International Journal of General Medicine

Dovepress
Taylor & Francis Group

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ORIGINAL RESEARCH

The Gut-Disc Axis: Mendelian Randomization Study Reveals Causal Effects of Gut Microbiota on Intervertebral Disc Degeneration Not Mediated by Systemic Inflammation

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Background: Intervertebral disc degeneration (IVDD) is a major cause of low back pain. Observational studies suggest a link with gut microbiota (GM), possibly mediated by systemic inflammation, but causality remains unclear.

Objective: To assess the suggestive associations of GM and inflammatory cytokines (ICs) on IVDD using Mendelian randomization (MR), and evaluate potential mediation by ICs.

Methods: We performed a two-sample MR analysis. Genetic instruments for GM came from MiBioGen (n=18,340), for 41 ICs from a meta-analysis (n=8337), and for IVDD from FinnGen (29,508 cases/227,388 controls). Inverse-variance weighted (IVW) was the primary method. Mediation was assessed via two-step MR, with sensitivity analyses (MR-Egger, MR-PRESSO).

Results: At the nominal significance level ($P<0.05$), 11 microbial taxa showed suggestive causal associations with IVDD risk, of which only Eubacterium coprostanoligenes group approached the Bonferroni-corrected threshold ($P=3.0\times10^{-5}$). Seven inflammatory cytokines showed suggestive associations with IVDD; however, none remained statistically statistically significant after Bonferroni correction. Mediation analysis revealed no statistically statistically significant indirect effect of cytokines linking gut microbiota to IVDD.

Conclusion: This study provides genetic evidence supporting a potential gut-disc axis. The lack of evidence for cytokine mediation does not exclude alternative pathways but indicates insufficient support for systemic cytokine-mediated mechanisms among those examined; future mechanistic studies should explore microbial metabolites and local immune activity.

Keywords: intervertebral disc degeneration, gut microbiota, inflammatory cytokines, Mendelian randomization, mediation analysis, causality

Introduction

Intervertebral disc degeneration (IVDD) is a predominant worldwide cause of low back pain and disability, conferring a substantial socioeconomic burden on healthcare systems.[1] While aging, mechanical stress, and genetic predisposition are well-established risk factors,[2] emerging evidence suggests the gut microbiota (GM) as a potential contributor to IVDD pathogenesis.[3] Observational studies have linked dysbiosis of the GM to an increased risk of IVDD, potentially through mechanisms involving systemic inflammation, metabolic dysfunction, or immune system modulation.[4] Nonetheless, inherent limitations of observational designs—such as residual confounding and reverse causality—preclude definitive conclusions regarding a causal relationship between GM and IVDD.[5]

Concurrently, the role of inflammatory cytokines (ICs) in IVDD progression has gained increasing attention.[6] Elevated levels of specific ICs are a hallmark of degenerated disc tissue, where they are believed to promote extracellular

https://doi.org/10.2147/IJGM.S615488
Received: 14 April 2026
Accepted: 3 June 2026
Published: 15 June 2026

matrix degradation, neurovascular ingrowth, and pain sensitization.[7] Given that GM composition is a known regulator of systemic host immune responses and circulating IC levels,[8] it has been hypothesized that ICs may act as key mediators in the putative gut-disc axis.[9] For instance, certain bacterial taxa can influence the production of prototypical cytokines and chemokines, which could subsequently disrupt the homeostasis of the disc microenvironment.[10]

To address these hypotheses and overcome the limitations of observational epidemiology, we employed a two-sample Mendelian randomization (MR) framework.[11] This method utilizes genetic variants as instrumental variables to infer causality while minimizing confounding and bias from reverse causation. Leveraging summary-statistics from large-scale genome-wide association studies (GWAS) of GM, ICs, and IVDD, we aimed to: (i) assess the causal effect of GM on IVDD risk; (ii) evaluate the causal influence of ICs on IVDD; and (iii) investigate the mediating role of ICs in the pathway from GM to IVDD.

Although a limited number of recent MR studies have explored associations between gut microbiota and IVDD, none have systematically incorporated a comprehensive panel of circulating inflammatory cytokines as candidate mediators within a unified two-step MR framework. Whether systemic inflammation constitutes the principal biological bridge linking gut dysbiosis to IVDD remains unresolved. Our study fills this gap by simultaneously evaluating 211 microbial taxa, 41 inflammatory cytokines, and IVDD outcomes within a single causal-inference framework. This study provides genetic evidence supporting the gut-disc axis hypothesis.

Methods

Study Design

In brief, our analytical framework consisted of three sequential steps (Figure 1): (Step 1) two-sample MR to assess the total causal effect of each gut microbial taxon on IVDD; (Step 2) two-sample MR to assess the causal effect of each inflammatory cytokine on IVDD; and (Step 3) two-step mediation MR to test whether cytokines mediate the microbiota-IVDD relationship.[12]

Data Sources

Genetic instruments for GM were obtained from the MiBioGen consortium GWAS, which included 18,340 individuals and provided summary statistics for 211 microbial taxa (spanning 9 phyla, 16 classes, 20 orders, 35 families, and 131 genera).[13] Summary statistics for 41 inflammatory cytokines were sourced from a GWAS meta-analysis comprising 8337 individuals of European ancestry.[14] Genetic association estimates for IVDD were obtained from the FinnGen consortium (R8 release)(https://www.finngen.fi/fi), including 29,508 cases and 227,388 controls. IVDD cases were identified using ICD-10 (M51), ICD-9 (722), and ICD-8 (725) codes. GWAS summary statistics were downloaded on 5th Nov 2025.

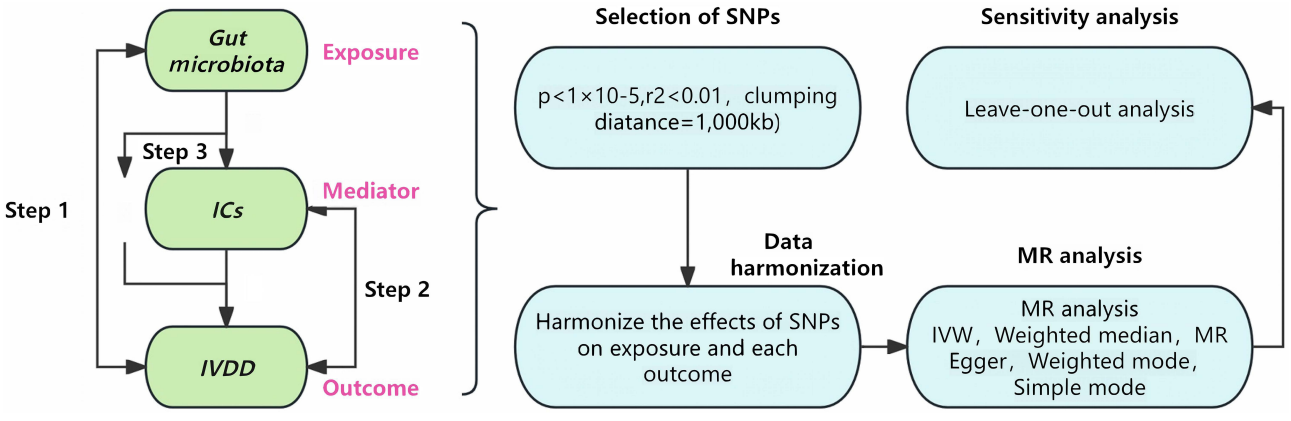

Figure 1 Study Design and Analytical Workflow. A schematic overview of the three-step Mendelian randomization (MR) analysis. Step 1: suggestive associations of GM on intervertebral disc degeneration (IVDD) were assessed. Step 2: suggestive associations of inflammatory cytokines (ICs) on IVDD were assessed. Step 3: A two-step MR mediation analysis was performed to investigate whether the identified ICs mediate the causal pathways from GM to IVDD.

Selection of Genetic Instruments

The locus-wide significance threshold ($P<1\times10^{-5}$) for GM is widely adopted in microbiome MR studies because few microbial taxa reach genome-wide significance, and overly stringent thresholds would leave most taxa without analyzable instruments.[15] For the included taxa, the number of SNPs per exposure ranged from 3 to 21 (mean ≈11), the minimum F-statistic was 19.5, and the mean F-statistic was 34.7, with cumulative R^2 between 0.5% and 3.2% per exposure (Table S1), indicating sufficient instrument strength and minimal weak instrument bias.[16] Data harmonization was conducted using the harmonise_data function in the TwoSampleMR R package. Effect alleles were aligned between exposure and outcome datasets based on the reference strand. Palindromic SNPs with intermediate allele frequencies (MAF > 0.42) were excluded to prevent strand-related ambiguity, while palindromic SNPs with MAF ≤ 0.42 were retained and aligned using allele frequency information. SNPs that could not be unambiguously harmonized were removed prior to MR analysis. To satisfy the second MR assumption (independence of instruments from confounders), all candidate SNPs were queried in the PhenoScanner V2 database to identify potential associations with known IVDD confounders (eg, body mass index, smoking, type 2 diabetes); SNPs showing genome-wide significant associations ($P < 5\times10^{-8}$) with these traits were excluded prior to downstream analysis.

Mendelian Randomization Analysis

Reverse MR analysis was conducted using IVDD as the exposure and the significant microbial taxa / cytokines as outcomes. SNPs associated with IVDD at genome-wide significance ($P<5\times10^{-8}$) were selected as instruments after clumping ($r^2<0.001$, kb=10,000).[17–20] The IVW method was used as the primary analysis; a P>0.05 in the reverse direction was interpreted as evidence against reverse causation.[21] Bonferroni correction was applied separately at each analytical level: for the GM–IVDD analysis, the corrected threshold was $P < 0.05/211 \approx 2.37\times10^{-4}$; for the IC–IVDD analysis, $P < 0.05/41 \approx 1.22\times10^{-3}$; and for the mediation analysis, $P < 0.05/77 \approx 6.5\times10^{-4}$. Findings with $P < 0.05$ but exceeding the corresponding Bonferroni threshold are reported as "suggestive" rather than statistically significant, consistent with established practice in microbiome MR studies where strict correction can be overly conservative given the correlated nature of microbial taxa.

Mediation Analysis

A two-step MR approach was used to quantify mediation effects. First, MR was performed to estimate the effect of GM on potential mediator ICs (Path A). Second, the effect of these ICs on IVDD was estimated (Path B). The 95% confidence intervals for the indirect effects (β_A × β_B) were estimated using the Delta method, which provides analytically derived standard errors via first-order Taylor expansion. The proportion mediated was calculated only when both the total effect and indirect effect were nominally significant; otherwise, the proportion mediated was considered statistically unstable and is not reported as a primary outcome.[22]

Bidirectional and Sensitivity Analyses

Reverse causation was assessed by testing the effects of genetic liability to IVDD on GM and ICs, using genome-wide statistically significant SNPs ($P<5\times10^{-8}$) as instruments. Sensitivity analyses included: (1) Cochran's Q test to assess heterogeneity; (2) MR-Egger regression and MR-PRESSO to detect and correct for horizontal pleiotropy; (3) Leave-one-out analysis to evaluate the influence of individual variants. All analyses were conducted in R (v4.2.1) using the TwoSampleMR (v0.5.7) and MR-PRESSO (v1.0) packages.[23–26]

Ethical Approval

This study was exempt from institutional ethical review. Specifically, this exemption is strictly based on items 1 and 2 of Article 32 of the Measures for Ethical Review of Life Science and Medical Research Involving Human Subjects (issued by the National Health Commission of China, dated February 18, 2023).

Result

Instrumental Variable Selection

Initially, we identified 224, 478, 1667, 280, and 125 SNPs associated with 210 GMs at the class, family, genus, order, and phylum levels, respectively, at a level of $P < 1 \times 10^{-5}$ (one gut microbiota was excluded due to no eligible SNPs). These 2774 SNPs were selected as IVs for the 210 GM taxa. Then, we identified 451 SNPs associated with 41 cytokines at a level of $P < 5 \times 10^{-6}$.

Suggestive Causal Associations Between GM and IVDD

Using the IVW method, we identified suggestive causal associations between 11 microbial taxa and IVDD risk at the nominal level ($P < 0.05$). None of these associations survived Bonferroni correction ($P < 2.37\times10^{-4}$); only the protective association of the Eubacterium coprostanoligenes group ($P = 3.0\times10^{-5}$) approached corrected significance. Therefore, with the exception of Eubacterium coprostanoligenes group, all microbiota–IVDD findings should be interpreted as suggestive rather than definitive (Figures 2 and 3, Table 1). The F-statistic for all SNPs exceeded 10, indicating no substantial weak instrument bias (Table S1).

Five taxa were identified as risk factors for IVDD: *genus Sutterella* (id.2896, OR = 1.18, 95% CI: 1.08–1.29, P = 0.0004), *genus Marvinbryantia* (id.2005, OR = 1.17, 95% CI: 1.07–1.29, P = 0.001), *genus Escherichia-Shigella* (id.3504, OR = 1.11, 95% CI: 1.01–1.23, P = 0.03), *phylum Verrucomicrobia* (id.3982, OR = 1.09, 95% CI: 1.01–1.18, P = 0.03), and *genus Allisonella* (id.2174, OR = 1.06, 95% CI: 1.00–1.11, P = 0.04).

Six taxa were identified as protective factors for IVDD: *genus Eubacterium coprostanoligenes group* (id.11375, OR = 0.81, 95% CI: 0.73–0.89, $P = 3.0\times10^{-5}$), *family Rhodospirillaceae* (id.2717, OR = 0.92, 95% CI: 0.87–0.98, P = 0.008), *order Rhodospirillales* (id.2667, OR = 0.93, 95% CI: 0.87–0.98, P = 0.014), *genus Bifidobacterium* (id.436, OR = 0.92, 95% CI: 0.85–0.99, P = 0.03), *genus Ruminococcaceae UCG011* (id.11368, OR = 0.94, 95% CI: 0.89–0.99, P = 0.03), and *genus Prevotella9* (id.11183, OR = 0.94, 95% CI: 0.88–1.00, P = 0.048).

Reverse MR analysis using IVDD-associated SNPs as instruments showed no significant reverse effect of IVDD on the identified gut microbial taxa (P>0.05), supporting the directional assumption from microbiota to IVDD rather than the reverse (Table S2), which helps to mitigate concerns about reverse causation.

Sensitivity analyses supported the robustness of the primary findings. No statistically significant heterogeneity was observed among the IVs for the gut microbiome traits (Table S3). We used the MR Pleiotropy RESidual Sum and Outlier (MR-PRESSO) method to assess horizontal pleiotropy, and the results indicated no evidence of pleiotropic bias (Table S4). Furthermore, leave-one-out analysis confirmed that no single SNP disproportionately drove the observed associations between GM and IVDD (Figure 4).

Exposure	Outcome	Method	nSNP	pval	OR (95% CI)
genus Eubacterium coprostanoligenes group	IVDD	IVW	13	3.03e−05	0.81 (0.73 – 0.89)
genus Sutterella	IVDD	IVW	11	3.67e−04	1.18 (1.08 – 1.29)
genus Marvinbryantia	IVDD	IVW	10	9.68e−04	1.17 (1.07 – 1.29)
family Rhodospirillaceae	IVDD	IVW	15	0.008	0.92 (0.87 – 0.98)
order Rhodospirillales	IVDD	IVW	14	0.014	0.93 (0.87 – 0.98)
genus Bifobacterium	IVDD	IVW	12	0.029	0.92 (0.85 – 0.99)
genus Escherichia Shigella	IVDD	IVW	10	0.030	1.11 (1.01 – 1.23)
genus Ruminococcaceae UCG011	IVDD	IVW	8	0.030	0.94 (0.89 – 0.99)
phylum Verrucomicrobia	IVDD	IVW	12	0.035	1.09 (1.01 – 1.18)
genus Allisonella	IVDD	IVW	8	0.037	1.06 (1.00 – 1.11)
genus Prevotella9	IVDD	IVW	15	0.048	0.94 (0.88 – 1.00)

Figure 2 Forest Plot of suggestive associations of GM on IVDD Risk. The suggestive associations of 11 gut microbial taxa statistically significantly associated with IVDD risk, as determined by the inverse-variance weighted (IVW) method, are displayed. Effect sizes are presented as odds ratios (ORs) with 95% confidence intervals (CIs). Taxa are categorized as risk factors (OR > 1) or protective factors (OR < 1).

https://doi.org/10.2147/IJGM.S615488

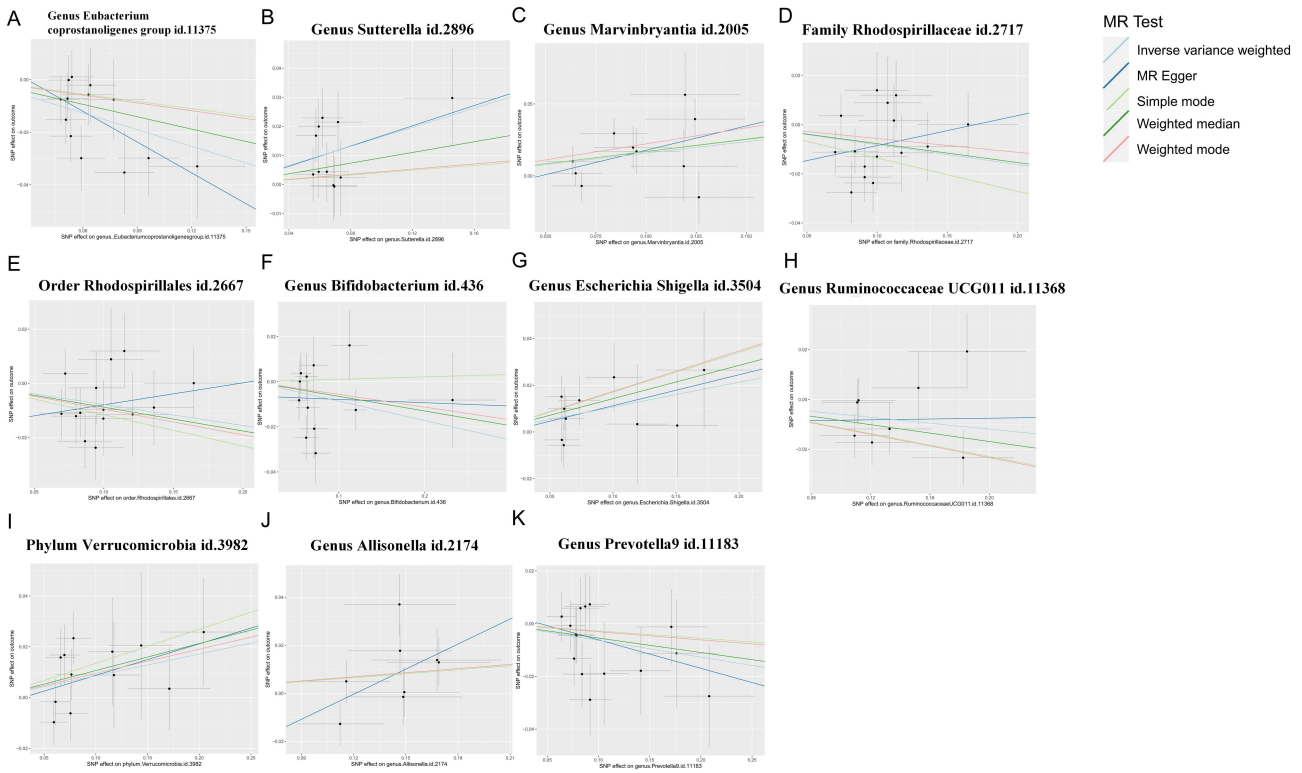

Figure 3 Scatter Plots of Genetic Associations for statistically significant GM and IVDD. Each point represents a single-nucleotide polymorphism (SNP). The slopes of the colored regression lines generated by different Mendelian randomization (MR) methods (including inverse-variance weighted, MR-Egger, simple mode, weighted median, and weighted mode) indicate the estimated causal effect. (**A**) Genus Eubacterium coprostanoligenes group. (**B**) Genus Sutterella. (**C**) Genus Marvinbryantia. (**D**) Family Rhodospirillaceae. (**E**) Order Rhodospirillales. (**F**) Genus Bifidobacterium. (**G**) Genus Escherichia-Shigella. (**H**) Genus Ruminococcaceae UCG011. (**I**) Phylum Verrucomicrobia. (**J**) Genus Allisonella. (**K**) Genus Prevotella9.

Suggestive Associations of Inflammatory Cytokines on IVDD

We next assessed the suggestive associations of 41 inflammatory cytokines on IVDD. IVW analysis revealed suggestive associations for seven cytokines. Genetically predicted levels of RANTES (OR = 1.07, 95% CI: 1.01–1.12, P = 0.013) and SDF-1α (OR = 1.09, 95% CI: 1.00–1.19, P = 0.042) were associated with increased odds of IVDD. In contrast, genetically predicted levels of IFN-γ (OR = 0.89, 95% CI: 0.83–0.95, P = 0.0007), IL-1β (OR = 0.89, 95% CI: 0.83–0.97, P = 0.005), MIP-1β (OR = 0.97, 95% CI: 0.95–1.00, P = 0.02), MIG (OR = 0.95, 95% CI: 0.91–0.99, P = 0.025), and IL-18 (OR = 0.96, 95% CI: 0.93–1.00, P = 0.036) were associated with decreased odds of IVDD (Figures 5 and 6, Table 2). The F-statistic for all SNPs exceeded 10, indicating no substantial weak instrument bias (Table S5).

It should be noted that none of these associations remained statistically statistically significant after Bonferroni correction for multiple testing (corrected significance threshold P < 0.0012). Sensitivity analyses supported the robustness of these findings. No statistically significant heterogeneity was detected among the IVs for cytokine traits (Table S6). The MR-PRESSO test revealed no evidence of horizontal pleiotropy (Table S7). Leave-one-out analysis indicated that no single SNP disproportionately influenced the results (Figure 7). It must be emphasised that none of the cytokine–IVDD associations survived Bonferroni correction. The following findings are therefore exploratory in nature and should not be interpreted as validated suggestive associations.

Mediation Analysis

We performed a two-step MR analysis to assess whether the suggestive associations of the 11 identified GM on IVDD were mediated by the 7 inflammatory cytokines suggestively associated with IVDD.

The overall findings from the mediation analysis were null. After applying a Bonferroni correction for the number of tested mediator-exposure-outcome combinations (corrected significance threshold P < [0.05/77] ≈ 0.00065), none of the

Table 1 Mendelian Randomization Analysis of the Causal Effects of Gut Microbiota on Intervertebral Disc Degeneration

Bacteria Taxa (Exposure)	No. of SNP	MR Method	β	OR (95% CI)	p-value
genus Sutterella id.2896	11	IVW	0.163894341	1.18 (1.08–1.29)	**0.000367143**
		Weighted median	0.091834597	1.10 (0.96–1.25)	0.167902609
		MR Egger	0.174293286	1.19 (0.81–1.74)	0.392164169
		Weighted mode	0.044135358	1.05 (0.84–1.30)	0.696172642
		Simple mode	0.041017424	1.04 (0.84–1.29)	0.71073143
genus Marvinbryantia id.2005	10	IVW	0.158303937	1.17 (1.07–1.29)	**0.000967851**
		Weighted median	0.169299987	1.18 (1.03–1.36)	**0.017288451**
		MR Egger	0.338462482	1.40 (0.88–2.22)	0.18832638
		Weighted mode	0.222484691	1.25 (0.93–1.67)	0.16731844
		Simple mode	0.171206186	1.19 (0.90–1.56)	0.251468991
genus Escherichia Shigella id.3504	10	IVW	0.106635285	1.11 (1.01–1.23)	**0.030273197**
		Weighted median	0.142584518	1.15 (1.02–1.31)	**0.024139701**
		MR Egger	0.13277237	1.14 (0.85–1.54)	0.412405268
		Weighted mode	0.173430922	1.19 (0.97–1.45)	0.123682334
		Simple mode	0.170468486	1.19 (0.95–1.48)	0.168767293
phylum Verrucomicrobia id.3982	12	IVW	0.085630678	1.09 (1.01–1.18)	**0.034735307**
		Weighted median	0.106300576	1.11 (1.00–1.24)	0.057286774
		MR Egger	0.124037789	1.13 (0.92–1.39)	0.27121849
		Weighted mode	0.095225595	1.10 (0.93–1.30)	0.294147404
		Simple mode	0.13459189	1.14 (0.95–1.37)	0.176750271
genus Allisonella Id.2174	8	IVW	0.054639431	1.06 (1.00–1.11)	**0.037141097**
		Weighted median	0.057258675	1.06 (0.99–1.14)	0.118381478
		MR Egger	0.347910545	1.42 (0.92–2.17)	0.162746248
		Weighted mode	0.057204084	1.06 (0.96–1.16)	0.268236762
		Simple mode	0.054554516	1.06 (0.95–1.17)	0.339276912
genus Eubacterium coprostanoligenes group id.11375	13	IVW	−0.21155005	0.81 (0.73–0.89)	**3.03286E-05**
		Weighted median	−0.156826576	0.85 (0.74–0.99)	**0.031421281**
		MR Egger	−0.39071807	0.68 (0.46–1.00)	0.075207433
		Weighted mode	−0.098523056	0.91 (0.72–1.15)	0.42710449
		Simple mode	−0.092183697	0.91 (0.72–1.16)	0.464153845
family Rhodospirillaceae id.2717	15	IVW	−0.081176075	0.92 (0.87–0.98)	**0.008465592**
		Weighted median	−0.077085324	0.93 (0.85–1.01)	0.087670899
		MR Egger	0.122031896	1.13 (0.87–1.47)	0.376536227
		Weighted mode	−0.056265032	0.95 (0.82–1.10)	0.466104047
		Simple mode	−0.135726285	0.87 (0.75–1.02)	0.103941293
order Rhodospirillales id.2667	14	IVW	−0.077758562	0.93 (0.87–0.98)	**0.013944643**
		Weighted median	−0.087835142	0.92 (0.84–1.00)	0.050942036
		MR Egger	0.081146878	1.08 (0.84–1.39)	0.536699377
		Weighted mode	−0.09508224	0.91 (0.79–1.04)	0.189847797
		Simple mode	−0.115512047	0.89 (0.77–1.03)	0.145604375
genus Bifidobacterium id.436	12	IVW	−0.086086468	0.92 (0.85–0.99)	**0.029011276**
		Weighted median	−0.064918925	0.94 (0.84–1.05)	0.249471132
		MR Egger	−0.014704759	0.99 (0.78–1.24)	0.902964025
		Weighted mode	−0.05631633	0.95 (0.83–1.07)	0.407457996
		Simple mode	0.010738091	1.01 (0.86–1.19)	0.900175223

(*Continued*)

Table 1 (Continued).

Bacteria Taxa (Exposure)	No. of SNP	MR Method	β	OR (95% CI)	p-value
genus Ruminococcaceae UCG011 id.11368	8	IVW	−0.059659018	0.94 (0.89–0.99)	**0.030351239**
		Weighted median	−0.084506001	0.92 (0.85–0.99)	**0.030919467**
		MR Egger	0.008001241	1.01 (0.73–1.40)	0.963384364
		Weighted mode	−0.116864203	0.89 (0.79–1.00)	0.084782496
		Simple mode	−0.114636033	0.89 (0.78–1.01)	0.125674554
genus Prevotella9 id.11183	15	IVW	−0.06341495	0.94 (0.88–1.00)	**0.048089579**
		Weighted median	−0.05470108	0.95 (0.87–1.03)	0.216257955
		MR Egger	−0.108018418	0.90 (0.75–1.08)	0.270183982
		Weighted mode	−0.030764261	0.97 (0.85–1.11)	0.660650223
		Simple mode	−0.027755564	0.97 (0.84–1.13)	0.720531078

Notes: No. of SNP is the number of SNPs being used as IVs. Bold text indicates a statistically significant p-value ($P < 0.05$).
Abbreviations: MR, Mendelian randomization; SNP, single-nucleotide polymorphism; IVW, inverse-variance weighted; OR, odds ratio; CI, confidence interval.

indirect effects were statistically statistically significant. The 95% confidence intervals for all indirect effect estimates included the null value (Table 3), indicating a lack of robust evidence to support the mediating role of these systemic inflammatory cytokines in the pathways from GM to IVDD.

Although none of the indirect effects reached statistical significance, point estimates and confidence intervals for all tested mediation pathways are presented in Table 3 for transparency. Overall, no robust evidence of mediation by the examined systemic cytokines was identified. The abundance of *genus Marvinbryantia* was associated with lower levels of MIG (β = −0.451, SE = 0.127), and lower MIG levels were in turn associated with a reduced risk of IVDD (β = −0.051, SE = 0.023). The resulting point estimate for the indirect effect was positive (β_indirect = 0.023), but its 95% confidence interval was wide and included zero (95% CI: −0.0008 to 0.0470), precluding a definitive conclusion.

Similarly, the observed suggestive association between *phylum Verrucomicrobia*, RANTES, and IVDD did not yield a statistically statistically significant mediation effect. Due to the non-statistically significant primary associations, the estimated proportion mediated for all pathways was highly unstable and should be interpreted with extreme caution.

Discussion

This two-sample Mendelian randomization study represents a comprehensive genetic investigation into the causal relationships between GM, inflammatory cytokines, and intervertebral disc degeneration. Our analysis yielded three principal findings. First, we identified several gut microbial taxa that exert suggestive associations on IVDD risk, providing genetic evidence supporting the hypothesis of a potential "gut-disc" axis.[27] Second, we found suggestive evidence that genetically proxied levels of several inflammatory cytokines are associated with IVDD. Third, and most notably, our mediation analysis did not find statistically statistically significant evidence that the systemic inflammatory cytokines we investigated act as mediators between the implicated GM and IVDD after rigorous multiple testing correction.

The identification of specific GM, such as the protective *Eubacterium coprostanoligenes group* and the risk-associated *Sutterella* and *Marvinbryantia*, aligns with a growing body of literature linking GM to musculoskeletal health.[28] *Eubacterium* species are known producers of short-chain fatty acids (SCFAs) such as butyrate, which could be hypothesised to contribute to the anti-inflammatory and anti-catabolic effects observed in various models, potentially partially explaining the protective signal observed here. However, as butyrate and other SCFA levels were not directly measured in this study, this mechanistic interpretation remains speculative and warrants experimental validation in future studies.[29] Conversely, *Sutterella* has been associated with mucosal inflammation and could potentially promote systemic low-grade inflammation.[30] Our use of MR strengthens the evidence for these relationships by minimizing the confounding and reverse causation that limit observational studies.

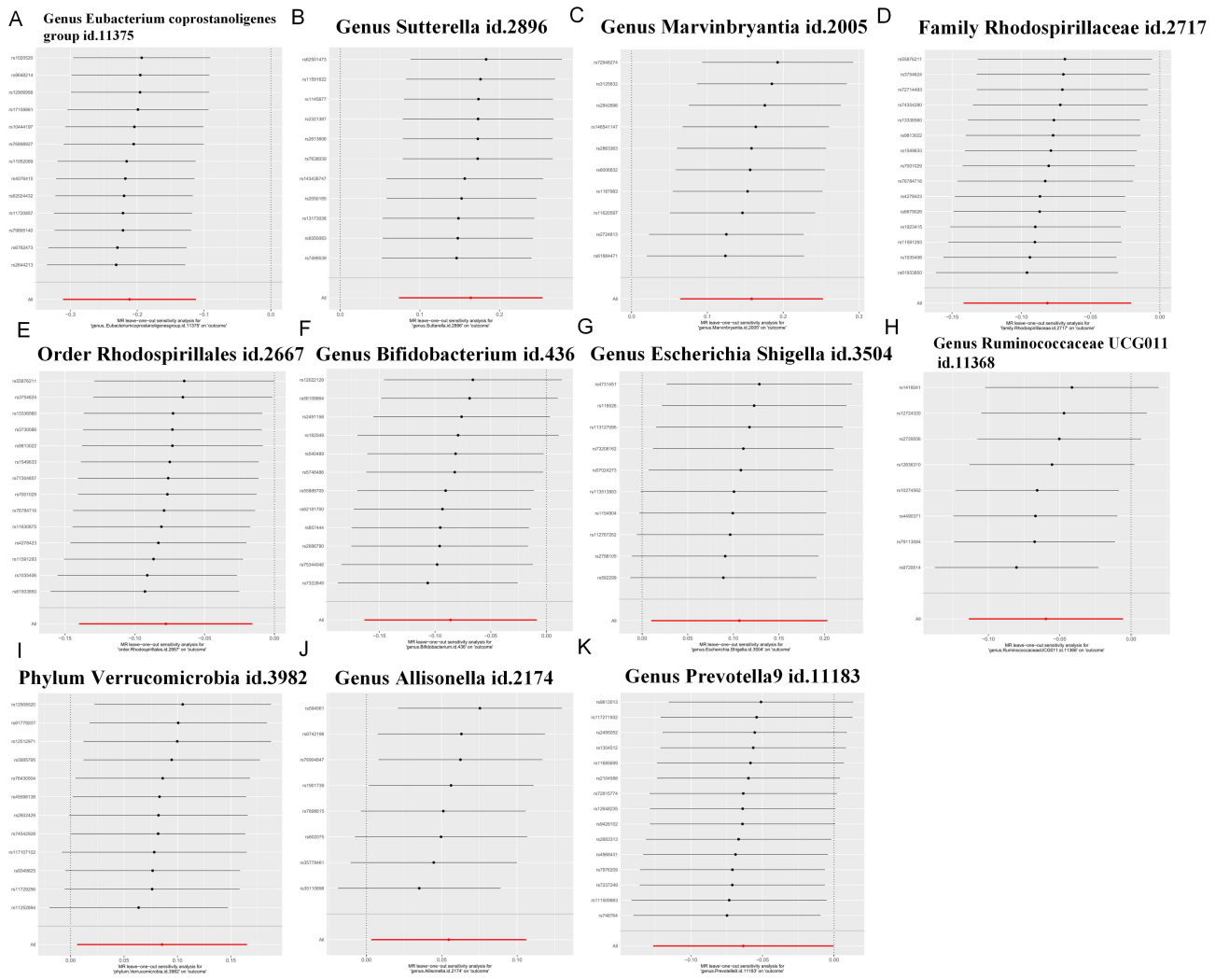

Figure 4 Leave-One-Out Sensitivity Analysis for GM on IVDD. Each panel shows the inverse-variance weighted (IVW) estimate and 95% confidence interval when iteratively removing one single-nucleotide polymorphism (SNP) at a time, confirming that no single SNP was driving the observed causal associations. (**A**) Genus Eubacterium coprostanoligenes group. (**B**) Genus Sutterella. (**C**) Genus Marvinbryantia. (**D**) Family Rhodospirillaceae. (**E**) Order Rhodospirillales. (**F**) Genus Bifidobacterium. (**G**) Genus Escherichia-Shigella. (**H**) Genus Ruminococcaceae UCG011. (**I**) Phylum Verrucomicrobia. (**J**) Genus Allisonella. (**K**) Genus Prevotella9.

Exposure	Outcome	Method	nSNP	pval	OR (95% CI)
IFN_G	IVDD	IVW	10	7.21e−04	0.89 (0.83 − 0.95)
IL_1B	IVDD	IVW	4	0.005	0.89 (0.83 − 0.97)
RANTES	IVDD	IVW	9	0.009	1.07 (1.02 − 1.12)
MIP_1B	IVDD	IVW	20	0.021	0.97 (0.95 − 1.00)
MIG	IVDD	IVW	10	0.025	0.95 (0.91 − 0.99)
IL_18	IVDD	IVW	11	0.036	0.96 (0.93 − 1.00)
SDF_1A	IVDD	IVW	6	0.042	1.09 (1.00 − 1.19)

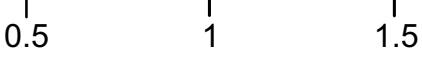

Figure 5 Forest Plot of Suggestive associations of Inflammatory Cytokines on IVDD. The suggestive associations of 7 inflammatory cytokines suggestively associated with IVDD risk (P < 0.05, pre-Bonferroni correction), as determined by the IVW method, are displayed. Effect sizes are presented as odds ratios (ORs) with 95% confidence intervals (CIs).

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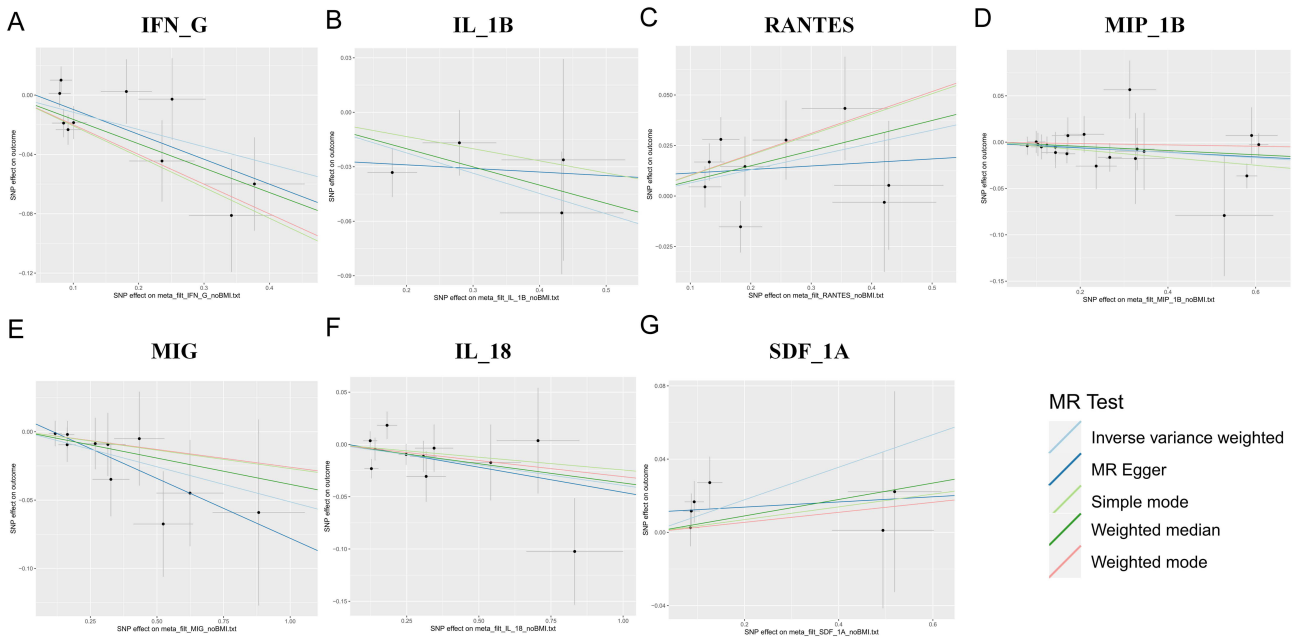

Figure 6 Scatter Plots of Genetic Associations for Suggestive Inflammatory Cytokines and IVDD. Each point represents a single-nucleotide polymorphism (SNP). The slopes of the colored regression lines generated by different Mendelian randomization (MR) methods illustrate the estimated causal direction. (**A**) IFN-γ. (**B**) IL-1β. (**C**) RANTES. (**D**) MIP-1β. (**E**) MIG. (**F**) IL-18. (**G**) SDF-1α.

It must be emphasised that none of the cytokine–IVDD associations survived Bonferroni correction; the following biological interpretations are therefore exploratory and should not be regarded as validated causal effects. The suggestive associations between cytokines like RANTES (a risk factor) and IFN-γ (a protective factor) with IVDD are biologically plausible. RANTES (CCL5) is a chemokine that recruits monocytes and T-cells to sites of inflammation and has been implicated in matrix degradation processes relevant to IVDD.[31] IFN-γ, typically considered a pro-inflammatory cytokine, may have complex, context-dependent roles, including immunoregulatory functions that could be protective in chronic degenerative settings.[32] However, the fact that these associations did not survive Bonferroni correction necessitates caution in their interpretation and highlights the need for replication in larger GWAS.

The most critical finding of our mediation analysis is the lack of statistically significant evidence for cytokine mediation. Despite identifying several suggestive causal paths for both exposures and mediators, the formal two-step MR

Table 2 Mendelian Randomization Analysis of the Suggestive Causal Effects of Inflammatory Cytokines on Intervertebral Disc Degeneration

ICs (Exposure)	No. of SNP	MR Method	β	OR (95% CI)	p-value
IFN-G	10	IVW	−0.116071808	0.89 (0.83–0.95)	0.000721352
		Weighted mode	−0.200199797	0.82 (0.69–0.96)	0.040482766
		Simple mode	−0.207523219	0.81 (0.68–0.98)	0.052697237
IL-1B	20	IVW	−0.111776542	0.89 (0.83–0.97)	0.004598071
		Weighted mode	−0.066838347	0.94 (0.83–1.06)	0.370371453
		Simple mode	−0.066838347	0.94 (0.81–1.08)	0.430379185
RANTES	6	IVW	0.065153332	1.07 (1.02–1.12)	0.009012153
		Weighted mode	0.103381989	1.11 (0.99–1.25)	0.119455769
		Simple mode	0.101287886	1.11 (0.99–1.24)	0.125403726

(*Continued*)

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Table 2 (Continued).

ICs (Exposure)	No. of SNP	MR Method	β	OR (95% CI)	p-value
MIP-1B	9	IVW	−0.028041633	0.97 (0.95–1.00)	0.02109614
		Weighted mode	−0.007395728	0.99 (0.96–1.03)	0.66803483
		Simple mode	−0.04154009	0.96 (0.90–1.02)	0.206316465
MIG	11	IVW	−0.05127649	0.95 (0.91–0.99)	0.024934396
		Weighted mode	−0.025924688	0.97 (0.89–1.06)	0.576217694
		Simple mode	−0.026934725	0.97 (0.89–1.06)	0.556609676
IL-18	4	IVW	−0.039160185	0.96 (0.93–1.00)	0.035998692
		Weighted mode	−0.030827582	0.97 (0.91–1.03)	0.336240867
		Simple mode	−0.02444671	0.98 (0.90–1.06)	0.573963639
SDF-1A	10	IVW	0.088923989	1.09 (1.00–1.19)	0.041507349
		Weighted mode	0.027354648	1.03 (0.88–1.21)	0.750482639
		Simple mode	0.034796268	1.04 (0.87–1.24)	0.715453298

Notes: No. of SNP is the number of SNPs being used as IVs. Bold text indicates a statistically significant p-value ($P < 0.05$).
Abbreviations: MR, Mendelian randomization; SNP, single-nucleotide polymorphism; IVW, inverse-variance weighted; OR, odds ratio; CI, confidence interval.

analysis did not identify any statistically statistically significant indirect effects after multiple testing correction. The confidence intervals for all mediation estimates included the null value. This suggests that the causal effect of these gut microbes on IVDD may operate through alternative pathways. Potential mechanisms not captured by our analysis include: (1) Local immune modulation: The GM may influence the immune cell populations within the disc micro-environment itself without altering systemic cytokine levels.[33] (2) Metabolic products: Microbial metabolites (eg, SCFAs, bile acids, tryptophan derivatives) could directly influence disc cell homeostasis, apoptosis, or matrix

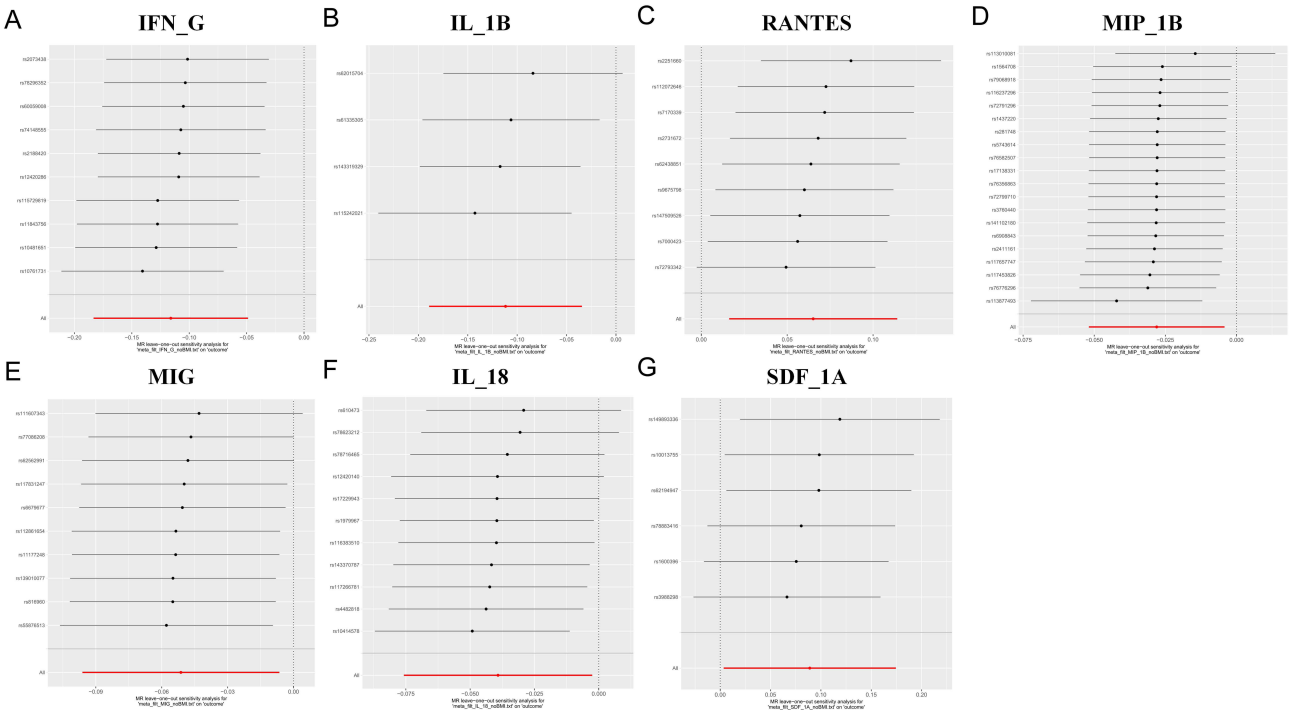

Figure 7 Leave-One-Out Sensitivity Analysis for Inflammatory Cytokines on IVDD. The plots suggests that the results were not disproportionately influenced by any individual single-nucleotide polymorphism (SNP). (**A**) IFN-γ. (**B**) IL-1β. (**C**) RANTES. (**D**) MIP-1β. (**E**) MIG. (**F**) IL-18. (**G**) SDF-1α.

Table 3 Two-Step MR Mediation Analysis of Inflammatory Cytokines in the Gut Microbiota-IVDD Pathway

exp	med	out	beta_EM	se_EM	beta_MO	se_MO	beta_EO	se_EO	med_effect	med_lowci	med_upperci	pro
Gut microbiota abundance (family Rhodospirillaceae id.2717)	meta_filt_RANTES_noBMI	finngen_R7_M13_INTERVERTEB	−0.209688589	0.090883331	0.065153332	0.024947738	−0.081176075	0.030831274	−0.01366191	−0.029148198	0.001824378	16.82997144
Gut microbiota abundance (genus Allisonella id.2174)	meta_filt_IL_1B_noBMI	finngen_R7_M13_INTERVERTEB	0.164098229	0.073635461	−0.111776542	0.039442365	0.054639431	0.026215899	−0.018342333	−0.038865035	0.00218037	−33.5697725
Gut microbiota abundance (genus Bifidobacterium id.436)	meta_filt_SDF_1A_noBMI	finngen_R7_M13_INTERVERTEB	0.143838998	0.07000368	0.088923989	0.04362385	−0.086086468	0.039428918	0.012790738	−0.004533263	0.030114738	−14.8580118
Gut microbiota abundance (genus Escherichia Shigella id.3504)	meta_filt_RANTES_noBMI	finngen_R7_M13_INTERVERTEB	−0.353593201	0.149481072	0.065153332	0.024947738	0.106635285	0.04922013	−0.023037775	−0.048792805	0.002717255	−21.60427034
Gut microbiota abundance (genus Marvinbryantia id.2005)	meta_filt_MIG_noBMI	finngen_R7_M13_INTERVERTEB	−0.450471376	0.127276378	−0.05127649	0.022866611	0.158303937	0.047975118	0.023098591	−0.000801994	0.046999176	14.59129281
Gut microbiota abundance (genus Prevotella9 id.11183)	meta_filt_MIG_noBMI	finngen_R7_M13_INTERVERTEB	0.163893165	0.083607946	−0.05127649	0.022866611	−0.06341495	0.032083234	−0.008403866	−0.019564604	0.002756872	13.25218458
Gut microbiota abundance (phylum Verrucomicrobia id.3982)	meta_filt_RANTES_noBMI	finngen_R7_M13_INTERVERTEB	0.219293963	0.110642858	0.065153332	0.024947738	0.085630678	0.04055576	0.014287732	−0.00344964	0.032025105	16.68529618
Gut microbiota abundance (unknown genus id.959)	meta_filt_IL_1B_noBMI	finngen_R7_M13_INTERVERTEB	0.19940981	0.075469192	−0.111776542	0.039442365	−0.071869486	0.027900034	−0.022289339	−0.044895037	0.000316359	31.01363334

synthesis.[34] (3) Direct translocation: In cases of increased intestinal permeability ("leaky gut"), microbial components or whole bacteria may translocate, potentially eliciting a localized immune response in paravertebral tissues or even the disc itself.[35] (4) Other unmeasured mediators: Pathways involving neurotransmitters, oxidative stress, or vascularization were not examined in this study.[36]

The major strength of this study is the application of MR, which provides a robust framework for assessing causality by leveraging genetic variants as instrumental variables.[37] The use of large, publicly available GWAS consortia data ensured substantial statistical power. We also conducted extensive sensitivity analyses to check for pleiotropy and heterogeneity, strengthening the validity of our primary results.

However, several limitations must be acknowledged. First, the statistical power for the mediation analysis was a key constraint. The GWAS for ICs had a relatively smaller sample size (n≈8337) compared to those for GM and IVDD, which likely resulted in weaker genetic instruments and reduced power to detect true mediation effects (Type II error).[38] The stringent Bonferroni correction, while conservative, further increased this risk. Second, the GWAS data for GM and ICs were derived from blood and fecal samples, respectively, and may not fully reflect the biological activity at the site of the intervertebral disc.[39] Third, despite our efforts to control for horizontal pleiotropy, residual pleiotropy remains a potential caveat in any MR study.[40] Fourth, Importantly, all GWAS datasets used in this study were derived from individuals of European ancestry. Therefore, the generalizability of our findings to other ethnic populations (eg, East Asian, African) remains uncertain, and replication in trans-ancestry MR studies is essential before any broader clinical extrapolation.[41]

Conclusion

In conclusion, our study provides novel genetic evidence that specific GM play a causal role in the development of IVDD, solidifying the concept of a gut-disc axis. However, it indicates no evidence of mediation by the examined systemic inflammatory cytokines was detected, rather than excluding the existence of such mediation. This important negative result shifts the focus of future research towards alternative mechanistic pathways, such as direct microbial metabolite actions, local immune regulation, or other biological processes. Future studies with larger GWAS for cytokines and more precise tissue-specific molecular data are needed to conclusively rule out or identify specific inflammatory mediators. Importantly, our findings do not provide direct evidence for clinical interventions, probiotic therapies, or biomarker applications. Rather, they highlight specific microbial taxa as priority candidates for future mechanistic and translational studies, particularly those focused on microbial metabolites and local immune microenvironments within the intervertebral disc. Ultimately, integrating multi-omics data (metagenomics, metabolomics) from well-phenotyped cohorts will be essential to unravel the precise mechanisms linking the gut microbiome to disc health and disease.

Data Access Statement

All data used in this present study are publicly available summary-level data from previously conducted genome-wide association studies (GWAS). Genetic instruments for gut microbiota were obtained from the MiBioGen consortium (https://mibiogen.gcc.rug.nl/). Genetic associations for inflammatory cytokines were sourced from the GWAS. Summary statistics for intervertebral disc degeneration were derived from FinnGen. Original datasets can be accessed and applied for via the links provided by the respective consortia or publications.

Ethics Statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. The data used in this study were publicly available summary-level statistics obtained from large-scale genome-wide association studies (GWAS) consortia. All original studies included in this analysis had been approved by their respective institutional review boards or ethics committees, and informed consent was obtained from all participants in those primary studies. As our research involved secondary analysis of de-identified, publicly available data and did not involve any new collection of human biological samples or individual-level data, additional ethical approval was not required for this specific study. This study was exempt from institutional ethical

review. Specifically, this exemption is strictly based on items 1 and 2 of Article 32 of the Measures for Ethical Review of Life Science and Medical Research Involving Human Subjects (issued by the National Health Commission of China, dated February 18, 2023). Our research complied with the principles set forth in the Declaration of Helsinki.

Author Contributions

YD and ZN conceived the study. ZY, QJ and ZL analyzed data. SC, JB and ZN drafted the manuscript. QJ, JB, and SC provided guidance and checked the manuscript.

All authors made a statistically significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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