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Article

Genetic Evidence Elucidates the Effect of Gut Microbiota on Diverticular Disease: A Bidirectional Mendelian Randomization Study

Pan Jiang ^{1,†}, Wenjing Yang ^{1,†}, Xianghan Cao ¹, Yushu Cao ¹, Yanfei Fang ¹, Hong Sheng ^{2,*‡} and Kan Wang ^{1,3,*‡}

¹ Department of Gastroenterology, Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou, 310016, China

² Department of gastroenterology, First People's Hospital of Xiangshan County, Ningbo, 315700, China

³ Inflammatory bowel disease center, Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou, 310016, China

[†] Pan Jiang and Wenjing Yang contributed equally to this work.

[‡] Hong Sheng and Kan Wang jointly supervised this work.

* Correspondence: Hong Sheng, Doctor, Department of gastroenterology, First People's Hospital of Xiangshan County, Ningbo, 315700, China. Email: xssh8086@163.com; Kan Wang, Doctor, Department of Gastroenterology, Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou, 310016, China. Email: paradisoj@zju.edu.cn

Abstract: Background: Observational studies have implicated the association between gut microbiota and diverticular disease. However, the causality remains unclear. We aim to evaluate the causal association of gut microbiota alterations and circulating metabolite level with the risk of diverticular disease. **Methods:** A bidirectional two-sample Mendelian randomization (MR) was conducted by using GWAS summary-level data of gut microbiota, circulating metabolites, and diverticular disease. The inverse-variance weighted (IVW) MR approach was applied as the primary analysis. The MR-Egger, weighted-median, and MR-PRESSO methods were supplemented as sensitivity analyses if applicable. The false discovery rate was adopted for multiple testing corrections. **Results:** In the forward MR analysis, a genetically determined higher abundance of *Ruminococcus torques* group was found to be causally associated with decreased risk of diverticular disease. With each one-unit increase in log-transformed relative abundance values of the *Ruminococcus torques* group, there was a significant decrease in the odds ratio (OR) and 95% confidence interval (CI) for diverticular disease risk, with an OR of 0.68 (95% CI: 0.56-0.82, $P=4.72 \times 10^{-5}$). A genetically determined increased abundance of *Oxalobacter* (OR=0.88, 95%CI: 0.80-0.97, $P=0.011$) was suggestively associated with a lower risk of diverticular disease. We did not find any significant association between circulating metabolites level and diverticular disease risk. Additionally, no association between genetic liability to diverticular disease and the relative abundance of microbiota was observed in reverse MR analysis. **Conclusions:** This study provides evidence for a causal association between genetically determined higher abundance of the *Ruminococcus torques* group and decreased risk of diverticular disease. We also suggest a potential protective role of *Oxalobacter* on diverticular disease.

Keywords: Gut microbiota; circulating metabolites; diverticular disease; Mendelian randomization study

Introduction

Diverticular disease, one of the most common gastrointestinal (GI) disorders, is reckoned as a heterogeneous disease, and the disease burden is increasing throughout the world.[1,2] The incidence of diverticular disease in the old has increased to 50%, while a rising trend of incidence and

hospitalization rates has also been found in the young.[3] Although diverticular disease is asymptomatic in most instances, it has been one of the most burdensome GI diseases in Western countries due to its high prevalence and complex complications, such as acute diverticulitis, abscess, fistula formation, and bleeding or perforation.[4,5] Therefore, it is needful to explore the pathogenesis of diverticular disease and the mechanisms by which an asymptomatic condition shifts to a symptomatic one.

Recent studies have emphasized the role of gut microbiota imbalance in the onset of diverticular disease. A pilot study reported that the abundance of *Clostridium* cluster IV decreased in the feces of patients with diverticulosis and symptomatic uncomplicated diverticular disease (SUDD) when compared with controls.[6] They also showed that six urine metabolites have the potential to discriminate patients with diverticula from controls.[6] Another study also observed a reduction of *Bacteroides fragilis*, *Collinsella aerofaciens*, and *Collinsella steroris* species in the feces samples of diverticular disease patients.[7] In addition to the increment of *Akkermansia muciniphila* in SUDD and diverticulosis, no other differences in the fecal microbial composition were identified between the patients and controls.[8] Besides, a case-control study found little evidence to support substantial associations between microbiota and diverticular disease.[9] However, the findings of conventional epidemiological studies may suffer from bias, such as residual confounding and reverse causation.

Mendelian randomization (MR) study is a widespread statistical method to evaluate causation between exposure and outcome.[10] By utilizing genetic variants as instrumental variables for exposure, the MR study can reduce residual confounding since genetic variants are randomly assigned at conception and therefore not associated with confounders.[10,11] In addition, the MR approach can diminish reverse causation because the allocation of genetic variants occurs earlier than the outcome.[11] Considering the inconclusive findings and limitations of the observational study, we conducted a two-sample bidirectional MR study to evaluate the potential causal association between gut microbiota alterations and diverticular disease risk. We also explored whether circulating metabolites level were causally associated with the risk of diverticular disease, as they may serve as diagnostic markers for diverticular disease.

Method

Study Design

The study overview and three main assumptions of MR design are displayed in **Figure 1**. First, a two-sample MR analysis was performed to evaluate the causal association of gut microbiota alteration and circulating metabolites level with the risk of diverticular disease, namely forward MR analysis. Then, a reverse MR analysis was conducted to explore whether genetic liability to diverticular disease has a potential effect on the relative abundance of gut microbiota.

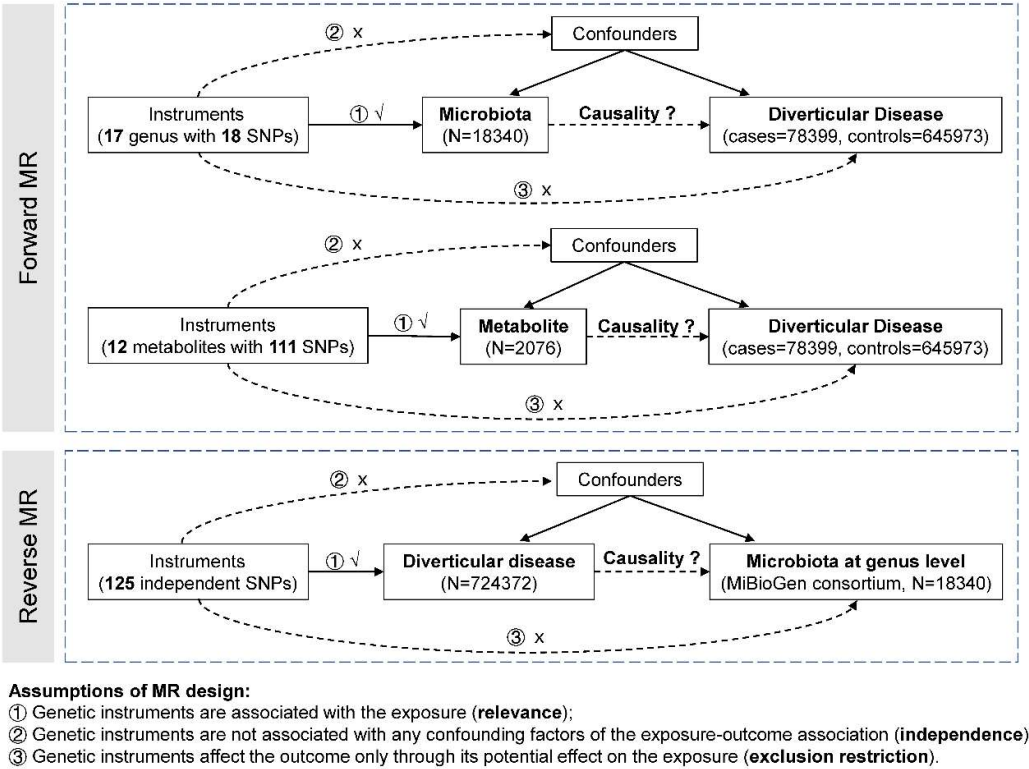


Figure 1. The study design of this study. MR, Mendelian randomization. SNP, single nucleotide polymorphism.

Summary Statistics and Instrumental Variants Selection for Gut Microbiota

To derive the genetic variants for gut microbiota, summary statistics of gut microbial taxa were first obtained from a large-scale multi-ethnic GWAS meta-analysis conducted by the MiBioGen consortium which included 18,340 individuals (mostly European ancestry) from 24 cohorts.[12] Briefly, the study included subjects with different ethnic backgrounds, ages, gender, and microbiome analysis methodology and profiled microbial composition by targeting three distinct variable regions of the 16S rRNA gene (i.e., V1-V2, V3-V4, and V4).[12] The direct taxonomic binning was used to classify taxonomic and a total of 211 microbial taxa were identified at six levels (namely phylum, class, order, family, and genus). Microbial quantitative trait locus (mbQTLs) mapping analysis was performed through Spearman's correlation analysis in which age, sex, technical covariates, and genetic principal components were employed as covariates. The details of the original GWAS have been described elsewhere.[12] The summary statistic of gut microbiota was also obtained from the MiBioGen consortium that has been described above.

Since the genus level is the lowest taxonomic level and we aim to identify each causal microbiota as specifically as possible, only the genus-level taxa were included in this study. After removing 12 unknown genera, a total of 119 genus-level taxa were retained for subsequent analysis. To obtain genetic instruments for gut microbiota, single nucleotide polymorphisms (SNPs) that are associated with each genus at a genome-wide significance ($P < 5 \times 10^{-8}$) were derived as candidate instrumental variables (IVs). Then, linkage disequilibrium (LD) pruning ($r^2 = 0.001$, clumping window = 10,000kb) was performed to exclude the SNP which has pleiotropy with other SNPs or phenotype traits. The 1000 genomes project European samples data were used as the reference panel to estimate LD.[13] Finally, 18 SNPs associated with 17 genus-level taxa were selected as IVs for the forward MR analysis (Table S1). The microbiota variance was explained by the IVs arranged from 0.18% to 1.01%. The detailed PMID and web link of the datasets were provided in the Table 1.

Table 1. The summary of data sources used in this study.

Exposure or Outcome	Participants Included in Analysis	Adjustments	Number of genetic instruments	PubMed ID and/or URL
Gut microbiota taxa	18340 European-descent individuals	Age and any necessary study-specific covariates	18 SNPs for 17 gut microbiota taxa	33462485 https://mibiogen.gcc.rug.nl/
Gut microbial metabolites	2076 European-descent individuals	Age, sex	111 SNPs for 12 gut microbial metabolites	23823483
Diverticular disease	78399 cases and 645973 controls of European ancestry	sex, age, and genetic principal components	125	37492107

Instrumental Variants Selection for Metabolites

Summary-level data of plasma metabolites was obtained from a genome-wide association study of the human metabolome, which developed an LC-MS-based platform that measures a total of 217 plasma metabolites and conducted metabolite profiling for 2076 European individuals in the Framingham Heart Study (FHS).[14] Genetic analyses were performed by leveraging normalized residuals of metabolite concentrations and adjusted for age and sex. The association between genetic variants and metabolite level was assessed through mixed effects models to adapt to pedigree data under an additive genetic model. Population stratification was adjusted in this genome-wide association analysis.[14]

We set a suggestive genome-wide significance ($P < 1 \times 10^{-5}$) to select potential IVs for circulating metabolites to acquire a higher variance explained.[15,16] This criterion has been adopted by previous studies.[17,18] We also performed LD pruning ($r^2=0.001$, clumping window=10,000kb) to obtain independent SNPs for these metabolites. Six to fourteen SNPs were eventually employed as IVs for 12 plasma metabolites, including betaine, carnitine, carnosine, choline, indole 3 propionate, niacinamide, pantothenic acid, propionic acid, ribose, serotonin, taurine and trimethylamine N-oxide

(TMAO). These IVs can explain 8.20%~54.26% genetic variance for these 12 plasma metabolites (Table S2).

Summary Statistics and Instrumental Variants Selection for Diverticular Disease

Recently, the largest-scaled GWAS analysis for diverticular disease was conducted by Wu et al., which contained 724,372 subjects coming from the UK Biobank (56,355 cases and 398,143 controls), the FinnGen Biobank (14,357 cases and 182,433 controls), and the BioVU (7,687 cases and 65,137 controls).[19] Briefly, the diagnostic criteria of diverticular disease in FinnGen and BioVU were consistent with the UK Biobank so that potential inaccuracy associated with self-report can be avoided. The GWAS analysis within each study was performed by adjusting for sex, age, and genetic principal components, and the estimates from different studies were combined by a meta-analysis.[19] The summary statistic of diverticular disease was subsequently generated.

We derived 142 SNPs that were associated with diverticular disease at the genome-wide significance ($P < 5 \times 10^{-8}$) from the GWAS mentioned above.[19] We also conducted LD pruning for these SNPs with the 1000 genomes project European samples data as the reference panel. After LD pruning ($r^2 = 0.01$, clumping distance = 10,000 kb), 125 SNPs were finally employed to perform the reverse MR analysis (Table S3). These SNPs can explain a 10.63% genetic variance of diverticular disease.

Statistical Analysis

In the forward MR analysis, the gut microbiota and circulating metabolite were used as exposure, and diverticular disease was utilized as the outcome. The Wald ratio and random effect inverse-variance weighted (IVW) MR methods were applied as the primary analyses when there was only one IV and more than two IVs for the exposure, respectively. For metabolite, three sensitivity analyses, including MR-Egger, Weighted median, and MR pleiotropy residual sum and outlier (MR-PRESSO) methods, were also performed since there were more than three SNPs for each metabolite. Although with less statistical power, MR-Egger regression can detect horizontal pleiotropy and give an estimate with adjustment for pleiotropic effects.[20] If the weight is derived from at least half of valid IVs, the weighted median approach can provide consistent estimates even if horizontal pleiotropy exists.[21] The MR-PRESSO method can identify potential pleiotropy and outliers and provide an estimate with the removal of the outliers.[22] In the reverse MR analysis, the diverticular disease was reckoned as exposure while the gut microbiota was considered as the outcome. As mentioned above, the IVW method was leveraged as the primary analysis while MR-Egger, weighted median, and MR-PRESSO methods were employed as sensitivity analysis. In addition, we also executed Cochrane's Q test to evaluate the heterogeneity among estimates of the used SNPs and calculated F statistics to exclude weak instruments ($F < 10$). All statistical tests were two-sided and a false discovery rate (FDR) was performed for multiple testing corrections. The FDR P value < 0.05 indicated robust evidence of causal association. The associations did not survive after FDR correction but a P value < 0.05 was thought to be suggestive evidence. The analyses of this study were implemented through "TwoSampleMR" (0.5.6) and "MR-PRESSO" (1.0) packages in the R software 4.2.2.

Results

The Association between Gut Microbiota and Diverticular Disease Risk

As shown in Figure 2, among the 17 genus-level taxa with genetic instruments, only 13 microbiota-diverticular disease associations could be found in the summary statistic of diverticular disease. The Wald ratio MR analysis demonstrated that a genetically determined higher abundance of *Ruminococcus torques* group was causally associated with decreased risk of diverticular disease. For per one-unit in log-transformed relative abundance values of *Ruminococcus torques* group, the odd ratios (OR) and 95% confidence interval (CI) of diverticular disease risk were 0.68 (95%CI: 0.56-0.82,

$P=4.72 \times 10^{-5}$). In addition, a genetically determined higher abundance of *Oxalobacter* (OR=0.88, 95%CI: 0.80-0.97, $P=0.011$) was suggestively associated with a lower risk of diverticular disease, but this association did not survive the FDR correction. F statistics illustrated there was no weak instrument (Table S1). Since all microbiota has less than three independent IVs, sensitivity analyses, such as MR-Egger, weighted median, and MR-PRESSO, were not applicable.

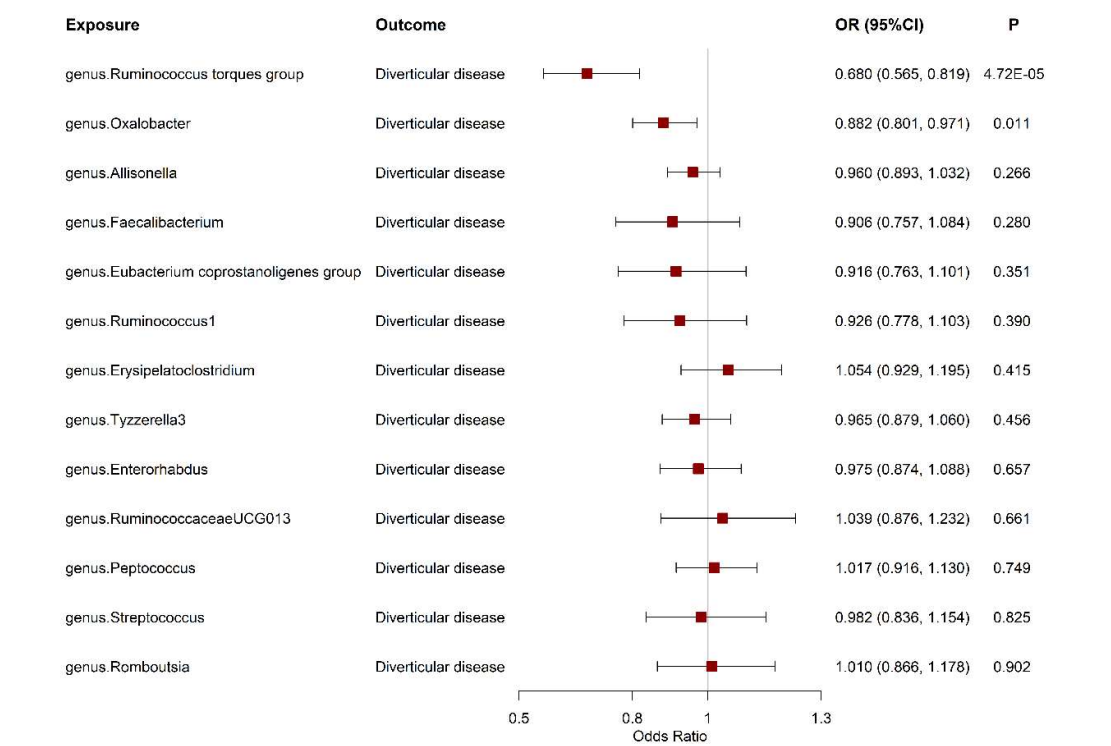


Figure 2. The association between gut microbiota and the risk of diverticular disease. OR, odds ratio. CI, confidence interval.

The Association between Circulating Metabolite Level and Diverticular Disease Risk

Among the 12 circulating metabolites, only choline level exhibited a nominal association with an increased risk of diverticular disease (OR=1.03, 95%CI: 0.99-1.06, $P=0.185$). No significant association was observed between genetically determined elevated metabolite concentration and the risk of diverticular disease (all $P>0.05$, Table 2). In addition, sensitivity analyses showed consistent estimates with the primary analysis. (Table 2) Cochrane’s Q test and F statistics indicated there was no heterogeneity and weak instrument, respectively (Table 2 and Table S2). Similarly, the MR-Egger and MR-PRESSO approaches did not detect any pleiotropy and outlier. (Table 2 and Table S4).

Table 2. The association between genetically determined metabolites level and the risk of diverticular disease.

Exposure	Outc	Method	Ns	O	L	U	pv	P_heterog	P_inter
Betaine	DD	MR Egger	12	1.	0.	1.	0.3	0.721	0.436
		Weighted	12	1.	0.	1.	0.3		
		Inverse variance	12	1.	0.	1.	0.6		
		Simple mode	12	1.	0.	1.	0.5		
		Weighted mode	12	1.	0.	1.	0.3		
Choline	DD	MR Egger	6	0.	0.	1.	0.6		0.447
		Weighted	6	1.	0.	1.	0.6		

Serotonin	DD	Inverse variance	6	1.	0.	1.	0.1	0.058	0.870		
		Simple mode	6	1.	0.	1.	0.9				
		Weighted mode	6	1.	0.	1.	0.8				
		MR Egger	7	1.	0.	1.	0.8	0.604			
		Weighted	7	1.	0.	1.	0.8				
		Inverse variance	7	1.	0.	1.	0.7				
Propionic acid	DD	Simple mode	7	1.	0.	1.	0.7	0.425	NA		
		Weighted mode	7	1.	0.	1.	0.7				
		Inverse variance	2	1.	0.	1.	0.4				
	Carnitine	DD	MR Egger	11	1.	0.	1.	0.1		0.862	0.270
			Weighted	11	1.	0.	1.	0.5			
			Inverse variance	11	1.	0.	1.	0.2			
Indole 3	DD	Simple mode	11	1.	0.	1.	0.7	0.366	0.803		
		Weighted mode	11	1.	0.	1.	0.8				
		MR Egger	12	0.	0.	1.	0.8				
		DD	Weighted	12	1.	0.	1.	0.7		0.619	0.154
			Inverse variance	12	1.	0.	1.	0.9			
			Simple mode	12	0.	0.	1.	0.5			
Ribose 5-P and	DD	Weighted mode	12	0.	0.	1.	0.6	0.370	0.110		
		MR Egger	7	0.	0.	1.	0.3				
		Weighted	7	0.	0.	1.	0.5				
	Taurine	DD	Inverse variance	7	0.	0.	1.	0.1		0.084	0.154
			Simple mode	7	0.	0.	1.	0.6			
			Weighted mode	7	0.	0.	1.	0.5			
Carnosine		DD	MR Egger	8	0.	0.	1.	0.4	0.742	0.471	
			Weighted	8	1.	0.	1.	0.5			
			Inverse variance	8	1.	0.	1.	0.3			
	TMAO	DD	Simple mode	8	1.	0.	1.	0.2	0.324		0.425
			Weighted mode	8	1.	0.	1.	0.7			
			MR Egger	13	1.	0.	1.	0.2			
Niacinamide		DD	Weighted	13	0.	0.	1.	0.2	0.300	NA	
			Inverse variance	13	1.	0.	1.	0.7			
			Simple mode	13	0.	0.	1.	0.6			
	Pantothenic acid	DD	Weighted mode	13	0.	0.	1.	0.3	0.324		0.120
			MR Egger	8	0.	0.	1.	0.4			
			Weighted	8	0.	0.	1.	0.3			
		DD	Inverse variance	8	1.	0.	1.	0.7	0.324		
			Simple mode	8	0.	0.	1.	0.5			
			Weighted mode	8	0.	0.	1.	0.5			
		DD	Inverse variance	2	1.	0.	1.	0.6	0.324		
			MR Egger	10	1.	0.	1.	0.2			
			Weighted	10	0.	0.	1.	0.1			
		DD	Inverse variance	10	0.	0.	1.	0.0	0.324		
			Simple mode	10	0.	0.	1.	0.1			
			Weighted mode	10	0.	0.	1.	0.2			

DD, diverticular disease. OR, odds ratio. CI, confidence interval.

The Effect of Diverticular Disease on Gut Microbiome

In the reverse MR analysis, we did not find any compelling evidence to support an association between genetic susceptibility to diverticular disease and the relative abundance of taxa at the genus

level (all $P>0.05$, **Table 3**). All sensitivity analyses demonstrated consistent association patterns with the IVW MR approach (**Table 3**). Furthermore, our calculations of F statistics and implementation of MR-Egger and MR-PRESSO analyses revealed no indications of weak instruments, pleiotropy, or outliers in these investigations (**Table S3 and S5**).

Table 3. The association between genetic liability to diverticular disease and the abundance of gut microbiota.

Expo	Outcome	Method	ns	bet	se	pv	P_heterog	P_inter
DD	Allisonella	MR Egger	77	0.2	0.1	0.2	0.556	0.214
		Weighted	77	0.0	0.1	0.8		
		Inverse variance	77	0.0	0.0	0.7		
		Simple mode	77	0.0	0.2	0.6		
		Weighted mode	77	0.0	0.1	0.9		
DD	Enterorhabdus	MR Egger	93	-	0.1	0.7	0.410	0.747
		Weighted	93	-	0.0	0.6		
		Inverse variance	93	-	0.0	0.9		
		Simple mode	93	-	0.1	0.7		
		Weighted mode	93	-	0.0	0.7		
DD	Erysipelatoclostridium	MR Egger	93	-	0.1	0.6	0.002	0.615
		Weighted	93	0.0	0.0	0.8		
		Inverse variance	93	0.0	0.0	0.9		
		Simple mode	93	0.1	0.1	0.4		
		Weighted mode	93	0.0	0.1	0.4		
DD	Eubacteriumcoprostan	MR Egger	94	-	0.0	0.4	0.577	0.775
		Weighted	94	-	0.0	0.1		
		Inverse variance	94	-	0.0	0.2		
		Simple mode	94	-	0.0	0.5		
		Weighted mode	94	-	0.0	0.3		
DD	Faecalibacterium	MR Egger	94	-	0.0	0.7	0.697	0.937
		Weighted	94	-	0.0	0.5		
		Inverse variance	94	-	0.0	0.4		
		Simple mode	94	0.0	0.0	0.9		
		Weighted mode	94	-	0.0	0.9		
DD	Oxalobacter	MR Egger	92	-	0.1	0.8	0.407	0.728
		Weighted	92	0.0	0.0	0.8		
		Inverse variance	92	0.0	0.0	0.6		
		Simple mode	92	-	0.2	0.9		
		Weighted mode	92	0.0	0.1	0.7		
DD	Peptococcus	MR Egger	93	-	0.1	0.8	0.913	0.944
		Weighted	93	0.0	0.0	0.6		
		Inverse variance	93	-	0.0	0.6		
		Simple mode	93	0.0	0.1	0.9		
		Weighted mode	93	0.0	0.1	0.8		
DD	Romboutsia	MR Egger	94	-	0.0	0.1	0.159	0.247
		Weighted	94	-	0.0	0.5		
		Inverse variance	94	-	0.0	0.1		
		Simple mode	94	0.0	0.1	0.9		
		Weighted mode	94	0.0	0.0	0.9		
DD	RuminococcaceaeUCG	MR Egger	94	-	0.0	0.4		0.531
		Weighted	94	-	0.0	0.8		

DD	Ruminococcus1	Inverse variance	94	-	0.0	0.6	0.602	0.512
		Simple mode	94	-	0.0	0.8		
		Weighted mode	94	-	0.0	0.8		
		MR Egger	94	-	0.0	0.5		
		Weighted	94	0.0	0.0	0.4		
DD	Ruminococcustorquesg	Inverse variance	94	0.0	0.0	0.8	0.677	0.860
		Simple mode	94	0.0	0.0	0.7		
		Weighted mode	94	0.0	0.0	0.5		
		MR Egger	94	-	0.0	0.8		
		Weighted	94	-	0.0	0.7		
DD	Streptococcus	Inverse variance	94	-	0.0	0.9	0.625	0.771
		Simple mode	94	-	0.0	0.5		
		Weighted mode	94	0.0	0.0	0.8		
		MR Egger	94	-	0.0	0.8		
		Weighted	94	-	0.0	0.9		
DD	Tyzzarella3	Inverse variance	94	-	0.0	0.1	0.612	0.056
		Simple mode	94	-	0.1	0.9		
		Weighted mode	94	-	0.0	0.7		
		MR Egger	93	0.3	0.1	0.0		
		Weighted	93	0.0	0.0	0.5		
		Inverse variance	93	0.0	0.0	0.1	0.146	
		Simple mode	93	0.1	0.1	0.5		
		Weighted mode	93	0.0	0.1	0.4		

DD, diverticular disease. OR, odds ratio. CI, confidence interval.

Discussion

In this study, we found significant causality between a genetically determined higher abundance of *Ruminococcus torques* group and the decreased risk of diverticular disease, and also a suggestive association of *Oxalobacter* with diverticular disease. These findings might have implications for the prevention and treatment of diverticular disease. However, we did not discover any association between circulating metabolites and diverticular disease. In addition, genetic liability to diverticular disease did not affect the relative abundance of genus-level taxa.

In line with our study, a case-control study investigated the effect of colonic microbiome in the pathogenesis of acute diverticulitis (AD) and found lower abundance of *Ruminococcus*, *Lachnospiraceae*, and *Faecalibacterium* in the AD patients.[23] In addition, bacteremia was found to be associated with diverticular disease in two cases of bacteremia with the anaerobic bacterium *Ruminococcus gnavus*.[24] However, a pilot study reported that the relative abundance of *Ruminococcus* is positively associated with bloating severity score in 28 subjects with symptomatic uncomplicated diverticular disease.[25] These conflicting findings may be attributed to the characteristics of study populations, residual confounding and reverse causation, and the low statistical power of traditional observational studies.

The precise mechanisms by which the *Ruminococcus torques* group was involved in the pathogenesis of diverticular disease remain incompletely understood. However, existing research provided some insights into potential explanations. The *Ruminococcus torques* group is a genus reclassified as *Mediterraneibater* in the family *Lachnospiraceae*, and it is also known as *Mediterraneibacter torques*.[26,27] The *Ruminococcus torques* group is involved in carbohydrate metabolism and the production of short-chain fatty acids (SCFAs).[28] SCFAs serve as the major energy source for intestinal epithelial cells (IECs) and possess anti-inflammatory and immunomodulatory properties.[29,30] In addition, SCFAs could regulate IECs functions by modulating their proliferation, differentiation, and subpopulations, to affect gut motility and enhance the gut barrier functions as well as host metabolism.[31] Therefore, the increase in *Ruminococcus torques* group abundance may

potentially reduce the risk of diverticular disease by regulating the gut microbial community and intestinal mucosal health through increased the level of SCFAs. Nevertheless, further research is needed to elucidate these mechanisms and evaluate the potential of *Ruminococcus torques* group to serve as a treatment strategy for diverticular disease.

This study also suggested a potential association between a higher abundance of *Oxalobacter* and a lower risk of diverticular disease, although this association did not reach statistical significance after correction for multiple testing. *Oxalobacter* plays a key role in oxalate metabolism, which has been deeply investigated in renal stones.[32,33] Furthermore, it has been also found to be associated with coronary artery disease, Grave's disease, and inflammatory bowel disease.[34–36] However, there is no research reporting the association of *Oxalobacter* with diverticular disease and the underlying mechanisms so far. This might be a worthy direction to be investigated and further well-designed studies are warranted to explore this association and the potential pathogenesis pathways.

The biggest strength of this study is the MR design, which could compensate for the limitations (i.e., reverse causation and residual confounding) of traditionally observational studies and provide the first evidence supporting the involvement of gut microbiota in the development of diverticular disease. Second, we used the most recent summary statistics of diverticular disease with the largest sample size which enhances the statistical power. We applied stringent criteria to select IVs for microbiota to strengthen the reliability of the evaluation and adopt a bidirectional MR framework to exclude reverse causality bias. Besides, the study population of this work was restricted to European ancestry, which could diminish the bias caused by population structure. However, this also limits the generalizability of our findings to other ethnicities.

In addition to this limitation, we have to acknowledge other limitations of this study. First, although no weak instrument and pleiotropy were identified, the few numbers of IVs could only explain a small genetic variance of gut microbiota and make us unable to perform additional sensitivity analyses to preclude potential bias, such as MR-Egger test and MR-PRESSO. Second, we only focused on the genus level in the analysis to discover the most specific bacterial taxa which may affect the risk of diverticular disease. Therefore, this study cannot preclude potential associations of other level of microbiota with diverticular disease. Finally, the effects of environmental factors on gut microbiota and diverticular disease need to be considered in further epidemiology studies to demonstrate the gene-environment interaction effect.

In conclusion, our MR study provides evidence for a causal association between a genetically determined higher abundance of the *Ruminococcus torques* group and a reduced risk of diverticular disease. Additionally, we suggest a potential protective role of *Oxalobacter*, though further validation is needed. These findings contribute to the understanding of the complex interactions between gut microbiota and diverticular disease and highlight potential therapeutic targets for its prevention and treatment.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org, Table S1. The instrumental variables of microbiota. Table S2. The instrumental variables of blood microbiota-associated metabolites. Table S3. The instrumental variables of diverticular disease. Table S4. The MR-PRESSO analysis of blood metabolites with diverticular disease. Table S5. The MR-PRESSO analysis of diverticular disease with microbiota.

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List of Abbreviations

MR, Mendelian randomization. OR, odds ratio. GI, gastrointestinal. SUDD, symptomatic uncomplicated diverticular disease. mbQTLs, Microbial quantitative trait locus. LD, linkage disequilibrium. IVW, inverse-variance weighted. MR-PRESSO, MR pleiotropy residual sum and outlier. FDR, false discovery rate. AD, acute diverticulitis. SCFAs, short-chain fatty acids. IECs, intestinal epithelial cells.

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