

Circulating Inflammatory Proteins and Intrahepatic Cholestasis of Pregnancy: A Two-Sample Bidirectional Mendelian Randomization Analysis

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Abstract:

Objective: This study aims to explore the causal relationship between circulating inflammatory proteins and Intrahepatic Cholestasis of Pregnancy (ICP) using a two-sample Mendelian randomization approach. **Methods:** Based on genome-wide association study (GWAS) datasets, genetic variations strongly associated with circulating inflammatory proteins were selected as instrumental variables. A variety of statistical models including Inverse Variance Weighted (IVW), MR-Egger, weighted median, and weighted mode were employed to assess the potential impact of circulating inflammatory proteins on ICP. Additionally, MR-Egger intercept tests were conducted to evaluate pleiotropy, while Cochran's Q-tests and leave-one-out analyses were used to assess the potential impact of circulating inflammatory proteins on ICP risk. Tests and leave-one-out analyses were used to assess heterogeneity and the robustness of the findings. **Results:** The analysis indicated a causal relationship between elevated levels of the inflammatory proteins and the risk of ICP. The analysis indicated a causal relationship between elevated levels of the inflammatory chemokine CCL23 (C-C Motif Chemokine 23, CCL23) and an increased risk of ICP (OR: 1.176, 95% CI: 1.027-1.346, P=0.019). Conversely, elevated levels of CUB Domain-containing Protein 1 (CDCP1) were associated with a decreased risk of ICP (OR: 0.821, 95% CI: 0.730-0.923, P=0.001). Furthermore, sensitivity analyses confirmed the association between specific circulating inflammatory proteins and ICP. No evidence of reverse causality was found in the bidirectional MR analysis. **Conclusion:** The findings of this study underscore the significant role of circulating inflammatory proteins in the pathogenesis of ICP, suggesting that modulation of the inflammatory pathway could be an effective strategy for the prevention or treatment of ICP.

Keywords:

Intrahepatic Cholestasis of Pregnancy, Circulating Inflammatory Proteins, Causal Relationship, Mendelian Randomization

1. Introduction

Intrahepatic cholestasis of pregnancy (ICP) is one of the most common liver disorders during pregnancy, especially in the second or third trimester [1]. The main clinical manifestations of ICP include intolerable itching of the skin, especially on the palms of the hands and soles of the feet, and a significant increase in serum bile acid concentration [2]. The main clinical manifestations of ICP include intolerable itching of the skin, especially on the palms and soles of the feet, and a significant increase in serum bile acids. Studies have shown that the development of ICP is associated with an obstruction of bile excretion, which leads to a build-up of toxic bile acids in the bloodstream, leading to the symptoms described above. The condition usually resolves quickly after delivery. Although ICP does not usually have long-term health effects on the mother, it can lead to a range of adverse maternal outcomes, such as preterm labor, placental abruption, and even intrauterine death [3]. In addition, fetuses of patients with ICP may face higher health risks, including fetal asphyxia and increased need for neonatal intensive care units (NICUs) [4]. The mechanism by which ICP occurs remains unknown. However, the mechanisms by which ICP occurs remain unclear.

In recent years, an increasing number of studies have focused on the role of inflammation in the pathogenesis of ICP. The inflammatory response is regulated by a complex of cells and mediators, including circulating inflammatory proteins such as cytokines, chemokines and other soluble receptors [5,6]. As small molecule proteins and cytokines that regulate the immune response and promote or inhibit the inflammatory process, circulating inflammatory proteins may play a role in ICP through multiple mechanisms [7]. These proteins not only directly affect the transport and metabolism of bile acids by hepatocytes, but may also be indirectly involved in the development of ICP by affecting placental function and regulation of progesterone levels, among other things [8,9]. The role of inflammation in the development of ICP can be seen in the following. Thus, an in-depth understanding of the role of inflammation and its associated circulating inflammatory proteins in ICP is important for unraveling the pathophysiological mechanisms of ICP and developing effective therapeutic and preventive strategies.

Mendelian Randomization (MR), a method that uses genetic variation as an instrumental variable to estimate the causal relationship between exposure and outcome, has shown unique strengths in studies that have resolved the relationship between circulating inflammatory proteins and ICP [10]. Because genetic variants are randomly assigned at birth, MR analysis reduces the problems of confounders and reverse causation that are common in traditional observational studies, providing us with more reliable causal inferences. MR analysis can reduce the problems of confounding and reverse causation that are common in traditional observational studies, providing us with more reliable causal inferences [11].

Therefore, this study employed a two-way two-sample MR approach to clarify the causal relationship between circulating inflammatory proteins ICP. We attempted to

utilize genetic variants significantly associated with specific circulating inflammatory proteins as instrumental variables (IVs), aiming to reduce potential interference and thus more accurately assess the effect of circulating inflammatory proteins on ICP risk. With this approach, we hope to reveal the role of circulating inflammatory proteins in the occurrence and development of ICP, and thus provide new strategies and theoretical basis for the prevention and treatment of ICP.

2. Methodology

2.1. Study design

The aim of this study is to analyze the association between circulating inflammatory proteins and ICP, where circulating inflammatory proteins are the exposure factors and ICP is the study outcome. To guarantee the stability and credibility of the findings, we will conduct multiple validity tests, heterogeneity analyses, and sensitivity checks, and estimate the efficacy of the tests. Three conditions need to be fulfilled for a MR study to be valid: (1) the instrumental variable (IV) needs to be strongly correlated with the exposure factor; (2) the association between the IV and the findings of the study should not be subjected to a confounding bias; and (3) the effect of the IV on the findings should be only through the exposure factor as a pathway [12]. The two-way two-sample MR analysis method adopted in this study can reveal the causal relationship between exposure factors and study outcomes more effectively and robustly than the traditional single-sample MR method by using independent sample data from different genome-wide association studies (GWAS) [13].

2.2. Data source

Our study used 91 circulating inflammatory proteins as exposure factors. By accessing the Genome-Wide Association Studies (GWAS) database provided by the Medical Research Council Integrated Epidemiology Unit (MRC IEU) in the UK, we retrieved data related to these inflammatory factors with registry numbers ranging from GCST90274758 to GCST90274848 (<https://www.ebi.ac.uk/gwas/>). The dataset included 11 cohorts totaling 14,824 participants of European ancestry [14]. The outcome variable of the study was intrahepatic cholestasis of pregnancy (ICP), and the GWAS summary data for ICP was provided by the FinnGen Collaboration, which included 940 cases of European descent and 122,639 control individuals (<https://r10.finnngen.fi/>).

2.3. Instrumental Variables (IVs) Selection

In the process of exploring the causal link between circulating inflammatory proteins and ICP, we conducted a rigorous screening of instrumental variables (IVs) to ensure the scientific validity and accuracy of the analysis. First, to ensure a strong association between IVs and the exposure factor-circulating inflammatory proteins-we selected single nucleotide polymorphisms (SNPs) with a P value of $<1.0 \times 10^{-5}$, a criterion that is often regarded as the threshold of significance for genome-wide association. Subsequently, to minimize the possibility of the influence of multiple related loci on the same chromosome, we removed SNPs with linkage disequilibrium. This step was accomplished by setting the random effect below a specific threshold ($r^2 < 0.001$, kb = 1 Mb), which ensured independence among the selected IVs. We also excluded IVs containing palindromic sequences to avoid analysis errors due to inconsistencies between effect alleles and effect sizes. To ensure

a low risk of instrumental variable bias in the selected IVs, we further excluded SNPs with an F statistic of less than 10. This criterion was determined by calculating the formula $[F = (R^2/(1 - R^2)) * ((N-K-1)/K)]$, where N is the sample size and K is the number of IVs. a high or a low value of F is directly correlated to the reliability of the IVs. Finally, we queried each SNP in the PhenoScanner database to exclude potential confounders and to ensure that the selected IVs affected ICP only through circulating inflammatory proteins, which fulfilled all the conditions of the two-sample MR randomization analysis.

2.4. MR analysis

In this study, we mainly performed MR analysis by random-effects inverse variance weighted (IVW) method with the aim of assessing the causal association between circulating inflammatory proteins and ICP. The IVW method is considered to be the preferred method for estimating the causal effect because of its high test efficacy. In addition, we used analytical methods such as Weighted Median, Weighted Mode, and MR-Egger regression, which can assess the stability and confidence of the causal relationship from different perspectives. In order to fully consider the heterogeneity and horizontal pleiotropy of genetic variation on the results, a series of stability and reliability tests were performed. Cochran's Q statistic and leave-one-out were used for sensitivity analyses, which helped to identify individual SNPs that had a significant effect on the results. The intercept of MR-Egger regression was used to test for the presence of horizontal pleiotropy, and thus to assess the robustness of the analyzed results. robustness of the analyzed results. All these statistical analyses were done in the R environment using Two Sample MR, aiming to ensure the reliability and robustness of the MR analysis results from multiple dimensions.

3. Result

3.1. Effect of circulating inflammatory proteins on ICP

A total of 2570 SNPs were selected as IVs for 91 circulating inflammatory proteins according to the selection criteria for IVs. Detailed information on the genetic variants used in the MR analyses is shown in Table S1. The F-statistics of the selected SNPs were all greater than 10, suggesting that the present study was not susceptible to weak instrumental variable bias. As shown in Figure 1 and Figure 2, IVW results of four circulating inflammatory proteins were shown to be associated with ICP risk, including: eosinophil chemokine, chemokine CCL23, interleukin 15 receptor α antibody, and CUB structural domain-containing protein 1. Among them, eosinophil chemokine (OR: 1.284, 95% CI: 1.092-1.509, P=0.002), chemokine C-C Motif Chemokine 23 (CCL23) (OR: 1.176, 95% CI: 1.027-1.346, P=0.019), and interleukin 15 receptor alpha antibody (IL-15RA) (OR: 1.141, 95% CI: 1.020-1.277, P=0.021) levels were positively associated with the risk of developing ICP. On the contrary, the level of CUB Domain-containing Protein 1 (CDCP1) (OR: 0.821, 95% CI: 0.730-0.923, P=0.001) was negatively associated with the risk of ICP.

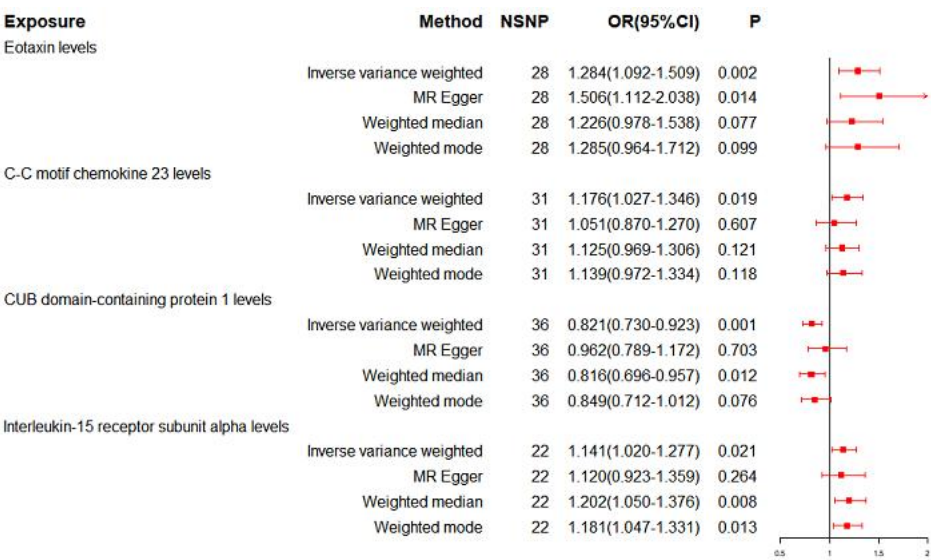


Figure 1. Forest plot of causal effects of circulating inflammatory proteins on ICP.

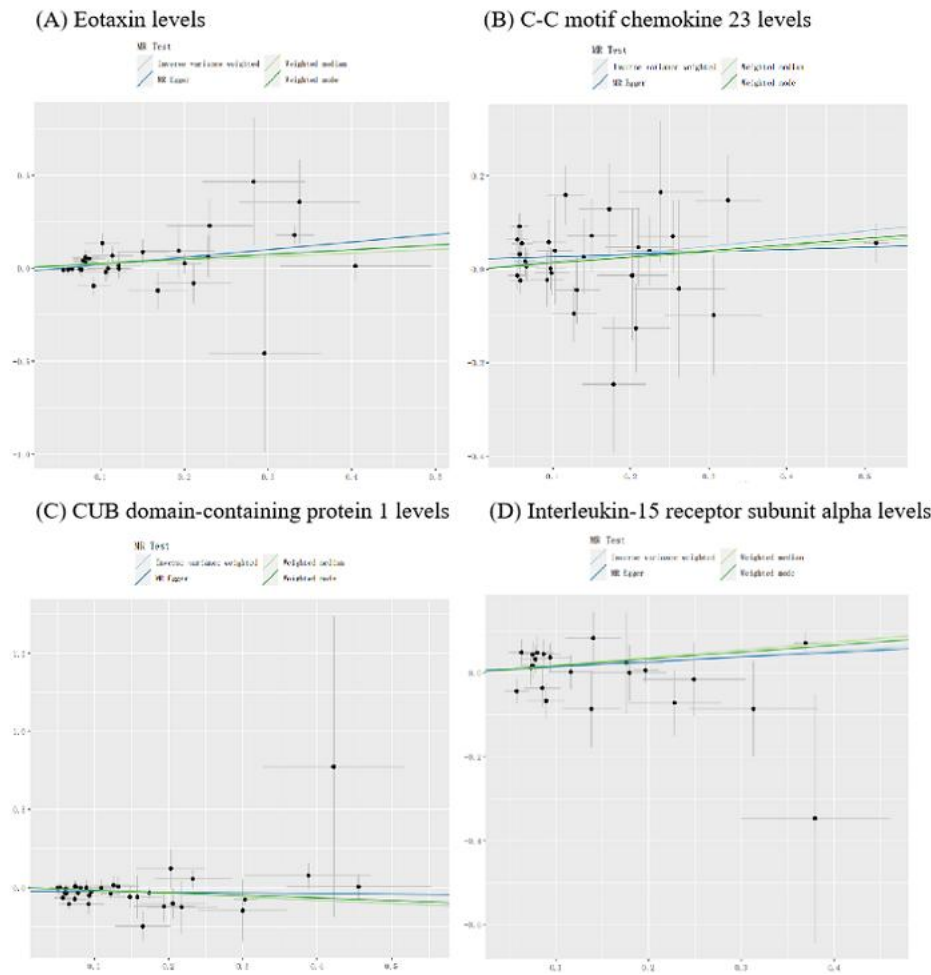


Figure 2. Scatter plot of the causal effect of circulating inflammatory proteins on ICP.

3.2. Sensitivity analysis

In order to verify the robustness of the results, a series of analyses including Cochran’s Q test, MR-Egger regression and leave-one-out method were performed, all of which did not reveal heterogeneity and pleiotropy. In Figure 3, Cochran’s IVW

Q test shows that there is no heterogeneity among the selected IVs. In Figure 4, the leave-one-out method showed no change in the results when removing SNPs one by one for MR analysis. According to the results of MR-Egger intercept analysis, the presence of horizontal pleiotropy was not detected for eosinophil chemokine (P=0.235), chemokine CCL23 (P=0.235), interleukin 15 receptor α -antibody (P=0.064), and CUB structural domain-containing protein 1 (P=0.813) (Table 1).

Table 1. Results of heterogeneity analysis and horizontal multiple validity test.

Circulating inflammatory proteins	Methods	Heterogeneity	Horizontal multiplicity
		MR-IVW	Egger intercept test
Eotaxin levels	Statistics	Q=35.729	-0.022
	P	0.121	0.235
C-C motif chemokine 23 levels	Statistics	Q=40.933	0.022
	P	0.088	0.116
CUB domain-containing protein 1 levels	Statistics	Q=40.690	-0.023
	P	0.234	0.064
Interleukin-15 receptor subunit alpha levels	Statistics	Q=23.450	0.004
	P	0.32	0.813

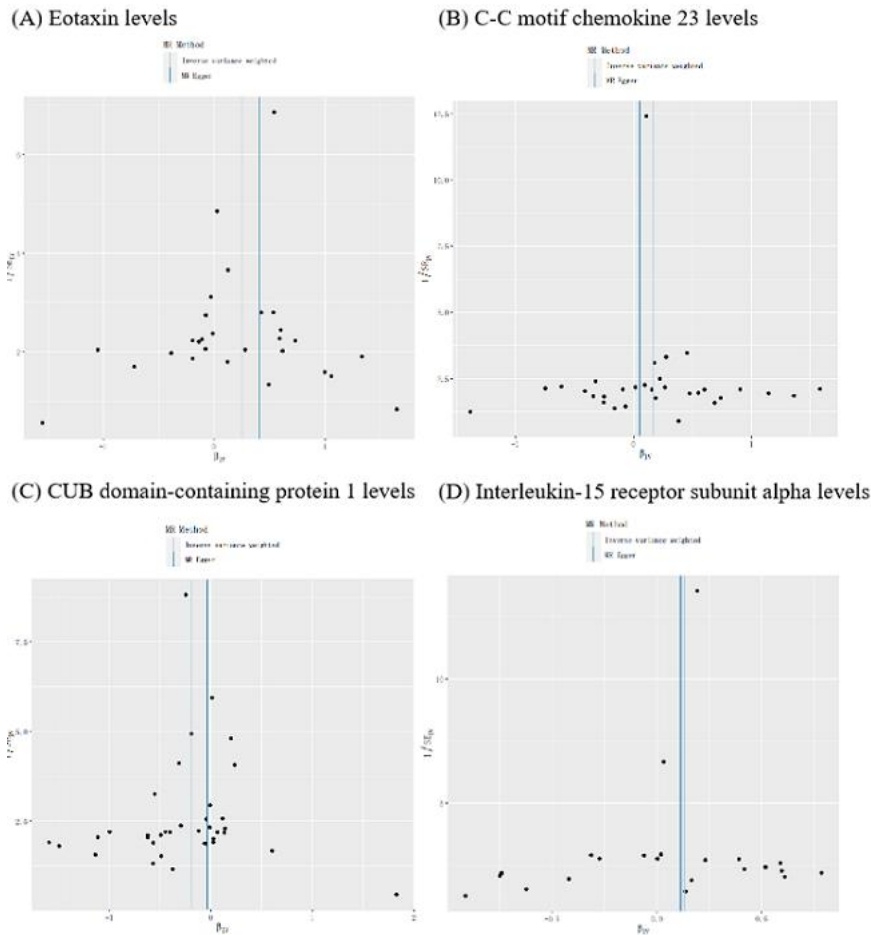


Figure 3. Funnel plot of causal effects of circulating inflammatory proteins on ICP.

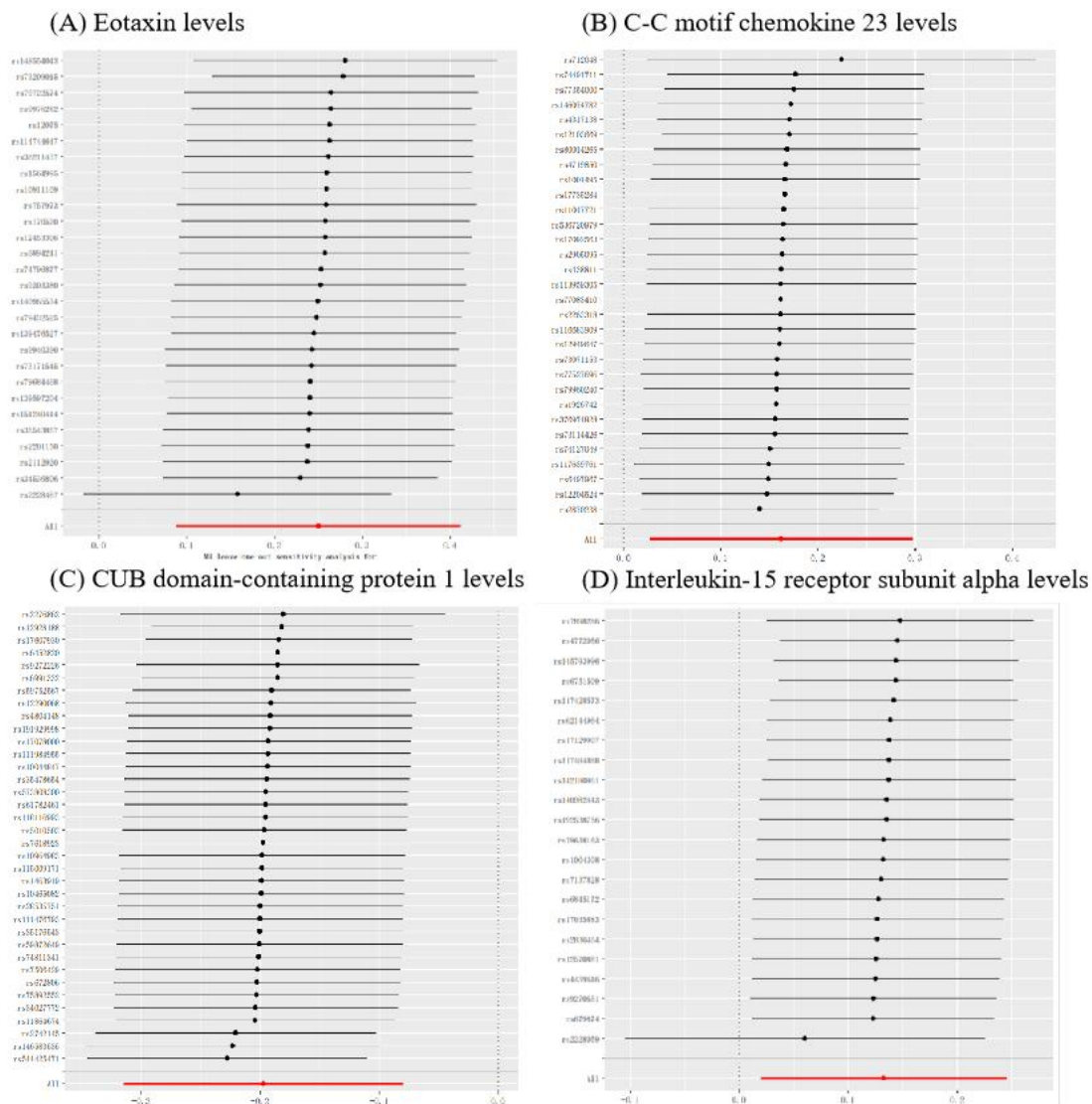


Figure 4. Leave-one-out plot of the causal effect of circulating inflammatory proteins on ICP.

3.3. Reverse MR analysis

We performed reverse MR analysis to infer whether there is a causal relationship between ICP and four circulating inflammatory proteins. We selected SNPs that were significantly associated with the risk of ICP as IVs (Table S2), and the primary analytical method, IVW, indicated that there was an inverse causal relationship between ICP and eosinophil chemokines (OR: 1.022, 95% CI: 1.002-1.042, $P=0.03$), and antibodies to interleukin 15 receptor α (OR: 1.022, 95% CI: 1.000-1.043, $P=0.046$) had an inverse causal relationship (Table S2), so the causal relationship between these two inflammatory proteins and ICP could not be determined (Figure 5).

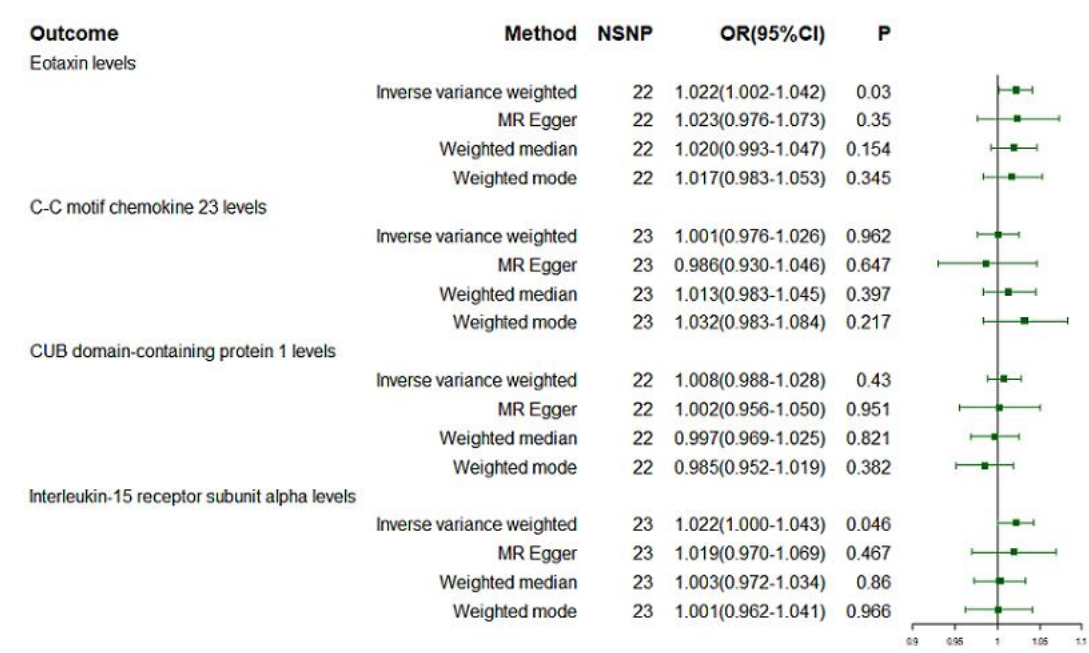


Figure 5. Forest plot of causal effects of ICP on circulating inflammatory proteins.

4. Discussion

We found that ICP risk was associated with levels of the chemokine CCL23 and CUB structural domain-containing protein 1. High levels of the chemokine CCL23 increased the risk of ICP development, and conversely, elevated levels of CUB structural domain-containing protein 1 were associated with a decreased risk of ICP.

ICP is a pregnancy-specific complication characterized by elevated serum levels of bile acids and itchy skin in pregnant women, but the mechanism by which ICP occurs is currently unknown [15]. Previous studies have pointed out that the inflammatory response plays an important role in the development of liver injury. For example, Inflammatory factors such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) are often abnormally elevated in patients with hepatic injury, which may contribute to the development of ICP by affecting the function of liver cells and promoting the abnormal secretion of bile acids [16,17,18]. In addition, these inflammatory proteins may also be associated with an increased risk of adverse pregnancy outcomes in ICP, including preterm labor and fetal distress [19]. Inflammation and Bile Acid Dysregulation. The mechanistic link between inflammation and bile acid dysregulation involves cytokine-mediated inhibition of bile acid transport proteins on hepatocyte membranes, leading to accumulation of bile acids in the liver and body circulation [20,21] . Studies have also suggested that anti-inflammatory therapy may be effective in patients with ICP. Anti-inflammatory treatments such as ursodeoxycholic acid have shown some efficacy in lowering serum bile acids and mother's symptoms by modulating cytokine levels [22,23]. These findings emphasize the potential for targeting inflammatory pathways as part of a comprehensive treatment strategy for ICP.

CCL23 is a chemokine with chemotactic activity towards monocytes and activated T-lymphocytes and can act by binding to specific receptors such as CCR1 [24]. If CCL23 is aberrantly expressed or activated during pregnancy due to certain stimuli (e.g., hormonal changes or genetic factors), it may increase the risk of ICP by promoting the accumulation of inflammatory cells (e.g., monocytes and lymphocytes)

in the liver, exacerbating the abnormal accumulation of bile acids and hepatic dysfunction. In addition, upregulation of CCL23 may induce hepatocytes and immune cells to release inflammation-related cytokines, such as IL-6 and TNF- α , etc., and the excessive release of these cytokines may disrupt the normal metabolism and excretion process of bile acids, leading to the development of ICP [25]. In addition, CCL23 affects the balance of the immune response by regulating the activity of immune cells and the production of cytokines, and this immune imbalance may lead to damage to the placenta and maternal tissues, exacerbating the development of ICP [26]. The development of ICP is exacerbated by this immune imbalance.

CUB structural domain-containing protein 1 (CDCP1) is a transmembrane glycoprotein expressed in a variety of cell types, which plays an important role in cell proliferation, migration, cell signaling, and interaction with the extracellular matrix [27]. Previous studies have found that CDCP1 is highly overexpressed in a variety of solid tumors such as breast, colon, and lung cancers [28]. Al-Awadhi et al. could reduce prostate cancer metastasis by inhibiting the expression of CPCP1 [29]. And our study found that the expression level of CDCP1 was negatively correlated with the risk of ICP, which implies that elevated levels of CDCP1 may help to reduce the occurrence of ICP. Currently, studies have not shown direct evidence that CDCP1 is associated with inhibition of inflammatory cell activation and reduction of liver inflammation in ICP or other liver diseases. Further studies are needed to explore and validate the role of CDCP1 in this specific context, especially how it affects inflammatory processes and cytokine release in liver disease.

In this study, we explored the causal relationship between circulating inflammatory proteins and ICP by two-sample bidirectional Mendelian randomization. First, compared with traditional observational studies, MR analysis is effective in reducing the interference caused by confounding factors and reverse causality. In addition, to rule out possible pleiotropic effects, the MR-Egger intercept, Cochran's Q test, and leave-one-out method were used in this study to verify the robustness of the causal relationships. Despite these advantages, our study has some limitations. First, as the study sample was mainly derived from a European population, the broad applicability of the results may be limited, especially in populations of other ethnicities. Second, the two-sample MR approach used focused primarily on the linear effects of ICP risk and may have failed to capture more complex nonlinear relationships. Finally, although this study provided a statistical association between circulating inflammatory proteins and ICP, further clarification is needed regarding the specific biological mechanisms underlying this relationship. Future studies need to explore the underlying mechanisms in detail with additional biomarkers and clinical trials.

5. Conclusions

In summary, we comprehensively assessed the potential causal relationship between circulating inflammatory proteins and ICP. This two-sample MR study provides conclusive evidence that the levels of two circulating inflammatory proteins are causally associated with ICP. This finding suggests that modulating inflammatory pathways may provide an effective strategy to prevent or treat ICP. This further emphasizes the importance of a deeper understanding of the mechanisms that regulate inflammation and provides a possible direction for the development of new therapeutic approaches in the future.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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References

- [1] Huri, M.; Seravalli, V.; Lippi, C, et al. Intrahepatic cholestasis of pregnancy - Time to redefine the reference range of total serum bile acids: A cross sectional study. *BJOG: an international journal of obstetrics and gynaecology*, 2022, 129(11), 1887-1896.
- [2] Walker KF, Chappell LC, Hague WM, et al. Pharmacological interventions for treating intrahepatic cholestasis of pregnancy. *The Cochrane database of systematic reviews*, 2020, 7(7), Cd000493.
- [3] Manzotti, C.; Casazza, G.; Stimac, T. Total serum bile acids or serum bile acid profile, or both, for the diagnosis of intrahepatic cholestasis of pregnancy. *The Cochrane database of systematic reviews*, 2020, 7(7), Cd012546.
- [4] Gao, X.X.; Ye, M.Y.; Liu, Y, et al. Prevalence and risk factors of intrahepatic cholestasis of pregnancy in a Chinese population. *Scientific reports*, 2020, 10(1), 16307.
- [5] Zhu, N.; Hou, J. Molecular mechanism of the anti-inflammatory effects of *Sophorae Flavescentis* Aiton identified by network pharmacology. *Scientific reports*, 2021, 11(1), 1005.
- [6] Otero, C.; Klagges C, Morales B, et al. *Anti-Inflammatory Chilean Endemic Plants. Pharmaceutics*, 2023, 15(3).
- [7] Huang, S.J. et al. "Serum profiles of inflammatory cytokines associated with intrahepatic cholestasis of pregnancy." *The journal of maternal-fetal & neonatal medicine: the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstetricians*, 2022, 35(25), 10072-10081.
- [8] Zhang, M.L.; Yang, Q.; Zhu, Y.D. et al. Nobiletin Inhibits Hypoxia-Induced Placental Damage via Modulating P53 Signaling Pathway. *Nutrients*, 2022, 14(11).
- [9] Schmitz, K.; Wilken-Schmitz, A.; Vasic, V, et al. Progranulin deficiency confers resistance to autoimmune encephalomyelitis in mice. *Cellular & molecular immunology*. 2020, 17(10), 1077-1091.
- [10] Davies, N.M.; Holmes, M.V.; Davey, S.G. Reading Mendelian randomisation studies: a guide, glossary, and checklist for clinicians. *BMJ (Clinical research ed)*, 2018, 362:k601.
- [11] Smith, G.D.; Ebrahim, S. "Mendelian randomization": can genetic epidemiology contribute to understanding environmental determinants of disease? *International journal of epidemiology*, 2003, 32(1), 1-22.

- [12] Li, Y.; Sundquist, K.; Zhang, N, et al. Mitochondrial related genome-wide Mendelian randomization identifies putatively causal genes for multiple cancer types. *EBioMedicine*. 2018, 88, 104432.
- [13] Shen, H.H.; Zhang, Y.Y.; Wang, X.Y, et al. Potential Causal Association between Plasma Metabolites, Immunophenotypes, and Female Reproductive Disorders: a Two -Sample Mendelian Randomization Analysis. *Biomolecules*. 2024, 16(14), 1.
- [14] Zhao, J.H.; Stacey, D.; Eriksson, N, et al. Genetics of circulating inflammatory proteins identifies drivers of immune-mediated disease risk and therapeutic targets. *Nat Immunol*. 2023, 24(9), 1540-1551.
- [15] Ibrahim, S.H.; Jonas, M.M.; Taylor, S.A. et al. Liver Diseases in the Perinatal Period: Interactions Between Mother and Infant. *Hepatology (Baltimore, Md)*. 2020, 71(4), 1474-1485.
- [16] Lee, H.L.; Jang, J.W.; Lee, S.W, et al. Inflammatory cytokines and change of Th1/Th2 balance as prognostic indicators for hepatocellular carcinoma in patients treated with transarterial chemoembolization. *Scientific reports*. 2019, 9(1), 3260.
- [17] Hämäläinen, S.T.; Turunen, K.; Mattila, K.J, et al. Intrahepatic cholestasis of pregnancy and associated causes of death: a cohort study with follow-up of 27-46 years. *BMC women's health*, 2018, 18(1), 98.
- [18] Meng, Z.; Liao, Y.; Peng, Z, et al. Bone Marrow Mesenchymal Stem-Cell-Derived Exosomes Ameliorate Deoxynivalenol-Induced Mice Liver Damage. *Antioxidants (Basel, Switzerland)*. 2023, 12(3).
- [19] Shao, Y. et al. Effect of Histone Deacetylase HDAC3 on Cytokines IL-18, IL-12 and TNF- α in Patients with Intrahepatic Cholestasis of Pregnancy. *Cellular physiology and biochemistry*, 2017, 42(4), 1294-1302.
- [20] Nessler, N.; Launey, Y.; Aninat, C, et al. Clinical review: the liver in sepsis. *Critical care (London, England)*. 2012, 16(5), 235.
- [21] Alonso-Peña, M.; Espinosa-Escudero, R.; Hermanns, H.M, et al. Impact of Liver Inflammation on Bile Acid Side Chain Shortening and Amidation. *Cells*. 2022, 11(24).
- [22] Buryova, H.; Chalupsky, K.; Zbodakova, O, et al. Liver protective effect of ursodeoxycholic acid includes regulation of ADAM17 activity. *BMC gastroenterology*, 2013, 13(155).
- [23] Ovadia, Caroline et al. "Ursodeoxycholic acid in intrahepatic cholestasis of pregnancy: a systematic review and individual participant data meta-analysis." *The lancet. Gastroenterology & hepatology*, 2021, 6(7), 547-558.
- [24] Forssmann, U.; Delgado, M.B.; Uguccioni, M. et al. CKbeta8, a novel CC chemokine that predominantly acts on monocytes. *FEBS letters*. 1997, 408(2), 211-6.
- [25] Paital, B.; Das, K.; Parida, S.K. Inter national social lockdown versus medical care against COVID-19, a mild environmental insight with special reference to India. *The Science of the total environment*, 1997, 728, 138914.
- [26] Ramasubramanian, R.; Kalhan, R.; Jacobs, D.R. Jr. et al. Lung Function and Gene Expression of Pathogen Recognition Pathway Receptors: the Cardia Lung Study. *Scientific reports*. 1997, 10(1), 9360.

- [27] Lin, Z.; Zhang, Z.; Zheng, H, et al. Molecular mechanism by which CDCP1 promotes proneural-mesenchymal transformation in primary glioblastoma. *Cancer cell international*, 2022, 22(1), 151.
- [28] Lim, S.A.; Zhou, J.; Martinko, A.J. et al. Targeting a proteolytic neoepitope on CUB domain containing protein 1 (CDCP1) for RAS-driven cancers. *The Journal of clinical investigation*, 2022, 132(4).
- [29] Al-Awadhi, F.H.; Salvador, L.A, Law BK, et al. a Novel Marine-Derived Serine Protease Inhibitor Targeting Invasive Breast Cancer. *Marine drugs*, 2017, 15(9).



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