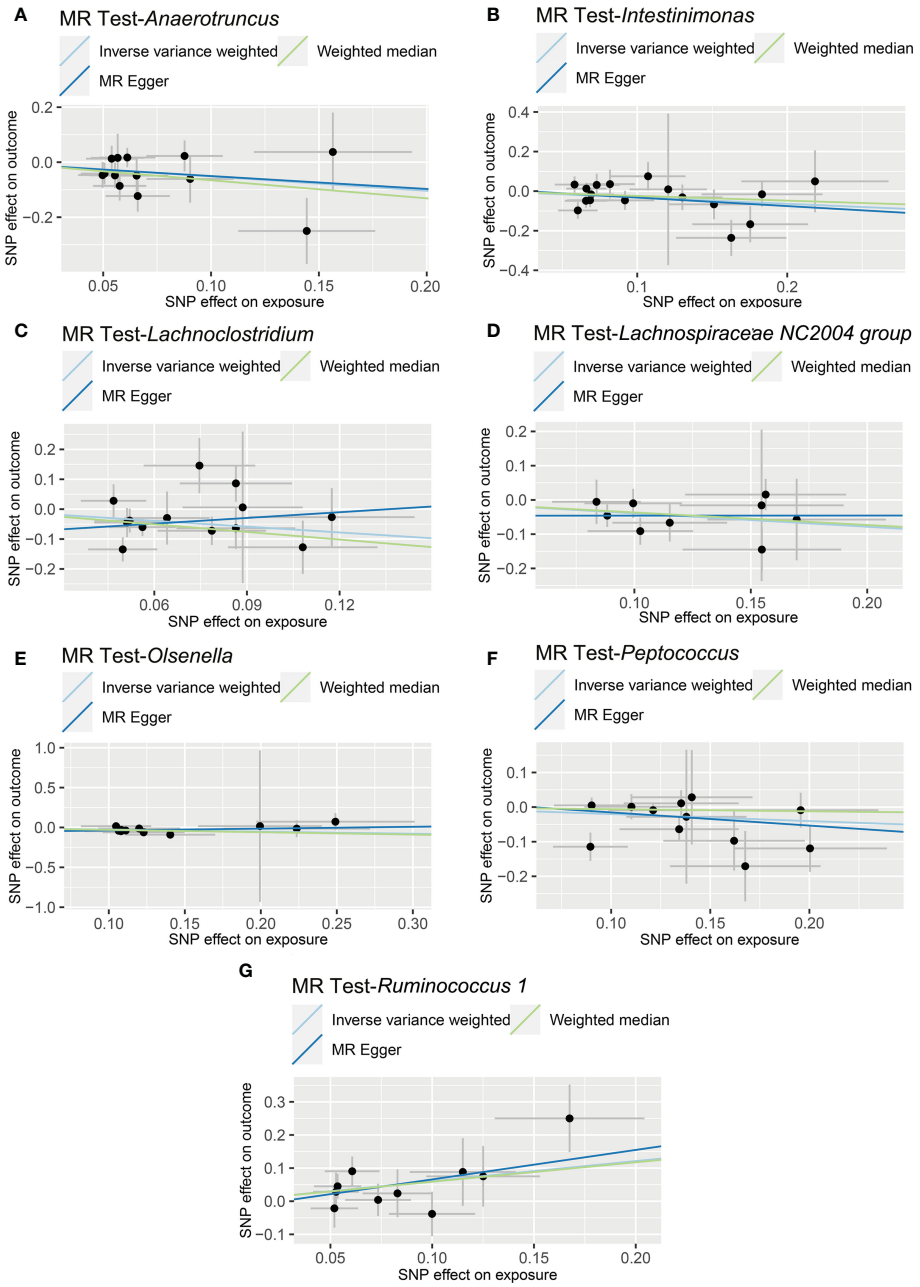

FIGURE 2
Causal relationship between gut microbiota and the risk of non-alcoholic fatty liver disease. Each point represents the SNP effects on *Anaerotruncus* **(A)**, *Intestinimonas* **(B)**, *Lachnoclostridium* **(C)**, *Lachnospiraceae NC2004 group* **(D)**, *Olsenella* **(E)**, *Peptococcus* **(F)**, *Ruminococcus 1* **(G)** and *non-alcoholic fatty liver disease*. MR, Mendelian randomization; SNP, single nucleotide polymorphism.

= 0.621, 95% CI: 0.398-0.970, p = 0.0364), was correlated with a lower risk of ALD (Table 2 and Figure 3); while, the genetically predicted *Ruminococcaceae UCG 002* level was associated with a higher risk of ALD (OR = 1.263, 95% CI: 1.014-1.572, p = 0.0371) (Table 2 and Figure 3). The results of the MR-PRESSO analysis were similar to those of the IVW method (Table 2). The IVW test, weighted median method, and MR-Egger test were all in the same direction, which strengthened the confidence in the true causal associations. Detailed statistics for the remaining genera are shown in Table S3. No significant heterogeneity was observed across these results (Table 2). MR-Egger regression confirmed that there was no horizontal pleiotropy between IVs and outcomes (Table 2). Moreover, neither outliers nor any indication of pleiotropy were observed in the MR-PRESSO analysis (Table 2). The F-statistics of IVs ranged between 18.53 and 31.28, indicating no evidence of weak instrument bias (Table S4).

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TABLE 2 Association of genetically predicted gut microbiota with alcoholic liver disease.

Methods	IVs	OR	95% CI	*p* value	Egger intercept, *p* value	Heterogeneity (Q, *p* value)	MR-PRESSO (Global test *p* value)
Ruminococcaceae UCG 002							
IVW	20	1.263	1.014-1.572	**0.0371**	-0.030, 0.196	13.997, 0.784	0.797
Weighted median	20	1.337	0.980-1.824	0.0670			
MR-Egger	20	1.812	1.024-3.206	0.0561			
MR-PRESSO	20	1.263	1.046-1.524	**0.0252**			
Lachnospira							
IVW	6	0.568	0.332-0.971	**0.0388**	0.149, 0.148	6.680, 0.246	0.278
Weighted median	6	0.672	0.355-1.273	0.2227			
MR-Egger	6	0.049	0.003-0.745	0.0956			
MR-PRESSO	6	0.568	0.332-0.971	**0.0454**			
Desulfovibrio							
IVW	10	0.744	0.574-0.964	**0.0252**	0.025, 0.532	4.043, 0.906	0.907
Weighted median	10	0.772	0.553-1.077	0.1272			
MR-Egger	10	0.584	0.271-1.260	0.2076			
MR-PRESSO	10	0.744	0.625-0.885	**0.0087**			
Ruminococcus torques group							
IVW	7	0.621	0.398-0.970	**0.0364**	0.006, 0.910	1.726, 0.943	0.945
Weighted median	7	0.571	0.319-1.022	0.0592			
MR-Egger	7	0.574	0.146-2.259	0.4633			
MR-PRESSO	7	0.621	0.489-0.789	**0.0080**			

IV, instrumental variables; OR, Odd Ratio; IVW, inverse variance weighted; Bold represents $p < 0.05$.

3.3 Causal effect of gut microbiota on viral hepatitis

As shown in Figure 4 and Table 3, we observed that *Alistipes* (OR = 1.720, 95% CI: 1.160-2.550, $p = 0.0069$) and *Ruminococcaceae NK4A214 group* (OR = 1.460, 95% CI: 1.063-2.006, $p = 0.0195$) were related to a higher risk of viral hepatitis. The results of the MR-PRESSO analysis supported the above findings. Detailed statistics for the remaining genera are shown in Table S5. None of the MR-Egger regression intercepts deviated from null, and no outliers were detected with the MR-PRESSO test, suggesting no evidence of horizontal pleiotropy (Table 3). Besides, the F statistic was larger than 10, and the Cochrane Q statistic results revealed no significant heterogeneity (Tables 3, S6).

3.4 Causal effect of gut microbiota-derived metabolites on liver diseases

As shown in Tables 4, S7, and Figure 5, alanine (OR = 19.586, 95% CI: 2.206-173.934, $p = 0.0076$) and phenyllactate (OR = 0.212, 95% CI: 0.065-0.689, $p = 0.0100$) were found to be negatively

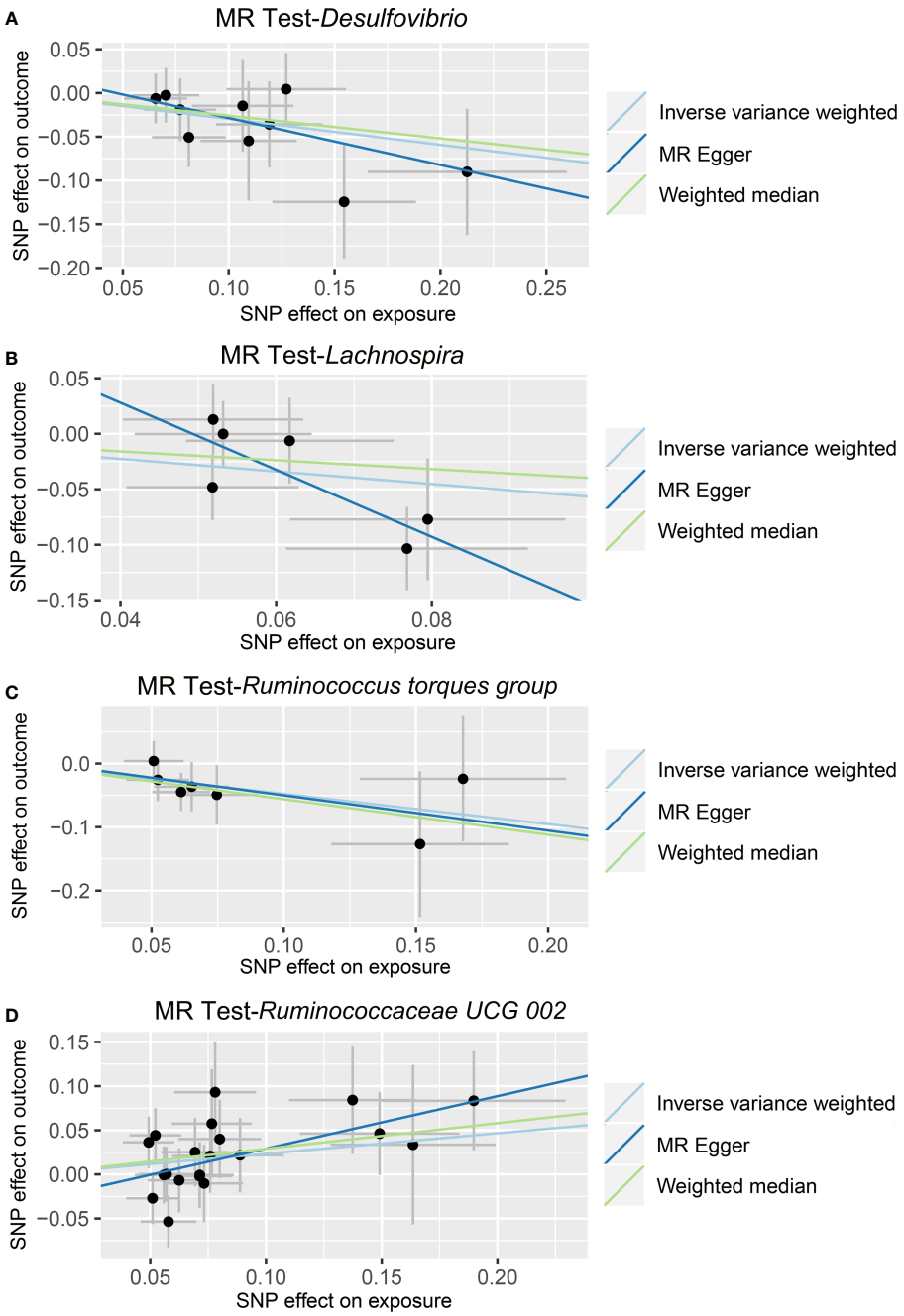

FIGURE 3
Causal relationship between gut microbiota and the risk of alcoholic liver disease. Each point represents the SNP effects on *Desulfovibrio* **(A)**, *Lachnospira* **(B)**, *Ruminococcus torques group* **(C)**, *Ruminococcaceae UCG 002* **(D)**, and alcoholic liver disease. MR, Mendelian randomization; SNP, single nucleotide polymorphism.

correlated with NAFLD, while stachydrine (OR = 2.228, 95% CI: 1.109-4.474, p = 0.0244) was found to be positively associated with NAFLD in the IVW and MR-PRESSO methods. The IVW estimate indicated that phenylacetate (OR = 0.496, 95% CI: 0.258-0.953, p = 0.0353) and ursodeoxycholate (OR = 0.662, 95% CI: 0.476-0.921, p = 0.0144) had a protective effect on ALD; while threonate (OR = 1.570, 95% CI: 1.028-2.397, p = 0.0370) exerts a detrimental influence on ALD (Tables 5, S8, and Figure 6). Besides, the IVW estimate of alanine (OR = 3.348, 95% CI: 1.052-10.655, p = 0.0408) and cholate (OR = 1.560, 95% CI: 1.046-2.327, p = 0.0293) showed its suggestive harmful effect against viral hepatitis; and threonate (OR = 0.621, 95% CI: 0.385-0.971, p = 0.0401) displayed its suggestive protective effect against viral hepatitis (Tables 6, S9, and Figure 7). No heterogeneity and horizontal pleiotropy were observed in these analyses (Tables 4–6). The F-statistics of IVs ranged between 17.64 and 88.97 (Tables S10–12).

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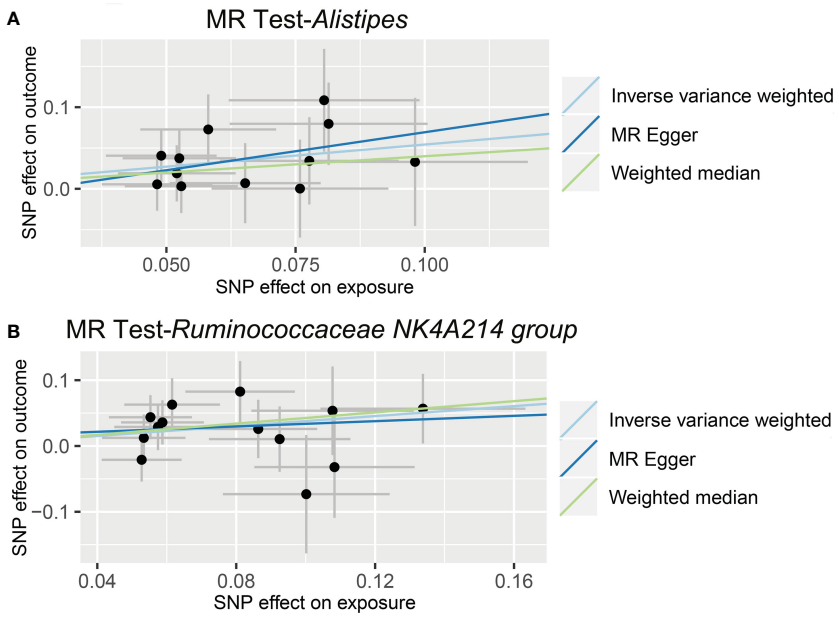

FIGURE 4
Causal relationship between gut microbiota and the risk of viral hepatitis. Each point represents the SNP effects on *Alistipes* **(A)**, *Ruminococcaceae NK4A214 group* **(B)**, and viral hepatitis. MR, Mendelian randomization; SNP, single nucleotide polymorphism.

4 Discussion

According to our knowledge, this study is the first to estimate the causal relationships between gut microbiota, gut microbiota-derived metabolites, and liver diseases using MR analysis. Our results revealed that multiple gut microbiota and metabolites play significant roles in the development of liver diseases, 5 suggestive microbial taxa (*Anaerotruncus*, *Intestinimonas*, *Lachnospiraceae NC2004 group*, *Peptococcus*, and *Ruminococcus 1*) and 3 suggestive metabolites (*Alanine*, *Phenyllactate*, and *Stachydrine*) in NAFLD, 4 suggestive microbial taxa (*Ruminococcaceae UCG 002*, *Lachnospira*, *Desulfovibrio*, and *Ruminococcus torques group*)

TABLE 3 Association of genetically predicted gut microbiota with viral hepatitis.

Methods	IVs	OR	95% CI	*p* value	Egger intercept, *p* value	Heterogeneity (Q, *p* value)	MR-PRESSO (Global test *p* value)
Alistipes							
IVW	12	1.720	1.160-2.550	**0.0069**	-0.024, 0.686	4.704, 0.920	0.951
Weighted median	12	1.490	0.891-2.494	0.1288			
MR-Egger	12	2.538	0.390-16.521	0.3527			
MR-PRESSO	12	1.720	1.330-2.225	**0.0017**			
Ruminococcaceae NK4A214 group							
IVW	13	1.460	1.063-2.006	**0.0195**	0.014, 0.735	7.206, 0.844	0.869
Weighted median	13	1.531	1.004-2.335	**0.0479**			
MR-Egger	13	1.224	0.429-3.493	0.7134			
MR-PRESSO	13	1.460	1.142-1.868	**0.0108**			

IV, instrumental variables; OR, Odd Ratio; IVW, inverse variance weighted; Bold represents $p < 0.05$.

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TABLE 4 Association of genetically predicted gut microbiota derived metabolites with non-alcoholic fatty liver disease.

Methods	IVs	OR	95% CI	*p* value	Egger intercept, *p* value	Heterogeneity (Q, *p* value)	MR-PRESSO (Global test *p* value)
Alanine							
IVW	33	19.586	2.206-173.934	**0.0076**	-0.005 0.896	66.480, 0.001	0.067
Weighted median	33	3.814	0.289-50.406	0.3095			
MR-Egger	33	33.147	0.010-110573	0.4041			
MR-PRESSO	33	19.584	2.206-173.934	**0.0118**			
Phenyllactate							
IVW	17	0.212	0.065-0.689	**0.0100**	-0.006, 0.873	22.769, 0.120	
Weighted median	17	0.384	0.093-1.588	0.1863			0.183
MR-Egger	17	0.289	0.005-15.218	0.5486			
MR-PRESSO	17	0.212	0.065-0.689	**0.0203**			
Stachydrine							
IVW	6	2.228	1.109-4.474	**0.0244**	-0.052, 0.237	13.221, 0.104	
Weighted median	6	2.211	0.898-5.444	0.0843			0.454
MR-Egger	6	3.148	0.228-43.387	0.4398			
MR-PRESSO	6	2.228	1.109-4.474	**0.0342**			

IV, instrumental variables; OR, Odd Ratio; IVW, inverse variance weighted; Bold represents $p < 0.05$.

and 3 suggestive metabolites (*Phenylacetate*, *Threonate*, and *Ursodeoxycholate*) in ALD, 2 suggestive microbial taxa (*Alistipes* and *Ruminococcaceae NK4A214 group*) and 3 suggestive metabolites (*Alanine*, *Cholate*, and *Threonate*) in viral hepatitis. Notably, the MR test *p* values for both gut microbiota and metabolites and liver diseases were greater than *p*FDR.

Anaerotruncus and *Intestinimonas* were revealed to be butyrate-producing bacterium in the intestine (36–39). *Intestinimonas* is generally recognized as beneficial bacteria with anti-inflammatory and anti-obesity properties (40). Rodriguez-Diaz et al. (41) found a significant decrease in the abundance of *Intestinimonas* in patients with NAFLD compared to the healthy population. Supplementation with Adzuki beans has been shown to significantly reduce high-fat diet-induced obesity and lipid accumulation, as well as lipopolysaccharide levels, and alleviate liver function impairment and hepatic steatosis (42). Besides, it significantly reversed the imbalance of gut microbiota caused by high-fat diets and significantly increased the abundance of *Lachnoclostridium* (42). As for *Olsenella*, Zhong et al. showed that probiotic-fermented blueberry juice significantly reduced low-density lipoprotein cholesterol levels and fat accumulation, ameliorated insulin resistance, and improved the abundance and diversity of intestinal microbial communities in high-fat diet mice (43). The blueberry juice-treated mouse showed a relatively high abundance of lean bacteria (*Olsenella* and *Bifidobacterium*) and a lower abundance of obesity-associated bacteria (*Oscillibacter* and *Alistipes*) compared to the high-fat diet-fed mouse (43). Interestingly, Li et al. revealed that the gut formation of propionic acid and acetic acid is related to an increase in *Olsenella* in pectin-fed mice (44). Recently, Pan et al. diagnosed 21 chronic hepatitis B and 42 NAFLD patients with the classic damp-heat (DH) syndrome group and identified 29 chronic hepatitis B and 28 NAFLD patients as the non-DH syndrome group. They found a decreased relative abundance of the *Lachnospiraceae NC2004 group* in patients with the DH syndrome compared to the non-DH syndrome (45). Taken together, these studies were in agreement with our MR analysis that this aforementioned genus plays a protective role in NAFLD. In contrast, Pung et al. demonstrated that Ulva prolifera polysaccharide greatly slowed high-fat diet-induced weight gain, ameliorated metabolic disturbances in high-fat diet-fed mice, and improved intestinal flora disorders, as evidenced by the growth in *Bifidobacterium* abundance and downregulation of *Ruminococcus 1* abundance (46). This implies that *Ruminococcus 1* may play a negative role in NAFLD.

Alistipes is mainly found in the intestines of healthy humans (47, 48). However, *Alistipes* has also been isolated from the bloodstream, appendiceal, and abdominal, highlighting its possible opportunistic pathogenic involvement in human

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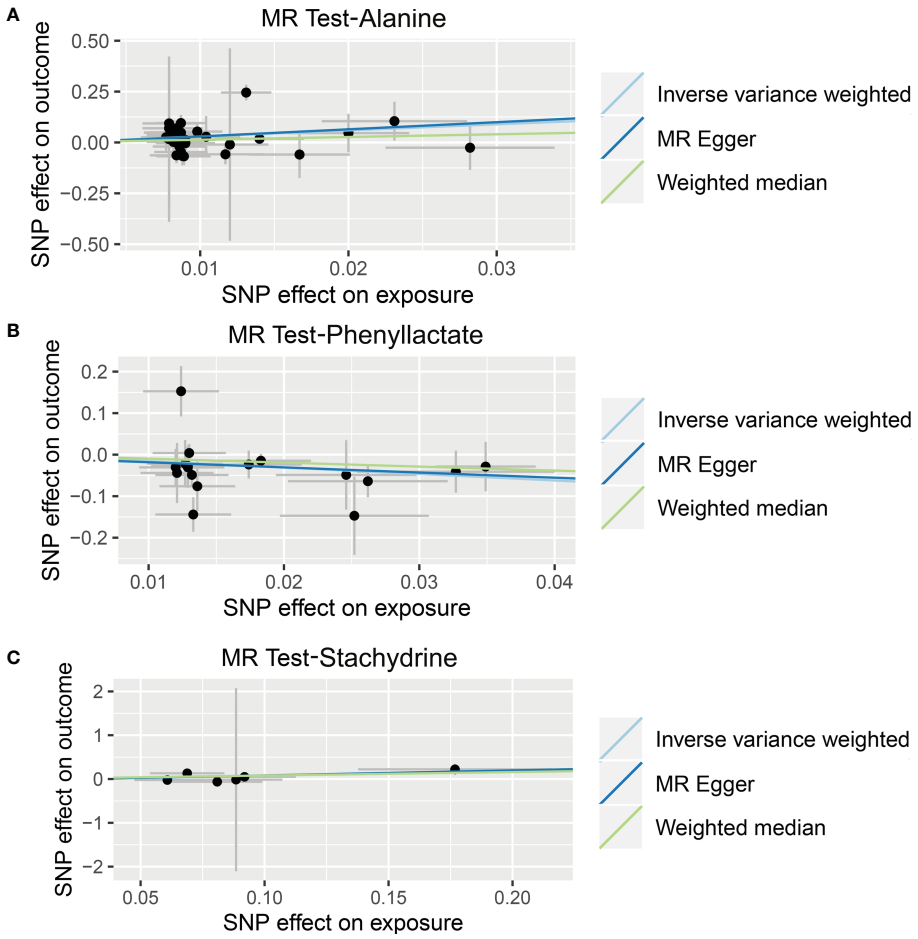

FIGURE 5
Causal relationship between gut microbiota-derived metabolites and the risk of non-alcoholic fatty liver disease. Each point represents the SNP effects on Alanine **(A)**, Phenyllactate **(B)**, Stachydrine **(C)**, and non-alcoholic fatty liver disease. MR, Mendelian randomization; SNP, single nucleotide polymorphism.

TABLE 5 Association of genetically predicted gut microbiota derived metabolites with alcoholic liver disease.

Methods	IVs	OR	95% CI	*p* value	Egger intercept, *p* value	Heterogeneity (Q, *p* value)	MR-PRESSO (Global test *p* value)
Phenylacetate							
IVW	9	0.496	0.258-0.953	**0.0353**	0.025, 0.274	4.928, 0.765	0.656
Weighted median	9	0.399	0.166-0.958	**0.0399**			
MR-Egger	9	0.335	0.133-0.841	0.0526			
MR-PRESSO	9	0.496	0.297-0.828	**0.0278**			
Threonate							
IVW	18	1.570	1.028-2.397	**0.0370**	-0.013, 0.333	6.562, 0.989	
Weighted median	18	1.885	1.018-3.490	**0.0436**			0.972

(Continued)

TABLE 5 Continued

Methods	IVs	OR	95% CI	*p* value	Egger intercept, *p* value	Heterogeneity (Q, *p* value)	MR-PRESSO (Global test *p* value)
MR-Egger	18	1.965	1.066-3.619	**0.0457**			
MR-PRESSO	18	1.570	1.206-2.042	**0.0037**			
Ursodeoxycholate							
IVW	11	0.662	0.476-0.921	**0.0144**	-0.004, 0.851	4.814, 0.903	
Weighted median	11	0.696	0.425-1.137	0.1480			0.931
MR-Egger	11	0.693	0.392-1.224	0.2381			
MR-PRESSO	11	0.662	0.527-0.833	**0.0055**			

IV, instrumental variables; OR, Odd Ratio; IVW, inverse variance weighted; Bold represents $p < 0.05$.

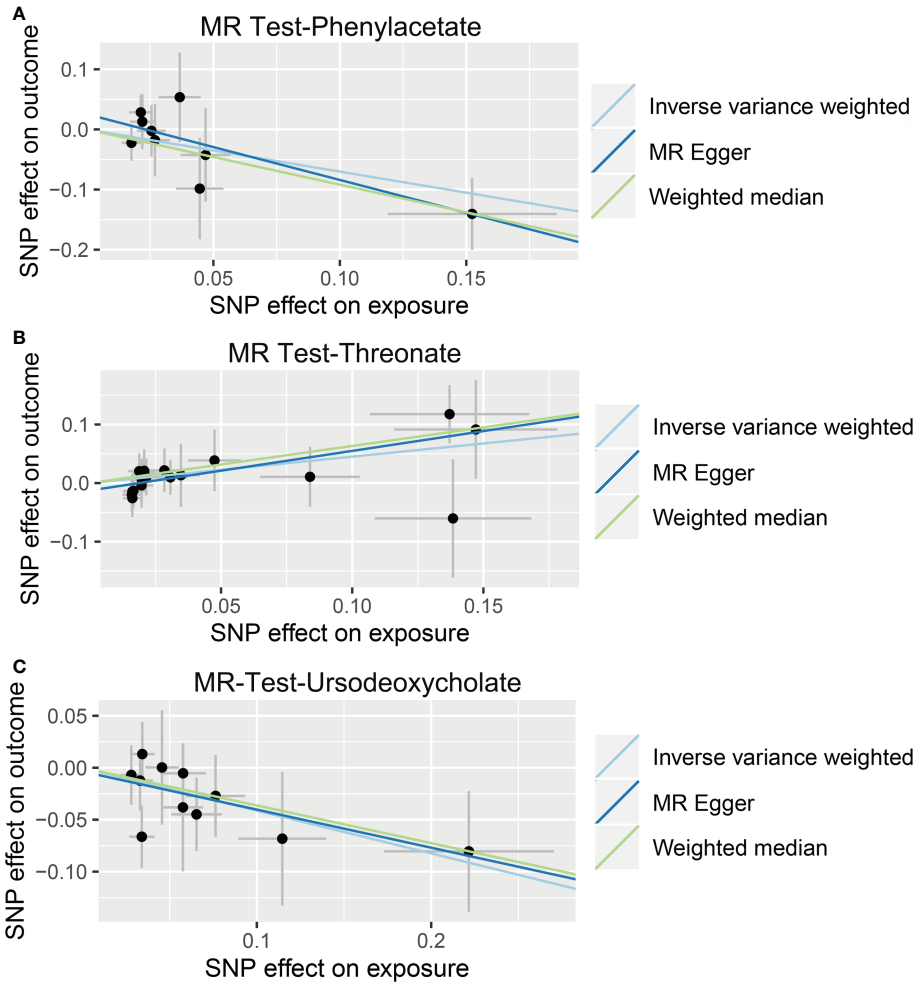

FIGURE 6
Causal relationship between gut microbiota-derived metabolites and the risk of alcoholic liver disease. Each point represents the SNP effects on Phenylacetate **(A)**, Threonate **(B)**, Ursodeoxycholate **(C)**, and alcoholic liver disease. MR, Mendelian randomization; SNP, single nucleotide polymorphism.

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TABLE 6 Association of genetically predicted gut microbiota derived metabolites with viral hepatitis.

Methods	IVs	OR	95% CI	*p* value	Egger intercept, *p* value	Heterogeneity (Q, *p* value)	MR-PRESSO (Global test *p* value)
Alanine							
IVW	37	3.348	1.052-10.655	**0.0408**	0.021, 0.323	31.059, 0.703	0.719
Weighted median	37	3.948	0.783-19.904	0.0962			
MR-Egger	37	1.098	0.005-29.948	0.6788			
MR-PRESSO	37	3.348	1.142-9.812	**0.0341**			
Cholate							
IVW	9	1.560	1.046-2.327	**0.0293**	-0.023, 0.475	14.834, 0.062	
Weighted median	9	1.291	0.798-2.086	0.2976			0.135
MR-Egger	9	1.989	0.936-4.223	0.1168			
MR-PRESSO	9	1.560	1.046-2.327	**0.0410**			
Threonate							
IVW	18	0.621	0.385-0.971	**0.0401**	-0.012, 0.406	10.826, 0.865	
Weighted median	18	0.709	0.346-1.453	0.3472			0.883
MR-Egger	18	0.769	0.387-1.528	0.4648			
MR-PRESSO	18	0.621	0.424-0.908	**0.0249**			

IV, instrumental variables; OR, Odd Ratio; IVW, inverse variance weighted; Bold represents $p < 0.05$.

disorders (48). Feng et al. found that *Alistipes* could promote the development of colorectal cancer *via* the interleukin-6/signal transducer and activator of transcription 3 pathway (49). As for *Ruminococcaceae UCG 002*, there was significant enrichment of *Ruminococcaceae UCG 002* abundance in prostate cancer patients compared to the healthy population, suggesting a pathogenic role (50). These studies support our conclusions. We found that *Ruminococcaceae UCG 002* and *Alistipes* play a pathogenic role in ALD and viral hepatitis, respectively.

Lachnospira was significantly lower in all disease cohorts (multiple sclerosis, inflammatory bowel disease, and rheumatoid arthritis) relative to healthy controls (51). Due to its reduced abundance, studies suggest that *Lachnospira* may have a protective effect under inflammatory conditions (51, 52). *Desulfovibrio* was negatively related to the host body mass index, waist, triglyceride, and uric acid, which are signs of obesity or metabolic disorders (53–56). The abundance of *Desulfovibrio* was positively related to the diversity of flora, favoring microbiome stability and host health (57, 58). Besides, *Desulfovibrio* was positively correlated with the beneficial bacteria *Oscillospira*, *Phascolarctobacterium*, *Prevotella*, *Coprococcus*, *Dialister*, *Ruminococcus*, *Akkermansia*, *Roseburia*, *Faecalibacterium*, and *Bacteroides* and negatively correlated with the harmful bacteria *Streptococcus*, *Clostridium*, *Escherichia*, *Klebsiella*, and *Ralstonia* (59–68). Previous studies have shown a positive correlation between the *Ruminococcus torques group* and short-chain fatty acid levels by studying some people who ingested less starch in order to lose weight (69). Recently, Wan et al. found that improvement in colitis was associated with a higher *Ruminococcus torques group*, suggesting that the *Ruminococcus torques group* may have another application as a potential probiotic in the anti-inflammatory response (70). The above studies revealed their beneficial role in human diseases and supported our findings.

This work also has some limitations. First, because only people of European heritage were included in the GWAS, the conclusions of this study might not apply to people of other racial or ethnic backgrounds. Second, the sequencing of the 16S rRNA genes only permitted resolution from the genus to the phylum level, not at a more specific level, and the results were skewed when certain

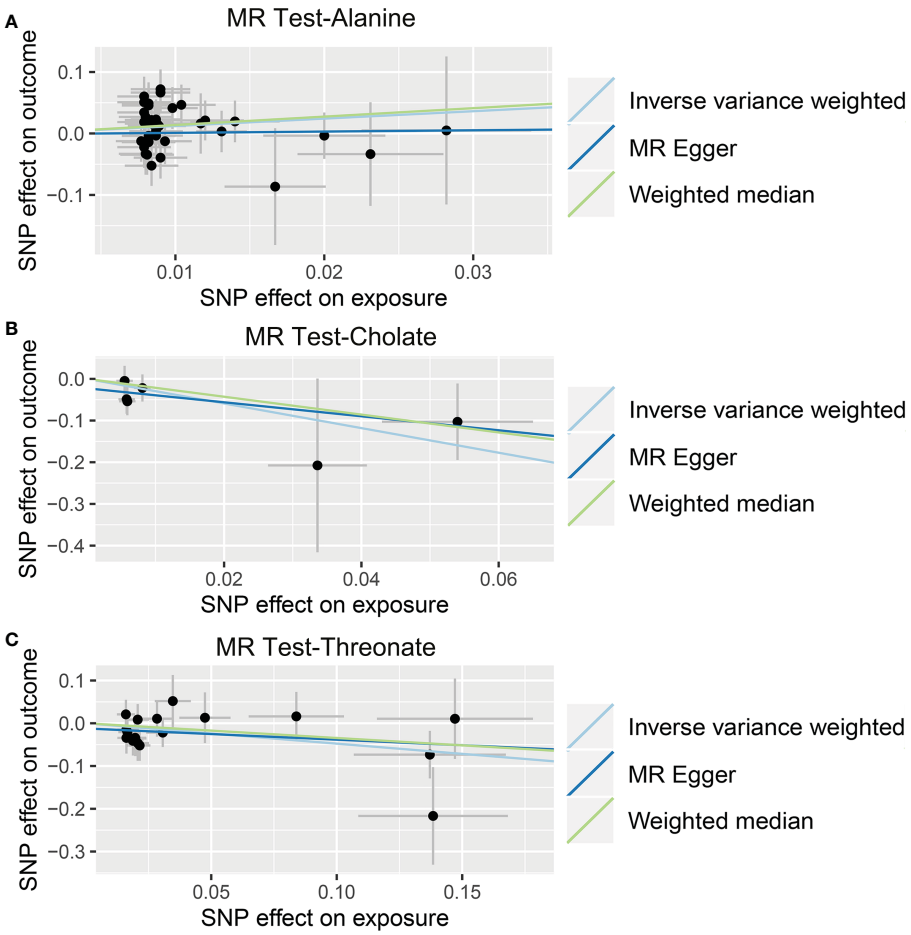

FIGURE 7
Causal relationship between gut microbiota-derived metabolites and the risk of viral hepatitis. Each point represents the SNP effects on Alanine **(A)**, Cholate **(B)**, Threonate **(C)**, and viral hepatitis. MR, Mendelian randomization; SNP, single nucleotide polymorphism.

specific species affected the risk of liver diseases. Third, our results are not significant after the Bonferroni adjustment. However, multiple statistical corrections may overlook GM taxa with a potential causal connection to liver diseases because they are excessively tight and cautious. Furthermore, although the Mendelian randomization analysis was comparable to the level of evidence from the RCT study, further animal experimental confirmation is necessary.

5 Conclusion

In conclusion, our research supported causal links between the gut microbiome and its metabolites and NAFLD, ALD, and viral hepatitis. It is necessary to conduct further population-based research on the potential mechanisms of gut microbiota and liver disease development.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

LilZ, LiuZ, TK, PW, RL, and WW conceived and designed the study. LilZ, LiuZ, TK, ZQ, KW, ZW, and LL were responsible for the collection and assembly of data, data analysis, interpretation, and writing the manuscript. RL, PW, and WW revised the manuscript. All the work was performed under RL, PW, and WW instructions. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1159148/full#supplementary-material

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