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Metabolite genetic characterization and bile acid metabolomics reveal deoxycholic acid protective effects on acute pancreatitis

Liuhui Wang[1]*, Xiaowu Dong[1]*, Qingtian Zhu[1]*, Zhihao Wang[1], Pin Li[1], Ziyi Zhao[1], Weiwei Chen[2], Chenchen Yuan[1], Wei Li[3], Bo Tu[4], Weijuan Gong[1], Lei Zhang[5], Guotao Lu[1], Weixuan Yang[6]

[1]*Pancreatic Center, Department of Gastroenterology, Yangzhou Key Laboratory of Pancreatic Disease, The Affiliated Hospital of Yangzhou University, Yangzhou University, Yangzhou 225000, China;*

[2]*School of Clinical Medicine, Yangzhou University, Yangzhou, Jiangsu 225100, China;*

[3]*Faculty of Pharmaceutical Sciences, Toho University, Funabashi, Chiba 274-8510, Japan;*

[4]*Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA;*

[5]*Department of clinical laboratory, Huai'an Hospital Affiliated to Yangzhou University (The Fifth People's Hospital of Huai'an)，Yangzhou University, Huai'an 223001, China;*

[6]*Department of Gastroenterology, Huai'an Hospital Affiliated to Yangzhou University (The Fifth People's Hospital of Huai'an)，Yangzhou University, Huai'an 223001, China;*

ABSTRACT: Abnormal bile acid metabolism is associated with various diseases, including acute pancreatitis (AP), although its beneficial effects have not been fully elucidated. This study aimed to investigate the relationship between deoxycholic acid (DCA) and AP. We designed a comprehensive analysis pipeline involving two-sample Mendelian randomization (MR) and targeted bile acid metabolomics based on a bi-center population to identify potential metabolic influencers of AP. These analyses were complemented by in vivo and in vitro experimental studies to assess the effects of metabolites, followed by integrated analysis and prediction of target molecules to explore the potential mechanisms underlying the protective effects of DCA. In MR studies, we identified a causal relationship between DCA and AP. A population-based targeted bile acid metabolomics showed that DCA levels were significantly reduced during the acute phase of AP compared with those in healthy controls and were associated with acute respiratory distress syndrome and infectious pancreatic necrosis. In the cholecystokinin-induced pancreatic acinar cell injury model and the caerulein-induced AP model, DCA administration alleviated pancreatic acinar cell injury, improved the pathological manifestations of pancreatic tissues, and reduced serum lipase and amylase levels. Integrated analysis of pancreatic transcriptomic data and DCA target proteins prediction by PharmMapper revealed significant alterations in the PPAR signaling pathway. Furthermore, Western blot and immunofluorescence assays confirmed that DCA likely mediates its protective effects through this mechanism. For the first time, we found that DCA is closely associated with the development of AP using a public database and a dual-center clinical cohort and that supplementation with DCA can improve acinar cell necrosis in AP, representing a promising novel strategy for the prevention and treatment of AP.

Keywords: Acute pancreatitis; Pancreatic acinar cells; Deoxycholic acid; Bile acid metabolomics.

* Liuhui Wang, Xiaowu Dong and Qingtian Zhu contributed equally to this work.
Corresponding author:
Lei Zhang: aoe3721@163.com; Guotao Lu: gtlu@yzu.edu.cn and Weixuan Yang: YWX15861719531@yzu.edu.cn

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1. Introduction

Acute pancreatitis (AP) is a common digestive system disorder characterized by inflammatory activation of the pancreatic tissue due to various etiologies**[1, 2]**. Metabolic disorders, such as obesity and hypertriglyceridemia, are well-established risk factors for AP, and growing evidence indicates that metabolic dysregulation plays a critical role in both its onset and progression**[3, 4]**. As end products of biochemical reactions, metabolites are essential for maintaining vital physiological processes**[5]**, highlighting the importance of understanding their involvement in diseases such as AP.

Mendelian randomization (MR), a genetic epidemiological approach, has been widely employed in recent years to assess causal effects between exposures and outcomes by utilizing genetic variants as instrumental variables**[6]**. Over the past decade, genome-wide association studies (GWAS), whole-genome sequencing, and exome sequencing have suggested potential genetic correlations between metabolite levels and inflammatory diseases, including AP**[7]**. These findings provide an opportunity for MR analysis to further identify comprehensive genetic determinants of metabolites and clarify the causal effects of metabolites on diseases. Previous studies have also employed GWAS and MR approaches to investigate causal relationships between gut microbiota, cholesterol metabolites, and pancreatic diseases**[8, 9]**.

Bile acids, endogenous derivatives of cholesterol metabolism, exhibit multiple biological functions beyond their well-established role in fat digestion and absorption**[10]**. Compared with existing treatment strategies for AP, modulating bile acid metabolism offers a unique multi-target mechanism of action. This includes regulating inflammatory responses, improving pancreatic microcirculation, and modulating the gut microbiota**[11]**. Further exploration of the role of bile acid metabolism in the pathogenesis of AP may, therefore, yield novel insights and strategies for its clinical management.

Our research group previously showed that chenodeoxycholic acid (CDCA) reduces acinar cell necrosis in AP**[12]**, and other studies have demonstrated that conjugated bile acids alleviate AP by suppressing inflammasome-mediated inflammation**[13]**. However, most previous studies have focused on the functional validation of specific bile acids, with limited emphasis on genetic correlates linking bile acids to disease. As key metabolites and immunomodulators involved in systemic glucose–lipid metabolism and immune homeostasis, bile acids have been shown to exhibit significant genetic correlations with phenotypes such as obesity and inflammatory diseases**[14]**. Nevertheless, the causal relationship between bile acid metabolites and AP remains unclear from a human genetic perspective. Thus, more comprehensive studies are needed to elucidate the role of bile acids in the initiation, progression, and treatment of AP.

Deoxycholic acid (DCA) is a free bile acid derived from cholic acid through the loss of an oxygen atom at the C-7 position. DCA is the third most abundant biliary bile acid, accounting for approximately 20% of total bile acids in humans**[15, 16]**. Studies have demonstrated that DCA supplementation can alleviate conditions such as acute liver injury, cardiac dysfunction, and necrotizing enterocolitis**[17-19]**. Clinical studies have reported DCA levels in patients with various etiologies of liver disease compared with those in healthy individuals**[20]**. Furthermore, research has shown that serum levels of multiple bile acids are significantly

elevated during the acute phase of biliary AP and are higher in patients with acute biliary AP than in those with alcoholic AP**[21]**.

Based on two large-scale metabolomic GWAS, this study employed MR analysis to screen metabolites causally associated with AP. Targeted metabolomic profiling was performed across two independent cohorts to examine changes in the levels of bile acid metabolites before and after the onset of AP. Furthermore, through in vitro experiments using mouse acinar cells and in vivo animal models, the role of the secondary bile acid DCA in AP was elucidated. The study also revealed potential mechanisms through which DCA may mitigate AP severity. These findings offer new strategic directions for the clinical treatment of AP.

2. Materials and methods

2.1 Study design

The overall study design is shown in Fig. 1. We established a comprehensive analysis pipeline: (1) to conduct metabolomics-based association studies to identify potential metabolites that may influence AP using the two-sample MR method; (2) identify bile acid metabolites that exhibit significant changes before and after AP by performing targeted bile acid metabolomics in two central populations; (3) verify the efficacy of differential metabolites in animal and cell models of AP through supplementation with the corresponding differential metabolites; and (4) conduct proteomics-based association studies to investigate proteins potentially mediating the effects of AP on metabolites by using a two-sample MR approach.

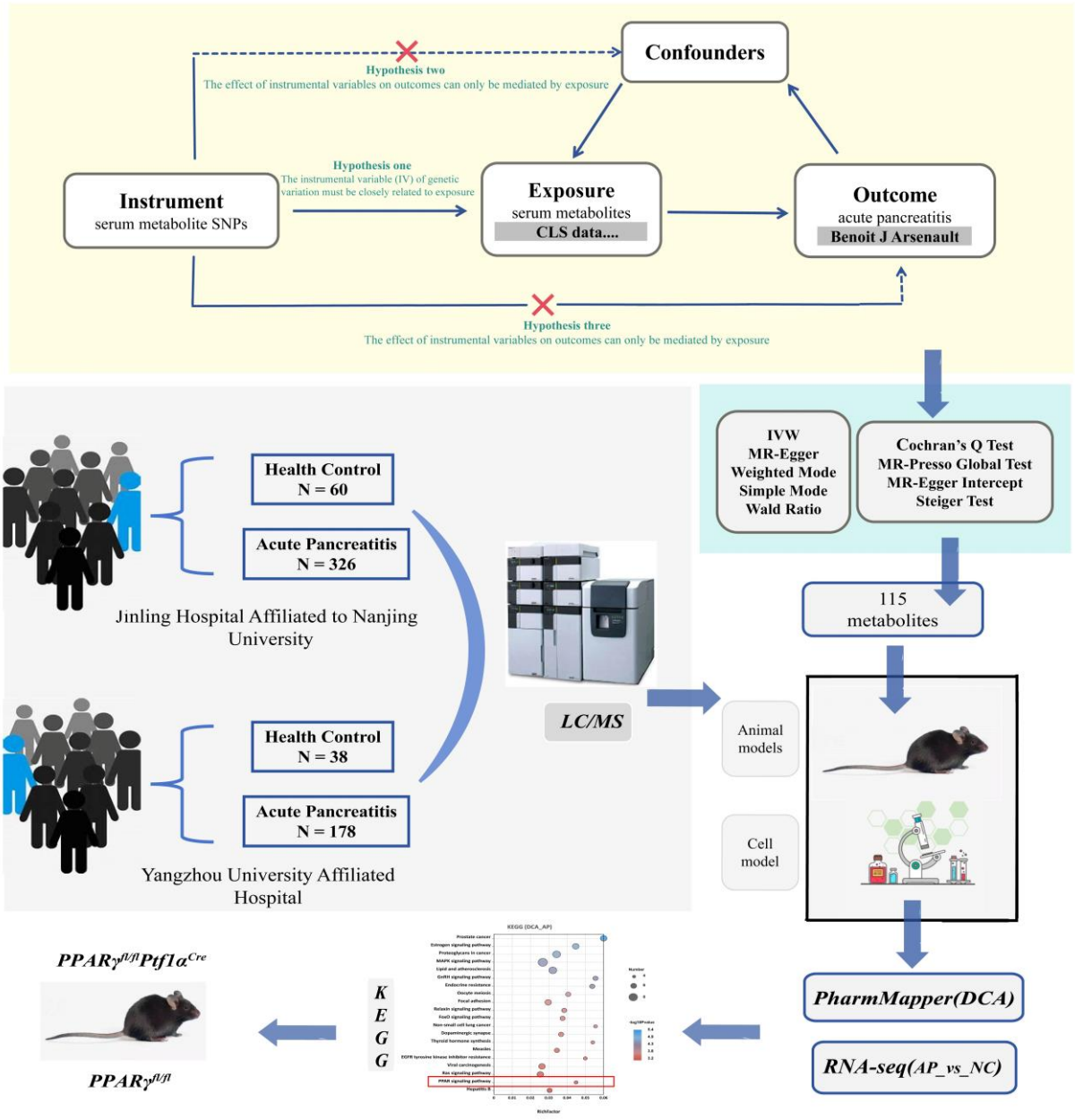

Fig.1. Flowchart of the study design

2.2 MR analysis

2.2.1 Data source

For this study, blood metabolite data were sourced from multiple large-scale studies. Specifically, genome-wide genotyping and circulating blood metabolite profiling were conducted using data from the Canadian Longitudinal Study on Aging**[22]**, which included 8,299 European participants. After stringent quality control, approximately 15.4 million single-nucleotide polymorphisms (SNPs) were identified for GWAS analysis. Additional genetic data were retrieved from the Metabolomics GWAS Server, comprising 7,824 European individuals, including 1,768 participants from the KORA F4 study in Germany and 6,056 individuals from the twin study in the United Kingdom (UK)**[23]**. Furthermore, allele frequency data for AP were derived from a meta-analysis of three cohorts (UK Biobank, Estonia Biobank, and FinnGen), which included a total of 10,630 cases and 844,679 controls of European ancestry**[24]**. All genetic data utilized in

this study adhered to ethical standards and received ethical approval from the relevant local ethics review committees.

2.2.2 Selection of instrumental variables

For each metabolite, instrumental variables were selected using the Plink software package based on data from the 1000 Genome Project. Specifically, (1) genetic variations associated with metabolites across the genome were identified under a significance threshold of $p < 5\times10^{-7}$ to assess the robustness of the findings; (2) linkage disequilibrium was used to evaluate the independence of SNPs by applying a threshold of $r^2 < 0.001$ within a distance of 10,000 kb; (3) related instrumental variables and palindromic SNPs were excluded; and (4) the strength of individual SNPs was quantified using the F-statistic, calculated as $F = R^2x * (N-K-1)/K * (1-R^2)$, with SNPs retained with an F-value greater than 10**[25]**.

2.2.3 Two-sample MR

The study primarily employed the inverse variance weighting (IVW) method as its primary strategy for MR analysis, supplemented by additional methods such as weighted median, MR Egger, weighted modality, simple modality, and Wald ratio to ensure comprehensive evaluation. In MR studies, the IVW method serves as the primary metric for assessing the causal relationship between exposure and outcome variables. It represents a weighted average of the Wald ratios for multiple instrumental variables, where the weights are typically the inverse of the variance of the estimated effect of each instrument on the outcome. This method is generally applied when a study incorporates multiple instrumental variables, assuming that all instruments are valid. When the analysis is restricted to a single SNP, the Wald ratio method is employed for evaluation**[26, 27]**. Consistent directional effects across different analytical approaches underscore the robustness of the findings. Multiple testing correction procedures were applied to adjust the significance levels for all p-values derived from both IVW and Wald ratio methods. A corrected $p < 0.05$ indicated a statistically significant causal association between the exposure and the outcome, whereas an uncorrected $p < 0.05$ indicated a plausible but less definitive potential causal relationship.

Multiple sensitivity analysis methods were performed to evaluate the robustness of the results. Heterogeneity was tested using Cochran's Q-statistic, while horizontal pleiotropy was evaluated using the MR-PRESSO test and MR Egger regression intercept. The Steiger test was applied to assess the directionality of the effects of instrumental variables on outcomes, mitigate potential reverse causality issues, and validate whether the observed results aligned with the initial hypothesis. A p-value greater than 0.05 in the Steiger test for an instrument variable suggests a possible reverse causal relationship.

2.3 Targeted metabolomics analysis of serum bile acids

2.3.1 Study participants

The population data were sourced from previous studies conducted by the research team. The discovery cohort originated from Jinling Hospital, affiliated with Nanjing University, and included 326 patients with AP and 60 healthy controls. Among the patients, 99 were classified as mild AP (MAP), 144 as moderate-to-severe

AP (MSAP), and 83 as severe AP (SAP). The validation cohort originated from Yangzhou University Affiliated Hospital and included 123 patients with AP and 38 healthy controls. Among the patients, 60 were classified as MAP, 45 as MSAP, and 18 as SAP. Basic demographic characteristics are presented in Supplementary Table S4.

2.3.2 Correlation analysis between metabolites and AP

According to the revised Atlanta classification in 2012**[28]**, AP severity was classified as MAP, MSAP, and SAP. MAP does not involve pancreatic fistula (OF) formation or local/systemic complications. MSAP is characterized by the presence of local/systemic complications alongside transient OF formation within 48 h. SAP involves persistent OF (single or multiple) or local/systemic complications lasting beyond 48 h. Local complications of AP include acute peripancreatic effusion, pancreatic pseudocyst, acute necrotic effusion, and wall necrosis.

2.3.3 2.3.3 Statistical analysis

The collected data were analyzed using R Studio and GraphPad Prism 9.0. Heatmaps of variables were generated using the R corrplot package. Normal continuous variables are expressed as means ± standard deviation, non-normal continuous variables are expressed as medians (interquartile range [IQR]), and categorical variables are expressed as frequencies (percentage). Normal continuous variables were analyzed using Student's two-tailed t-test or one-way analysis of variance, whereas non-normal continuous variables were analyzed using the Mann–Whitney U test or Kruskal–Wallis test. Univariate and multivariate logistic regression models were established after adjusting for age, sex, and body mass index (BMI) to evaluate the association between acute necrotic collection (ANC), acute respiratory distress syndrome (ARDS), acute kidney injury, infectious pancreatic necrosis (IPN), shock, acute peripancreatic fluid accumulation, and serum DCA. Statistical significance was defined as $p<0.05$ (two-tailed).

2.4 Experimental model

2.4.1 Experimental animals and ethics

C57BL/6 wild-type mice aged 6–8 weeks, weighing between 20 and 25 g were purchased from GemPharmatech Co., Ltd., (Nanjing, China). Pancreatic acinar cell PPARγ specific knockout ($Ptf1\alpha^{Cre}PPAR\gamma^{fl/fl}$) mice were generated by intercrossing $Ptf1\alpha^{Cre}$ mice with $PPAR\gamma^{flox/flox}$ and Gsdmdflox/flox mice. All mice were of C57BL/6 background. Prior to the experiment, all mice were housed under specific pathogen-free conditions with controlled temperatures (25 ± 2 ° C), a 12-h light/dark cycle, and ad libitum access to food and water. All animal experiments adhered to the "Principles for the Care of Experimental Animals" and were approved by the Experimental Animal Ethics Committee of Yangzhou University Affiliated Hospital (ID: 202407027). All animal experiments were performed in accordance with ARRIVE guidelines.

2.4.2 Cell model

The pancreatic acinar cell (PAC) injury model was established using cholecystokinin (CCK). Fresh PACs were isolated from mice by intravenous injection of collagenase. Cells were exposed to 10 μM CCK for 6 h, with or without DCA (0.1, 0.5, 1, and 5 μM). Cell injury was assessed by measuring lactate dehydrogenase (LDH) levels in the cell culture supernatant.

2.4.3 Staining of live and dead cells

Necrotic cell death was determined using 4 μm propidium iodide for dying/dead cells and 2 μm Calcein-AM for live cells.

2.4.4 Animal model

MAP was induced by the intraperitoneal injection of caerulein (200 μg/kg, 10 times, at 1-h intervals; Nanjing Peptide Co., Ltd., Nanjing, China). Mice were randomly divided into five groups: normal control group, caerulein-only, low-dose caerulein DCA (10 mg/kg), medium-dose caerulein DCA (20 mg/kg), and high-dose caerulein DCA (40 mg/kg). DCA (item number, HY-N0593-100 mg; MCE Co. Ltd., New Jersey, USA) was dissolved in Dimethyl sulfoxide (DMSO) and injected intraperitoneally after the second caerulein injection. The control group received an equal volume of PBS. All groups of mice were euthanized 12 h after the first injection of caerulein.

2.4.5 Blood and tissue collection

Mice were anesthetized with intraperitoneal injection of pentobarbital sodium (50 mg/kg) prior to euthanasia. Serum was collected at different time points for amylase (Amylase Assay Kit [item number 100000060]; Biosino Bio Technology, Beijing, China) and lipase measurements (Lipase assay kit, cat. A054-1-1; Nanjing Jiancheng Co., Ltd., China), following the manufacturer's instructions. The pancreatic tissue was immediately harvested, with some portion of the tissue fixed in 4% paraformaldehyde for morphological study and the remaining stored at -80 °C for further analysis.

2.4.6 Histopathological assessment

Following dehydration, waxing, and embedding, the pancreas and lung tissues were stained with hematoxylin and eosin. Pancreatic damage was assessed by evaluating the severity of edema, inflammatory cell infiltration, and acinar cell necrosis, while lung tissue damage was assessed by evaluating the severity of neutrophil infiltration, alveolar thickness, and alveolar congestion. All histopathological scoring evaluations were conducted by two blinded pathologists.

2.4.7 Western blot analysis

Protein extraction was performed using RIPA lysis buffer (Beyotime, Beijing, China), supplemented with protease and phosphatase inhibitors (diluted 1:50, Beyotime). The protein concentrations were determined using a Bicinchoninic Acid assay kit (Beyotime). Equal amounts of protein samples were separated by gel electrophoresis, transferred to polyvinylidene fluoride membranes, and blocked with 5% skim milk for 2 h at room temperature. The membranes were then incubated overnight at 4 °C with the following primary antibodies: anti-GAPDH (1:5,000 dilution), anti-PPARγ (1:1,000 dilution), and

anti-P-PPARγ (1:1,000 dilution). This was followed by incubation with appropriately diluted secondary antibodies for 2 h at room temperature. After washing with Tris-buffered saline with Tween-20, protein bands were visualized using an ECL Plus chemiluminescence system, and grayscale values were quantified and comparatively analyzed using ImageJ software.

2.4.8 Immunofluorescence examination

Paraffin-embedded pancreatic tissue slices (thickness: 5 μm) were rehydrated in xylene, followed by rehydration in decreasing concentrations of ethanol. Next, antigen repair was performed at high temperatures for 20 min in a citrate buffer (10 mm, pH 6.0) and washed with PBS (3× for 5 min each). Sections were blocked with 5% goat serum for 1 h and incubated overnight at 4 °C with the following primary antibodies: anti-amylase (1:250) and anti-p-PPARγ (1:100). The next day, the sections were incubated with a fluorescein-labeled secondary antibody (diluted 1:200) at room temperature in a dark environment for 1 h. Cell nuclei were subsequently stained with DAPI for 10 min, and the sections were sealed with a cover glass. Images were captured using a confocal laser scanning microscope (400×).

3. Results

3.1 MR reveals a causal relationship between DCA and AP

3.1.1 Two-sample MR

Using GWAS and MR analysis, we identified 115 metabolites with potential causal relationships with AP after excluding unknown metabolites and metabolite ratios. Following multiple test correction, three metabolites demonstrated significant causal relationships with AP, namely campesterol, deoxycholate, and N-acetylglycine (Fig. 2). Deoxycholate, the salt form of the secondary bile acid DCA, was chosen for subsequent AP-related investigations. All SNPs had F-values >10, indicating the robustness of the results. Furthermore, consistent directional effect estimates across multiple analytical methods enhanced the reliability of the findings. Detailed MR analysis results for different methodologies and additional data are presented in Supplementary Tables S1–S3.

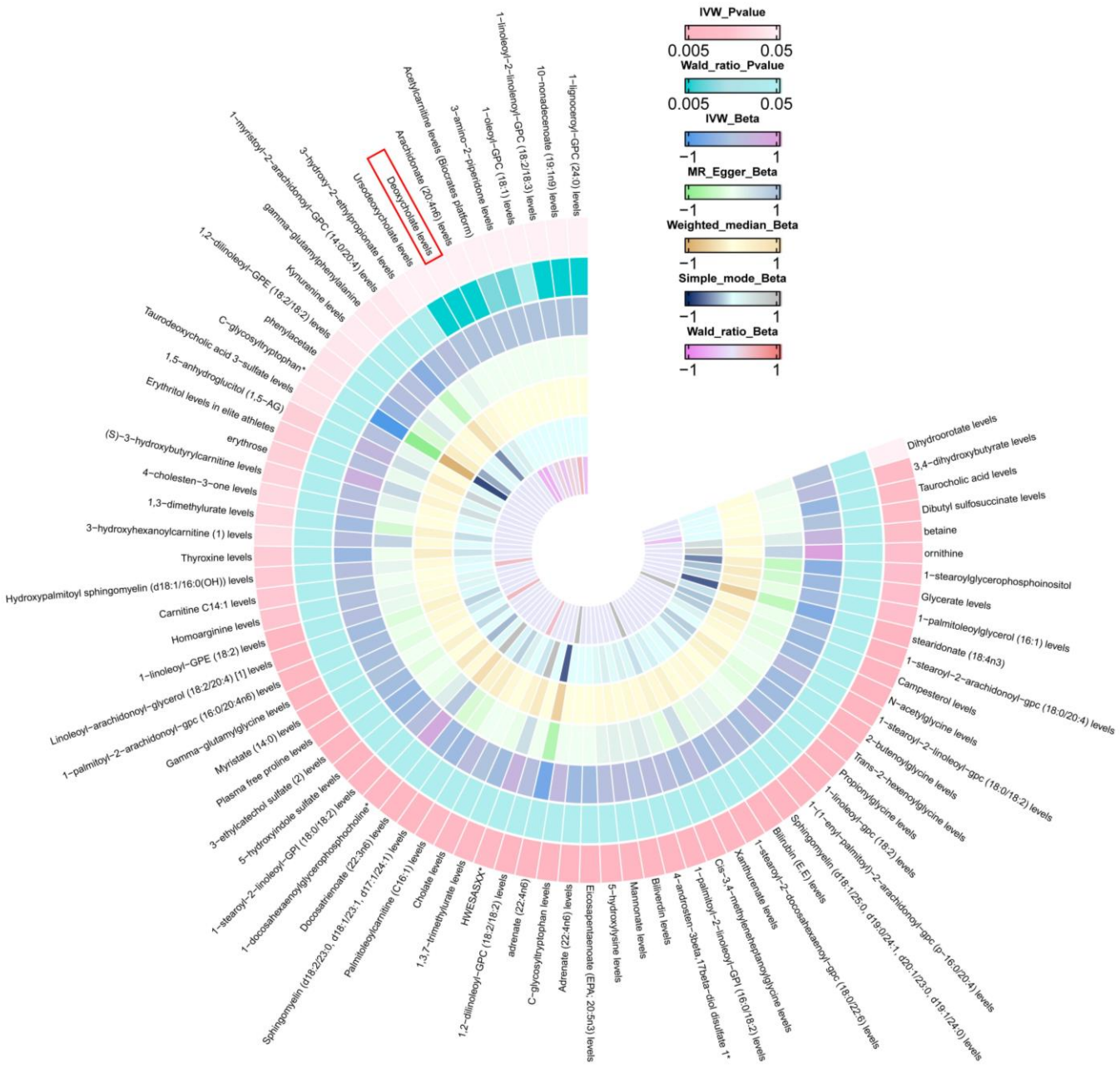

Fig. 2. Overall circular heatmap of Mendelian randomization (MR) results

3.1.2 Sensitivity analysis of MR

After determining significant estimates, sensitivity analysis was performed to identify potential biases in the MR hypothesis. Heterogeneity was detected using Cochran's Q-test ($p < 0.05$), while pleiotropy was evaluated using the Egger intercept. Outliers were identified using the MR-PRESSO test. Leave-one-out analysis was conducted to evaluate the influence of individual SNPs on pooled IVW estimates. Finally, Steiger directional testing performed to confirm the directionality of the association between exposure and outcomes. The forest plot and specific sensitivity analysis of the causal relationship between identified metabolites and AP risk are shown in Fig. 3 and Supplementary Table S1–S3.

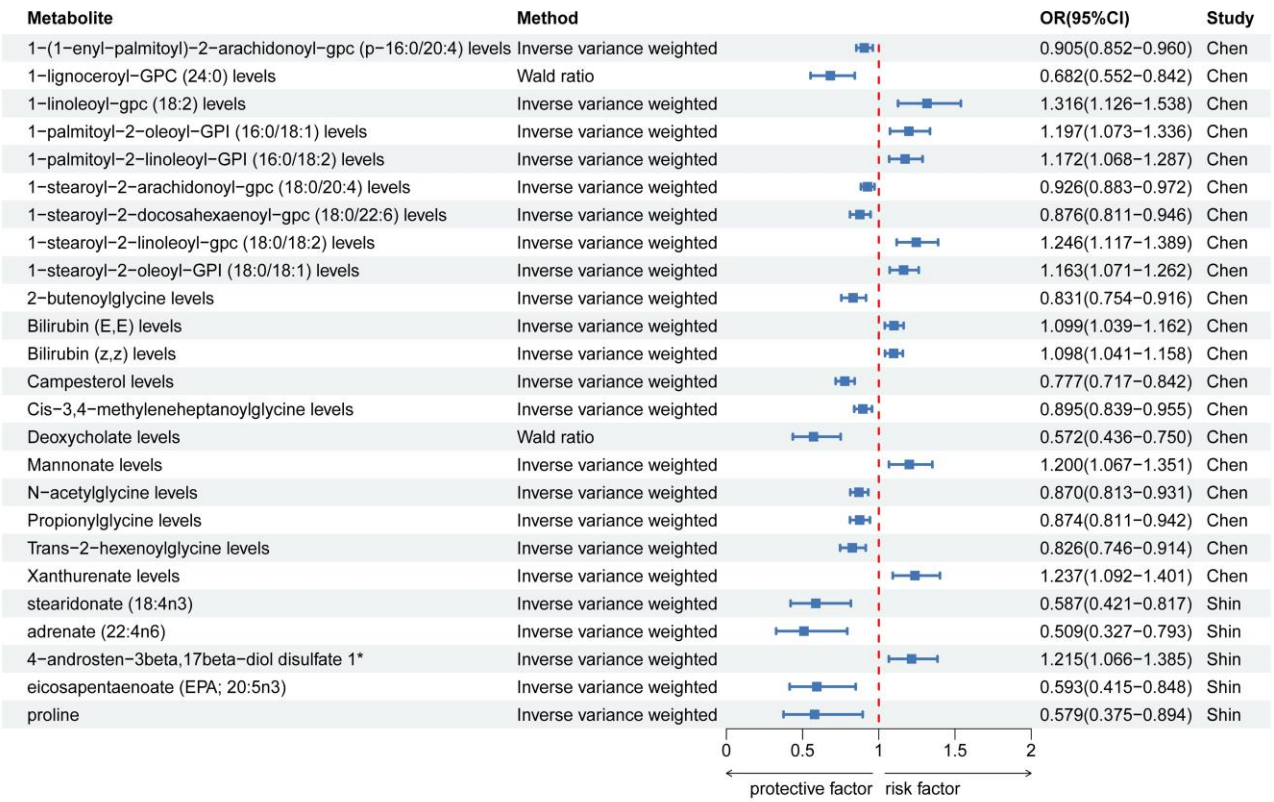

Fig. 3. forest plot of Mendelian randomization (MR) results

3.2 Bile acid metabolomics reveal a significant decrease in serum DCA levels during the acute phase of AP

Targeted bile acid metabolomics analysis was performed on serum samples obtained from the included population. A flowchart of inclusion and exclusion criteria is shown in Fig. 4A. After excluding metabolites with >50% missing values, 17 bile acids met the criteria. Volcano plots were used to plot the fold changes in serum bile acid metabolite levels across different severity levels of AP (Fig. 4B). The heatmap displays the average normalized concentrations of various metabolites (Fig. 4C). Quantitative analysis demonstrated a significant decrease in serum DCA concentrations during the acute phase of AP compared with that in the control group (Fig. 4D). In addition, we divided patients with acute-phase AP into three groups based on the time from admission to onset, and the results showed that DCA levels demonstrated a decreasing trend in the early stages of AP onset (Fig. 4F). Validation in an independent cohort from Yangzhou University Affiliated Hospital (38 healthy controls and 178 patients with acute-phase AP) ensured the reliability of our findings. Consistent with the Nanjing cohort, patients with acute-phase AP exhibited significantly reduced DCA levels (Figs. 4E and 4G).

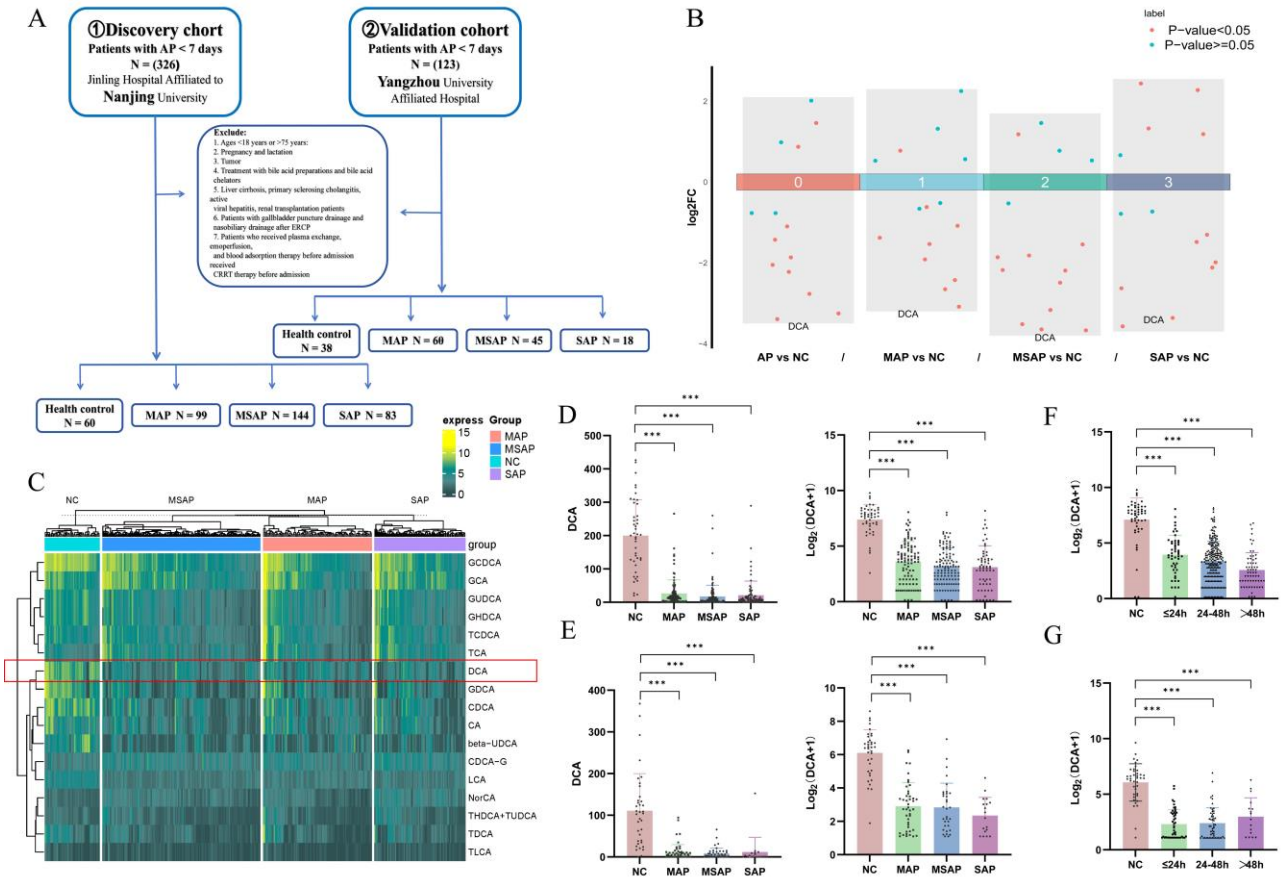

Fig. 4. Bile acid metabolomics demonstrating a significant reduction in serum DCA levels during the acute phase of AP.

(A) Schematic diagram of the cohort selection process. Two cohorts from Nanjing University Affiliated Jinling Hospital and Yangzhou University Affiliated Hospital were included. **(B)** Multiple volcano plots demonstrating the changes in serum DCA levels at different severity levels of AP compared with those in the healthy control group. **(C)** Heatmap displays the concentration of bile acid metabolites analyzed via bile acid targeted metabolomics. **(D)** Bar chart displays serum DCA and normalized concentrations in patients with MAP (n=99), MSAP (n=144), and SAP (n=83) and healthy controls (n=60). **(E)** Serum DCA and normalized concentrations of patients with AP and healthy controls in the validation cohort derived from Yangzhou University Affiliated Hospital. **(F)** Serum DCA and normalized concentrations in patients with acute-phase AP (classified according to different onset and admission times: <24 h, 24-48 h, and >48 h). **(G)** Serum DCA and normalized concentrations in patients with AP and healthy controls (classified according to different onset and admission times) derived from the validation cohort at Yangzhou University Affiliated Hospital. Numbers represent the mean and standard error of the mean (SEM). *** $p < 0.001$.

3.3 Serum DCA levels are associated with IPN and ARDS in patients with AP

We next examine the association between serum DCA levels and AP-related clinical outcomes. The results showed that serum DCA levels were lower in patients with ANC than in those without ANC, and this difference was also evident in patients with ARDS and those with IPN (Fig. 5A). Subsequently, we performed univariate logistic regression (Fig. 5B), which showed that decreased serum DCA levels were a risk factor for IPN and ARDS in patients with AP. After adjusting for age, sex, and BMI, multivariate logistic regression

confirmed these associations (Fig. 5C). In addition, we analyzed the correlation between serum DCA levels and AP-related clinical indicators such as C-reactive protein (CRP), procalcitonin (PCT), and interleukin-6 (IL-6) (Fig. 5D), and the results showed that serum DCA levels were correlated with CRP and PCT.

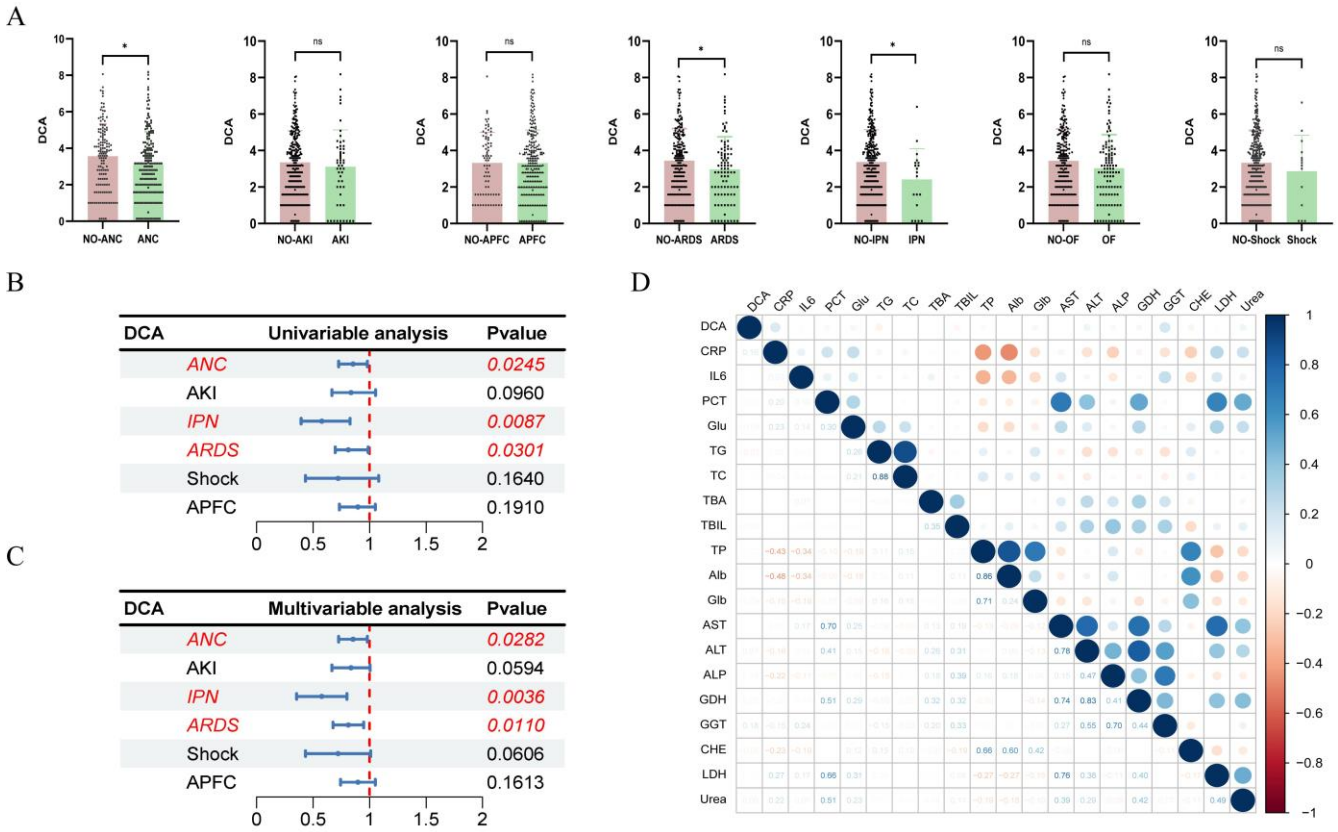

Fig. 5. Serum DCA levels are associated with infectious pancreatic necrosis (IPN) and acute respiratory distress syndrome (ARDS) in patients with AP.

(A) Association analysis between serum DCA levels and pancreatic necrosis (ANC), ARDS, acute kidney injury (AKI), IPN, shock, acute peripancreatic fluid accumulation (APFC), and organ failure (OF). **(B)** Forest plot of ANC, AKI, IPN, ARDS, shock, and APFC with serum DCA in patients with AP, derived using univariate logistic regression. **(C)** Multivariate logistic regression analysis, adjusted for age, gender, body mass index, and etiology. **(D)** Heatmap displays the correlation between DCA levels and other relevant clinical indicators of AP. C-reactive protein (CRP), interleukin-6 (IL-6), procalcitonin (PCT), blood glucose (Glu), triglycerides (TGs), total cholesterol (TC), total bile acids (TGA), total bilirubin (TBIL), total protein (TP), albumin (Alb), globulin (Glb), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), glutamate dehydrogenase (GDH), glutamyl transpeptidase (GGT), cholinesterase (CHE), lactate dehydrogenase (LDH), urea. Numbers represent the mean and standard error (SE),* $p < 0.05$. ns, No statistical significance.

3.4 DCA protects against acinar cell necrosis in AP in vivo and in vitro

Next, we evaluated the pharmacological effects of DCA in an acinar cell model in vitro. The results showed that administration of 0.5 μM DCA significantly reduced the release of LDH (Fig. 6A). Furthermore, AM/PI (Calcein-AM and propidium iodide) staining revealed that administration of 0.5 μM DCA reduced the

number of necrotic cells (Figs. 6B,C). Subsequently, the pharmacological effects of DCA in a caerulein-induced animal model were evaluated in vivo. Intraperitoneal administration of DCA at different doses revealed that 20 mg/kg conferred the most significant protective effect, as reflected by amylase and lipase activity. Histological analyses via hematoxylin and eosin staining and amylase immunofluorescence of the pancreatic tissue further confirmed that 20 mg/kg DCA mitigated pancreatic injury (Figs. 6D-G).

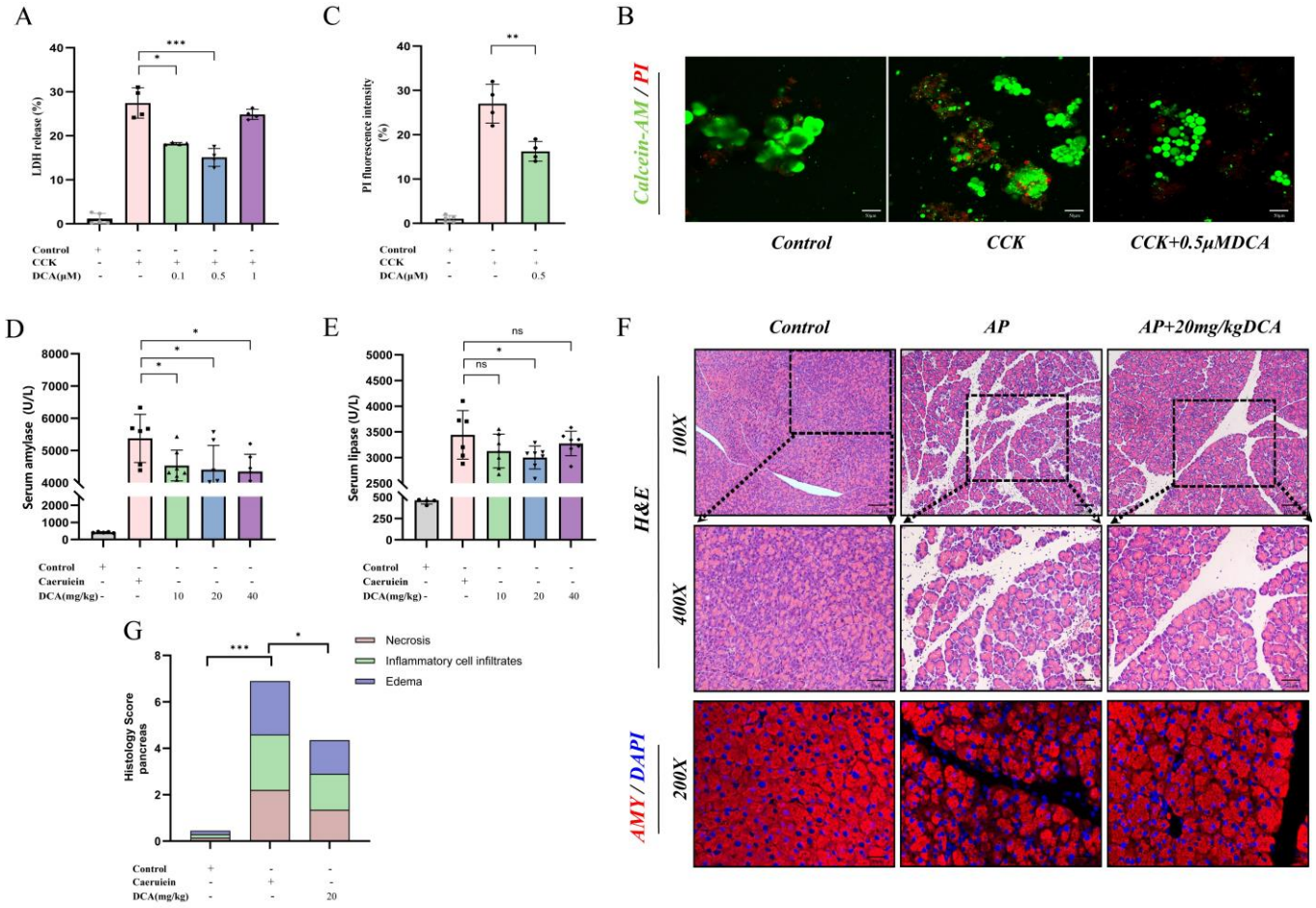

Fig. 6. DCA protects against acinar cell necrosis in AP both in vivo and vitro.

Detection of cell damage by LDH levels after treatment with different concentration gradients of DCA (0.1, 0.5, 1, and 5 μM). **(B, C)** Immunofluorescence imaging of calcein-AM (green) /PI (red)–stained acinar cells (scale bar: 50 μm) to quantify phosphatidylinositol in damaged acinar cells (n=4). **(D, E)** Changes in serum amylase and lipase activity. **(F)** Hematoxylin and eosin (H&E) staining images of pancreatic tissues obtained from AP mice (scale bar: 100 and 50 μm), and immunofluorescence imaging of amylase activity (scale bar: 50 μm). Amylase(red); DAPI(blue). **(G)** Histopathology score (edema, inflammation, and necrosis; n=7). Numbers indicate the mean and standard error (SE). ***$p < 0.001$, and *$p < 0.05$. ns, not statistically significant.

3.5 DCA exerts its protective effects via the PPAR signaling pathway

To investigate the mechanism underlying the protective effects of DCA, RNA sequencing was performed on pancreatic tissues obtained from caerulein-induced AP mice. Additionally, potential targets of DCA were predicted using PharmMapper. By integrating drug targets with differentially expressed genes in pancreatic

tissues between the AP and control groups, 52 overlapping targets were identified (Fig. 7A). Based on these overlapping targets between DCA and AP, Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis was conducted, which revealed significant alterations in the PPAR signaling pathway (Fig. 7B). Subsequently, Western blot analysis was employed to validate changes in the PPAR signaling pathway following AP induction. Treatment with DCA significantly increased the phosphorylation levels of PPARγ (Figs. 7C-D). Consistent with the Western blot analysis results, immunofluorescence staining also demonstrated a marked increase in PPARγ phosphorylation after DCA treatment (Fig. 7E). Western blot analysis confirmed that the expression of PPARγ in acinar cell was significantly decreased in Ptf1α^{Cre}PPARγ$^{fl/fl}$ mice.(Fig. S1). Cell death rates were measured via LDH assay in wild-type and Ptf1α^{Cre}PPARγ$^{fl/fl}$ mice following the establishment of the CCK model and subsequent DCA administration. The results indicated that DCA exerts its effects through the PPAR signaling pathway.

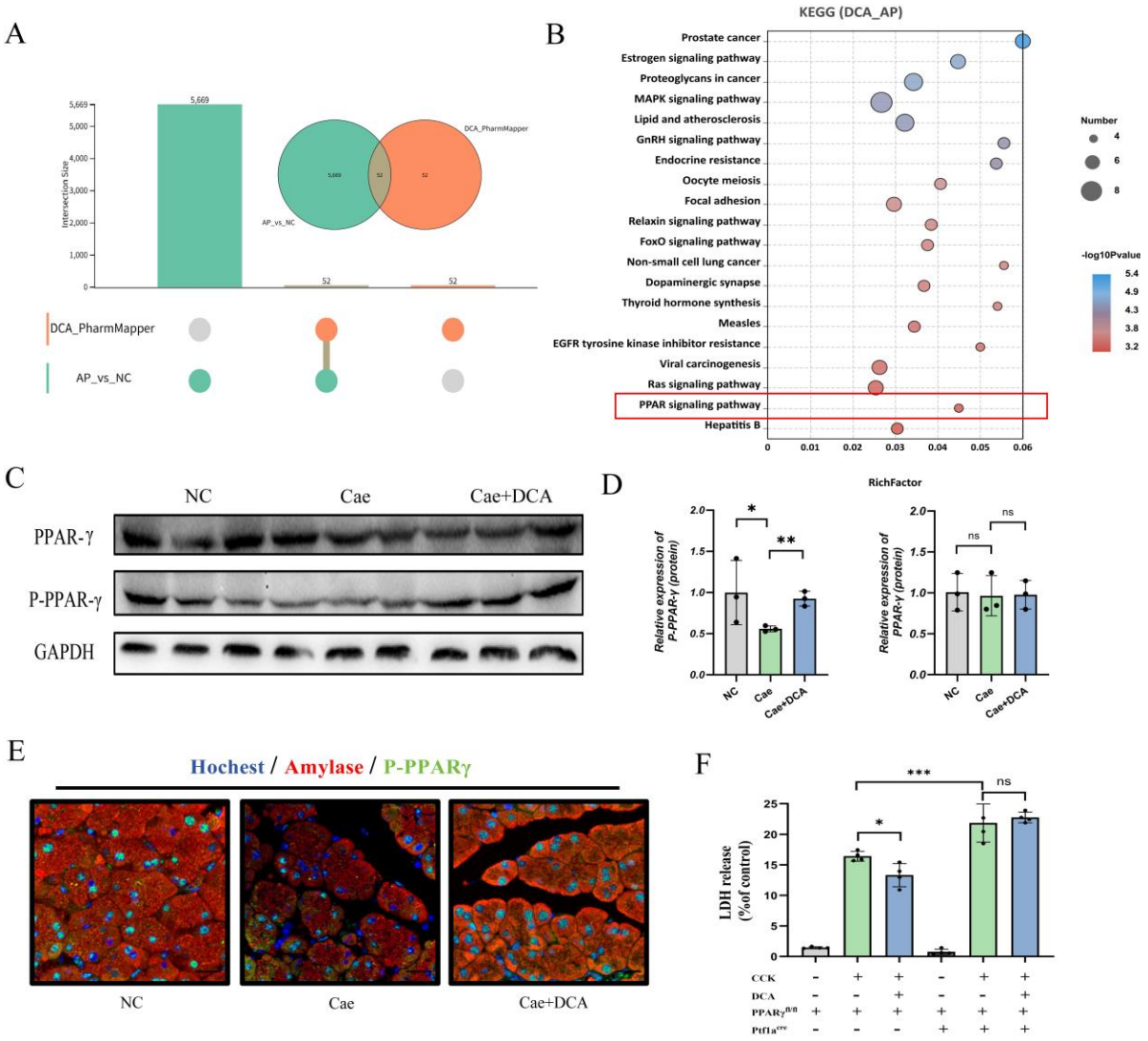

Fig. 7. DCA exerts its protective effects via modulation of the PPAR signaling pathway.

Venn diagram of drug targets and differentially expressed genes (DEGs) of AP. **(B)** Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis. **(C, D)** Relative protein expression of the PPAR signaling pathway in pancreatic tissues of normal, caerulein-induced AP, and DCA-treated AP

mice. GAPDH was used as a control for protein loading (n=3 for each group). **(E)** Immunofluorescence imaging of amylase activity and P-PPARγ. Amylase(red), P-PPARγ(green), Hochest(blue). **(F)** Detection of cell damage by LDH levels after treatment between wild-type and Ptf1α^{Cre}PPARγ$^{fl/fl}$ mice. ***$p < 0.001$, and *$p < 0.05$. ns, not statistically significant.

4. Discussion

In this study, we initially screened for potential pathogenic metabolites associated with AP using public databases. Subsequent targeted bile acid metabolomics analysis based on two cohorts revealed a significant reduction in DCA levels during the acute phase of AP, which correlated with pancreatic necrosis and ARDS. Supplementation with DCA alleviated pancreatic injury in both in vivo and in vitro models. integration of KEGG analysis of DCA targets and differentially expressed genes before and after AP induction, combined with Western blot analysis and immunofluorescence staining, suggested that DCA may exert its protective effects by modulating the PPAR signaling pathway to regulate inflammatory responses. Collectively, these findings indicate that modulation of DCA levels may represent a promising therapeutic strategy for mitigating pancreatic injury in patients with AP.

Bile acids are synthesized from cholesterol in the liver and undergo continuous enterohepatic circulation involving secretion, transport, and reabsorption. They are secreted into the bile ducts, with some of the acids partially stored in the gallbladder and the remainder entering the small intestine. In the gut, microbial enzymes further metabolize bile acids**[29]**. At the terminal ileum, most of the unconjugated bile acids (e.g., cholic acid [CA], CDCA, DCA, and ursodeoxycholic acid [UDCA]) are reabsorbed via active or passive transport into the portal vein and subsequently taken up by the liver. Studies have shown that gut microbiota significantly influences pancreatic health**[30]**. A bidirectional interaction system termed the “gut–pancreas axis” has been proposed, wherein beneficial gut bacteria help treat pancreatitis by maintaining intestinal integrity and regulating gut barrier permeability**[31]**. As a secondary bile acid, DCA has demonstrated notable protective effects in necrotizing enterocolitis and liver injury models. Related bile acids such as CDCA and UDCA have also shown protective effects in AP models**[12]**. In lipopolysaccharide and nigericin-stimulated bone marrow–derived macrophages, DCA significantly reduced the production of inflammatory cytokines, including IL-1β, IL-6, and tumor necrosis factor alpha**[13]**. Therefore, we proposed that DCA may act as a key mediator connecting the liver, gut, and pancreas.

This study employed targeted bile acid metabolomics to quantify bile acid metabolites in patients with AP. After quality control, 17 bile acids were included for analysis, consistent with previous cohort studies. Bile acid synthesis is tightly regulated by a negative feedback mechanism mediated by the nuclear receptor FXR, which is most highly expressed in the liver**[32]**. In the hepatic tissue, FXR induces the expression of small heterodimer partner (SHP), which interacts with liver receptor homolog-1 to suppress CYP7A1 gene expression**[33]**. Fredrik et al. indicated that the most potent ligand for FXR is CDCA, followed by CA, DCA, and lithocholic acid**[34]**. Our previous research demonstrated altered mRNA expression of CYP7A1-a key enzyme in bile acid synthesis—in AP mouse models. Specific modulation of FXR expression reduced acinar

cell necrosis and attenuated AP severity **[12]**. Collectively, these findings suggest that decreased serum DCA levels are associated with dysregulated hepatic bile acid synthesis.

Studies have proposed that different types of bile acids exert divergent effects in various in vitro and in vivo models of AP, likely due to their distinct physicochemical properties**[35]**. For instance, in acinar cell models, hydrophobic bile acids exhibit greater efficacy than hydrophilic acids in mitigating CCK-induced cell injury. However, in mouse models of AP induced by caerulein or L-arginine, the same bile acids may demonstrate contrasting effects. In this study, DCA was characterized as a hydrophobic bile acid. The modulation of AP severity by bile acids appears to depend on their inherent hydrophobic or hydrophilic properties, as well as the specific pathogenesis of pancreatitis. In addition, there may still be other minor factors-such as individual variations in gut microbiota—that could impede the effective exertion of DCA's actions. However, in contrast, the present study has prioritized the control of factors with relatively greater impacts, including physiological factors (e.g., baseline body weight, mouse strain, etc.) and environmental factors (e.g., light cycle, drinking water, feed, drug administration procedures, etc.). This ensures that the research was conducted under conditions where the overall experimental context remained relatively consistent.

PPARs (peroxisome proliferators-activated receptors), belonging to the ligand-activated receptor subfamily within the nuclear receptor superfamily, are nuclear hormone receptors activated by fatty acids and their derivatives. The PPAR signaling pathway is a critical transcriptional regulatory mechanism involved in lipid metabolism, inflammatory responses, and cardiovascular diseases**[36]**. Previous studies have revealed that FXR participates in fatty acid oxidation via PPARα and its target genes. PPARα also regulates numerous genes related to lipid homeostasis and bile acid biosynthesis by suppressing CYP7A1 transcription**[37]**. Furthermore, FXR induces SHP expression, which acts as a negative regulator by interacting with other nuclear receptors and transcription factors. Two independent studies have identified interactions between both PPARα and PPARγ with SHP, noting that SHP enhances the transcriptional activity mediated by PPARα and PPARγ**[38, 39]**.

This study has several limitations. First, the stringent screening criteria excluded many metabolites for which sufficient SNPs could not be extracted. Even after relaxing the criteria, some metabolites still yielded an insufficient number of SNPs, potentially introducing bias. Second, unmeasured confounding factors—particularly pleiotropic effects and epigenetic regulation—may still influence MR results. And Conclusions derived from Mendelian randomization (MR) studies are mostly exploratory; therefore, subsequent MR studies with larger sample sizes are required to provide supplementary validation. The animal model employed in this study exhibits inherent limitations, including physiological disparities, genetic background differences, and controlled environmental conditions, which may not fully recapitulate human disease pathophysiology and, thus, could affect the translational efficacy of the therapeutic strategy. These limitations reflect common challenges in translational medicine, and further validation in specific human populations is warranted in future studies.

5. Conclusions

In summary, this study showed that DCA is closely associated with the development of AP in a public database and a dual-center clinical cohort and that supplementation with DCA can improve acinar cell necrosis in AP. Targeted regulation of DCA can alleviate pancreatic injury and prevent the aggravation of AP, which may represent a novel strategy for treating pancreatic injury in patients with AP.

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References

1. Mederos MA, Reber HA, Girgis MD: Acute Pancreatitis: A Review. *Jama* 2021, 325(4):382-390.
2. Iannuzzi JP, King JA, Leong JH, Quan J, Windsor JW, Tanyingoh D, Coward S, Forbes N, Heitman SJ, Shaheen AA *et al*: Global Incidence of Acute Pancreatitis Is Increasing Over Time: A Systematic Review and Meta-Analysis. *Gastroenterology* 2022, 162(1):122-134.
3. Fan Z, Zhang Y, Li J, He W, Bai X, Cai Y, Li N, Xie F, Wen L, Akshintala VS *et al*: Global burden and characterization of hypertriglyceridemia-induced acute pancreatitis: results from a systematic review and a multi-center cohort study. *Science China Life Sciences* 2025.
4. Tayier D, Wang K, Yuan Y, Yang Z: Research Status on the Effects of Obesity on Acute Pancreatitis. *Obesity Reviews* 2025, n/a(n/a):e70007.
5. Fan Y, Pedersen O: Gut microbiota in human metabolic health and disease. *Nature reviews Microbiology* 2021, 19(1):55-71.
6. Larsson SC, Butterworth AS, Burgess S: Mendelian randomization for cardiovascular diseases: principles and applications. *European Heart Journal* 2023, 44(47):4913-4924.
7. Hemani G, Tilling K, Davey Smith G: Orienting the causal relationship between imprecisely measured traits using GWAS summary data. *PLoS genetics* 2017, 13(11):e1007081.
8. Yan C, Bao J, Jin J: Exploring the interplay of gut microbiota, inflammation, and LDL-cholesterol: a multiomics Mendelian randomization analysis of their causal relationship in acute pancreatitis and non-alcoholic fatty liver disease. *Journal of translational medicine* 2024, 22(1):179.
9. Wang B, Dron JS, Wang Y, Choi SH, Huffman JE, Cho K, Wilson PWF, Natarajan P, Peloso GM: Lipid levels and risk of acute pancreatitis using bidirectional Mendelian randomization. *Scientific reports* 2024, 14(1):6267.
10. Mohanty I, Allaband C, Mannochio-Russo H, El Abiead Y, Hagey LR, Knight R, Dorrestein PC: The changing metabolic landscape of bile acids - keys to metabolism and immune regulation. *Nature reviews Gastroenterology & hepatology* 2024, 21(7):493-516.
11. Jia W, Li Y, Cheung KCP, Zheng X: Bile acid signaling in the regulation of whole body metabolic and immunological homeostasis. *Science China Life sciences* 2024, 67(5):865-878.
12. Zhu Q, Yuan C, Dong X, Wang Y, Li B, Tu B, Chen W, Xu X, Gong W, Xiao W *et al*: Bile acid metabolomics identifies chenodeoxycholic acid as a therapeutic agent for pancreatic necrosis. *Cell reports Medicine* 2023, 4(12):101304.
13. Zhang Z-y, Guo X-l, Liu J-t-y, Gu Y-j, Ji X-w, Zhu S, Xie J-y, Guo F: Conjugated bile acids alleviate acute pancreatitis through inhibition of TGR5 and NLRP3 mediated inflammation. *Journal of translational medicine* 2024, 22(1):1124.
14. Choucair I, Mallela DP, Hilser JR, Hartiala JA, Nemet I, Gogonea V, Li L, Lusis AJ, Fischbach MA, Tang WHW *et al*: Comprehensive Clinical and Genetic Analyses of Circulating Bile Acids and Their Associations With Diabetes and Its Indices. *Diabetes* 2024, 73(8):1215-1228.
15. Hofmann AF: Enterohepatic Circulation of Bile Acids. In: *Comprehensive Physiology.* edn.; 2011: 567-596.

16. Chiang JY: Bile acid metabolism and signaling. (2040-4603 (Electronic)).
17. Zhang L, Zheng Z, Huang H, Fu Y, Chen T, Liu C, Yi Q, Lin C, Zeng Y, Ou Q *et al*: Multi-omics reveals deoxycholic acid modulates bile acid metabolism via the gut microbiota to antagonize carbon tetrachloride-induced chronic liver injury. *Gut microbes* 2024, 16(1):2323236.
18. Kim DM, Liu J, Whitmore MA, Tobin I, Zhao Z, Zhang G: Two intestinal microbiota-derived metabolites, deoxycholic acid and butyrate, synergize to enhance host defense peptide synthesis and alleviate necrotic enteritis. *Journal of animal science and biotechnology* 2024, 15(1):29.
19. Wang H, Wang J, Cui H, Fan C, Xue Y, Liu H, Li H, Li J, Li H, Sun Y *et al*: Inhibition of fatty acid uptake by TGR5 prevents diabetic cardiomyopathy. *Nature metabolism* 2024, 6(6):1161-1177.
20. Ma Z, Wang X, Yin P, Wu R, Zhou L, Xu G, Niu J: Serum metabolome and targeted bile acid profiling reveals potential novel biomarkers for drug-induced liver injury. *Medicine* 2019, 98(31):e16717.
21. Maleszka A, Dumnicka P, Matuszyk A, Pędziwiatr M, Mazur-Laskowska M, Sporek M, Ceranowicz P, Olszanecki R, Kuźniewski M, Kuśnierz-Cabala B: The Diagnostic Usefulness of Serum Total Bile Acid Concentrations in the Early Phase of Acute Pancreatitis of Varied Etiologies. *International journal of molecular sciences* 2017, 18(1).
22. Chen Y, Lu T, Pettersson-Kymmer U, Stewart ID, Butler-Laporte G, Nakanishi T, Cerani A, Liang KYH, Yoshiji S, Willett JDS *et al*: Genomic atlas of the plasma metabolome prioritizes metabolites implicated in human diseases. *Nature genetics* 2023, 55(1):44-53.
23. Shin SY, Fauman EB, Petersen AK, Krumsiek J, Santos R, Huang J, Arnold M, Erte I, Forgetta V, Yang TP *et al*: An atlas of genetic influences on human blood metabolites. *Nature genetics* 2014, 46(6):543-550.
24. Bourgault J, Abner E, Manikpurage HD, Pujol-Gualdo N, Laisk T, Gobeil É, Gagnon E, Girard A, Mitchell PL, Thériault S *et al*: Proteome-Wide Mendelian Randomization Identifies Causal Links Between Blood Proteins and Acute Pancreatitis. *Gastroenterology* 2023, 164(6):953-965.e953.
25. Si S, Liu H, Xu L, Zhan S: Identification of novel therapeutic targets for chronic kidney disease and kidney function by integrating multi-omics proteome with transcriptome. *Genome medicine* 2024, 16(1):84.
26. Pagoni P, Korologou-Linden RS, Howe LD, Davey Smith G, Ben-Shlomo Y, Stergiakouli E, Anderson EL: Causal effects of circulating cytokine concentrations on risk of Alzheimer's disease and cognitive function. *Brain, behavior, and immunity* 2022, 104:54-64.
27. Sun J, Zhao J, Jiang F, Wang L, Xiao Q, Han F, Chen J, Yuan S, Wei J, Larsson SC *et al*: Identification of novel protein biomarkers and drug targets for colorectal cancer by integrating human plasma proteome with genome. *Genome medicine* 2023, 15(1):75.
28. Banks PA, Bollen TL, Dervenis C, Gooszen HG, Johnson CD, Sarr MG, Tsiotos GG, Vege SS: Classification of acute pancreatitis--2012: revision of the Atlanta classification and definitions by international consensus. *Gut* 2013, 62(1):102-111.
29. Guan B, Tong J, Hao H, Yang Z, Chen K, Xu H, Wang A: Bile acid coordinates microbiota homeostasis and systemic immunometabolism in cardiometabolic diseases. *Acta pharmaceutica Sinica B* 2022, 12(5):2129-2149.
30. Jia W, Xie G, Jia W: Bile acid-microbiota crosstalk in gastrointestinal inflammation and carcinogenesis. *Nature reviews Gastroenterology & hepatology* 2018, 15(2):111-128.
31. Zhang C, Li G, Lu T, Liu L, Sui Y, Bai R, Li L, Sun B: The Interaction of Microbiome and Pancreas in Acute Pancreatitis. *Biomolecules* 2023, 14(1).
32. Clifford BL, Sedgeman LR, Williams KJ, Morand P, Cheng A, Jarrett KE, Chan AP, Brearley-Sholto MC, Wahlström A, Ashby JW *et al*: FXR activation protects against NAFLD via bile-acid-dependent reductions in lipid absorption. *Cell metabolism* 2021, 33(8):1671-1684.e1674.
33. Halilbasic E, Claudel T, Trauner M: Bile acid transporters and regulatory nuclear receptors in the liver and beyond. *Journal of hepatology* 2013, 58(1):155-168.
34. Wahlström A, Sayin SI, Marschall HU, Bäckhed F: Intestinal Crosstalk between Bile Acids and Microbiota and Its Impact on Host Metabolism. *Cell metabolism* 2016, 24(1):41-50.

35. Tran QT, Sendler M, Wiese ML, Doller J, Zierke L, Gischke M, Glaubitz J, Tran VH, Lalk M, Bornscheuer UT *et al*: Systemic Bile Acids Affect the Severity of Acute Pancreatitis in Mice Depending on Their Hydrophobicity and the Disease Pathogenesis. *International journal of molecular sciences* 2022, 23(21).
36. Li T, Chiang JY: Regulation of bile acid and cholesterol metabolism by PPARs. (1687-4757 (Print)).
37. Montaigne DA-OX, Butruille LA-O, Staels BA-O: PPAR control of metabolism and cardiovascular functions. (1759-5010 (Electronic)).
38. Nishizawa H, Yamagata K Fau - Shimomura I, Shimomura I Fau - Takahashi M, Takahashi M Fau - Kuriyama H, Kuriyama H Fau - Kishida K, Kishida K Fau - Hotta K, Hotta K Fau - Nagaretani H, Nagaretani H Fau - Maeda N, Maeda N Fau - Matsuda M, Matsuda M Fau - Kihara S *et al*: Small heterodimer partner, an orphan nuclear receptor, augments peroxisome proliferator-activated receptor gamma transactivation. (0021-9258 (Print)).
39. Kassam A, Capone Jp Fau - Rachubinski RA, Rachubinski RA: The short heterodimer partner receptor differentially modulates peroxisome proliferator-activated receptor alpha-mediated transcription from the peroxisome proliferator-response elements of the genes encoding the peroxisomal beta-oxidation enzymes acyl-CoA oxidase and hydratase-dehydrogenase. (0303-7207 (Print)).