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Original Article

Potential causal association between gut microbiota, inflammatory cytokines, and acute pancreatitis: A Mendelian randomization study

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ABSTRACT

Background: Acute pancreatitis (AP) ranks among the most frequently encountered gastrointestinal diseases in the emergency department. Recent studies have increasingly emphasized the substantial connection among gut microbiota, inflammatory cytokines, and AP.

Methods: A two-sample Mendelian randomization (MR) study was conducted using summary statistics of gut microbiota (GM) from the largest available meta-analysis of genome-wide association studies conducted by the MiBioGen consortium (n=18,340). For cytokines, the data were obtained from a study that investigated genome variant associations with 41 inflammatory cytokines and growth factors (n=8293). The summary statistics of AP were obtained from the FinnGen consortium version R5 data (3022 cases and 195,144 controls). The inverse variance weighted (IVW) method was used as the main analysis, with MR–Egger and weighted median as complementary analytical methods. Sensitivity analyses were performed using Cochran's Q-test, MR–Egger intercept test, leave-one-out analyses, and MR–PRESSO. In addition, we employed the reverse MR analysis and MR Steiger method to estimate the orientations of exposure and outcome.

Result: Among the 211 examined GM taxa, the IVW method revealed that Bacteroidales (odds ratio [OR]=1.412, 95% confidence interval [CI]:1.057 to 1.885, P=0.019), *Eubacterium fissicatena* group (OR=1.240, 95% CI:1.045 to 1.470, P=0.014), and Coprococcus3 (OR=1.481, 95 % CI:1.049 to 2.090, P=0.026) exhibited a positive association with AP. Conversely, Prevotella9 (OR=0.821, 95% CI:0.680 to 0.990, P=0.038), RuminococcaceaeUCG004 (OR=0.757, 95% CI:0.577 to 0.994, P=0.045), and Ruminiclostridium6 (OR=0.696, 95% CI:0.548 to 0.884, P=0.003) displayed a negative correlation with AP. Among the 41 inflammatory cytokines, only macrophage colony-stimulating factor (M_CSF, OR=0.894, 95% CI:0.847 to 0.943, P=0.037) exhibited a negative association with AP. Sensitivity analyses revealed no evidence of pleiotropy or heterogeneity. Nevertheless, the mediation analysis showed that M_CSF did not act as a mediating factor.

Conclusion: This two-sample MR study revealed causal associations between specific GM and inflammatory cytokines with AP, respectively. However, inflammatory cytokines did not appear to act as mediating factors in the pathway from GM to AP.

Introduction

Acute pancreatitis (AP), a prevalent gastrointestinal ailment necessitating urgent hospital intervention, is delineated as a sudden inflammatory onset within the pancreatic region, marked notably by heightened abdominal distress and a surge in pancreatic enzymes.[1] The estimated incidence of AP is 34 cases per 100,000 person-years, and it is on the rise globally.[2] Although the majority of patients experience mild, self-limiting pancreatitis with a low mortality rate of 1%–3%, approximately 20% of patients develop moderate or severe AP, which can have a mortality rate as high as 15%–35%.[3] The therapeutic

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trajectory for AP poses not only intricate clinical dilemmas but also imparts a substantial economic strain on societal and individual resources.[4]

The gut microbiome, comprising a complex community of various microorganisms, stands pivotal in the orchestration of both human health equilibrium and disease pathogenesis.[5] Perturbations in this microbial consortium, manifesting as dysbiosis, have been implicated in a spectrum of conditions ranging from metabolic disorders such as obesity and diabetes to inflammatory pathologies and neoplastic conditions of the colon.[6] Recent research has indicated a link between dysbiosis and the severity of AP, suggesting an active role in its pathogenesis.[7] Multiple studies have revealed that AP patients exhibit distinct GM compared to healthy controls, characterized by reduced richness and diversity of the predominant fecal microbiota.[8–10] GM-derived Nicotinamide mononucleotide (NMN) translocates to the pancreas, where it is converted to Nicotinamide adenine dinucleotide (NAD^+). This process prompts the NAD^+-dependent deacetylase SIRT3 to deacetylate and increase PRDX5 levels, ultimately mitigating pancreatic damage.[11] Furthermore, probiotics have been found to offer significant health benefits by stabilizing the gut barrier, boosting anti-inflammatory responses, and reducing acinar cell injury, via a reduction in oxidative stress.[12]

The progression of AP is associated with additional increases in the expression of TNF-α, interleukin (IL)-1, IL-6, and various other inflammatory cytokines.[13] Steele et al.[14] discovered that CXC chemokine receptor 2 (CXCR2) plays a critical role in the development of AP, and its severity could be reversed through inhibition of CXCR2. Previous studies have shown that the activation of Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) in myeloid cells and the expression of IL-6 in macrophages mediate the development of AP in mice.[15] Additionally, Baumann et al.[16] found that the activation of NF-κB in pancreatic cells is essential for the development of pancreatitis. Sendler et al.[17] initially discovered that infiltrating macrophages promote inflammation and simultaneously induce a Th2 cell-mediated response via IL-18, inhibition of NLR family pyrin domain containing 3 (NLRP3) reduces both systemic inflammatory response syndrome and compensatory anti-inflammatory response syndrome. GM plays an important role in regulating the host immune system by modulating inflammatory cytokines.[18] We hypothesize that inflammatory cytokines may act as mediators in the pathway from GM to AP.

Mendelian randomization (MR), a potent epidemiological tool, leverages summary data from GWAS datasets to dissect causal relationships between variables, capitalizing on congenital genetic variant allocation to circumvent biases stemming from confounding factors or reverse causality.[19] Preceding MR explorations have discerned associations between factors such as gallstone pathology, serum calcium concentrations, lipid profiles, and smoking habits with escalated AP risk.[20,21] Wang et al.[22] have also investigated the relationship between GM and AP. Nevertheless, a methodical scrutiny evaluating whether inflammatory cytokines as mediators in the pathway from GM to AP remains conspicuously absent. Within this investigative framework, our study employs a comprehensive MR approach, aspiring to unravel the intricate causative interplay between GM, inflammatory cytokines, and AP. Elucidating foundational understanding of pathogenic mechanisms could contribute to the development of potential prophylactic strategies.

Methods

MR design

We conducted a two-sample MR study to assess the causal relationship between GM, inflammatory cytokines and AP. MR relies on three fundamental assumptions: (1) Genetic variants are closely associated with the exposure; (2) Genetic variants are unrelated to potential confounding factors; and (3) Genetic variants affect the outcome solely through the exposure.[23] In our study, GM served as the exposure, while AP was considered the outcome, and inflammatory cytokines acted as mediating factors. The procedure is illustrated in Figure 1: evaluate the causal effect of GM on AP; evaluate the causal effect of inflammatory cytokines on AP; and evaluate the mediation effect of inflammatory cytokines in the causal association between GM and AP. Our reporting of the study results meets the requirements of the Strengthening the Reporting of Observational Studies in Epidemiology using MR (STROBE-MR) guidance (Supplementary Table S1).[24]

GWAS summary statistics

We obtained GM data from the MiBioGen consortium, representing a vast investigation involving 18,340 participants from 24 cohorts across 11 countries, encompassing various ethnicities.[25] A total of 211 taxa (131 genera, 35 families, 20 orders, 16 classes, and 9 phyla) were included. Taking into account inter-population differences in GM composition, the GWAS analysis yielded 122,110 variants from these 211 taxa. Cohorts included in the study were adjusted for gender and age covariates. Summary-level statistics from this association study are publicly accessible at https://mibiogen.gcc.rug.nl. The data on inflammatory cytokines were derived from a study that established associations between genomic variants and 41 inflammatory cytokines and growth factors in a cohort of 8293 Finnish individuals.[26] This study combined the results from The Cardiovascular Risk in Young Finns Study (YFS) and the FINRISK surveys. Additionally, we acquired GWAS summary statistics for AP from FinnGen Round 5 (https://r5.finngen.fi/), involving 3022 cases and 195,144 controls (ICD-10, K80-K87, https://risteys.finngen.fi/phenocode/PANCREATITIS).

Instrumental variables (IVs) selection

To ensure data robustness and result accuracy, we imposed several criteria for single nucleotide polymorphisms (SNPs): (1) SNPs linked to GM classification met genome-wide significance ($P < 5 \times 10^{-8}$), but due to limited SNP availability, we used a more inclusive threshold ($P < 1 \times 10^{-5}$).[27]; (2) To optimize the available instruments for each cytokine, we selected a SNP with a P-value of 5×10^{-6} as the threshold.[28]; (3) Linkage disequilibrium (LD) clumping was employed based on European-based 1000 Genome Projects, with SNPs displaying large P-values ($R^2 \geq 0.001$) eliminated.[29]; (4) SNPs were assessed for instrumental strength using the F-statistic, with those below 10 (indicating insufficient strength) excluded.[30]; (5) If

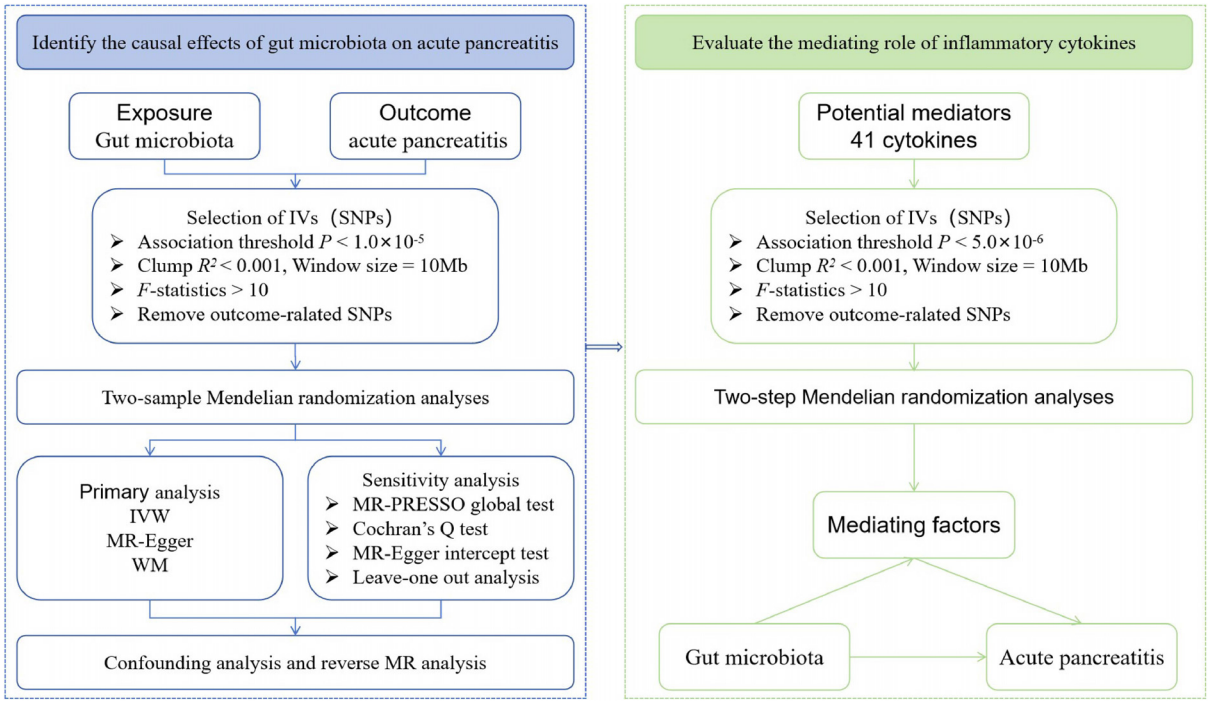

Figure 1. Overview of the current MR study.
IVW: Inverse variance weighted; IVs: Instrumental variables; MR: Mendelian randomization; MR-PRESSP: MR-Pleiotropy Residual Sum and Outlier; SNPs: Single nucleotide polymorphisms; WM: Weighted median.

SNPs associated with exposure were absent in the outcome data, suitable proxies were identified (R^2 >0.800) at SNPs (https://snipa.helmholtz-muenchen.de/snipa3/index.php). If no suitable proxy was found, the SNPs were discarded; and (6) GM associated with the outcome ($P < 1 \times 10^{-5}$) and those associated with fewer than three SNPs were excluded.

MR analysis and sensitivity analysis

For the elementary analysis, we utilized standard inverse variance weighted (IVW) estimates, combining the Wald ratio of each SNP on the outcome to obtain a pooled causal estimate.[31] Additionally, we performed supplementary MR analyses, such as MR–Egger regression and the weighted median (WM), to enhance result robustness across various scenarios. MR–Egger regression tests for considerable heterogeneity and unbalanced pleiotropy, but requires a larger sample size.[32] The WM approach delivers consistent effect estimates when at least 50% of the weighted variance from horizontal pleiotropy is valid.[33] Associations with $P \leq 0.050$ were considered nominally significant. Sensitivity analyses are crucial in MR studies for detecting potential gene pleiotropy and estimating MR result robustness. We employed Cochran's Q statistic, MR–Egger intercept tests, leave-one-out (LOO) analyses, and MR–Pleiotropy Residual Sum and Outlier (MR-PRESSO) to assess heterogeneity, pleiotropy, and result robustness.[34–36]

Confounding analysis, reverse MR analysis, and steiger test

To assess potential confounders, we examined whether GM-related SNPs were concurrently associated with common risk factors that might influence MR estimates, such as smoking, body mass index, whole-body fat mass, waist circumference, elevated triglycerides, short-chain fatty acid (SCFAs), type 2 diabetes, and inflammatory bowel disease.[20,21] We used the Phenoscanner V2 website (http://www.phenoscanner.medchsl.cam.ac.uk/) for this analysis. If the correlation between SNPs and these potential confounders reached a threshold of $P < 1 \times 10^{-5}$, IVW was performed again after removing these SNPs to verify the robustness of the findings.[37,38]

We conducted a reverse MR analysis to investigate reverse causality. We considered AP as the "exposure," with GM or inflammatory cytokines linked to AP as the "outcome." In addition, we utilized the MR Steiger approach to estimate the orientation of each extracted IV in terms of exposure and outcome, with "TRUE" indicating an expected association.[39]

Mediation analysis

Through the two-sample analysis, we incorporated GM and inflammatory cytokines exhibiting notable causal effects on AP into the mediation analysis. We investigated whether GM causally influenced inflammatory cytokines. If such an effect existed, we conducted multiple MR analyses to examine whether inflammatory cytokines served as the mediation factors in the pathway from GM to AP.

Statistical analysis

Analysis was executed using R software (version 4.0.4). We performed the MR analysis using the R package "Two-Sample. MR" version 0.5.6, which cater to Two-Sample MR methods for inference of cause and effect. A P-value threshold of <0.05 was considered indicative of a potential causative effect. Based on prior research, a P-value <0.05 is considered indicative of a potential causal relationship, whereas a False Discovery Rate

(FDR) value <0.05 is regarded as evidence of a statistically significant causal relationship.

Result

IVs selection

In the process of selecting IVs relevant to AP, criteria were established that targeted SNPs exhibiting genome-wide associations across all studied traits. This rigorous selection process resulted in the identification of 2320 IVs for the 211 GM taxa and 424 IVs for the 41 inflammatory cytokines, having dismissed those SNPs affected by LD and others not meeting the requirements. From this set, 107 SNPs that met genome-wide significance and represented 9 microbial taxa were retained for subsequent MR analysis.. These taxa included Bacteroidia (class level), Bacteroidales (order level), and Prevotella9, *Eubacterium fissicatena* group, Slackia, ErysipelotrichaceaeUCG003, RuminococcaceaeUCG004, Ruminiclostridium6, and Coprococcus3 (genus level) (Figure 2).

Cause effect for GM and inflammatory cytokines on AP

After FDR, the IVW analysis revealed potential associations between certain microbial taxa and AP (Supplementary Table S2). Specifically, Bacteroidia, Bacteroidales, *E. fissicatena* group, and Coprococcus3 exhibited positive associations with AP. Conversely, Prevotella9, RuminococcaceaeUCG004, and Ruminiclostridium6 were negatively correlated with AP (Figure 2). Among the three MR analysis methods, Slackia and ErysipelotrichaceaeUCG003 displayed inconsistent effect directions. Scatter plots visually represented the causal relationship between GM and AP across these methods (Supplementary Figure 1). Cochran's *Q* test results indicated no significant IV heterogeneity (Table 1). MR–Egger intercept and MR–PRESSO analyses (MR–Egger intercept >0.05 and global MR–PRESSO >0.05) suggested no substantial horizontal pleiotropy among the IVs associated with AP (Table 1). Additionally, leave-one-out analysis corroborated the stability of our overall findings (Supplementary Figure 2). The Order Bacteroidales is classified under the class Bacteroidia. Among the 41 inflammatory factors, only macrophage colony-stimulating factor (M_CSF) and pancreatitis exhibited a negative correlation (odds ratio [OR]=0.894, 95% confidence interval [CI]=0.847 to 0.943, *P*=0.037) after FDR, without significant IV heterogeneity and horizontal pleiotropy (Figure 3, Supplementary Table S3).

Confounding analysis, reverse MR analysis, and steiger test

We explored potential confounding factors that might influence the GM–AP association. Specifically, we investigated factors such as smoking, BMI, whole-body fat mass, waist circumference, elevated triglycerides, SCFAs, type 2 diabetes, and inflammatory bowel disease. Using the Phenoscanner V2 website, we identified two SNPs linked to Crohn's disease-related phenotypes and weight-related phenotypes, respectively. Causality remained significant after removing these SNPs for genus. Ruminiclostridium6 (IVW OR=0.72, 95% CI:0.57 to 0.93, *P*=0.0102)

Exposure	Nsnp	Method	*P* value	OR(95% CI)
class.Bacteroidia	13	IVW	0.019	1.412 (1.057−1.885)
		MR−Egger	0.493	1.276 (0.650−2.503)
		WM	0.257	1.260 (0.845−1.878)
order.Bacteroidales	13	IVW	0.019	1.412 (1.057−1.885)
		MR−Egger	0.493	1.276 (0.650−2.503)
		WM	0.252	1.260 (0.849−1.870)
genus.Prevotella9	15	IVW	0.038	0.821 (0.680−0.990)
		MR−Egger	0.772	0.921 (0.533−1.591)
		WM	0.085	0.790 (0.604−1.033)
genus.*Eubacterium fissicatena* group	9	IVW	0.014	1.240 (1.045−1.470)
		MR−Egger	0.324	1.616 (0.666−3.921)
		WM	0.089	1.209 (0.971−1.505)
genus.Slackia	6	IVW	0.047	0.767 (0.590−0.996)
		MR−Egger	0.353	2.487 (0.454−13.621)
		WM	0.080	0.742 (9.530−1.037)
genus.ErysipelotrichaceaeUCG003	16	IVW	0.019	0.756 (0.598−0.956)
		MR−Egger	0.442	1.281 (0.693−2.370)
		WM	0.327	0.854 (0.623−1.171)
genus.RuminococcaceaeUCG004	11	IVW	0.045	0.757 (0.577−0.994)
		MR−Egger	0.270	0.398 (0.086−1.848)
		WM	0.433	0.878 (0.635−1.215)
genus.Ruminiclostridium6	15	IVW	0.003	0.696 (0.548−0.884)
		MR−Egger	0.854	0.945 (0.522−1.710)
		WM	0.026	0.698 (0.508−0.958)
genus.Coprococcus3	9	IVW	0.026	1.481 (1.049−2.090)
		MR−Egger	0.812	1.274 (0.185−8.757)
		WM	0.175	1.373 (0.869−2.171)

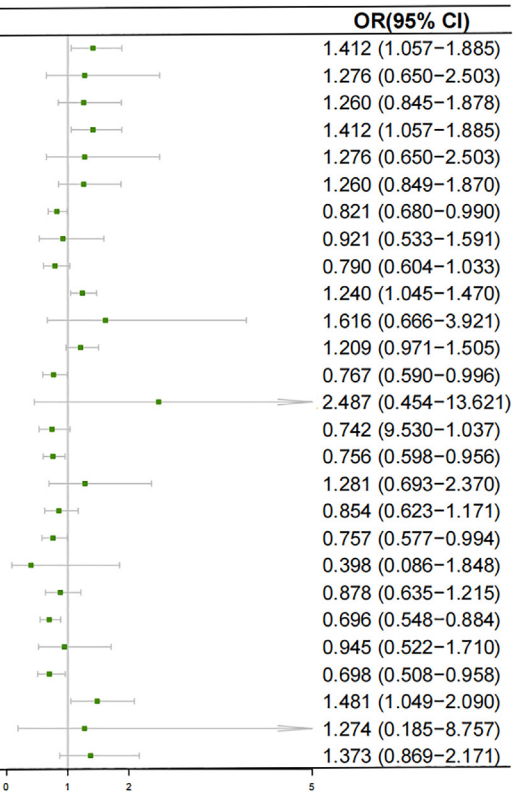

Figure 2. Causal effects for GM on acute pancreatitis performed by MR.
CI: Confidence interval; GM: Gut microbiota; IVW: Inverse variance weighted; MR: Mendelian randomization; Nsnp: Number of single nucleotide polymorphism; OR: Odds ratio; WM: Weighted median.

Table 1
Sensitivity analysis of the causal association between GM and AP.

Exposure	Outcome	Method	Heterogeneity			Horizontal pleiotropy	MR-PRESSO
			Q	*Q*_df	*Q*_*P*-value	Egger_intercept_*P*-value	*P*-value
class.Bacteroidia	AP	IVW	9.9165	12	0.6233	0.7503	0.6664
		MR-Egger	9.8100	11	0.5476		
order.Bacteroidales	AP	IVW	9.9165	12	0.6233	0.7503	0.6644
		MR-Egger	9.8100	11	0.5476		
genus.Prevotella9	AP	IVW	10.1245	14	0.7530	0.6673	0.7702
		MR-Egger	9.9311	13	0.6996		
genus.*Eubacterium fissicatena* group	AP	IVW	2.3661	8	0.9677	0.5694	0.9688
		MR-Egger	2.0098	7	0.9593		
genus.Slackia	AP	IVW	3.7929	5	0.5796	0.2418	0.6092
		MR-Egger	1.9086	4	0.7526		
genus.ErysipelotrichaceaeUCG003	AP	IVW	16.2879	15	0.3632	0.0924	0.4000
		MR-Egger	13.0261	14	0.5245		
genus.RuminococcaceaeUCG004	AP	IVW	14.1013	10	0.1684	0.4254	0.2032
		MR-Egger	13.0877	9	0.1587		
genus.Ruminiclostridium6	AP	IVW	6.9449	14	0.9368	0.2899	0.9402
		MR-Egger	5.7275	13	0.9555		
genus.Coprococcus3	AP	IVW	4.9645	8	0.7614	0.8809	0.7738
		MR-Egger	4.9403	7	0.6672		

AP: Acute pancreatitis; GM: Gut microbiota; IVW: Inverse variance weighted; MR: Mendelian randomization; MR-PRESSO: MR-Pleiotropy Residual Sum and Outlier.

and genus. RuminococcaceaeUCG004 (IVW OR=0.71, 95% CI:0.54 to 0.94, *P*=0.016) (Supplementary Table S4). Notably, the remaining SNPs showed no association with the analyzed confounders. Concerning inflammatory cytokines, it was found that SNPs of M_CSF were causally associated with AP, independent of confounding factors. In the reverse MR analysis, there was no evidence of a causal effect of AP on significant GM taxa and M_CSF (Supplementary Tables S5 and S6). There was also no evidence of a causal effect of M_CSF on significant GM taxa (Supplementary Table S7). However, during the MR–PRESSO analysis of AP and genus *E. fissicatena* group, an outlier was identified. After excluding this outlier, no causal link between AP and the bacterial genus was observed. Furthermore, by using the MR–Steiger method, we also found no inverse association between genetically predicted GM-related IVs, M_CSF-related IVs, and AP risk (Supplementary Table S8).

Mediation analysis

Our results indicate no causal relationship between the gut microbiota (GM) associated with acute pancreatitis (AP) and the inflammatory cytokines linked to AP, as shown in Figure 4. Both Cochran's *Q* and MR–Egger regression tests show no evidence of heterogeneity or horizontal pleiotropy. Additionally, the MR–PRESSO test identified no outliers (Supplementary Table S9).

Discussion

This study employed a two-sample MR study to investigate the causal link between GM, inflammatory cytokines and AP, aiming to minimize the influence of confounding variables. Our findings indicated positive correlations between certain GM genera (Bacteroidia, *E. fissicatena* group, and Coprococcus3) and AP, while other genera (Prevotella9, RuminococcaceaeUCG004, and Ruminiclostridium6) displayed negative associations. M_CSF and pancreatitis exhibited a negative correlation.

A number of observational studies have reported GM dysbiosis is related to the progress of AP.[40,41] Tsuji et al.[42] found that commensal organisms, in synergy with low doses of cerulein, can exacerbate the severity of experimental AP via the Nucleotide-binding Oligomerization Domain-containing Protein 1 (NOD1) pathway. It was found that the proportions of Bacteroidales are higher in patients with AP, which is consistent with the result of our study.[8–10] Bacteroidales has been considered to be the most common microorganism responsible for infected pancreatic necrosis, and treatment with antibiotics targeting Bacteroidales is effective in infectious pancreatic necrosis.[43] Bacteroidales was also found to be correlated with other pancreatic diseases, such as diabetes, pancreatic cancer, and so on.[44,45] In addition, we find that *E. fissicatena* group was associated with AP. A previous study showed Bacteroidales and Eubacterium involved in bile acid oxidation and epimerization disrupt the enterohepatic circulation, leading to the formation of gallstones, which was the most commom aetiology of AP.[46] There have been relatively few previous studies of Coprococcus3, but Coprococcus3 has been reported to be positively associated with the risk of obstructive sleep apnea and migraine with aura.[47,48] GM dysbiosis might not be the initial cause of AP, but can progress with the disease and lead to worsening of AP.

In this study, part of the GM identified to be negatively associated with AP were SCFA-producing bacteria, including Prevotella9 (genus level), RuminococcaceaeUCG004 (genus level), and Ruminiclostridium6 (genus level).[49] SCFAs mainly include acetic acid, butyric acid, and propionic acid, which are metabolites recognized as potential mediators involved in maintaining intestinal homeostasis, immunity, and anti-inflammation.[50,51] Pan et al.[52] demonstrated that butyrate had multifaceted protective effects on mice with caerulein-induced AP, including attenuation of pancreatic edema and acinar necrosis, prevention of neutrophil recruitment, and inhibition of pro-inflammatory cytokines production in the pancreas and colon. Zhang et al.[53] Found sodium butyrate can effectively attenuate pancreatic injury in SAP mice by inhibiting NF-κB signaling pathway and reducing HMGB1 expression. The higher enrichment of Prevotella

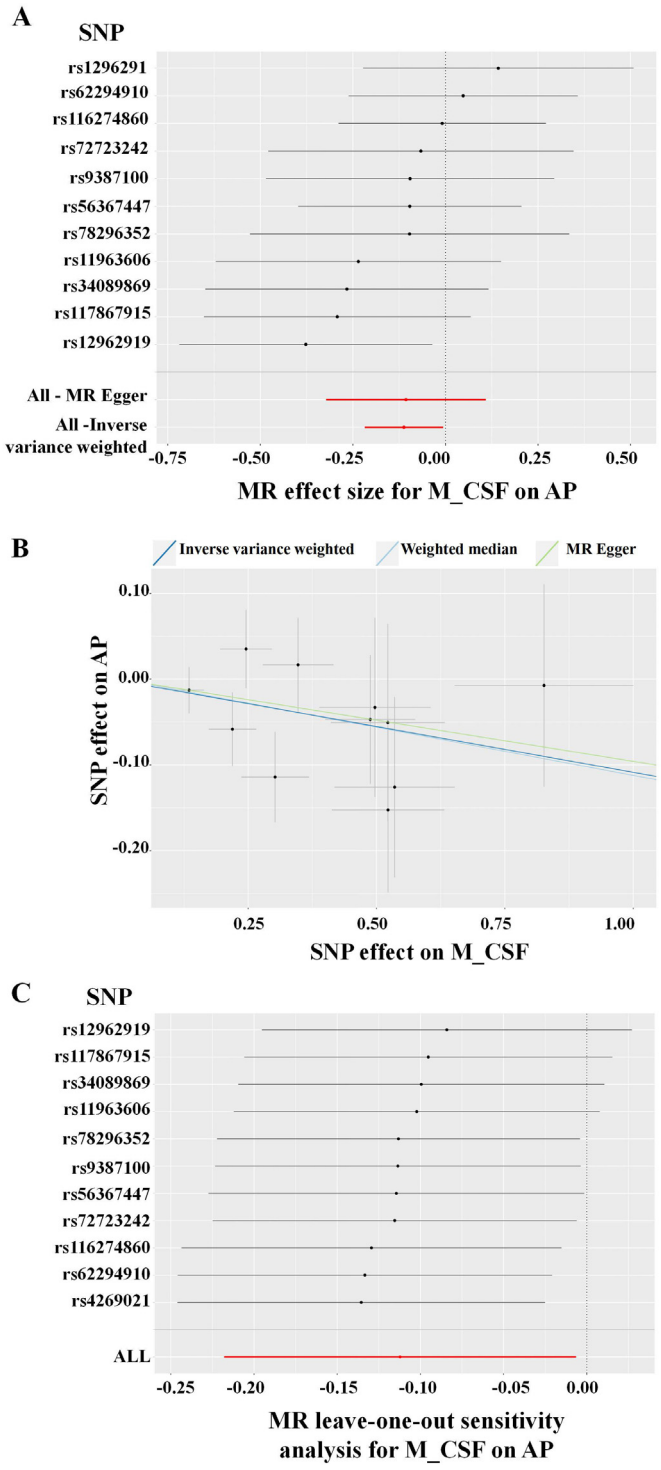

Figure 3. Causal effects for M_CSF on AP performed by MR. A: Forest Plot for MR Effect Size for M_CSF on AP (x-axis, log OR). B: Scatter plot showing SNP Effects on M_CSF (x-axis, beta coefficients) and AP (Y-axis, beta coefficients). C: MR leave-one-out sensitivity analysis for M_CSF on AP (x-axis, log OR).
AP: Acute pancreatitis; M_CSF: Macrophage colony-stimulating factor; MR: Mendelian randomization; SNP: Single nucleotide polymorphisms. M_CSF.

and Ruminococcaceae in healthy controls compared to patients with AP implies that the depletion of SCFA-producing bacteria may be associated with a compromised intestinal barrier, which in turn exacerbates the severity of AP.[54] We hypothesized that the reduction of these beneficial bacteria due to a compromised intestinal barrier may promote bacterial translocation, thereby facilitating the development of AP.[55] Further studies are needed to elucidate the exact relationship between GM dysbiosis and AP, thus providing new therapeutic strategies to improve clinical outcomes in AP.

The concept that severe pancreatitis results from excessive inflammation was introduced three decades ago. Unresolved inflammation contributes to the development of pancreatitis.[56] Injured acinar cells express inflammatory cytokines and chemokines that contribute to the development of AP.[57] Animal models have demonstrated that inhibiting NLRP3 reduces both the systemic inflammatory response syndrome and compensatory anti-inflammatory response syndrome in severe pancreatitis.[58] Moreover, pretreatment with a combination of antibiotics results in attenuated pancreatic injury after inducing AP by inhibiting the pancreatic NLRP3 pathway.[59] Alongside other inflammatory cytokines, GM-CSF can regulate the activation and expansion of neutrophils. GM-CSF can drive the development of neutrophils and monocytes, playing a crucial role in regulating the inflammatory response.[60] Our study identified GM-CSF as a protective factor against pancreatitis. In contrast to our findings, Lin et al.[61] reported that increased GM-CSF expression induced by STAT5 was responsible for the augmentation of neutrophils and the more severe pancreatic inflammation observed in chronic pancreatitis. Therefore, further exploration is warranted to understand the role of GM-CSF in the pathogenesis of AP.

While our study strengthens the evidence for a causal relationship between GM, inflammatory cytokines, and AP, several limitations should be considered. The small sample size and limited IVs at the gene level restricted our analysis to the genus level. Moreover, the lack of demographic data (e.g., age, gender, and etiology) in the original study due to the use of summary statistics rather than raw data in the analyses meant that the different etiologies leading to AP were not analyzed separately in this study, which may have had an impact on the results. Future research should consider etiology-specific MR analysis. In addition, our findings may not generalize to other ethnic groups, as the majority of cohorts in our study were of European descent. Replication of the study in other populations will ensure generalizability of the current findings. Finally, despite our exploration of the mediating effect of inflammatory cytokines between varying GM abundances and AP, the mechanism by which the GM influences the pathogenesis of AP requires further investigation, given that inflammatory cytokines do not serve as mediators.

This study explored whether inflammatory cytokines could serve as mediators in the pathway between GM and AP. While we initially hypothesized a mediating role of inflammatory cytokines, our findings did not support this hypothesis, indicating that other mechanisms might be involved in connecting GM and AP.

In conclusion, our MR study supports a causal link between GM, inflammatory cytokines and AP. Increasing certain genera, such as Prevotella9, RuminococcaceaeUCG004, and Ruminiclostridium6, may alleviate AP, while suppressing others, including Bacteroidales, *E. fissicatena* group, and Coprococcus3, may represent potential AP treatment targets. Although there was a negative correlation between M_CSF and pancreatitis, it

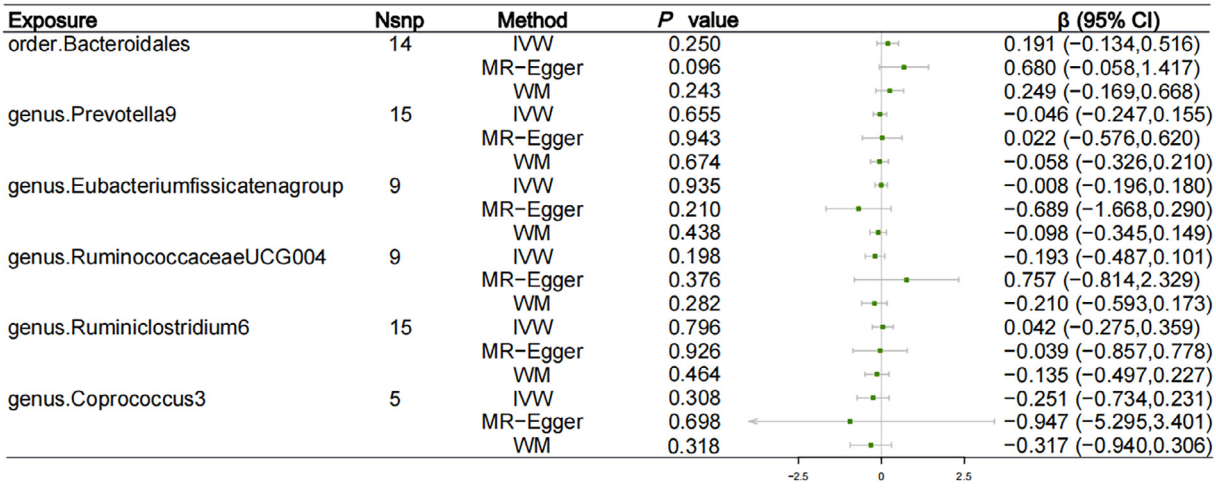

Figure 4. Causal effects for GM on M_CSF performed by MR.
CI: Confidence interval; GM: Gut microbiota; IVW: Inverse variance weighted; MR: Mendelian randomization; Nsnp: Number of single nucleotide polymorphism; WM: Weighted median.

appears that inflammatory cytokines do not play a mediating role in the pathway from GM to AP.

CRediT Authorship Contribution Statement

Xiaofeng Wang: Writing – original draft, Conceptualization. **Yiwen Qiu:** Data curation. **Ying Di:** Writing – original draft. **Hou Shaohua:** Formal analysis. **Wei Wu:** Data curation. **Weiyi Wang:** Formal analysis. **Huan Liu:** Writing – review & editing, Conceptualization. **Pu Li:** Writing – review & editing.

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Ethics statement

We obtained our data from publicly available summary statistics of GWAS. All of the GWAS included in this study had previously received ethical approval from their respective institutions. No new data were collected, and as such, no additional ethical approval was necessary.

Conflict of 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.

Data Availability Statement

Publicly available datasets were analyzed in this study.

Supplementary Materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jointm.2024.10.004.

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