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ORIGINAL RESEARCH

Causal Association Between Circulating Inflammatory Proteins and Autoimmune Liver Disease: a Bidirectional Two-Sample Mendelian Randomization Study

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Introduction: To investigate whether there is a direct causal relationship between circulating inflammatory proteins and autoimmune liver disease (AILD).

Materials and Methods: We collected genetic data for various AILD from the Genome Wide Association Studies (GWAS) dataset. The latest research provides GWAS data for 91 proteins associated with inflammation. Perform bidirectional two sample Mendelian randomization (MR) analysis using inverse variance weighted (IVW) to determine the causal relationship between inflammatory proteins and AILD, and use Mendelian randomization Egger method (MR Egger), weighted median (WM), and weighted mode as supplementary evaluations. In addition, we conducted sensitivity analysis.

Results: Positive MR analysis showed that CDCP1 (OR=1.363, p=0.0465) and IL-18 (OR=1.416, p=0.0477) were associated with higher including autoimmune hepatitis (AIH) risk. Higher CXCL11 (OR=1.574, p=9.23×10-5) were associated with an increased risk of primary biliary cholangitis (PBC). Lower levels of three inflammatory proteins were associated with increased risk of PBC. TNFSF12 (OR=1.827, p=0.0001, p_adj_fdr=0.0063), CD6 isoform (OR=1.126, p=0.0389), CCL20 (OR=1.880, p=0.0395) are associated with increased risk of primary sclerosing cholangitis (PSC). Reverse MR imaging showed that PBC may promote the expression levels of CCL4 (OR=1.023, p=0.0201) and OSM (OR=1.022, p=0.0236). PSC may promote the expression of five inflammatory proteins. Sensitivity analysis further excluded the effects of heterogeneity and horizontal pleiotropy.

Conclusion: This study indicates a potential association between circulating inflammatory proteins and AILD, which may become a new diagnostic indicator or drug target for clinical application in the prevention and treatment of AILD. However, further investigation is needed.

Keywords: autoimmune liver disease, Mendelian randomization, circulating inflammatory protein, autoimmune hepatitis, primary biliary cholangitis, primary sclerosing cholangitis

Introduction

Autoimmune liver diseases (AILD) are a spectrum of liver pathologies triggered by autoimmune dysregulation, characterized by lymphocytic infiltration of the liver, elevated levels of circulating immunoglobulins, increased transaminases, and the production of specific autoantibodies.[1] Based on distinct histopathological features and the expression of autoantibodies, AILD can be categorized into autoimmune hepatitis (AIH), primary biliary cholangitis (PBC), and primary sclerosing cholangitis (PSC).[2,3] Initially, AILD may be asymptomatic. However, as the disease progresses, it can lead to liver fibrosis, cirrhosis, and even hepatocellular carcinoma.[4,5] Currently, the incidence of AILD is on the rise, with global rates of AIH ranging from 0.4 to 2.39 per 100,000 people, PBC from 0.84 to 2.75, and PSC from 0.1 to 4.39.[1]

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Moreover, there is currently no cure for AILD. Despite aggressive treatment, the response rate is suboptimal,[6,7] and long-term pharmacological therapy often leads to systemic adverse effects such as infections, osteoporosis, and diabetes mellitus.[8] Consequently, AILD has become a severe chronic liver condition, imposing a significant economic burden on society.

The etiology of AILD is multifactorial, involving environmental triggers and genetic anomalies that result in chronic inflammation and T-cell mediated liver damage.[9,10] Recent research has revealed the significant role of inflammatory proteins in the development of AILD.[11,12] Therefore, focusing on these inflammatory elements could be beneficial. Inflammatory proteins in circulation may provide valuable prognostic information to improve the outcomes of AILD, representing a substantial potential strategy for its prevention and treatment. However, comprehensive preclinical and clinical studies are still lacking, and the clinical significance of these inflammatory proteins remains unclear. Observational studies may be confounded by potential biases and reverse causality. The relationship between inflammatory proteins and AILD observed in previous observational studies requires further investigation.

Mendelian randomization (MR) is an innovative method in genetic epidemiology that employs genetic variants (typically single nucleotide polymorphisms) as instrumental variables (IVs) to establish causal relationships between risk factors (exposures) and diseases (outcomes), essentially mimicking randomized controlled trials (RCTs).[13] By leveraging genetic diversity, the MR method aims to elucidate the causality between exposures and outcomes, effectively addressing inherent biases such as confounding, reverse causality, and biased sampling in epidemiological studies, while circumventing the practical limitations of randomized trials.[14] Although MR has broad applications, it has not yet been explored as a tool to investigate the potential causal relationship between AILD and circulating inflammatory proteins. Therefore, we conducted a bidirectional MR analysis to assess the causal relationship between changes in inflammatory protein levels and the likelihood of developing AILD.

Materials and Methods

Study Design

We employed a two-sample MR study to investigate the causal relationship between inflammatory proteins and AILD. Obtaining valid results from MR analysis hinges on fulfilling three key assumptions. Firstly, the genetic instrumental variable (IV) must have a strong association with the exposure, which is the risk factor of interest. Secondly, the genetic variant must be independent of unmeasured confounders that could affect the exposure-outcome association. Lastly, it is presumed that the IV affects the outcome solely through its association with the exposure, thereby minimizing the potential for pleiotropy effects. This study has been proofread based on the STrengthening the Reporting of OBservational studies in Epidemiology - Mendelian Randomization (STROBE-MR) checklist to ensure the transparency and reproducibility of our findings.[15] Figure 1 depicts the flowchart of the MR study process.

Data Source

The GWAS summary data for AIH included 484,413 European controls and 821 European patients (GWAS ID: ebi-a-GCST90018785). The GWAS summary data for PBC included 16,489 European controls and 8,021 European patients (GWAS ID: ebi-a-GCST90061440), and the genetic data for PSC included 12,019 European controls and 2,871 European patients (GWAS ID: ieu-a-1112). The data for the 91 circulating inflammatory proteins were obtained from a recent GWAS conducted by Zhao et al.[16] This study provided summary statistics for the genetic associations with these proteins, which we used as the exposure data in our Mendelian randomization analysis. The GWAS included a large cohort of European ancestry participants, ensuring robust genetic associations. At the same time, it ensures that the samples between the exposure and outcome did not overlap.

IV Selection

In adherence to the three assumptions underlying MR analysis, we initially selected single nucleotide polymorphisms (SNPs) associated with the exposure and ensured that the chosen SNPs were significantly associated with the target exposure at the genome-wide significance threshold ($P < 5\times10^{-8}$) to meet the first assumption. Secondly, we set the

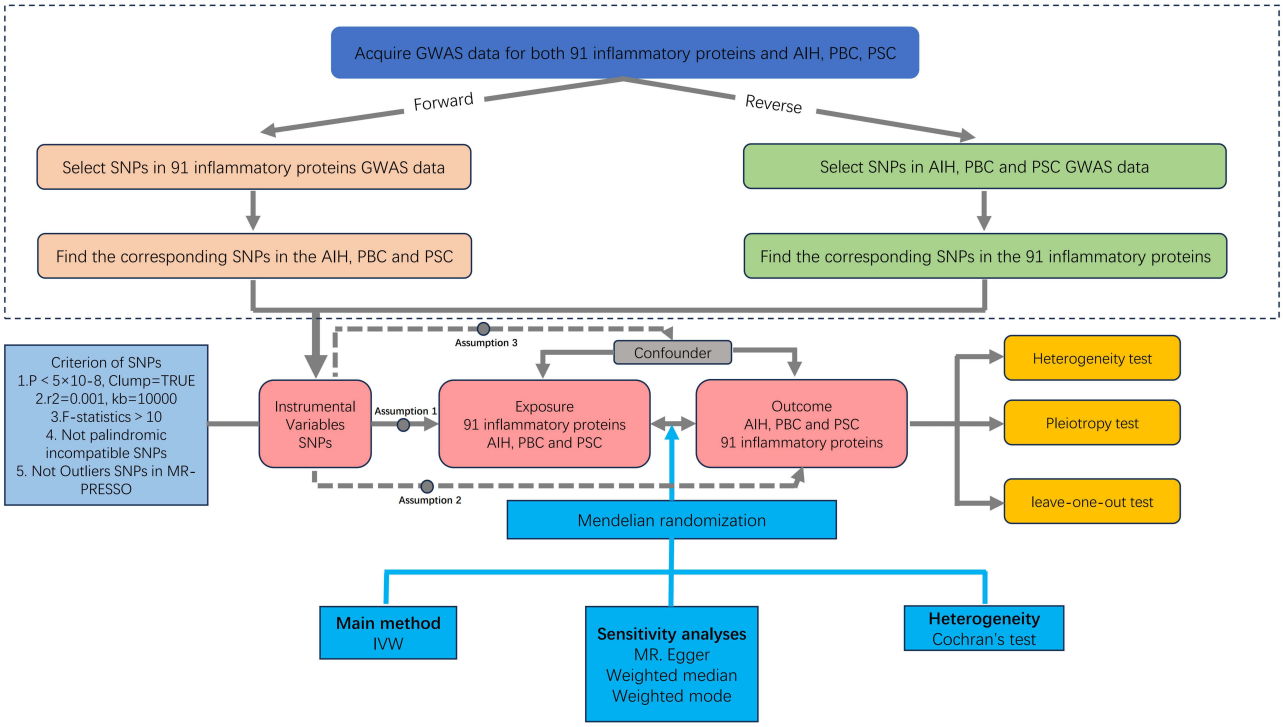

Figure 1 Overview of the assumptions of the Mendelian randomization design and the study design.
Abbreviation: GWAS, genome-wide association study; IVs, instrumental variables; AIH, autoimmune hepatitis; PBC, primary biliary cholangitis; PSC, primary sclerosing cholangitis; SNPs, single-nucleotide polymorphisms.

linkage disequilibrium (LD) window to kb = 10000kb, r2 < 0.001 to ensure the independence of the selected genetic variants. The third assumption is the unidirectional effect: the IV should only affect the outcome through the exposure and not directly influence the outcome itself. Adhering to these principles ensures the logical consistency of causal analysis. However, in the case of reverse causality, where AIH is considered the exposure and inflammatory proteins the outcome, the number of independent SNPs with a P-value below the 5×10^−8 threshold was insufficient for MR analysis. Therefore, a more lenient genome-wide significance threshold of P < 5×10^−6 was established to select SNPs closely associated with inflammatory proteins. Before each MR analysis, a Steiger test was conducted to avoid reverse causality. Only SNPs with "TREU" results were included. The MR pleiotropy residual and outlier (MR-PRESSO) test was employed to identify and remove any outliers in the data.[17] Subsequently, for each SNP, the F-statistic was determined by performing beta2 /se2 (BETA2/SE2). An F-statistic greater than 10 was considered to have sufficient strength for the selected IV.[18] In this study, all F-statistics met the criterion of F > 10. Finally, to enhance data quality, we also removed palindromic SNPs that are genomically mirror-symmetric. Detailed information about all IVs for exposures is provided in the supplementary materials.

MR analysis and sensitivity analysis.

The primary analysis utilized the multiplicative random effects inverse variance weighted (IVW) method, which combines the cumulative causal estimates from the Wald ratios derived from each IV,[19] and it offers the greatest statistical power in the absence of pleiotropy. Supplementary analyses were conducted using the Mendelian Randomization Egger (MR-Egger),[20] Weighted Median (WM),[21] and Weighted Mode methods[22] to ensure the validity and robustness of the study results. These MR methods are explained in detail in the corresponding references. False Discovery Rate (FDR) adjustment was applied to correct the results of the IVW method.[23] Cochran Q test was used to assess the heterogeneity of MR results. A p-value less than 0.05 was considered indicative of heterogeneity, leading to the use of a random effects model for analysis.[24] Additionally, we assessed pleiotropy by employing the MR-Egger intercept test and the MR-PRESSO global test. P-values exceeding 0.05 for both the MR-Egger regression and the MR-PRESSO global test indicate the absence of horizontal pleiotropy.[25] Ultimately, to ascertain whether individual SNPs

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were the sole contributors to the causal effect, we conducted leave-one-out analyses.[26] Statistical analyses were performed using R software (version 4.0.2) along with the TwoSampleMR (version 0.5.6) and MR-PRESSO packages, which can be accessed at the following website: https://cloud.r-project.org.

Results

Detailed Information of Included SNPs

Specific information regarding the 91 circulating inflammatory proteins can be found in Supplementary Table 1. Detailed SNP information for the MR analysis and reverse MR analysis of AIH, PBC, PSC, and the 91 circulating inflammatory proteins is presented in Supplementary Tables 2–7. All SNPs demonstrated "TRUE" results in the Steiger test, indicating the absence of reverse causality (Supplementary Tables 2–7). This comprehensive collection includes a variety of detailed information such as genetic loci, effect alleles (EA), and effect allele frequencies (EAF). Notably, the F-statistics for the selected IVs exceed 10. By systematically selecting SNPs, consistency in association with the target exposure is ensured. Additionally, efforts have been made to effectively manage the relationship between the outcome variables and potential confounders within acceptable parameter ranges. Consequently, all selected SNPs can be considered reliable and robust instrumental variables. Meanwhile, the baseline levels of 91 inflammatory proteins can be found at this website: https://www.nature.com/articles/s41590-023-01588-w/figures/8.

Exploring the Causal Links Between Inflammatory Proteins and AIH, PBC, PSC Through MR

The MR analysis results examining the causal relationships between the 91 circulating inflammatory proteins and AIH, PBC, and PSC are depicted in Figure 2 and Supplementary Tables 8–10. Utilizing the IVW method, we identified two outcomes with p-values less than 0.05. The IVW method revealed that elevated levels of CUB domain-containing

Exposure	Outcome	nSNP	Method	OR(95%CI)	P−value
CDCP1	AIH	5	Inverse variance weighted	1.363(1.005, 1.849)	0.046
IL−18	AIH	4	Inverse variance weighted	1.416(1.004, 1.999)	0.048
CXCL11	PBC	2	Inverse variance weighted	1.574(1.254, 1.976)	<0.001
IL−12β	PBC	6	Inverse variance weighted	0.872(0.799, 0.952)	0.002
TRANCE	PBC	2	Inverse variance weighted	0.686(0.518, 0.908)	0.009
CXCL10	PBC	3	Inverse variance weighted	0.762(0.586, 0.992)	0.044
TNFSF12	PSC	2	Inverse variance weighted	1.827(1.338, 2.495)	<0.001
CD6 isoform	PSC	2	Inverse variance weighted	1.126(1.006, 1.261)	0.039
CCL20	PSC	2	Inverse variance weighted	1.880(1.031, 3.430)	0.040
PBC	CCL4	39	Inverse variance weighted	1.023(1.004, 1.043)	0.020
PBC	OSM	38	Inverse variance weighted	1.022(1.003, 1.042)	0.024
PSC	M−CSF	17	Inverse variance weighted	1.027(1.010, 1.045)	0.002
PSC	IL−15Rα	16	Inverse variance weighted	1.030(1.010, 1.050)	0.003
PSC	PD−L1	16	Inverse variance weighted	1.019(1.001, 1.037)	0.035
PSC	CD40LR	17	Inverse variance weighted	1.022(1.001, 1.042)	0.036
PSC	LIF	17	Inverse variance weighted	1.020(1.000, 1.040)	0.049

P<0.05 was considered statistically significant

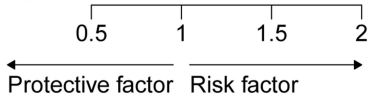

Figure 2 MR results between 91 circulating inflammatory proteins and AIH, PBC, PSC.

Abbreviation: AIH, Autoimmune hepatitis; PBC, Primary biliary cholangitis; PSC, Primary sclerosing cholangitis; CDCP1, CUB domain-containing protein 1; IL-18, Interleukin-18; CXCL11, C-X-C motif chemokine 11; IL-12β, Interleukin-12 subunit beta; TRANCE, TNF-related activation-induced cytokine; CXCL10, C-X-C motif chemokine 10; TNFSF12, Tumor necrosis factor ligand superfamily member 12; CD6 isoform, T-cell surface glycoprotein CD6 isoform; CCL20, C-C motif chemokine 20; CCL4, C-C motif chemokine 4; OSM, Oncostatin-M levels (CD6); M-CSF, Macrophage colony-stimulating factor 1; IL-15Rα, Interleukin-15 receptor subunit alpha; PD-L1, Programmed cell death 1 ligand 1; CD40LR, CD40L receptor; LIF, Leukemia inhibitory factor.

protein 1 (CDCP1) (OR = 1.363, 95% CI = 1.005–1.849, p = 0.0465) and Interleukin-18 (IL-18) (OR = 1.416, 95% CI = 1.004–1.999, p = 0.0477) are associated with an increased risk of AIH. According to the IVW results, higher levels of C-X-C motif chemokine 11 (CXCL11) (OR = 1.574, 95% CI = 1.254–1.976, p = 9.23×10^-5) were associated with an increased risk of PBC. After adjustment for the FDR, the p-value remained statistically significant with p_adj_fdr = 0.0027. Conversely, elevated levels of Interleukin-12 subunit beta (IL-12β) (OR = 0.872, 95% CI = 0.799–0.952, p = 0.0022), TNF-related activation-induced cytokine (TRANCE) (OR = 0.686, 95% CI = 0.518–0.908, p = 0.0085), and C-X-C motif chemokine 10 (CXCL10) (OR = 0.762, 95% CI = 0.586–0.992, p = 0.0437) were associated with a decreased risk of PBC. Notably, after FDR adjustment, the elevated levels of IL-12β (p_adj_fdr = 0.0022) still showed a significant association with a reduced risk of PBC. The findings revealed causal associations between PSC and three inflammatory proteins: Tumor necrosis factor ligand superfamily member 12 (TNFSF12) (OR = 1.827, 95% CI = 1.338–2.495, p = 0.0001, p_adj_fdr = 0.0063), T-cell surface glycoprotein CD6 isoform (CD6 isoform) (OR = 1.126, 95% CI = 1.006–1.261, p = 0.0389), and C-C motif chemokine 20 (CCL20) (OR = 1.880, 95% CI = 1.031–3.430, p = 0.0395), with elevated levels associated with an increased risk of PSC. In addition, we have summarized the positive results of MR analysis of circulating inflammatory proteins and AIH, PBC, PSC, as shown in Supplementary Table 11.

Supplementary scatter plots (Supplementary Figure 1) and forest plots (Figure 2) display the causal effects of each SNP in genes encoding the circulating inflammatory proteins that showed positive results on the risk of AIH, PBC, and PSC.

Exploring the Causal Links Between AIH, PBC, PSC and Inflammatory Proteins Through MR

The results of the reverse MR analysis are presented in Supplementary Tables 12–14. In the inverse MR analysis, no association was found between AIH and circulating inflammatory proteins. Regarding the impact of PBC on inflammatory proteins, two positive results were identified. PBC may promote the expression levels of C-C motif chemokine 4 (CCL4) (OR = 1.023, 95% CI = 1.004–1.043, p = 0.0201) and Oncostatin-M levels (CD6) (OSM) (OR = 1.022, 95% CI = 1.003–1.042, p = 0.0236). Furthermore, a positive causal relationship was observed between PSC and five circulating inflammatory proteins: Macrophage colony-stimulating factor 1 (M-CSF) (OR = 1.027, 95% CI = 1.009–1.045, p = 0.0024), Interleukin-15 receptor subunit alpha (IL-15Rα) (OR = 1.030, 95% CI = 1.010–1.050, p = 0.003), Programmed cell death 1 ligand 1 (PD-L1) (OR = 1.019, 95% CI = 1.001–1.037, p = 0.0346), CD40L receptor (CD40LR) (OR = 1.022, 95% CI = 1.001–1.042, p = 0.0364), and Leukemia inhibitory factor (LIF) (OR = 1.019, 95% CI = 1.000–1.040, p = 0.0491). In addition, the positive results of reverse MR analysis of circulating inflammatory proteins and AIH, PBC, PSC are shown in Supplementary Table 15.

Sensitivity Analysis

We observed that the IVW and MR-Egger heterogeneity tests confirmed the absence of statistically significant heterogeneity among the genetic instruments studied (all p-values > 0.05) (Table 1). Furthermore, the lack of statistically significant difference between the MR-Egger Egger intercept and 0 (all p-values > 0.05) indicated no evidence of horizontal pleiotropy (Table 1). In the leave-one-out sensitivity analysis (Supplementary Figure 2), no single SNP significantly disrupted the overall effect of inflammatory proteins on AIH, PBC, and PSC. Similarly, no single SNP significantly disrupted the overall effect of AIH, PBC, and PSC on inflammatory proteins. Due to the limited number of IVs available for positive proteins that meet the criteria, they do not meet the conditions for leave-one-out analysis. Therefore, only four figures are provided. The symmetry and funnel plot absence of bias in the results added credibility to the study's findings (Supplementary Figure 3). While our funnel plots and sensitivity analyses suggest that the data are unbiased, we acknowledge the importance of considering potential biases in the interpretation of our results. Future studies with larger sample sizes and more comprehensive data sets could provide further validation of our findings and help to minimize the impact of any potential biases.

Table 1 Heterogeneity and Directional Horizontal Pleiotropy Tests of Circulating Inflammatory Cytokines on AIH, PBC and PSC

Exposure	Outcome	Heterogeneity Test				Directional Horizontal Pleiotropy Test		
		IVW		MR Egger		Egger Intercept	SE	p-value
		Q	Q_ p-value	Q	Q_ p-value			
CDCP1	AIH	1.6438	0.8009	1.3633	0.7142	−0.0287	0.0542	0.6330
IL-18	AIH	0.2035	0.9770	0.0722	0.9646	−0.0430	0.1185	0.7518
CXCL11	PBC	0.6269	0.4285	NA	NA	NA	NA	NA
IL-12β	PBC	5.0231	0.4131	1.6261	0.8041	−0.0344	0.0187	0.1391
TRANCE	PBC	0.8669	0.3518	NA	NA	NA	NA	NA
CXCL10	PBC	0.0041	0.9980	0.0035	0.9528	0.0008	0.0332	0.9851
TNFSF12	PSC	0.0457	0.8307	NA	NA	NA	NA	NA
CD6 isoform	PSC	0.1671	0.6827	NA	NA	NA	NA	NA
CCL20	PSC	1.5700	0.2102	NA	NA	NA	NA	NA
PBC	CCL4	46.4184	0.1641	46.3423	0.1396	−0.0015	0.0062	0.8067
PBC	OSM	43.4824	0.2147	43.4455	0.1839	−0.0011	0.0061	0.8622
PSC	M-CSF	15.6146	0.4802	15.3686	0.4252	0.0030	0.0061	0.6312
PSC	IL-15Rα	12.5159	0.6396	12.4436	0.5707	0.0019	0.0072	0.7920
PSC	PD-L1	11.8973	0.6868	9.5936	0.7913	0.0099	0.0065	0.1513
PSC	CD40LR	21.4926	0.1603	20.6273	0.1492	NA	NA	NA
PSC	LIF	13.1917	0.6587	10.3068	0.8000	0.0116	0.0068	0.1101

Abbreviations: AIH, Autoimmune hepatitis; PBC, Primary biliary cholangitis; PSC, Primary sclerosing cholangitis; CDCP1, CUB domain-containing protein 1; IL-18, Interleukin-18; CXCL11, C-X-C motif chemokine 11; IL-12β, Interleukin-12 subunit beta; TRANCE, TNF-related activation-induced cytokine; CXCL10, C-X-C motif chemokine 10; TNFSF12, Tumor necrosis factor ligand superfamily member 12; CD6 isoform, T-cell surface glycoprotein CD6 isoform; CCL20, C-C motif chemokine 20; CCL4, C-C motif chemokine 4; OSM, Oncostatin-M levels (CD6); M-CSF, Macrophage colony-stimulating factor 1; IL-15Rα, Interleukin-15 receptor subunit alpha; PD-L1, Programmed cell death 1 ligand 1; CD40LR, CD40L receptor; LIF, Leukemia inhibitory factor.

Discussion

In this study, we employed a bidirectional MR approach to elucidate the causal relationships between circulating inflammatory proteins and AILD (including AIH, PBC, and PSC). The results suggest that certain cytokines appear to regulate disease susceptibility in various ways, with complex interactions at play. However, further research is needed to establish the causal effects.

CDCP1, also known as CD318, is a type I transmembrane glycoprotein that is widely upregulated in various malignancies, including liver and pancreatic tumors.[27] Most previous studies on CDCP1 have been limited to its role in oncogenesis, suggesting that CDCP1 is essential for cancer cell survival and metastasis through intrinsic mechanisms within tumor cells.[28,29] To date, changes in CDCP1 expression in the context of AIH have not been reported. However, CDCP1 has been implicated in related immune diseases. It is identified as a novel ligand for CD6, a surface marker and key regulatory molecule of T cells.[30] Polymorphisms in CD6 are associated with multiple sclerosis (MS)[31] and autoimmune uveitis,[32] and CDCP1 has been shown to promote T cell infiltration, thereby contributing to the development of autoimmune uveitis.[33] Both diseases involve a significant role of autoreactive T cells in their pathogenesis. Concurrently, mounting evidence suggests that abnormal T cell (eg, CD4+ T cell) infiltration and activation play a crucial role in the pathogenesis of AIH.[34,35] This may partially explain why CDCP1 is a risk factor for AIH in our study. Future preclinical and clinical research is needed to confirm the role of CDCP1 in the development of AIH. IL-18, a cytokine closely related to immune responses and a member of the IL-1 family,[36] was found in our forward analysis to be associated with an increased risk of AIH when elevated. IL-18 may play a role in AIH by promoting Th1 cell-mediated reactions, potentially related to the secretion of interferon-gamma (IFN-γ).[37] In a mouse model of AIH, dendritic cells in the spleen and liver produce large amounts of IL-18, leading to increased levels of IL-18 in the serum. Administration of anti-IL-18R in vivo can suppress the increase of C-X-C motif chemokine receptor 3 positive

(CXCR3+) T cells in the spleen and the progression to fatal AIH.[38] Given the significant role of IL-18 in AIH, monoclonal antibodies, inhibitors, and drugs targeting IL-18 may represent a potential therapeutic approach for AIH.

CXCL11 is a chemokine that binds to the CXCR3 receptor. Studies have indicated that the expression levels of CXCL11 in patients with PBC correlate with disease activity. After treatment with ursodeoxycholic acid (UDCA) in PBC patients, the levels of markers such as CXCL11 remain elevated in those who have an incomplete response to UDCA, suggesting that any degree of persistent abnormality may be associated with a poor prognosis.[39] In the therapeutic strategy for PBC, modulating related chemokines, including CXCL11, is one of the potential avenues for treatment.[40] This is consistent with our research findings. CXCL11 may serve as one of the biomarkers for PBC disease activity, aiding in the assessment of disease severity and therapeutic efficacy. Our study results reveal that IL-12β, TRANCE, and CXCL10 may be protective factors for PBC, which is inconsistent with previous study findings. IL-12β is one of the subunits of IL-12. IL-12 promotes Th1 cell differentiation by activating the STAT4 signaling pathway and induces the production of IFN-γ. In PBC, IFN-γ can activate macrophages and cytotoxic T cells, leading to damage of bile duct epithelial cells.[41] Additionally, IL-12β promotes the release of inflammatory factors (such as TNF-α and IL-6) by activating signaling pathways such as NF-κB and STAT4, exacerbating liver inflammation and fibrosis, and driving disease progression in PBC.[42] TRANCE, a cytokine belonging to the tumor necrosis factor (TNF) superfamily, plays an important role in immune regulation and bone metabolism by binding to its receptor RANK. TRANCE regulates the interaction between dendritic cells (DCs) and T cells by activating the RANK signaling pathway, promoting autoimmune responses. In PBC, TRANCE may enhance Th1 and Th17 immune responses, leading to damage of bile duct epithelial cells.[41] CXCL10, a chemokine produced by various cells (such as hepatocytes, bile duct epithelial cells, and immune cells), recruits Th1-type T cells to the liver and bile duct regions by binding to the receptor CXCR3, promoting inflammatory responses and bile duct injury. Serum and liver tissue levels of CXCL10 are significantly elevated in PBC patients and are associated with disease activity.[43] Further research is needed in the future to clarify the causal interactions between the aforementioned factors and PBC.

Studies have shown that under conditions of cholestasis, the expression of TNFRSF12A (the receptor for TNFSF12, also known as TWEAK) is significantly increased in hepatocytes and human cholangitis. The TWEAK/Tnfrsf12a axis plays a crucial role in liver injury induced by cholestasis by promoting hepatocyte pyroptosis through the activation of the NFκB signaling pathway.[44] This finding suggests that TNFSF12 may play an important role in the pathogenesis of PSC, which is a special type of cholestatic liver disease. Our research also indicates that TNFRSF12 may be a risk factor for PSC. These findings lay the groundwork for future exploration of the role of TNFSF12 in PSC and may contribute to the development of new therapeutic strategies. CD6 is a glycoprotein expressed on the surface of T cells and belongs to the scavenger receptor family. CD6 regulates the interaction between T cells and antigen-presenting cells (APCs), affecting the activation and differentiation of T cells. The isoforms of CD6 may participate in the pathogenesis of PBC by altering its binding ability with the ligand Activated Leukocyte Cell Adhesion Molecule (ALCAM), affecting the immune response of T cells.[45] Similarly, no studies have discussed the role of CCL20 in PSC. CCL20 has a strong chemoattractant effect on lymphocytes and a weaker effect on neutrophils. It acts on target cells by binding and activating the chemokine receptor CCR6, playing an important role in the formation and function of mucosal lymphoid tissues. Through its role in the chemotaxis, activation, and migration of immune cells, it may play a role in the pathogenesis of PSC. However, more research is needed to clarify the specific role and mechanism of CCL20 in PSC.

In reverse MR analysis, we found that PBC may be associated with the expression levels of CCL4 and OSM. CCL4 is an important chemokine that participates in the process of liver inflammation and fibrosis by recruiting monocytes, macrophages, and T cells to the site of inflammation.[46] In PBC, the elevation of CCL4 may exacerbate the inflammatory response around the bile duct, leading to damage to bile duct epithelial cells and disease progression.[47] In addition, OSM is an IL-6 family cytokine that promotes liver inflammation and fibrosis by activating the STAT3 signaling pathway. The elevation of OSM may further promote the pathological process of PBC, suggesting its potential as a therapeutic target.[48] There is a causal relationship between PSC and five circulating inflammatory proteins (M-CSF, IL-15R α, PD-L1, CD40LR, and LIF). These proteins play important roles in immune regulation and inflammatory response: M-CSF can

promote monocyte differentiation into macrophages, enhance inflammatory response and tissue damage.[49] IL-15R α mainly regulates the activity of NK cells and T cells, and participates in liver immune response.[50] PD-L1 participates in the regulation of immune tolerance by inhibiting T cell activity, and its elevation may be related to the immune escape mechanism of PSC.[51] CD40LR participates in the interaction between B cells and T cells, promoting the release of inflammatory factors.[52] LIF regulates the differentiation and function of immune cells and may be involved in the inflammatory and fibrotic processes of PSC.[53] These findings suggest that the pathogenesis of PSC involves abnormal activation of multiple inflammatory pathways, and treatment strategies targeting these proteins may bring new hope to PSC patients.

Nonetheless, it is important to acknowledge that this study has certain limitations. Firstly, the MR method assumes a linear relationship between genetic variants, cytokine levels, and disease risk, which may not always hold true in complex biological systems. Secondly, it is important to note that our study's findings are based on data from European populations. While this approach reduces the influence of confounding factors such as population stratification, it also limits the extrapolation of our results to other ethnic groups. Different populations may have distinct genetic backgrounds, environmental exposures, and disease susceptibilities, which could influence the observed associations. Therefore, our findings should be interpreted with caution and further validated in diverse populations to ensure their generalizability. Third, due to the complexity of disease progression, MR studies can only reduce confounding factors to a certain extent and cannot completely eliminate them. Fourthly, in this study, we focused our analysis on the relationship between 91 circulating inflammatory proteins and AILD. It is important to note that there are still additional circulating inflammatory proteins associated with the disease that were not included in our analysis. In the future, the connection between circulating inflammatory proteins and AILD should be more comprehensively validated. Lastly, although we have established causal relationships, the exact biological mechanisms behind these associations require further experimental confirmation. Future research should consider conducting relevant basic experimental studies, such as cell experiments and animal model experiments, to further explore the biological basis of the causal relationships identified in this study.

Conclusion

Our findings highlight a significant causal association between inflammatory proteins and the pathogenesis of AILD (AIH, PBC, and PSC). However, while these results provide valuable insights into the potential roles of these proteins, they do not fully explain the complex and multifaceted effects of these mediators in disease progression. Its potential mechanisms and broader impacts require further research.

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Data Sharing Statement

The original contributions presented in the study are included in the article/supplementary material. For further inquiries, please contact the corresponding author.

Ethics Statement

The data in this study were obtained from published studies, of which all data had been approved by the institutional review committee. The ethical application for this study was approved by the Ethics Committee of Xingtai People's Hospital [Approval number: 2025【014】].

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Author Contributions

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

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Disclosure

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