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Dissecting Causal Relationships Between Gut Microbiota, 1400 Blood Metabolites, and Intervertebral Disc Degeneration

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Objective: The precise mechanisms driving intervertebral disc degeneration (IVDD) development remain unclear, but evidence suggests a significant involvement of gut microbiota (GM) and blood metabolites. We aimed to investigate the causal relationships between GM, IVDD, and blood metabolites using Mendelian randomization (MR) analysis.
Methods: We utilized the summary statistics of GM from the MiBioGen consortium, 1400 blood metabolites from the genome-wide association studies (GWAS) Catalog, and IVDD data from the FinnGen repository, which are sourced from the largest GWAS conducted to date. Employing bidirectional MR analyses, we investigated the causal relationships between GM and IVDD. Additionally, we conducted 2 mediation analyses, 2-step MR and multivariable MR (MVMR), to identify potential mediating metabolites.
Results: Five bacterial genera were causally associated with IVDD, while IVDD did not show a significant causal effect on GM. In the 2-step MR analysis, Eubacteriumfissicatenagroup, RuminococcaceaeUCG003, Lachnoclostridium, and Marvinbryantia genera, along with metabolites X-24949, Pimeloylcarnitine/3-methyladipoylcarnitine (C7-DC), X-24456, histidine, 2-methylserine, Phosphocholine, and N-delta-acetylornithine, were all significantly associated with IVDD (all $p < 0.05$). MVMR analysis revealed that the associations between Eubacteriumfissicatenagroup genus and IVDD were mediated by X-24949 (8.1%, $p = 0.024$); Lachnoclostridium genus and IVDD were mediated by histidine (18.1%, $p = 0.013$); and RuminococcaceaeUCG003 genus and IVDD were mediated by C7-DC (-7.5%, $p = 0.041$).
Conclusion: The present MR study offers evidence supporting the causal relationships between several specific GM taxa and IVDD, as well as identifying potential mediating metabolites.

Keywords: Gastrointestinal microbiome, Intervertebral disc degeneration, Metabolomics, Mendelian randomization analysis

INTRODUCTION

Intervertebral disc degeneration (IVDD) constitutes a multifactorial pathophysiological process, serving as the pathological foundation for various spinal degenerative disorders and playing a pivotal role in the onset of low back pain.[1,2] As the disease progresses, it may ultimately lead to a loss of labor and potentially impose a significant socioeconomic burden. Although the precise mechanisms driving IVDD development remain unclear, evidence indicates a significant involvement of gut microbiota (GM) and blood metabolites in its progression.[3-7] The human GM plays a crucial role in regulating aspects such as host me-

tabolites and maintaining immunological homeostasis, among others. Dysbiosis in the GM has been linked to metabolic, immune, neurological, and musculoskeletal disorders.[8-10] Hence, we hypothesized that there might exist causal relationships between the GM, metabolites, and IVDD. Our aim was to elucidate these relationships and pinpoint potential metabolites that could serve as early diagnostic markers and clinical treatment targets.

Mendelian randomization (MR) is a powerful method utilized to uncover potential causal relationships between exposure and outcome.[11] It operates by leveraging genetic variations associated with the exposure as instrumental variables (IVs) to evaluate the connection between exposure and outcome.[12] Moreover, a growing body of evidence highlights the utility of utilizing human genetic data related to gut microbial characteristics in clinical investigations.[13] This approach allows us to apply MR as a tool for inferring causal relationships between GM and IVDD. In our study, we conducted bidirectional MR analyses and 2 mediation analyses using summary statistics derived from the most extensive and current genome-wide association studies (GWAS) of GM, blood metabolites, and IVDD. This enabled us to dissect the associations among these factors comprehensively.

MATERIALS AND METHODS

1. Study Design and Data Sources

Fig. 1 illustrates the study design, underscoring that the causal interpretations derived from MR estimates are contingent upon 3 fundamental assumptions. Specifically, the genetic variants, serving as IVs or single nucleotide polymorphisms (SNPs), are required to (1) strongly predict the exposures, (2) be associated with the outcome exclusively through these exposures, and (3) not be connected to any confounders that could affect the exposure-outcome relationship.[12] Ethical approval for each GWAS included in this study can be accessed through the respective original articles.

Genetic instruments for analyzing GM were sourced from the MiBioGen consortium (https://mibiogen.gcc.rug.nl), renowned for conducting the most comprehensive genome-wide meta-analysis that integrates human genome-wide genotypes with fecal 16S rRNA sequencing data.[14] This extensive analysis encompassed 18,340 individuals across 24 cohorts, mainly of Eu-

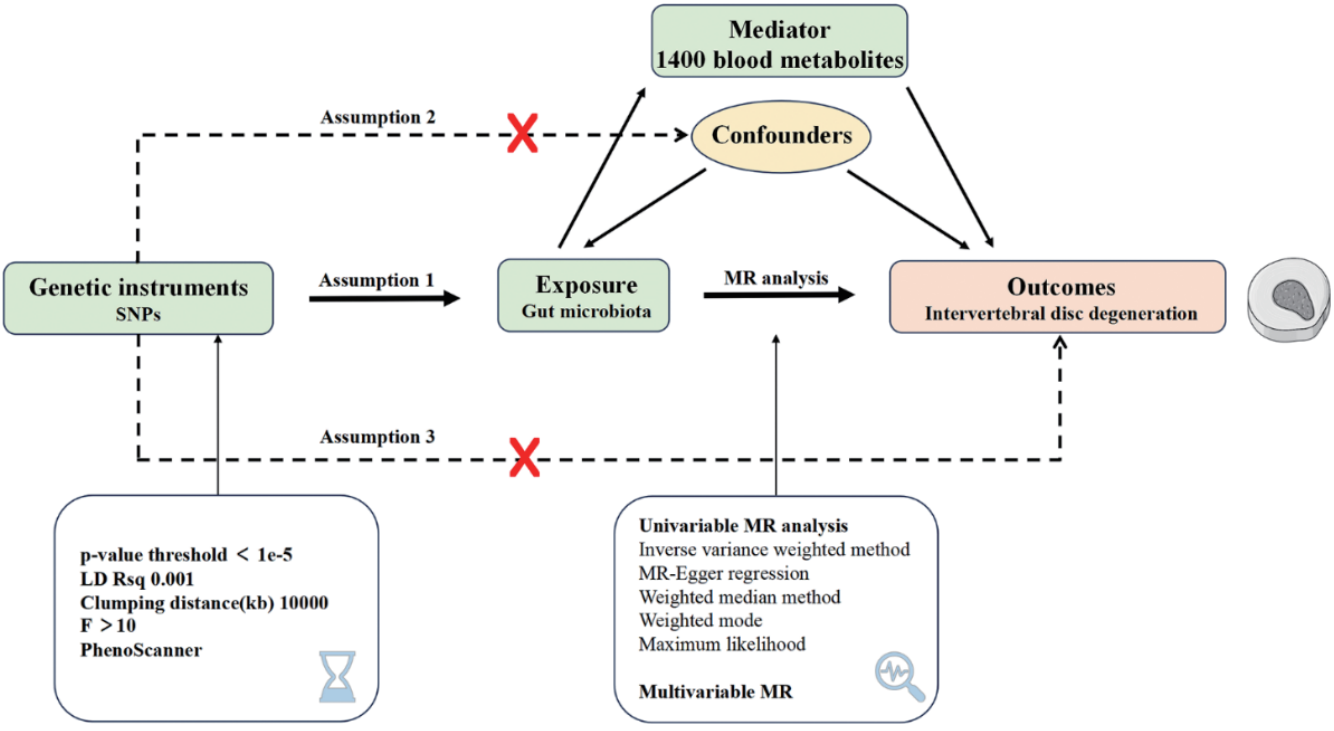

Fig. 1. Assumptions and design of the bidirectional and mediation Mendelian randomization (MR) analyses. Initially, a 2-sample bidirectional MR analysis was conducted to explore the causality between gut microbiota and intervertebral disc degeneration (IVDD). Subsequently, a 2-step MR approach was utilized to identify potential mediators (step 1: the impact of metabolites on IVDD; step 2: the influence of gut microbiota on metabolites), which was then validated through multivariable MR. SNP, single nucleotide polymorphism; LD, linkage disequilibrium.

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ropean ancestry. At the lowest taxonomic level examined, genus, 131 genera were identified with a mean abundance greater than 1%, including 12 unidentified genera. Consequently, 119 known genus-level taxa were selected for inclusion in this analysis. Summary statistics for 1400 blood metabolites were obtained from the GWAS Catalog (https://www.ebi.ac.uk/gwas/), representing an unparalleled resource of genetic associations.[15] The outcome data were sourced from the FinnGen Consortium's R10 release, an extensive genomic database with detailed phenotypic information. IVDD was identified using ICD-10 M51, ICD-9 722, and ICD-8 275 codes. Specifically, we focused on data labeled as "Other intervertebral disc disorders" within this dataset. Selecting this specific label was crucial to ensure an accurate and relevant definition of IVDD for our study.[16]

2. Instrumental Variable

To select IVs, the following quality control procedures were implemented: First, SNPs with a p-value below the locus-wide significance threshold (1×10^{-5}) were identified as IVs strongly linked to the GM and blood metabolites.[13] For reverse analysis, SNPs associated with IVDD meeting the conventional GWAS threshold ($p < 5 \times 10^{-8}$) were selected. Second, to ensure IV independence, linkage disequilibrium among SNPs was assessed using clumping ($R^2 < 0.001$, clumping distance = 10,000 kb). Third, screened SNPs were harmonized with the outcome's GWAS summary statistics, excluding palindromic and ambiguous alleles. Fourth, the F-statistic of each SNP was calculated to assess its statistical strength, discarding those with F-statistics < 10 to mitigate weak IV bias.[17] Fifth, SNPs in the harmonized dataset strongly linked to the outcome ($p < 1 \times 10^{-5}$) were manually reviewed and excluded. Sixth, the MR-PRESSO test was utilized to assess potential horizontal pleiotropy and identify outliers. The refined list of SNPs, post-outlier elimination, was then utilized for further MR analysis.

3. Two-Sample Bidirectional MR

We first conducted 2-sample bidirectional MR analyses to explore the causal relationship between the GM and IVDD. For effect estimates, we used the conventional MR approach: the inverse-variance-weighted (IVW) method. Results were reported as beta (β) values with standard errors for continuous outcomes, and odds ratios (ORs) with 95% confidence intervals (CIs) for binary outcomes; p-values below 0.05 were considered nominally significant. In addition, we performed 4 complementary methods: MR Egger, the weighted median method, the weighted mode method, and maximum likelihood.

4. Two-Step MR and MVMR

We further adopted 2 mediation approaches, 2-step MR (TSMR) and multivariable MR (MVMR),[18,19] to analyze the direct and indirect effects of the GM and 1400 blood metabolites on IVDD. TSMR assumes there is no interaction between the exposure and mediator. In addition to the basic effect estimates of GM on IVDD (β) obtained from univariate MR analyses, 2 additional estimates were calculated: (1) the causal effect of the mediator (1400 blood metabolites) on IVDD (β2), and (2) the causal effect of the exposure (the 5 bacterial genera identified in the primary MR analysis) on the mediator (β3).

Finally, we utilized MVMR as an additional method to validate the roles of metabolites identified in TSMR. MVMR estimates the controlled direct effect of the exposure on the outcome, which in our study includes the effect of metabolites on IVDD adjusting for bacteria (β2*), and the effect of bacteria on IVDD, adjusting for metabolites (β1*).[20] The indirect effect, representing the causal effect of the GM on IVDD via mediators, can then be estimated using the product of coefficients method ($\beta 3 \times \beta 2^*$). Consequently, the proportion mediated can be calculated as the "indirect effect divided by the total effect" ($[\beta 3 \times \beta 2^*]/\beta$). Our analytic process was in accordance with the STROBE-MR guidelines.[21]

5. Statistical Analysis

We conducted sensitivity analyses using the MR-Egger regression, leave-one-out, and MR-PRESSO methods. The MR-Egger regression and MR-PRESSO were utilized to test for and correct potential horizontal pleiotropy in our selected IVs. MR-Egger regression was employed to account for horizontal pleiotropy by relying on the InSIDE (Instrument Strength Independent of Direct Effect) assumption, which ensures the strength of genetic variants' effect on the exposure is independent of their effect on the outcome.[22] MR-Egger estimates both the causal effect (slope) and pleiotropy (intercept), with a non-zero intercept indicating pleiotropic bias. The p-value from the MR-Egger regression intercept test was applied to evaluate horizontal pleiotropy, and the method provided bias-adjusted causal estimates and robustness assessments. Additionally, MR-PRESSO was used to detect and eliminate outliers among the IVs. MR-PRESSO includes 3 components: the Global Test to identify overall pleiotropy, the Outlier Test to flag and remove pleiotropic IVs, and the Distortion Test to assess the impact of outlier correction on causal estimates.[23] By systematically addressing pleiotropy, MR-PRESSO enhanced the reliability of our causal estimates. Furthermore, Cochrane Q statistic was applied to assess the variabil-

ity of SNP estimates across each MR association, and MVMR analysis was implemented to adjust for confounding risk factors, effectively minimizing their influence on the causal inference. The statistical methods employed in our study have been thoroughly reviewed and rigorously validated by a statistician to ensure their accuracy and reliability.

To control for multiple comparisons in our analysis, we applied the false discovery rate (FDR) correction using the q-value approach. The q-value provides an estimate of the proportion of false positives among the set of significant results, allowing for a balance between statistical power and the control of false discoveries. This method was chosen due to its suitability for large-scale genomic data and its ability to provide interpretable significance thresholds.[24] All IVW results were adjusted for multiple testing using the FDR method, with a significance threshold set at q-value <0.1. Results indicating a p-value <0.05 but a q-value ≥ 0.1 were considered suggestive of an association. All MR analyses were conducted in R ver. 4.3.2 (R Foundation for Statistical Computing, Vienna, Austria), utilizing the "TwoSampleMR" and "MendelianRandomization" packages. FDR q-values were estimated using the "p.adjust" function in R.

RESULTS

1. Selection of IVs

The number of SNPs used as IVs was 1,256 for 119 bacterial genera in the MiBioGen consortium, 28,860 for the 1400 blood metabolites in the GWAS Catalog, and 170 for IVDD in the FinnGen repository. All IVs exhibited F-values greater than 10, demonstrating their robustness against bias introduced by weak IVs. Details about the selected IVs and the manually excluded SNPs are provided in Supplementary Tables 1-4.

2. Causal Effects of GM on IVDD

When evaluating the causal effects of GM on IVDD, 4 bacterial genera were found to be negatively associated with IVDD, while 3 genera showed positive associations with IVDD using the IVW method (Fig. 2, Supplementary Table 5). Although the Eubacteriumbrachygroup and RuminococcaceaeUCG011 genera were linked causally to IVDD, the MR-Egger method indicated an opposite direction, suggesting that the causality might be invalid.[22] Among the other 5 genera, the Eubacteriumcoprostanoligenesgroup genus demonstrated the strongest effect on the risk of IVDD (OR, 0.842; 95% CI, 0.769–0.922; $p<0.001$). The IVW results for the Eubacteriumcoprostanoligenesgroup genus remained significant after corrections for multiple testing (FDR q-value = 0.025). In the reverse MR analysis, we found no significant causal effect of IVDD on GM (Table 1). Furthermore, these results were deemed reliable, showing no evidence of pleiotropy, as confirmed by a sensitivity analysis (Supplementary Tables 6, 7).

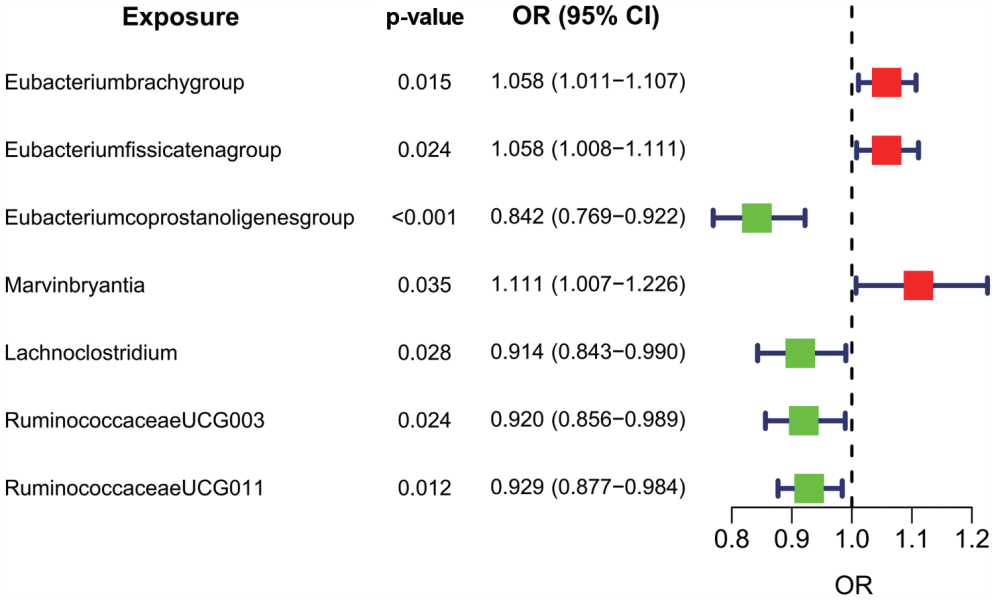

Fig. 2. Mendelian randomization analyses demonstrate the causal effect of gut microbiota on intervertebral disc degeneration. The squares, colored in red and green, indicate positive and negative odds ratios (ORs), respectively, derived from the inverse-variance-weighted analysis (truncated at a $p<0.05$). CI, confidence interval.

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Table 1. Mendelian randomization analyses demonstrating the causal effects of intervertebral disc degeneration (IVDD) on gut microbiota

IVDD	Method	No. of SNPs	OR (95% CI)	p-value	q-value
Eubacteriumfissicatenagroup	IVW	33	0.850 (0.721–1.002)	0.053	0.267
	MR Egger	33	0.869 (0.325–2.322)	0.781	
	Weighted median	33	0.966 (0.767–1.217)	0.770	
	Weighted mode	33	1.002 (0.656–1.530)	0.992	
	ML	33	0.853 (0.722–1.008)	0.062	
Eubacteriumcoprostanoligenesgroup	IVW	35	0.975 (0.903–1.052)	0.509	0.636
	MR Egger	35	0.869 (0.566–1.334)	0.524	
	Weighted median	35	0.952 (0.853–1.063)	0.379	
	Weighted mode	35	0.952 (0.768–1.179)	0.653	
	ML	35	0.974 (0.902–1.052)	0.506	
Marvinbryantia	IVW	34	1.062 (0.953–1.184)	0.275	0.636
	MR Egger	34	0.868 (0.468–1.613)	0.658	
	Weighted median	34	1.068 (0.934–1.221)	0.333	
	Weighted mode	34	1.061 (0.822–1.369)	0.653	
	ML	34	1.064 (0.969–1.169)	0.192	
Lachnoclostridium	IVW	34	1.012 (0.938–1.091)	0.765	0.765
	MR Egger	34	1.018 (0.664–1.561)	0.935	
	Weighted median	34	1.031 (0.931–1.142)	0.554	
	Weighted mode	34	1.073 (0.860–1.339)	0.537	
	ML	34	1.012 (0.938–1.092)	0.763	
RuminococcaceaeUCG003	IVW	34	1.030 (0.947–1.120)	0.496	0.636
	MR Egger	34	0.999 (0.620–1.610)	0.998	
	Weighted median	34	1.007 (0.894–1.134)	0.909	
	Weighted mode	34	0.979 (0.799–1.200)	0.840	
	ML	34	1.030 (0.946–1.121)	0.495	

OR, 95% CI, and p-values were derived from Mendelian randomization analysis, while q-values were calculated using the false discovery rate method.
SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval; IVW, inverse-variance-weighted; MR, Mendelian randomization; ML, maximum likelihood.

3. Mediation Analyses of 1400 Blood Metabolites

In the TSMR analysis (Fig. 3), it was found that a total of 84 blood metabolites were causally associated with IVDD (Supplementary Table 8). In the sensitivity analysis, the MR-PRESSO test identified 5 SNPs with pleiotropy (rs6851940, rs4517148, rs147750540, rs2393791, rs3809095). After removing these SNPs, we found that the causal relationships between 2 blood metabolites and IVDD changed ($p > 0.05$). In the leave-one-out analysis, we identified one pleiotropic IV (rs139446584). After removing this IV, the causal relationship between the corresponding blood metabolite and IVDD also changed ($p > 0.05$). Additionally, although there were 14 blood metabolites causally associated with IVDD, the MR-Egger method showed opposite trends, suggesting that the causal relationship may not hold.[22] Among the remaining 67 blood metabolites, after multiple testing correction, the IVW result for 2-methylserine showed significance (FDR $q = 0.065$). Although the Cochrane Q test indicated heterogeneity for 5 metabolites ($p < 0.05$) (Supplementary Table 6), this heterogeneity did not affect the overall result. The presence of heterogeneity may be attributed to the data coming from different analytical platforms or experiments, etc.

Of the 5 bacterial genera causally associated with IVDD, 4 were significantly correlated with 9 of the 67 metabolites mentioned above (Table 2, Supplementary Table 9). However, for Eubacteriumfissicatenagroup with Phosphocholine and Lachnoclostridium with Phosphate/N-palmitoyl-sphingosine (d18:1

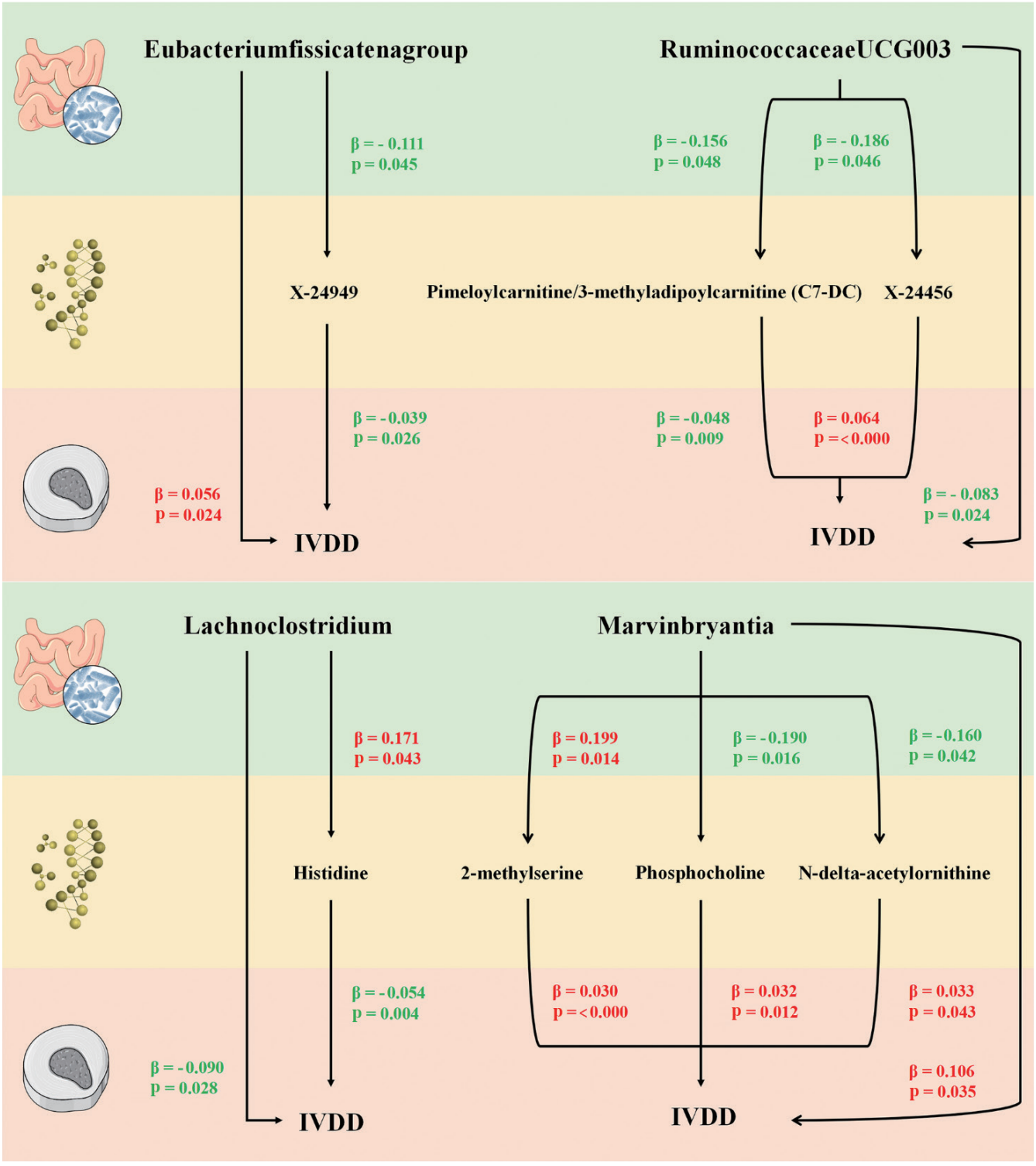

Fig. 3. The diagram illustrates the mediation pathway of "gut microbiota - blood metabolites - intervertebral disc degeneration" in a 2-step Mendelian randomization. Beta values (β) represent the causal effect estimates obtained through the inverse-variance-weighted method (truncated at $p < 0.05$). Characters highlighted in red and green denote positive and negative associations, respectively. IVDD, intervertebral disc degeneration.

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Table 2. Mendelian randomization analyses demonstrating the causal effects of gut microbiota on blood metabolites

Exposure	Mediator	Method	No. of SNPs	Beta ± SE	p-value	p_h	$p_{intercept}$
Eubacteriumfissicatenagroup	Phosphocholine	IVW	8	0.136 ± 0.052	0.009	0.479	0.510
		MR Egger	8	-0.049 ± 0.269	0.862	0.419	
	X-24949	IVW	8	-0.111 ± 0.056	0.045	0.751	0.831
		MR Egger	8	-0.174 ± 0.288	0.567	0.650	
Lachnoclostridium	Histidine	IVW	14	0.171 ± 0.085	0.043	0.828	0.108
		MR Egger	14	0.687 ± 0.309	0.046	0.950	
	Phosphate/N-palmitoyl-sphingosine (d18:1 to 16:0)	IVW	14	0.202 ± 0.087	0.019	0.522	0.322
		MR Egger	14	-0.112 ± 0.316	0.730	0.529	
RuminococcaceaeUCG003	Pimeloylcarnitine/3-methyladipoylcarnitine	IVW	14	-0.156 ± 0.079	0.048	0.295	0.565
		MR Egger	14	-0.310 ± 0.272	0.277	0.254	
	X-24456	IVW	14	-0.186 ± 0.093	0.046	0.205	0.783
		MR Egger	14	-0.273 ± 0.324	0.416	0.159	
Marvinbryantia	2-methylserine	IVW	10	0.199 ± 0.081	0.014	0.754	0.295
		MR Egger	10	0.544 ± 0.318	0.126	0.799	
	Phosphocholine	IVW	10	-0.190 ± 0.079	0.016	0.548	0.186
		MR Egger	10	-0.622 ± 0.309	0.079	0.673	
	N-delta-acetylornithine	IVW	10	-0.160 ± 0.079	0.042	0.947	1.000
		MR Egger	10	-0.160 ± 0.308	0.618	0.908	

Beta, standard errors (SE), and p-values were derived from the Mendelian randomization analysis. Heterogeneity testing in the IVW method was conducted using Cochran Q statistic.
SNP, single nucleotide polymorphism; p_h, p-value for heterogeneity; $p_{intercept}$, p-value for the intercept of the MR-Egger regression; IVW, inverse-variance-weighted; MR, Mendelian randomization.

Table 3. Multivariable Mendelian randomization analyses of the causal effects between gut microbiota, blood metabolites and intervertebral disc degeneration

Exposure	Mediator	Direct effect (β1* ± SE)	Direct effect (β2* ± SE)	p-value	Indirect effect (β3 × β2* ± SE)	Proportion mediated (β3 × β2*/β)
Eubacteriumfissicatenagroup	X-24949	0.034 ± 0.025	-0.041 ± 0.018	0.024	0.005 ± 0.003	0.081
Lachnoclostridium	Histidine	-0.002 ± 0.051	-0.095 ± 0.038	0.013	-0.016 ± 0.010	0.181
RuminococcaceaeUCG003	Pimeloylcarnitine/3-methyladipoylcarnitine	-0.100 ± 0.035	-0.040 ± 0.019	0.041	0.006 ± 0.004	-0.075
	X-24456	-0.087 ± 0.034	0.032 ± 0.022	0.140	-0.006 ± 0.005	0.072
Marvinbryantia	2-methylserine	0.001 ± 0.051	0.062 ± 0.044	0.163	0.012 ± 0.010	0.116
	Phosphocholine	-0.006 ± 0.051	0.015 ± 0.028	0.596	-0.003 ± 0.005	-0.027
	N-delta-acetylornithine	-0.004 ± 0.050	0.007 ± 0.018	0.685	-0.001 ± 0.003	-0.011

Beta (β1* and β2*), standard errors (SEs), and p-values were derived from multivariable Mendelian randomization analysis. β1* and β2* represent the controlled direct effects of each pair of bacteria and metabolite on intervertebral disc degeneration (IVDD) after adjusting for each other.
β3, the causal effect of exposure on the mediator; the indirect effect (β3 × β2*) signifies the effect of exposure on IVDD via the corresponding mediator; β, the total effect of exposure on IVDD; proportion mediated is calculated as the "indirect effect/total effect."

to 16:0), although they were causally related, the MR-Egger method indicated opposite directions, suggesting that the causality might be invalid.[22] The Lachnoclostridium genus, identified as a protective taxon against IVDD (OR, 0.914; 95% CI, 0.843–0.990; p = 0.028), was associated with increased levels of histidine. The RuminococcaceaeUCG003 genus also demonstrated protective effects against IVDD (OR, 0.920; 95% CI, 0.856–0.989; p = 0.024) by downregulating Pimeloylcarnitine/

3-methyladipoylcarnitine (C7-DC) and X-24456. However, the Eubacteriumfissicatenagroup (OR, 1.058; 95% CI, 1.008–1.111; p = 0.024) and Marvinbryantia (OR, 1.111; 95% CI, 1.007–1.226; p = 0.035) genera were found to have detrimental effects on IVDD. Specifically, the Eubacteriumfissicatenagroup genus was associated with detrimental effects on IVDD through downregulation of X-24949 levels. Similarly, the Marvinbryantia genus exhibited detrimental effects on IVDD by upregulating 2-methylserine and downregulating Phosphocholine and N-delta-acetylornithine. Moreover, these results were deemed reliable, with no evidence of pleiotropy, as confirmed by a sensitivity analysis (Supplementary Tables 6, 7).

We conducted MVMR to validate the mediating effects of blood metabolites uncovered in TSMR (Table 3). We calculated the indirect effect and proportion mediated by these metabolites and found that the roles of X-24949, histidine, and C7-DC remained significant after adjusting for GM. Overall, we observed an indirect effect of X-24949 in the associations between the Eubacteriumfissicatenagroup genus and IVDD, with a mediated proportion of 8.1% (p = 0.024); Histidine in the association between the Lachnoclostridium genus and IVDD with a mediated proportion of 18.1% (p = 0.013); and C7-DC in the association between the RuminococcaceaeUCG003 genus and IVDD with a mediated proportion of -7.5% (p = 0.041). The effects of X-24456, 2-methylserine, Phosphocholine, and N-delta-acetylornithine were insignificant after adjusting for the GM.

DISCUSSION

In this large-scale MR study, we found that 5 bacterial genera were causally associated with IVDD, while IVDD did not exhibit a significant causal effect on the GM. The IVW results for the Eubacteriumcoprostanoligenesgroup genus remained significant after corrections for multiple testing. Through a 2-step MR analysis, we identified several bacterial genera, including Eubacteriumfissicatenagroup, RuminococcaceaeUCG003, Lachnoclostridium, and Marvinbryantia, as well as metabolites such as X-24949, C7-DC, X-24456, histidine, 2-methylserine, Phosphocholine, and N-delta-acetylornithine, which were significantly associated with IVDD. Moreover, the IVW results for 2-methylserine remained significant after corrections for multiple testing. Additionally, our MVMR analysis revealed that the associations between Eubacteriumfissicatenagroup and IVDD were mediated by X-24949, between Lachnoclostridium and IVDD by histidine, and between RuminococcaceaeUCG003 and IVDD by C7-DC.

The Eubacteriumcoprostanoligenesgroup genus was observed to have a notable negative impact on IVDD in our study, exhibiting significant potency. These anaerobic gram-positive bacteria are capable of converting cholesterol into coprostanol, rendering it unabsorbable and thereby influencing cholesterol levels.[25] Previous research has highlighted cholesterol's role in promoting IVDD through apoptosis and pyroptosis in NP cells, as well as extracellular matrix metabolism.[26] However, our study did not find any evidence of the Eubacteriumcoprostanoligenesgroup genus directly regulating cholesterol levels. The RuminococcaceaeUCG003 genus, a member of the Ruminococcaceae family, along with many bacteria within this family, are known to be general butyrate-producing bacteria. However, butyrate alleviates inflammatory response and nuclear factor-kappa B (NF-κB) activation in human degenerated intervertebral disc tissues.[27] Therefore, the influence of RuminococcaceaeUCG003 on IVDD might be mediated by butyrate. In this study, we found that the RuminococcaceaeUCG003 genus also demonstrated protective effects against IVDD by downregulating C7-DC and X-24456. C7-DC, an important acylcarnitine, may play a significant role in IVDD. Acylcarnitines are esterified forms of carnitine and are essential for transporting long-chain fatty acids (FAs) across the mitochondrial inner membrane for β-oxidation.[28] In metabolic disorders, the concentration of acylcarnitines is often altered, suggesting that C7-DC may influence the energy balance of intervertebral disc cells by regulating FA oxidation.[29-31] In the context of IVDD, the metabolic regulatory role of C7-DC may help slow down cellular energy depletion and degenerative changes. Overall, the role of C7-DC in IVDD warrants further investigation, particularly regarding its impact on FA metabolism and metabolic risks. Additionally, based on the existing results, the mediated proportion of C7-DC (-7.5%) suggests that it may play a certain role in the degenerative process of IVDD. We believe that although the clinical relevance of this effect needs further validation, it provides new directions for future research on C7-DC as a potential therapeutic target for IVDD. Lachnoclostridium genus is believed to be causally linked to a variety of diseases. In this study, it was also identified as a protective taxon against IVDD, mediated by increased levels of histidine. The metabolic product of histidine, histamine, is known to possess anti-inflammatory effects, primarily by regulating the NF-κB signaling pathway, which plays a central role in many inflammatory responses. In IVDD, proinflammatory cytokines such as interleukin (IL)-6, tumor necrosis factor-α and IL-1β activate NF-κB, triggering inflammation and degenerative changes in intervertebral

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disc tissue.[32,33] Studies have shown that histidine can inhibit NF-κB activation and reduce the degradation of IkBα, thereby alleviating the inflammatory response.[34] Therefore, histidine may help alleviate the degenerative process of IVDD by inhibiting the NF-κB pathway and reducing the production of inflammatory mediators such as IL-6. However, the precise underlying mechanism still requires further investigation.

The Eubacteriumfissicatenagroup genus is part of potentially disease-related bacteria that add to the risk of metabolic disorders and intestinal inflammation.[35] However, it is also recognized as a group of butyrate-producing bacteria and beneficial bacteria that suppress intestinal inflammation.[36] In this study, the Eubacteriumfissicatenagroup genus was found to be associated with detrimental effects on IVDD through the downregulation of X-24949 levels. Marvinbryantia represents a cellulose-degrading bacterial genus, comprising solely of one known species called Marvinbryantia formatexigens, which naturally resides within the human gut.[37] Marvinbryantia formatexigens is known as a gut acetogen that produces acetate in the human gut. Interestingly, a key pathway for producing butyrate in the human GM involves using externally sourced acetate to generate butyrate through butyryl-CoA: acetate Co-A transferase.[38] This suggests that the production of fecal butyrate may depend on acetate generation from Marvinbryantia formatexigens. However, butyrate alleviates inflammatory response and NF-κB activation in human degenerated intervertebral disc tissues.[27] Therefore, the influence of Marvinbryantia on IVDD might also be mediated by butyrate. Our study identifies Marvinbryantia genus as exerting detrimental effects on IVDD by upregulating 2-methylserine while concurrently downregulating Phosphocholine and N-delta-acetylornithine. Nevertheless, the precise mechanism underlying this influence necessitates further investigation.

To further enhance the clinical relevance of this work, these findings could be applied to the prevention and personalized treatment of IVDD by targeting the identified bacterial genera and metabolites. For instance, modulating the Eubacteriumcoprostanoligenesgroup genus, which negatively impacts IVDD, could provide a novel strategy for prevention by altering its metabolic pathways to reduce cholesterol accumulation. On the other hand, enhancing the protective effects of butyrate-producing bacteria, such as RuminococcaceaeUCG003, may alleviate inflammatory responses in the intervertebral discs by increasing butyrate production, thus promoting disc health. Additionally, the metabolites identified in this study, such as 2-methylserine and histidine, could serve as potential biomarkers for early diagnosis and personalized treatment plans for IVDD. Future studies should explore how these bacterial and metabolic alterations can be leveraged to develop more precise microbiome-based interventions, such as probiotics or metabolic modulators, to improve treatment outcomes and slow disease progression in IVDD patients.

Nevertheless, this study was subject to several limitations. To begin with, one limitation of our study is the assumption of linearity in the MR analysis, which may not fully capture the complexity of biological systems. Nonlinear relationships, threshold effects, and interactions between bacterial genera and host factors could play significant roles. Future research should incorporate nonlinear MR models and interaction analyses, and network-based approaches could provide further insights into these complex dynamics, enhancing our understanding of causal pathways. Furthermore, the MiBioGen consortium characterizes microbiome profiles using 16S ribosomal RNA gene sequencing, limited to genus-level taxonomic classification. However, metagenomic sequencing offers detail at the species level. Previous MR study of the GM found that higher taxonomic units sometimes yielded more significant p-values, indicating similar functionalities among species.[39] Additionally, disparities in genetic variant distribution among ethnic or racial groups may introduce population stratification, potentially skewing study outcomes.[40] Hence, caution should be exercised when extending findings to other demographic groups. Furthermore, we applied the FDR correction, which is particularly suitable for genome-wide studies involving thousands of comparisons, as it strikes a balance between detecting true positives and controlling false discoveries. Unlike the overly conservative Bonferroni correction, which can reduce statistical power and miss subtle associations, FDR maintains sensitivity while providing interpretable significance thresholds.[41] Although some results with marginal q-values (e.g., $q = 0.095$) fall within the FDR-controlled range, we recognize the importance of validating these findings in independent datasets. Future studies will focus on replicating these associations in diverse populations to enhance their reliability and generalizability. Additionally, future research should carefully consider the interaction effects between GM and IVDD in MR analyses to provide a more comprehensive understanding of these complex relationships.

NOTES

Supplementary Materials: Supplementary Tables 1-9 for this article is available at https://doi.org/10.14245/ns.2449172.586.

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