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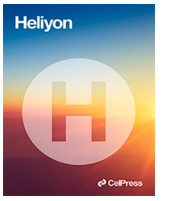

Causal effects of gut microbiota on scoliosis: A bidirectional two-sample mendelian randomization study

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ABSTRACT

Background: Recent studies have shown altered gut microbiome composition in patients with scoliosis. However, the causal effect of gut microbiota on scoliosis remains unknown.
Methods: A Mendelian randomization (MR) study was conducted to quantify the impact of 191 gut microbiome taxa's instrumental variables from the MibioGen Genome-wide association study (GWAS) on scoliosis risk using data from the FinnGen GWAS (1168 cases and 16,4682 controls). Inverse variance weighted (IVW) was the main method, and MR results were verified by sensitive analysis.
Results: Bilophila, Eubacterium (eligens group), *Prevotella*9, and *Ruminococcus*2 were discovered to have a protective effect on the risk of scoliosis. *Ruminococcaceae* UCG009, *Catenibacterium*, *Coprococcus*2, *Eubacterium* (ventriosum group), *Lachnospiraceae* (FCS020 group), *Ruminiclostridium*6, and *Mollicutes* RF9 may increase the occurrence of scoliosis. Heterogeneity ($P > 0.05$) and pleiotropy ($P > 0.05$) analysis confirmed the robustness of the MR results.
Conclusion: Our study identified four protective bacteria taxa on scoliosis and seven microbiota that may increase scoliosis occurrence. Further MR analysis is required to corroborate our findings, using a more sophisticated technique to obtain estimates with less bias and greater precision or GWAS summary data with more gut microbiome and scoliosis patients.

1. Background

Scoliosis is spinal curvature with a Cobb angle over 10° in the coronal plane of a standing orthopantomogram of the spine [1]. Over 60 % of all cases are regarded as idiopathic, even though scoliosis can have many causes [1]. Multiple organ damage, such as spinal cord compression, respiratory failure, and cardiovascular disease, can result from severe scoliosis [2]. The etiologies of scoliosis include genetic, metabolic, biomechanical, neurological, and environmental factors [3]. However, the exact cause of scoliosis remains unclear.

The human gut microbiome, made up of bacteria that live in the gastrointestinal tract, is thought to be the second brain and contributes to the development of various diseases [4]. Several studies indicate that gut microbiome dysregulation could impair hormone homeostasis and trigger the development of metabolic disorders [5,6]. Moreover, even before the onset of scoliosis, metabolic dysregulation and hormonal alterations were identified in patients with adolescent idiopathic scoliosis (AIS) and low bone mineral density [7–9]. Therefore, interest in the effect of gut microbiome on scoliosis is increasing. Identifying the different

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components of gut microbiome between patients with scoliosis and healthy people may deepen our understanding of the pathogenesis of scoliosis and explore potential treatments.

Mendelian randomization (MR) studies use genetic variation rather than exposure to analyze causal connections with outcomes, avoiding the impact of potential confounding factors and reverse causality [10]. Studies using MR analysis to investigate the causal connection between the gut microbiome and musculoskeletal diseases (such as ankylosing spondylitis) have been widely conducted [11]. However, no investigation demonstrated how gut microbiome influences scoliosis by MR analysis.

Herein, we collected the genetic variants from a large Genome-wide association study (GWAS) analysis of gut microbiome from MiBioGen and of scoliosis from FinnGen consortiums. To investigate the causative relationship and offer a theoretical foundation for more study into the intricate mechanisms of scoliosis, we chose gut microbiome taxa as the exposure and scoliosis as the outcome to perform a two-sample MR analysis. Furthermore, establishing the causal effect of gut microbiota on patients with scoliosis may lead to the development of novel biomarkers and diagnostic and therapeutic approaches.

2. Methods

2.1. Data sources

We collected the genetic variants of gut microbiome structure from a large-scale association analysis in 2021 from the international consortium MiBioGen [12]. The data sources included 16S rRNA gene sequencing profiles of 18,340 people from 24 cohorts. Most of the participants in the study (n = 13,266) were of European ancestry. Furthermore, microbiome trait loci mapping data revealed many host-microbiome quantitative trait locus associated with the relative abundance among gut bacteria taxa. After removing genera with a relative abundance of less than 1 % and those with unknown taxonomy, 9 phyla, 16 classes, 20 orders, 31 families, and 115 genera were included for the following analysis.

Genetic statistics of scoliosis were obtained from FinnGen consortium R5 released in 2021 to match the race of exposure (gut microbiome) [13]. The large GWAS of Finns contained 1168 cases and 164,682 controls, resulting in 16,380,270 single nucleotide polymorphisms (SNPs) for analysis after adjusting for factors including age, sex, and genotyping batch.

2.2. Principles of MR analysis

MR uses genetic variation as an instrumental variable (IV) to infer causality between exposure and outcome, effectively overcoming the bias caused by the confounding reverse causality problem. The IVs must be chosen to obey three rules to ensure credibility before MR analysis: (1) IVs were not associated with confounding factors, (2) IVs were associated with exposure factors, (3) IVs should have no association with outcome variables, and IVs could only be associated with outcomes through exposure. We obey these three principles and selected the situable SNPs as the IVs for the futher analysis.

2.3. Inclusion and exclusion of IVs

SNPs usually serve as IVs in MR. The IVs selection met the following criteria to ensure robustness and reliability for MR analysis: (1) SNPs of each bacteria taxa with a p-value lower than the locus-wide significance threshold ($1.0*10^{-5}$); (2) The minor allele frequency of each SNP is higher than 0.01; (3) Based on the 1000 Genomes project European samples data, only the SNPs with $R2<0.001$ (clumping window: 10,000 kb) were preserved to avoid the linkage disequilibrium between the SNPs; (4) The palindromic SNPs were excluded to control the same allele between the exposure and outcome; (5) F statistic revealed the strength of SNPs and was calculated by the formula:

$$F = \frac{R^2 \times (N - 1 - K)}{(1 - R^2) \times K}$$

where R^2 represents the percentage of variation in exposure that can be accounted for by SNPs, N represents the sample size, and K stands for the quantity of SNPs. The F value of each SNP should be > 10 to eliminate the weak instrumental bias, increasing the accuracy of the MR analysis.

2.4. MR estimates and statistical analysis

We conducted a two-sample MR analysis to estimate the causality between the gut microbiome and scoliosis. The MR analysis method included inverse variance weighting (IVW), MR Egger, weighted median (WM), simple mode, and weighted mode. The IVW method involves transforming to a weighted regression of the instrumental variable outcome effects on the exposure effects to provide an overall estimate of the influence of the gut microbiome on the development of scoliosis. In the absence of horizontal pleiotropy, IVW can provide unbiased estimates by avoiding the effects of confounding variables. When the SNPs have pleiotropy, MR-Egger is utilized because it might be heavily influenced by outlying genetic factors and produce erroneous results. The WM can offer reliable estimates of the causal effects using most genetic variants. Moreover, even if some IVs do not satisfy the criteria of the MR method for deducing the causality, the weighted mode approach is still viable. We also carried out reverse two-sample MR analysis on the bacteria taxa that were discovered to be causally connected with scoliosis to demonstrate the causal association between gut microbiota and scoliosis. In

the reverse MR analysis, we chose scolisois as exposure and the identified causal bacterial genus as outcome using SNPs that are associated with scolisois as IVs.

MR pleiotropy residual sum and outlier (MR-PRESSO), a sensitivity analysis to find outliers representing possible pleiotropic biases and rectify horizontal pleiotropy. Heterogeneity was examined using Cochrane's Q test, and a Q value > 0.05 indicated no significant heterogeneity. The pleiotropy was further estimated by the horizontal pleiotropy test. Finally, we performed a leave-one-out sensitivity analysis to confirm the stability and accuracy of the MR results and assess the possible SNPs with substantial influence.

All statistical analyses were conducted using R version 4.2.2. The "Two-sample MR" and "MRPRESSO" R packages were used for statistical analysis in R version 4.2.2. The statistical threshold for causal effect evidence was set at $P < 0.05$.

Table 1
MR estimates for the association between gut microbiota and Scoliosis. MR method, method used for Mendelian randomization analysis; nSNP, the number of SNPs selected for MR analysis; OR, odds ratio; OR-low, the lower limit of the confidence interval of OR; OR-high, the upper limit of the confidence interval of OR.

Level	Exposure	MR method	nSNP	OR	OR-low	OR-high	P-value
order	Mollicutes RF9	MR Egger	13	2.146026	0.6171339	7.462606	0.25500351
order	Mollicutes RF9	Weighted median	13	1.417218	0.8796256	2.283365	0.1518773
order	Mollicutes RF9	Inverse variance weighted	13	1.484121	1.0086558	2.183713	0.04509796
order	Mollicutes RF9	Simple mode	13	1.408144	0.6614325	2.99784	0.39208554
order	Mollicutes RF9	Weighted mode	13	1.435081	0.725491	2.838707	0.31977038
genus	Bilophila	MR Egger	13	0.3337719	0.02809411	3.9653752	0.4034062
genus	Bilophila	Weighted median	13	0.5466563	0.29830442	1.0017722	0.05066924
genus	Bilophila	Inverse variance weighted	13	0.6088069	0.37382919	0.9914845	0.04611175
genus	Bilophila	Simple mode	13	0.4720084	0.14159757	1.5734164	0.245112
genus	Bilophila	Weighted mode	13	0.5268883	0.17402389	1.595248	0.27906396
genus	Catenibacterium	MR Egger	4	0.8102634	0.008388756	78.262721	0.9363299
genus	Catenibacterium	Weighted median	4	1.542112	0.992695798	2.395607	0.05393234
genus	Catenibacterium	Inverse variance weighted	4	1.5958028	1.107961012	2.298444	0.01204771
genus	Catenibacterium	Simple mode	4	1.4168283	0.808203082	2.483785	0.31076973
genus	Catenibacterium	Weighted mode	4	1.4080384	0.800817383	2.475686	0.32013779
genus	Coprococcus2	MR Egger	8	1.408307	0.01085509	182.70949	0.89481125
genus	Coprococcus2	Weighted median	8	2.143047	1.06711901	4.303784	0.03214457
genus	Coprococcus2	Inverse variance weighted	8	1.360555	0.76768976	2.411275	0.29163854
genus	Coprococcus2	Simple mode	8	2.455936	0.70550615	8.549353	0.20086533
genus	Coprococcus2	Weighted mode	8	2.420656	0.70966638	8.256801	0.2007802
genus	Eubacterium eligens group	MR Egger	6	0.1250411	0.008920933	1.7526506	0.19759786
genus	Eubacterium eligens group	Weighted median	6	0.474921	0.206955988	1.0898452	0.07891977
genus	Eubacterium eligens group	Inverse variance weighted	6	0.4684879	0.23081191	0.9509083	0.03578419
genus	Eubacterium eligens group	Simple mode	6	0.5317552	0.158387076	1.7852692	0.35362395
genus	Eubacterium eligens group	Weighted mode	6	0.490182	0.151063579	1.5905781	0.28846773
genus	Eubacterium ventriosum group	MR Egger	15	1.089312	0.1888102	6.284625	0.925240045
genus	Eubacterium ventriosum group	Weighted median	15	1.846501	1.0749129	3.171946	0.026304056
genus	Eubacterium ventriosum group	Inverse variance weighted	15	1.692376	1.1421809	2.507604	0.008724147
genus	Eubacterium ventriosum group	Simple mode	15	2.811029	0.9799604	8.063471	0.075149593
genus	Eubacterium ventriosum group	Weighted mode	15	2.78745	0.963678	8.062732	0.079396727
genus	Lachnospiraceae FCS020 group	MR Egger	12	1.814395	0.6267731	5.252344	0.29770934
genus	Lachnospiraceae FCS020 group	Weighted median	12	1.740227	1.0128277	2.990036	0.04483969
genus	Lachnospiraceae FCS020 group	Inverse variance weighted	12	1.591546	1.068235	2.371219	0.0223425
genus	Lachnospiraceae FCS020 group	Simple mode	12	2.496088	1.0024819	6.21503	0.07512877
genus	Lachnospiraceae FCS020 group	Weighted mode	12	2.477977	0.9170933	6.695469	0.10109618
genus	Prevotella9	MR Egger	15	0.4680446	0.1969686	1.1121861	0.10927851
genus	Prevotella9	Weighted median	15	0.687193	0.4535988	1.0410836	0.07672202
genus	Prevotella9	Inverse variance weighted	15	0.6868752	0.5105647	0.9240698	0.01307301
genus	Prevotella9	Simple mode	15	0.6480044	0.3094901	1.3567792	0.26911676
genus	Prevotella9	Weighted mode	15	0.7029154	0.3717051	1.3292528	0.29649127
genus	Ruminiclostridium6	MR Egger	15	2.050483	0.8028679	5.236827	0.15724039
genus	Ruminiclostridium6	Weighted median	15	1.276291	0.7814107	2.084589	0.32975126
genus	Ruminiclostridium6	Inverse variance weighted	15	1.47435	1.010588	2.150932	0.04394051
genus	Ruminiclostridium6	Simple mode	15	1.226206	0.5175824	2.905011	0.65019525
genus	Ruminiclostridium6	Weighted mode	15	1.226206	0.5324933	2.823664	0.63920524
genus	Ruminococcaceae UCG009	MR Egger	12	0.9783813	0.2753956	3.475836	0.97370821
genus	Ruminococcaceae UCG009	Weighted median	12	1.6603339	1.0954315	2.51655	0.01686751
genus	Ruminococcaceae UCG009	Inverse variance weighted	12	1.3934449	1.0244035	1.895433	0.03454982
genus	Ruminococcaceae UCG009	Simple mode	12	1.9455947	0.9205587	4.112001	0.10914314
genus	Ruminococcaceae UCG009	Weighted mode	12	1.9455947	0.9804237	3.860921	0.0834604
genus	Ruminococcus2	MR Egger	15	0.6880487	0.2513309	1.8836162	0.47971292
genus	Ruminococcus2	Weighted median	15	0.5386832	0.3228839	0.8987119	0.01783877
genus	Ruminococcus2	Inverse variance weighted	15	0.833241	0.5553776	1.2501234	0.37809362
genus	Ruminococcus2	Simple mode	15	0.5374991	0.2255983	1.2806185	0.18281564
genus	Ruminococcus2	Weighted mode	15	0.5221005	0.260337	1.0470622	0.08854644

3. Results

3.1. The selection of IVs related to gut microbiome

After considering linkage disequilibrium effects, palindrome, and weak instrumental bias, 2095 SNPs were employed as IVs for 191 bacterium species (Supplementary Table 1). These taxa included 9 phyla (106 SNPs), 16 classes (184 SNPs), 20 orders (227 SNPs), 31 families (353 SNPs), and 115 genera (1225 SNPs) (Supplementary Table 2). The key SNP data, including effect allele, beta, Standard Error (SE), and p-value, were thoroughly gathered for further analysis. Each SNP displayed sufficient validity with an F value greater than 10 (range: 16.91−88.43).

3.2. The estimates of scoliosis with gut microbiome

Supplementary Table 3 illustrates the full result of the MR analysis for the casual relationship between gut microbiome and scoliosis. As illustrated in Table 1 and Fig. 1, one order and 10 genera had causality with scoliosis by MR analysis. The IVW method revealed a positive causal association of *Mollicutes* RF9 (odds ratio (OR) = 1.48, 95 % confidence interval (CI): 1.01−2.18, P = 0.045), *Catenibacterium* (OR = 1.60, 95 % CI: 1.11−2.30, P = 0.012), *Eubacterium* (ventriosum group) (OR = 1.69, 95 % CI: 1.14−2.51, P = 0.009), *Lachnospiraceae* (FCS020 group) (OR = 1.59, 95 % CI: 1.07−2.37, P = 0.022), *Ruminiclostridium*6 (OR = 1.47, 95 % CI: 1.01−2.15, P = 0.044) and *Ruminococcaceae* UCG009 (OR = 1.39, 95 % CI: 1.02−1.90, P = 0.035) on scoliosis. The WM method further demonstrated the positive effects of *Coprococcus*2 (OR = 2.14, 95 % CI: 1.07−4.30, P = 0.032), *Eubacterium* (ventriosum group) (OR = 1.85, 95 % CI: 1.07−3.17, P = 0.026), *Lachnospiraceae* (FCS020 group) (OR = 1.74, 95 % CI: 1.01−2.99, P = 0.045), and *Ruminococcaceae* UCG009 (OR = 1.66, 95 % CI: 1.10−2.52, P = 0.017) on scoliosis risk. In contrast, *Ruminococcus*2 showed a negative effect on scoliosis by the WM method (OR = 0.54, 95 % CI: 0.32−0.90, P = 0.018). Meanwhile, *Bilophila* (OR = 0.61, 95 % CI: 0.37−0.99, P = 0.046), *Eubacterium* (eligens group) (OR = 0.47, 95 % CI: 0.23−0.95, P = 0.036), and *Prevotella*9 (OR = 0.69, 95 % CI: 0.51−0.92, P = 0.013) may be a protective factor for scoliosis after analysis by the IVW method.

We then conducted a sensitivity analysis for the MR results (Supplementary Table 4). The horizontal pleiotropy test revealed no statistical significance in *Mollicutes* RF9 (P = 0.553), *Bilophila* (P = 0.64), *Catenibacterium* (P = 0.80), *Coprococcus*2 (P = 0.99), *Eubacterium* (eligens group) (P = 0.37), *Eubacterium* (ventriosum group) (P = 0.62), *Lachnospiraceae* (FCS020 group) (P = 0.80), *Prevotella*9 (P = 0.37), *Ruminiclostridium*6 (P = 0.46), *Ruminococcaceae* UCG009 (P = 0.58), and *Ruminococcus*2 (P = 0.69) for scoliosis (Table 2). Likewise, Cochran's Q test revealed no heterogeneity in *Mollicutes* RF9 (IVW: P = 0.24; MR-Egger: P = 0.20), *Bilophila* (IVW: P = 0.15; MR-Egger: P = 0.12), *Catenibacterium* (IVW: P = 0.88; MR-Egger: P = 0.75), *Coprococcus*2 (IVW: P = 0.19; MR-Egger: P = 0.13), *Eubacterium* (eligens group) (IVW: P = 0.24; MR-Egger: P = 0.25), *Eubacterium* (ventriosum group) (IVW: P = 0.70; MR-Egger: P = 0.65), *Lachnospiraceae* (FCS020 group) (IVW: P = 0.76; MR-Egger: P = 0.69), *Prevotella*9 (IVW: P = 0.67; MR-Egger: P = 0.66),

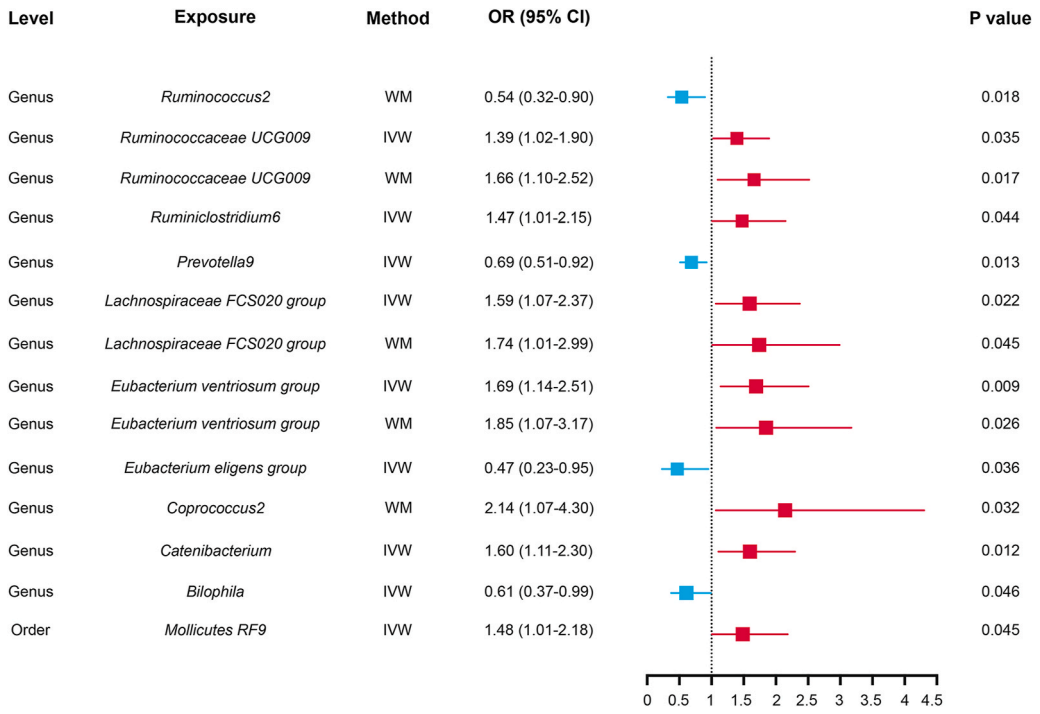

Fig. 1. MR results and forest plot of gut microbiome with a causal relationship to scoliosis. IVW, Inverse variance weighting; WM, weighted median; OR, odds ratio; CI, confidence interval.

Table 2
Sensitivity analysis between gut microbiome and scoliosis. MR, Mendelian randomization; IVW, Inverse variance weighting; MR-PRESSO, MR pleiotropy residual sum and outlier; IVW_Q, the Q value of heterogeneity analysis for the IVW method; IVW_Q_P, the P value of heterogeneity analysis for the IVW method; MR Egger_Q, the Q value of heterogeneity analysis for the MR Egger method; MR Egger_Q_P, the P value of heterogeneity analysis for the MR Egger method; Pleiotropy_P, the P value of pleiotropy analysis; MRPRESSO_P, the P value of MRPRESSO analysis.

Level	Exposure	IVW_Q	IVW_Q_P	MR Egger_Q	MR Egger_Q_P	Pleiotropy_P	MRPRESSO_P
order	Mollicutes RF9	15.08337	0.2369087	14.587	0.2021956	0.553097	0.094
genus	Bilophila	17.00041	0.1495817	16.64278	0.1188951	0.6363765	0.171
genus	Catenibacterium	0.6509941	0.8846655	0.5659713	0.7535306	0.7980646	0.889
genus	Coprococcus2	9.908582	0.1938135	9.908258	0.128569	0.9892768	0.207
genus	Eubacterium eligens group	6.796259	0.2362391	5.398787	0.2487705	0.3664344	0.339
genus	Eubacterium ventriosum group	10.77424	0.7036732	10.51858	0.6510772	0.621583	0.699
genus	Lachnospiraceae FCS020 group	7.444456	0.7620337	7.376502	0.6894866	0.7996301	0.763
genus	Prevotella9	11.24689	0.6665413	10.39191	0.6616156	0.3719928	0.674
genus	Ruminiclostridium6	8.29612	0.8733353	7.728601	0.8608209	0.4646719	0.895
genus	Ruminococcaceae UCG009	11.35687	0.4138684	11.00581	0.3570657	0.5846691	0.411
genus	Ruminococcus2	19.1335	0.1599108	18.88967	0.1265642	0.6887451	0.152

*Ruminiclostridium*6 (IVW: P = 0.87; MR-Egger: P = 0.86), *Ruminococcaceae* UCG009 (IVW: P = 0.41; MR-Egger: P = 0.36), and *Ruminococcus*2 (IVW: P = 0.16; MR-Egger: P = 0.13) for scoliosis (Table 2).

We found possible outliers of the IVs of *Catenibacterium*, *Coprococcus*2, *Prevotella*9, and *Ruminococcus*2 in scatter plots (Fig. 2). However, MR-PRESSO analysis showed no significant outliers for each order or genus (global test P > 0.05). Therefore, there was inadequate support for horizontal pleiotropy in the relationship between the gut bacteria taxa and scoliosis. Moreover, the leave-one-out plots further confirmed the robustness and reliability of the results (Supplementary Fig. 1). Finally, the inverse MR analysis revealed no causal association between scoliosis and all 11 bacteria taxa (Supplementary Table 5).

4. Discussion

In this study, we used the two-sample MR analysis with the gut microbiome IVs from the large GWAS by the MiBioGen consortium and the FinnGen consortium R5 aggregated data for scoliosis to evaluate the causal relationship between gut microbiome and scoliosis. The result revealed that *Bilophila*, *Eubacterium* (eligens group), Prevotella9, and *Ruminococcus*2 protected against scoliosis. However, we also discovered *Mollicutes* RF9, *Catenibacterium*, *Coprococcus*2, *Eubacterium* (ventriosum group), *Lachnospiraceae* (FCS020 group), *Ruminiclostridium*6, and *Ruminococcaceae* UCG009 may increase the incidence of scoliosis.

Numerous studies have been conducted on the impact of gut microbiota on bone metabolism, and microbiome alternation was regarded as a potential therapy to maintain or promote bone health. Microbiota may promote bone remodeling by activating insulin-like growth factor 1 [14]. *Bifidobacteria* and *Lactobacillus* have an anti-inflammatory function, which can improve vitamin D absorption and decrease osteoclast development, reducing bone loss resulting from ovariectomy in mice [15,16]. In contrast, 16S rRNA gene sequencing revealed that the *Proteobacteria* phylum of bacteria, such as *Pseudomonas* and *Enterobacter*, were enhanced in post-menopausal osteopenia [17]. Both probiotics and prebiotics positively affect bone loss reversal *in vivo*, expanding the therapeutic options towards osteoporosis [18,19]. Accumulating evidence indicates that dysbiosis is involved in musculoskeletal diseases. A large cohort study involving 1427 participants demonstrated a correlation between *Streptococcus* abundance and increasing inflammation in knee joints, resulting in a higher risk of knee pain and osteoarthritis [20]. The percentage of constituent microbial DNA from gram-negative microorganisms (such as *Betaproteobacteria* and *Burkholderiales*) in patients with osteoarthritis showed a significant increase compared to disease-free controls (P = 0.02) [21]. Ankylosing Spondylitis is characterized as an autoimmune spine inflammation with inflammasome activation and metabolism dysregulation. A recent study revealed a richness of *Bacteroides*, *Parabacteroides*, *Eubacterium*, and *Prevotella* in patients with ankylosing spondylitis [22]. Meanwhile, there were higher levels of adherent and invasive mucosa-associated bacteria in patients with ankylosing spondylitis, which were associated with the expression of inflammasome components [11]. Reduced gut microbiome diversity indicated a potential association between microbiota and fibromyalgia, a chronic widespread pain with unknown causes. An abundance of Phylum *Firmicutes* may increase the levels of glutamate, promoting neuropathic pain in fibromyalgia development [23].

Scoliosis can be divided into congenital, syndromic, and idiopathic. Since the data sources of the onset age of scoliosis in Finn consortium R5 appeared a peak in adolescents aged under 20 while AIS was considered as the most common spine deformity, the pathological mechanism of scoliosis in our study participants remains unclear [24]. Until now, few studies focused on the role of gut microbiome on scoliosis. A single study among Chinese people using 16S rRNA sequencing on 51 patients with AIS revealed more *Prevotella*, *Gelria*, and *Desulfovibrio* compared to 34 controls [25]. In contrast, the AIS group had lower concentrations of *Parasutterella, Tyzzerella,* and *Phascolarctobacterium* [25]. However, genus *Prevotella*9 has a protective effect on scoliosis according to the result of the two-sample MR analysis we performed in this study (OR = 0.69, 95 % CI: 0.51−0.92, P = 0.013). The disparities between the examined human species and the strain level variety of *Prevotella* may cause these contradicting results. Substantial evidence indicated that the abundance of *Prevotella* is higher in the gut microbiota of non-Westernized populations than in Westernized populations [26,27]. *Prevotella* species have been defined for 57 isolates with different biological functions that are publicly available now [28]. Patients with AIS develop low bone mineral density before the onset of scoliosis, while osteoporosis is an independent risk factor for the progression of scoliosis in patients with AIS [7]. People who consume a high-fiber diet have a *Prevotella*-rich gut microbiome, which

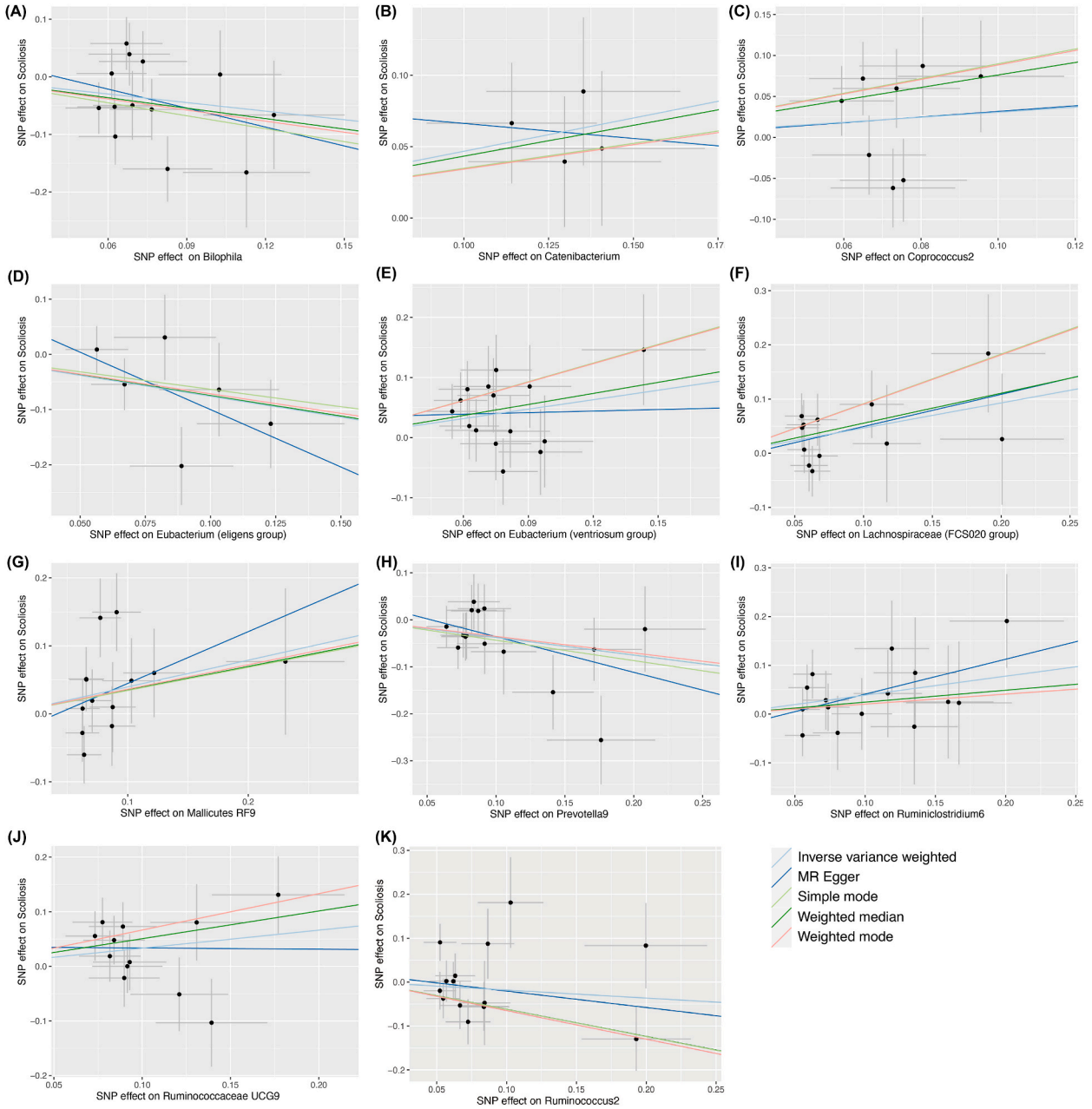

Fig. 2. Scatter plots for the causal association between gut microbiota and scoliosis. Each panel demonstrates the correlation between the SNP effect on scoliosis and each gut microbiota by performing five methods(bottom right corner of the figure) characterized by five different colors. (A) SNP effect on *Bilophila* and scolisis. (B) SNP effect on *Catenibacterium* and scolisis. (C) SNP effect on *Coprococcus*2 and scolisis. (D) SNP effect on *Eubacterium* (eligens group) and scolisis. (E) SNP effect on *Eubacterium* (ventriosum group) and scolisis. (F) SNP effect on *Lachnospiraceae* (FCS020 group) and scolisis. (G) SNP effect on *Mollicutes* RF9 and scolisis. (H) SNP effect on *Prevotella*9 and scolisis. (I) SNP effect on *Ruminiclostridium*6 and scolisis. (J) SNP effect on *Ruminococcaceae* UCG009 and scolisis. (K) SNP effect on *Ruminococcus*2 and scolisis. SNP, Single Nucleotide Polymorphism.

enhances weight loss and reduces cholesterol levels [29–31], possibly increasing bone mineral density and maintaining skeletal homeostasis, which inhibits the formation and reduces the risk of scoliosis. In newborns with meningitis or enterocolitis, *Prevotella*9 revealed a negative association with lipopolysaccharide (LPS) infection, an endotoxin-promoting bacteria diffusion, and inflammatory response, which may cause systemic metabolic disorders [32]. Fluctuations in serum hormone levels, such as melatonin, calmodulin, and leptin, were discovered in patients with AIS [33–35]. The anti-inflammatory effect of *Prevotella*9 may keep the body's hormone levels stable and reduce the risk of AIS. The relationship between other potential gut bacteria identified in this study and scoliosis has

not received much research to date. Further research is needed to determine the mechanisms through which various gut taxa affect scoliosis favorably or unfavorably.

This study is the first MR analysis on scoliosis pathology with gut microbiome. The gold standard for determining causality is the randomized control trials (RCTs), in which subjects are randomly divided into a control group or an experimental group to study the effects of a given factor. However, RCTs are very difficult to complete, require a lot of manpower and resources, and sometimes because of ethical issues, research on a certain factor is almost impossible. MR has been widely applied to reveal the causal relation between gut microbiota and diseases, including metabolic diseases, rheumatoid arthritis, preeclampsia-eclampsia, etc [36–38]. Thus, MR study using the Mendel's second law (the law of independent assortment) is similar to randomization in RCTs and if we follow the basic rules of MR, it truly can show the casuality between the variable (gut microbiome) and the outcome (scoliosis) [39,40]. The benefit of this study is that the two-sample MR analysis reduced the effect of confounding factors usually in observational studies, which increased the reliability of the causal relationship between gut microbiome and scoliosis. However, our study had some limitations. First, the study's results may not be easily extrapolated to other ethnic groups because most participants in the GWAS data sources were of European ancestry. Also, the two samples could differ according to population characteristics such as age, sex, and socio-economic background. Such differences may affect the validity of causal inferences. Second, since more SNPs should be included as IVs to make sure the feasibility of sensitivity analysis and horizontal pleiotropy detection, SNPs included in the analysis did not meet the standard significance threshold ($P = 5*10^{-8}$), which may weaken the reproducibility of the results. Finally, due to the lack of epidemiological studies, there was insufficient evidence to deduce the molecular mechanism behind the gut microbiome and scoliosis.

In summary, our study revealed four protective bacteria taxa on scoliosis and seven microbiota that may increase the incidence of scoliosis. Further research is required to determine the positive or negative association between different bacteria taxa and the risk of scoliosis to meet the biological plausibility and clinical viability, which is favorable to examining the pathology and potential treatments for scoliosis.

5. Conclusion

In this study, we found seven microbiota (*Mollicutes* RF9, *Catenibacterium*, *Coprococcus*2, *Eubacterium* (ventriosum group), and *Lachnospiraceae* (FCS020 group), *Ruminiclostridium*6, and *Ruminococcaceae* UCG009) that may raise the risk of scoliosis as well as four protective bacteria taxa (*Bilophila*, *Eubacterium* (eligens group), Prevotella9, and *Ruminococcus*2) that may reduce the risk of the disease. It will take more sophisticated MR analysis to obtain estimates with less bias and more precision or GWAS summary data with more gut microbiota and scoliosis patients in order to confirm our findings.

Ethics approval and consent to participate

The large-scale GWAS summary statistic of the gut microbiota and scoliosis used in this study is collected from previous studies. All subjects provided informed consent in all relevant original studies in accordance with the Methods.

Consent for publication

Not applicable.

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Author contribution statement

Bowen Lai: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper. Heng Jiang: Conceived and designed the experiments. Yuan Gao: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper. Xuhui Zhou: Performed the experiments.

Data availability statement

Data included in article/supp. Material/referenced in article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e21654.

List of Abbreviations

MR	Mendelian randomization
GWAS	Genome-wide association study
IVW	Inverse variance weighting
AIS	Adolescent idiopathic scoliosis
IV	Instrumental variable
SNPs	Single Nucleotide Polymorphisms
WM	Weighted median
MR-PRESSO	MR pleiotropy residual sum and outlier
SE	Standard Error
OR	Odds ratio
CI	Confidence interval
LPS	Lipopolysaccharide.

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