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Exploring the casual association between gut microbiome, circulating inflammatory cytokines and chronic pancreatitis

A Mendelian randomization analysis

Xiaoqiu Yang, BS[a], Hao Xu, MS[b], Xiaolu Liang, MD[b], Guojia Yuan, MS[b], Qiaoping Gao, BS[b], Xiaoyu Tan, BS[b], Yongguang Yang, MD[b], Yi Xiao, MS[b], Zhanren Huang, MS[b], Wei Dai, MD[b], Xiaoguang Liu, MD[b,*]

Abstract

It has been established that gut dysbiosis contributed to the pathogenesis of digestive disorders. We aimed to explore the causal relationships between intestinal microbiota, circulating inflammatory cytokines and chronic pancreatitis (CP). Summary statistics of genome-wide association studies (GWAS) of intestinal microbiome was retrieved from the MiBioGen study and the GWAS data of 91 circulating inflammatory cytokines and CP were obtained from the GWAS catalog. The 2-sample bidirectional Mendelian randomization (MR) analysis was performed between gut microbiota, circulating inflammatory cytokines and CP, in which the inverse variance weighted (IVW) method was regarded as the primary analysis approach. To prove the reliability of the causal estimations, multiple sensitivity analyses were utilized. IVW results revealed that genetically predicted 2 genera, including Sellimonas and Eubacteriumventriosumgroup, and plasm C-C motif chemokine 23 (CCL23) level were positively associated with CP risk, while genus Escherichia Shigella, Eubacteriumruminantiumgroup and Prevotella9, and plasma Caspase 8, Adenosine Deaminase (ADA), and SIR2-like protein 2 (SIRT2) level, demonstrated an ameliorative effect on CP. Leave-one-out analysis confirmed the robustness of the aforementioned causal effects and no significant horizontal pleiotropy or heterogeneity of the instrumental variables was detected. However, no association was found from the identified genera to the CP-related circulating inflammatory cytokines. Besides, the reverse MR analysis demonstrated no causal relationship from CP to the identified genera and circulating inflammatory cytokines. Taken together, our comprehensive analyses offer evidence in favor of the estimated causal connections from the 5 genus-level microbial taxa and 4 circulating inflammatory cytokines to CP risk, which may help to reveal the underlying pathogenesis of CP.

Abbreviations: ADA = adenosine deaminase, AP = acute pancreatitis, CCL23 = C-C motif chemokine 23, CP = chronic pancreatitis, FMT = fecal microbiota transplantation, GWAS = genome-wide association studies, IVs = instrumental variables, IVW = inverse variance weighted, LD = linkage disequilibrium, MR = Mendelian randomization, MR-PRESSO = MR-pleiotropy residual sum and outlier, SCFAs = short-chain fatty acids, SIRT2 = SIR2-like protein 2, SNPs = single nucleotide polymorphisms.

Keywords: chronic pancreatitis, circulating inflammatory cytokines, genome-wide association study, gut microbiota, Mendelian randomization, single nucleotide polymorphism

1. Introduction

Chronic pancreatitis (CP) is characterized by persistent pancreatic inflammation and the onset of parenchyma fibrosis. Continuous and irreparable localized, segmental, or widespread damages are caused by the chronic inflammation in the both exocrine and endocrine pancreatic tissues. On clinical manifestations, acute pancreatitis, epigastric pain, pancreatic exocrine dysfunction, and diabetes mellitus, which result from progressive damage to the pancreas, can be the problem of CP patients.[1,2] When it worse, CP is one of the major risk

XY and HX contributed equally to this work.

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The datasets generated during and/or analyzed during the current study are publicly available.

No additional ethics approval or informed consent was required due to our study was based on public databases.

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[a] Department of Anesthesiology, Affiliated Hospital of Guangdong Medical University, Zhanjiang, Guangdong Province, People's Republic of China, [b] Department of Hepatobiliary and Pancreatic Surgery, Affiliated Hospital of Guangdong Medical University, Zhanjiang, Guangdong Province, People's Republic of China.

** Correspondence: Xiaoguang Liu, Department of Hepatobiliary and Pancreatic Surgery, Affiliated Hospital of Guangdong Medical University, Zhanjiang, 524001, Guangdong Province, People's Republic of China (e-mail: xiaoguanggdmu@hotmail.com).*

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factors for pancreatic cancer, which carries a dismal prognosis, and approximately 5% of CP patients will develop such malignancy.[3] Conventional managements of CP include lifestyle modifications, medications, endoscopic intervention and surgical intervention.[4]

In terms of etiology, although alcohol use is still believed to be the dominant factor for CP pathogenesis, it is not the only provoking agent. Additionally, tobacco consumption acts as a significant contributing factor. In recent years, the onset and progression of many digestive diseases is influenced by interactions between the host and intestinal microbiota, which change the immune system and complex biological processes. The pancreatic duct structure, which anatomically connects the pancreas to the digestive system directly, inevitably results in a link with the gut microbiota. Pancreas-Microbiota Cross Talk has been established[5] and it is proved that the gut microbiota is crucial in regulating pancreatic functioning. In the course of acute pancreatitis (AP), there is a bidirectional modulation between the intestinal micropopulation and activated NOD-like receptor thermal protein domain associated protein 3.[6] Besides, a fecal microbiota signature with high specificity for the diagnosis of pancreatic ductal adenocarcinoma was uncovered.[7] For CP, one of the hallmarks is unremitting inflammation. The emergence of inflammatory mediator, such as reactive oxygen species, cooperating with oxidative stress reaction acts an essential role in the pathogenesis of AP and CP. The regulating neurogen of immunological responses induced by gut to pancreas axis and intestinal bacterial metabolites, such as short-chain fatty acids (SCFAs), could contribute to conferring the inflammatory process of pancreas. Certainly, the factors from pancreatic disorders might carry a significant influence on the functioning species and abundance of the intestinal microbiota.[8] Besides, multiple cytokine profiles have been demonstrated to promote the progression of CP, including IL 1β, IFN-γ, fractalkine, C-reactive protein and TGF-β and so on.[9,10] These changes on the microbial composition and circulating inflammatory factor may serve as the novel biomarker of pancreatic fibrosis.[11] Nevertheless, the causal associations for specific gut microbiome and CP are still elusive.

A statistical method known as Mendelian randomization (MR) analysis seeks to use the innate characteristics of common genetic variations to explain observational association findings. In our study, we explored the causal relationship between the intestinal microbiota, plasm proteins and CP through carrying out a thorough bidirectional 2-sample MR analysis. Manipulation of the gut microbiome is a potential approach to improve a series of human disorders and cancers and microbiome-based therapeutics is showing a decent future prospect.[12,13] Hence, our research can aid in the development of novel therapeutic modalities, including probiotic treatment, dietary modifications, and fecal microbiota transplantation (FMT). Future advances in the field of microbiome-based management will offer the chances for medical practitioners to exploit the fecal microbiota to work out the pancreatic diseases, including CP.

2. Materials and methods

2.1. Study design

As a genetic approach, MR analysis based on single nucleotide polymorphisms (SNPs) explores the causal effects of exposure on outcome utilizing the random distribution of genetic variants, which is chiefly applied to causal inference in genetic association and genomics studies. The SNPs believed to the instrumental variables (IVs) for MR analysis should adhere to the following fundamental premises: The SNPs and the issue of exposure need to be strongly correlated. There should be no link between SNPs and the outcome through confounding factors. The SNPs should not have a direct effect on the outcome. To explore the casual connections between gut microbiota at the feature level of genus, plasm proteins and CP, the comprehensive bidirectional 2-sample MR analyses were conducted and the study design was presented in Figure 1. "TwoSampleMR" and "MR-PRESSO" packages were utilized to complete our comprehensive analyses in R program (version 4.3.1).

2.2. GWAS summary statistics for gut microbiome (Exposure-1)

The SNPs related to the human gut microbiome composition were obtained from a GWAS dataset of the international consortium MiBioGen (https://mibiogen.gcc.rug.nl/), a summary statistics based on the substantial GWAS meta-analysis.[14] The 24 cohorts included MiBioGen study have made adjustments for sex and age as covariates in the calculation process. The date of data download was July 20,2023 and the microbial taxa at the level of genus were retained.

2.3. GWAS summary statistics for circulating inflammatory cytokines (Exposure-2)

Genetic instruments for plasm proteins were extracted from the large-scale GWAS summary statistics (accession numbers GCST90274758 to GCST90274848), which were derived from a genome-wide protein quantitative trait locus study mapping for 91 plasma proteins measured using the Olink Target Inflammation panel in 11 cohorts totaling 14,824 European ancestry participants.[15] All of the 91 metabolites were selected for our genetic investigation as Exposure-2.

2.4. Outcome data

As the outcome in MR analysis, SNPs data for CP were extracted from the large-scale GWAS summary statistics, the GWAS catalog (GWAS ID: ebi-a-GCST90018821, Trait name: CP; Published by Sakaue S et al, https://www.ebi.ac.uk/gwas). This outcome data of CP contains 1424 cases and 476,104 controls of European ancestry and the number of SNPs is 24,195,431.

2.5. Genetic instruments selection

we applied a more lenient threshold, P value of 1×10^{-5}, to screen the SNPs associated with each genus-level bacterial taxon and plasm proteinsas the candidate IVs for sequent MR analysis, which could enhance the feasibility of MR analysis.[16–18] Afterward, to avoid bias within the causal estimates, independent IVs were further selected for every taxon utilizing linkage disequilibrium (LD) analysis, in which each collection of SNPs were clumped with LD $r^2 < 0.001$ and distance > 10,000 kb in the PLINK clumping algorithm and the SNPs absent from LD reference pane were also excluded.[19] The LD reference panel was established utilizing the 1000 Genomes Project European sample.[20] Furthermore, to measure the statistical strength of the link between the candidate IVs and the associated taxon or proteins, the F-statistic for each SNP was determined and IVs with a F-statistic < 10 were excluded. A detailed description of the F-statistic computation was found elsewhere.[21]

2.6. MR estimates and reverse MR analysis

Subsequently, the effect variants of the screened IVs related to each bacterial taxon and plasm proteins and the SNPs for CP were harmonized and any alleles that were incompatible or

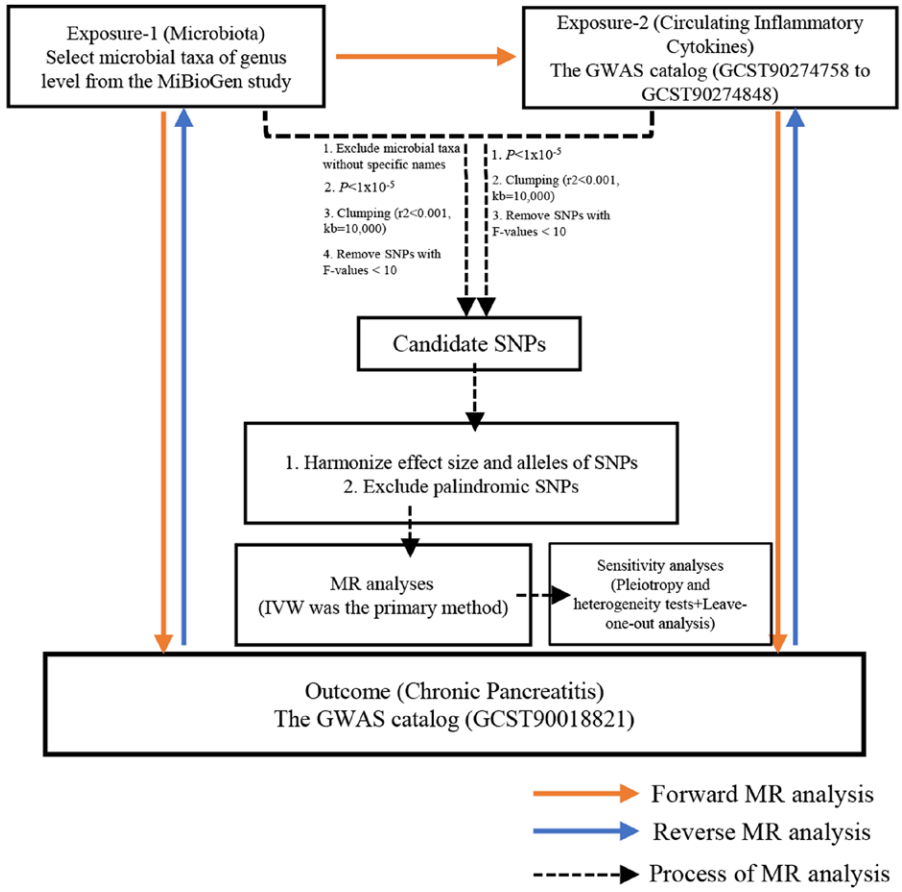

Figure 1. Workflow illustrating the 2-sample bidirectional MR analysis for exploring the casual associations between gut microbiota, plasm proteins and chronic pancreatitis. GWAS = genome-wide association study, MR = Mendelian randomization, SNP = single nucleotide polymorphism.

palindromic with intermediate allele frequency were removed. The 2-sample MR analyses were carried out for the bacterial taxa or plasm protein with at least 3 IVs. The inverse variance weighted (IVW) method was acknowledged as the dominant statistical analysis method,[22] which was supplemented with robust multiple MR methods, including weighted median, Weighted mode, MR-Egger regression, and MR-pleiotropy residual sum and outlier (MR-PRESSO) approaches. The causal associations are considered statistically significant if the *P* value of the IVW method is <.05. In weighted median method, to achieve accordant assumptions of causal effects, the requirement that half of SNPs being significant IVs is necessary. In the MR-PRESSO method, NbDistribution was set to 1000 and it is possible to identify potential outliers and provide outlier-corrected causal estimates. To examine the contribution of the pathogenesis of CP to the identified bacterial taxon and plasm proteins, we further performed the reverse MR analyses. The procedures of the reverse MR analysis followed the identical steps of the above-mentioned forward MR analysis.

2.7. Sensitivity analyses

The existence significant heterogeneity of IVs could skew the causal assumptions in summary-level MR analysis, weakening the validity of the causal inferences. Therefore, Cochran Q test (IVW method) and Rucker Q test (MR-Egger method) were employed to measure the heterogeneity of IVs for each microbial taxon. $P < .05$ suggested the existence of significant heterogeneity. In the MR-Egger method, if the corresponding *P* value was >.05, we assumed that the horizontal pleiotropy of the IVs was not noticeable enough to have an influence on the causal inferences. If the horizontal pleiotropy was identified, MR-PRESSO outlier test was further performed to eliminate any probable outlier SNPs. Moreover, MR-PRESSO global test was carried out before and after eliminating outliers to confirm that the IVs with pleiotropy were excluded ($P > .05$). Subsequently, "leave-one-out" analysis was undertaken to determine the impact of each instrumental SNP on the MR conclusions and the outliers should be excluded to make sure the robustness of causal estimates.

3. Results

3.1. Overview

In this study, the selected level of gut microbiota composition was genus, and 131 genera were extracted. As a results, 119 genus-level taxa, excluding 12 unidentified taxa, were acquired. Then the SNPs of them were screened by multiple steps and a total of 1531 eligible SNPs were consequently included for MR analysis. The details of genus-level taxa and according IVs are listed in Supplementary Table S1, http://links.lww.com/MD/M281. Besides, the concert types of plasm protein were presented in Supplementary Table S2, http://links.lww.com/MD/M282.

3.2. MR results from the genus-level gut microbiome to CP

In MR analyses, the full results for 5 MR methods between the genus-level taxa and CP were shown in Supplementary

Table S3, http://links.lww.com/MD/M283. According to the IVW method estimates, genetically predicted genus Escherichia Shigella (OR = 0.68, 95% CI: 0.48–0.97, P_{IVW} = 0.03), Eubacteriumruminantiumgroup (OR = 0.79, 95% CI: 0.66–0.96, P_{IVW} = 0.02), Prevotella 9 (OR = 0.72, 95% CI: 0.58–0.90, P_{IVW} < 0.01) were unfavorable for an increased risk of CP, indicating a protective impact of the above genera on CP, while Sellimonas (OR = 1.36, 95% CI: 1.07–1.74, P_{IVW} = 0.01), Eubacteriumventriosumgroup (OR = 1.50, 95% CI: 1.09–2.06, P_{IVW} = 0.01) indicated an unfavorable influence on CP (all P < .05; Table 1 and Fig. 2A). Furthermore, these significant associations remained stable in weighted median and MR-PRESSO methods (Table 1). The corresponding scatter plots of the associations of these 5 microbial taxa with CP were shown in Figure 3A–E.

3.3. MR results from the 91 plasm proteins to CP

Noticeably, the genetically predicted C-C motif chemokine 23 (CCL23) level (OR = 1.21, 95% CI: 1.06–1.37, P_{IVW} = 0.004) indicated a favorable influence on CP risk in IVW method estimates and this causality were supported by the other for MR methods (P < .05; Table 2 and Fig. 2B). On the other hands, the genetically predicted Caspase 8 level (OR = 0.69, 95% CI: 0.55–0.87, P_{IVW} = 0.001), Adenosine Deaminase (ADA) level (OR = 0.90, 95% CI: 0.85–0.96, P_{IVW} = 0.001), SIR2-like protein 2 (SIRT2) level (OR = 0.75, 95% CI: 0.57–0.96, P_{IVW} < 0.05) were a protective impact on CP (all P < .05; Table 2 and Fig. 2B). And these significant associations remained stable in partial other MR methods (Table 2). The corresponding scatter plots of the associations of these 5 microbial taxa with CP were shown in Figure 3F–I.

3.4. Exploring the casual association from CP-related gut microbiome to CP-related plasma proteins

Changes in intestinal flora can affect body metabolism and substance synthesis. Therefore, to explore possible mechanism of the identified genus-level taxa genetically related to CP, we further carried out the 2-sample forward MR analysis from the identified 5 CP-related gut taxa to CP-related plasm proteins. Unfortunately, the significant causal link from the identified 5 CP-related gut taxa to CP-related plasm proteins were not found (P_{IVW} > 0.05, Supplementary Table S4, http://links.lww.com/MD/M284).

3.5. Reverse mendelian randomization

Based on the aforementioned approaches of genetic instruments selection, a total of 32 eligible SNPs for CP were included in reverse MR analysis (P_{IVW} > 0.05, Supplementary Table S5, http://links.lww.com/MD/M285). Then, the reverse MR analyses from CP to the identified 5 bacterial taxon and 4 plasm proteins were conducted. According to the IVW method, there was not statistically significant causality from CP to the identified 5 bacterial taxon and 4 plasm proteins (P_{IVW} > 0.05, Supplementary Table S6–7, http://links.lww.com/MD/M286, http://links.lww.com/MD/M287).

Table 1

Association of genetically predicted gut microbiota with chronic pancreatitis.

Methods	IVs	OR	95%CI	*P* value	*P* value*	Heterogeneity (*Q***; *P* value***)	MR-PRESSO (Global test, *P* value****)
Sellimonas							
IVW	11	1.36	1.07–1.74	.01	.07	17.98, .06	.09
Weighted median		1.33	1.03–1.73	.03			
MR-Egger		0.33	0.09–1.28	.14			
Weighted mode		1.31	0.88–1.97	.21			
MR-PRESSO		1.36	1.07–1.74	.03			
Escherichia.Shigella							
IVW	15	0.68	0.48–0.97	.03	.28	19.96, .13	.49
Weighted median		0.56	0.36–0.87	.01			
MR-Egger		0.38	0.13–1.11	.10			
Weighted mode		0.49	0.25–0.97	.06			
MR-PRESSO		0.68	0.48–0.97	<.05			
Eubacterium ruminantium group							
IVW	19	0.79	0.66–0.96	.02	.44	17.51, .49	.14
Weighted median		0.80	0.61–1.06	.12			
MR-Egger		1.02	0.53–1.95	.96			
Weighted mode		0.79	0.44–1.42	.43			
MR-PRESSO		0.79	0.65–0.96	.03			
Eubacterium ventriosum group							
IVW	17	1.50	1.09–2.06	.01	.54	16.04, .45	.96
Weighted median		1.65	1.04–2.61	.03			
MR-Egger		2.34	0.56–9.74	.26			
Weighted mode		1.54	0.76–3.11	.24			
MR-PRESSO		1.50	1.09–2.06	.02			
Prevotella9							
IVW	19	0.72	0.58–0.90	<.01	.10	9.90, .94	.48
Weighted median		0.65	0.48–0.89	<.01			
MR-Egger		0.72	0.40–1.31	.30			
Weighted mode		0.60	0.35–1.04	.09			
MR-PRESSO		0.72	0.61–0.85	<.01			

* *P* value of the intercept of the MR-Egger.

** *Q*-value of Cochran Q test.

*** *P* value of the heterogeneity test.

**** *P* value of the MR-PRESSO global test.

95% CI = 95%, confidence interval, IVW = inverse variance weighted, IVs = instrumental variables, MR = Mendelian randomization, MR-PRESSO = MR-pleiotropy residual sum and outlier, OR = odd ratio.

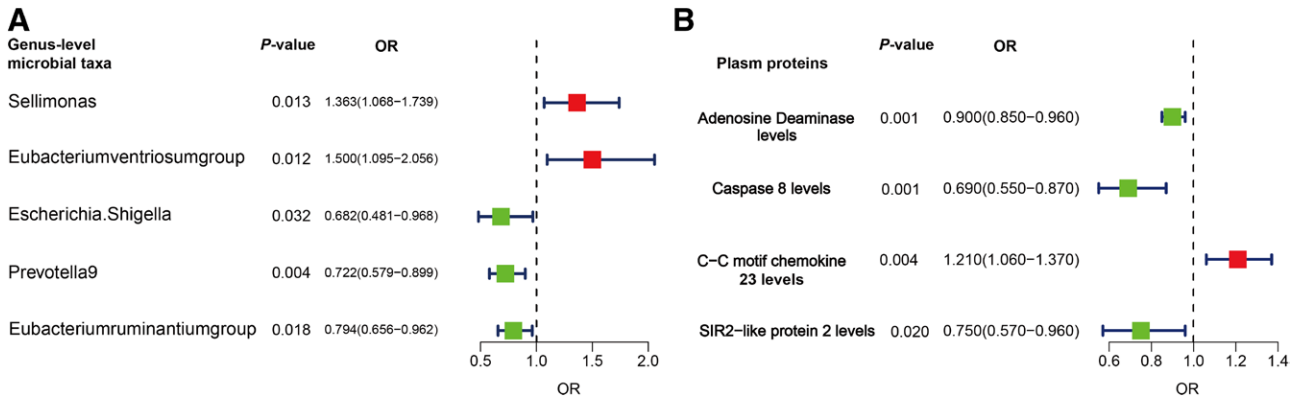

Figure 2. Forest plots illustrating the results of 2-sample MR analyses in significance level (IVW $P < .05$) with IVW method, in which the 5 genus-level taxa and 4 circulating proteins were identified. IVW = inverse variance weighted, MR = Mendelian randomization, OR = odd ratio.

3.6. Sensitivity analysis

The according *P* values of heterogeneity test and the intercept terms for the 5 identified genus-level taxa and 4 plasm proteins were all > 0.05, revealing no notable horizontal pleiotropy and heterogeneity of these significant estimates (Table 1 and 2, Supplementary Figure 1, http://links.lww.com/MD/M278 and 2, http://links.lww.com/MD/M279). Moreover, leave-one-out analysis of these 5 bacterial taxa revealed that no SNPs with dominant effects on the MR inference was identified, which confirmed the reliability and stability of the causal effects of the 5 identified associations (Supplementary Figure 3, http://links.lww.com/MD/M280).

4. Discussion

Using a thorough MR analysis, our study revealed that the genetically estimated 5 genus-level taxa, in which Sellimonas, Eubacteriumventriosumgroup indicated an unfavorable influence on CP, whereas Escherichia Shigella, Eubacteriumruminantiumgroup and Prevotella 9 was detrimental for an increased risk of CP, indicating a protective impact of the above 3 genera on CP. Additionally, the reliability and consistency of our results were further testified utilizing the multiple methods of sensitivity analyses. To the best of our knowledge, this is the initial MR study to thoroughly explore the effect of intestinal microbiota on CP in a causal way. Even with advancements in endoscopic and radiological techniques, diagnosing CP, especially the pathogenesis of CP in an earlier disease, can still be difficult, as they are more likely to be lack of symptom and not show typical signs of the disease on routine imaging.[2] Knowing the identified microbial taxa related to CP in a causal way may aid in the early diagnosis of this underlying illness. Genus_Sellimonas was reported to be associated with breast cancer and chronic granulomatous disease.[23,24] Prevotella_9 was related to the production of SCFAs.[25] A study demonstrated that compared to patients with ulcerative colitis without depression, patients with ulcerative colitis and depression had more Sellimona but less Prevotella_9 in fecal microbial community, suggesting the abundance of these 2 taxa may be related to the mental state of patients.[26] Increasing prevalence of anxiety and depression in CP patients was observed[27] and a MR analysis revealed that genetic liability to depression was associated with an increased risk of CP.[28] Combined with our MR results, we believed that changes in the gut microbiota of CP may be partly due to its accompanying black mood or depression.

The gut microbiota has been well recognized as a crucial immunomodulation regulator, which contribute to host metabolism, immunological homeostasis and immune diseases in human.[29,30] Besides, it has been established that the pancreatic injury and fibrosis in CP may be driven by host immune activation.[31] Actually, substantial evidence has established links between gut microbiota dysbiosis and pancreatic disorders. Damage-associated molecular patterns-mediated cytokine activation, which results in the migration of gut microbiota organisms into the circulation and the regulation of innate immune responses in the intestinal cells, is what drives the inflammation that underlies pancreatitis.[32] CP is linked to considerable dysbiosis of intestinal microbiota, including a significant decrease in diversity and richness and increased abundance of opportunistic pathogens.[33,34] The study using 16s RNA gene sequencing technique demonstrated that a severely reduced microbial diversity was shown in the CP patients, in which the bacteria that produce SCFAs such as Faecalibacterium reduced, whereas the increased abundance of facultative pathogenic organisms, such as Enterococcus, Streptococcus, and Escherichia Shigella was detected.[34] As the main energy source of intestinal epithelial cells, SCFAs can promote the proliferation and differentiation of epithelial cells, reduce cell apoptosis, and maintain the mechanical barrier of intestinal mucosa.[35] Additionally, it is shown that, in the composition of gut microbiomes in the CP patients, the abundances of Escherichia Shigella and other genera were relatively high and that of Faecalibacterium was low.[36] The plasma concentration of bile acid increased in CP patients, which may be due to an impaired bile acid circulation resulting from gut microbiota alteration.[37] Therefore, patients with CP may have reduced level of some taxa that may be helpful for intestinal barrier function and similar results have been confirmed by other studies.[38] In addition, the exocrine function of the pancreas has the ability to modify the makeup of the gut microbiota.[8] Steatorrhea caused by CP also can result in the alteration of host gut microbiota. Nevertheless, our reverse MR study demonstrated there was no the causal estimates from CP to the 5 identified bacterial taxa in MR analysis.

Furthermore, we found a causal contributing connection of CCL23 in CP pathogenesis. CCL23 is a known chemoattractant for resting T cells, monocytes and dendritic cells via their CCR1 receptor, promoting migration to sites of inflammation.[39] Additionally, positive correlation was found between plasma CCL23 levels and established indicators of the severity of systemic mastocytosis disease.[40] Reflexively, plasma Caspase 8, ADA and SIRT2 level negatively related to CP risk in our study. Caspase-8 is involved in the regulation of cell death mechanisms and immune responses in conditions like infection, autoimmunity, and T cell signal transduction. In humans, immunodeficiency, inflammatory bowel disease, and autoimmune lymphoproliferative syndrome can result from a lack of Caspase-8.[41] In human, ADA is an enzyme involved

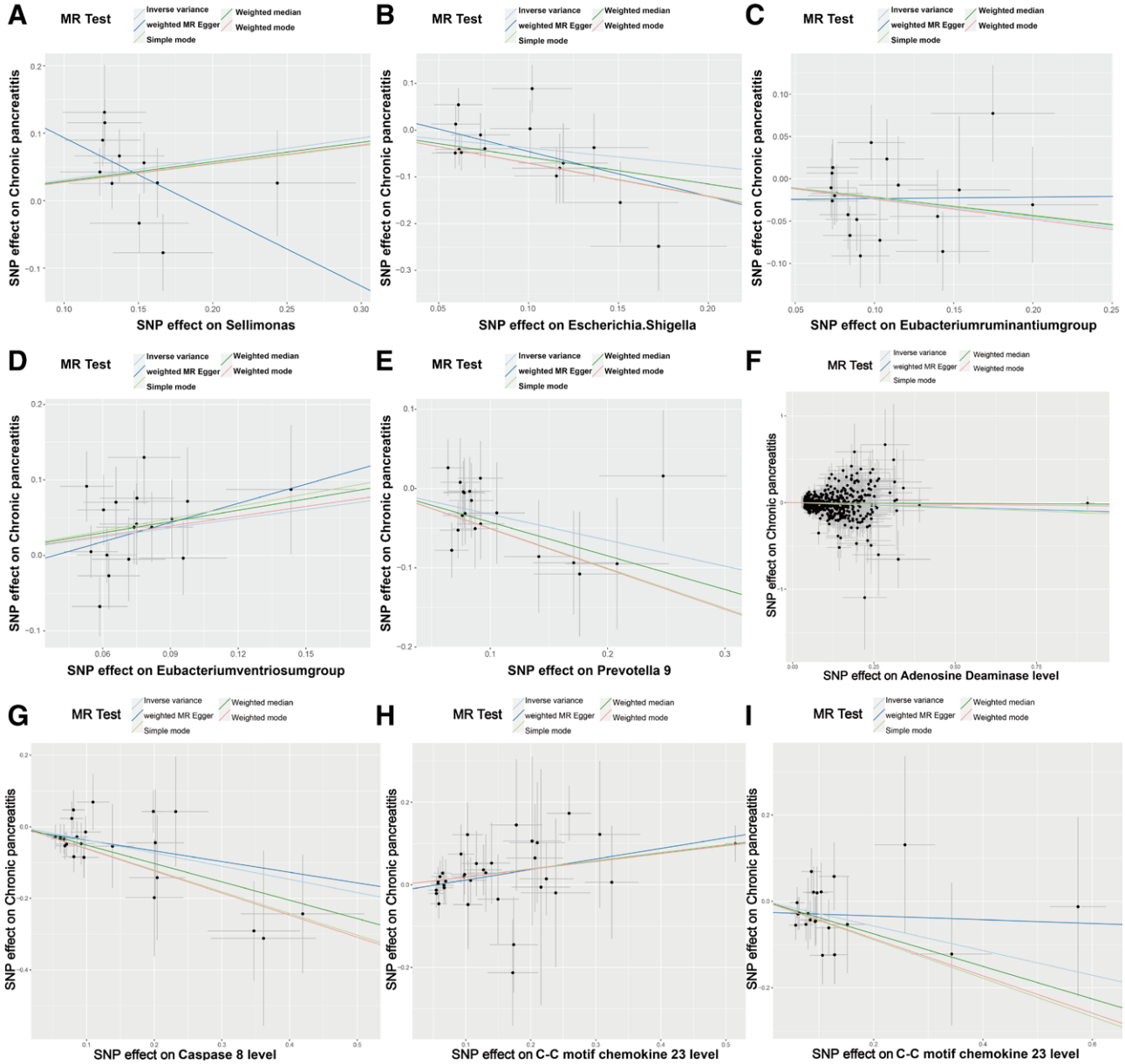

Figure 3. The scatter plots of the causal associations of the 5 identified genus-level microbial taxa with chronic pancreatitis. The scatter point represents the SNP effect of microbial taxa to chronic pancreatitis. The slope of each line corresponds to the estimated effect from different models. (A) Sellimonas; (B) Escherichia Shigella; (C) Eubacteriumruminantiumgroup; (D) Eubacteriumventriosumgroup; (E) Prevotella 9; (F) Adenosine Deaminase level; (G) Caspase 8 level; (H) C-C motif chemokine level; (I) SIR2-like protein 2 level. MR = Mendelian randomization, SNP = single nucleotide polymorphism.

in purine metabolism and the production of immune cells. If this enzyme is mutated or lacking, the complex immune deficiency and dysfunction of T cells, B cells, and natural killer cells may occur.[42] The function of ADA in the pathogenesis of autoimmune diseases, such as rheumatoid arthritis, have been noticed.[43] SIRT2 were associated with red cell distribution width and red cell distribution width was an important hematological parameter prognostic marker for acute pancreatitis.[44,45]

There is growing recognition of the role of the microbiome in pancreatic disorders and altering the bacterial ecology in the gut has the potential to be a potent therapeutic modality for CP. Experimental data demonstrated that FMT from CP mouse increased the fibrosis of pancreas through raising the infiltration level of $CD4^+$T cells and macrophage.[11] It has been proposed that FMT coordinates immunological responses in the gut mucosa and pancreas-resident immune system.[6,46] Using Ganoderma lucidum strain S3, the pancreatitis in mice can be relieved by reducing the inflammation mediator level and enhancing the antioxidant activity which was related to the increased abundance of the advantageous taxa.[47] In addition, administration of inonotus obliquus polysaccharide could improve intestinal microecology and then generated a therapeutic effect on CP in mice.[48,49] In our project, there are some limitations that baseline information for participants was lacking and *P* value instead of FDR was applied as threshold to determine the causality, which may result in more false positives.

5. Conclusions

In summary, this MR study illuminates a possible causative involvement of gut microbiota in the pathophysiology of CP. The detection of gut microbiota could be a practical method of disease screening to identify groups with a higher risk of

Table 2

Association of genetically predicted plasma proteins with chronic pancreatitis.

Methods	IVs	OR	95%CI	*P* value	*P* value*	Heterogeneity (*Q***; *P* value***)	MR-PRESSO (Global test, *P* value****)
Adenosine deaminase level							
IVW	562	0.90	0.85–0.96	.001	.91	552.02, .60	.619
Weighted median		0.98	0.85–1.13	.81			
MR-Egger		0.90	0.80–1.00	.05			
Weighted mode		0.96	0.83–1.11	.58			
MR-PRESSO		0.90	0.87–1. 93	.001			
Caspase 8 level							
IVW	23	0.69	0.55–0.87	.001	.74	16.34, .80	.77
Weighted median		0.60	0.44–0.82	.002			
MR-Egger		0.74	0.47–1.18	.22			
Weighted mode		0.54	0.25–1.15	.12			
MR-PRESSO		0.69	0.60–0.79	.001			
C-C motif chemokine 23 level							
IVW	34	1.21	1.06–1.37	.004	.32	18.68, .98	.99
Weighted median		1.21	1.03–1.43	.02			
MR-Egger		1.30	1.08–1.55	.01			
Weighted mode		1.21	1.03–1.42	.03			
MR-PRESSO		1.21	1.15–1.27	.001			
SIR2-like protein 2 level							
IVW	21	0.75	0.57–0.96	.02	.36	14.35, .81	.85
Weighted median		0.69	0.48–0.98	.04			
MR-Egger		0.96	0.55–1.68	.88			
Weighted mode		0.65	0.35–1.19	.18			
MR-PRESSO		0.75	0.64–0.86	.015			

* *P* value of the intercept of the MR-Egger.

** *Q*-value of Cochran Q test.

*** *P* value of the heterogeneity test.

**** *P* value of the MR-PRESSO global test.

95% CI = 95%, confidence interval, IVW = inverse variance weighted, IVs = instrumental variables, MR = Mendelian randomization, MR-PRESSO = MR-pleiotropy residual sum and outlier, OR = odd ratio.

CP. This would have an implication to clinicians that early stool examination might be a feasible practice for disease screening to recognize populations at a higher risk of CP. Furthermore, gut microbiota conditioning may be a future CP treatment. Therefore, it is necessary to conduct more researches to testify our MR results and uncover the underlying mechanism.

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Author contributions

Conceptualization: Wei Dai, Xiaoguang Liu.
Data curation: Xiaoyu Tan, Yongguang Yang, Yi Xiao.
Formal analysis: Xiaoqiu Yang, Qiaoping Gao, Zhanren Huang.
Investigation: Xiaolu Liang, Guojia Yuan, Qiaoping Gao, Xiaoyu Tan, Zhanren Huang.
Methodology: Xiaoqiu Yang, Hao Xu, Xiaolu Liang, Guojia Yuan, Qiaoping Gao, Xiaoyu Tan, Yongguang Yang, Zhanren Huang.
Project administration: Xiaoyu Tan, Yongguang Yang, Yi Xiao.
Resources: Hao Xu, Yongguang Yang, Yi Xiao, Wei Dai.
Software: Hao Xu.
Supervision: Xiaoguang Liu.
Validation: Xiaolu Liang, Guojia Yuan.
Writing – original draft: Xiaoqiu Yang, Hao Xu.
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