ImmunoTargets and Therapy Dovepress

Open Access Full Text Article ORIGINAL RESEARCH

The Effect of Circulating Inflammatory Proteins on Endometriosis: A Mendelian Randomization Study

Yunfang Wei[1,*], Xianlei Zhao[2,*], Linxia Li[1]

[1]Department of Obstetrics & Gynecology, Shanghai Seventh People's Hospital Affiliated to Shanghai University of Traditional Chinese Medicine, Shanghai, 200137, People's Republic of China; [2]School of Life Sciences, Fudan University, Shanghai, 200438, People's Republic of China

*These authors contributed equally to this work

Correspondence: Linxia Li, Email qiyuanfuchanke@126.com

Background: Endometriosis is a complex gynecological condition in which endometrial fragments are implanted outside the uterus, causing pain and infertility. Although immune mediators play a vital role in endometriosis, their exact etiology remains elusive. Using Mendelian randomization (MR), this study aimed to assess the causal relationship between inflammatory proteins and endometriosis.

Methods: Genetic variants associated with inflammatory proteins were filtered from a genome-wide protein quantitative trait locus study under stringent thresholds. These variants were used as instrumental variables (IVs) to evaluate the causal effects of these inflammatory proteins on endometriosis. A two-sample MR analysis was performed with endometriosis from the UK Biobank as the outcome, and a sensitivity analysis was performed to mitigate potential confounding factors. Analyses were replicated in an independent endometriosis cohort from the FinnGen, followed by a meta-analysis of MR results from both cohorts. Finally, we assessed the causality between inflammatory proteins and the endometriosis subtypes.

Results: Independent MR analysis revealed that the genetically higher levels of CXCL5 were linked to a lower chance of having endometriosis. The causal link remained significant in the meta-analysis. Furthermore, the causality of CXCL5 expression has been identified in ovarian and pelvic peritoneal endometriosis.

Conclusion: Our MR analysis indicated that CXCL5 was associated with a decreased risk of endometriosis, suggesting that CXCL5 might have a protective effect against endometriosis. This enhances our understanding of the involvement of chemokines in endometriosis pathology and provides insights for future studies to explore the detailed mechanisms underlying CXCL5 in endometriosis.

Keywords: endometriosis, inflammatory proteins, Mendelian randomization, CXCL5, causal inference

Introduction

Endometriosis (EMs) is a prevalent disorder characterized by endometrium-like tissues located outside the uterus.[1] It afflicts individuals with persistent pelvic pain, dysmenorrhea and infertility, impacting around 10% of women of childbearing age worldwide.[1,2] Despite multifactorial contributions to its pathogenesis, including hormonal, immunological, genetic and epigenetic factors, the precise etiology of EMs remains elusive.[3,4]

Numerous hypotheses have been proposed to explain endometriosis onset and progression. Among these, retrograde menstruation, in which endometrial fragments are shed into the pelvic cavity, is widely acknowledged to be a causative factor.[5] This ectopic debris attaches to distant tissues, circumvents immune clearance and proliferates into endometriotic lesions.[6] Recently, the immunological origins of endometriosis have gained increasing interest, as endometriotic fragments provoke a pro-inflammatory response in ectopic tissues.[7] Even under a heightened inflammatory response, endometriosis patients are unable to remove these lesions, hinting at deeper immune dysfunction underlying this phenomenon. Notably, the plasma from patients with endometriosis demonstrates dysregulation of inflammatory proteins.[8] For example, increased concentrations of pro-inflammatory cytokines, including IL-1β, TNF-α, and IL-6, have been observed in the peritoneal fluids and ectopic lesions of endometriotic individuals, thereby contributing to

Received: 21 August 2024
Accepted: 17 October 2024
Published: 1 November 2024

inflammation and fibrosis.[9–12] Conversely, cytokines produced in stressed tissues lead to perturbations at the endocrine, neuronal and immune levels, thus exerting an inevitable influence on the pathogenesis of endometriosis.[7] For instance, the Th1 cytokine milieus, which includes cytokines such as interferon-gamma (IFN-γ), has been shown to suppress the progression of endometriosis in murine models.[13] However, causal links between inflammatory mediators and endometriosis have not yet been determined. This inconsistency may stem from insufficient sample size, flaws in the research design and unmeasured confounding variables.

Mendelian randomization (MR) offers an alternative method for establishing causal relationships that are not easily discerned through conventional observational studies, particularly in cases where randomized controlled trials are impractical.[14] By utilizing randomly allocated genetic variants as instrumental variables (IVs), MR investigates the causal link between two factors, thereby mitigating confounding bias and reverse causality.[15,16] Here, we performed comprehensive MR analysis to elucidate the causal connections between inflammatory proteins and endometriosis. Our findings implicate CXCL5 in the etiology of endometriosis, with genetically elevated levels of CXCL5 inversely correlated with the risk of endometriosis.

Methods

Study Design

We performed two-sample MR analysis to assess the causal connections between inflammatory mediators and endometriosis. Single nucleotide polymorphisms (SNPs) were used as IVs based on three criteria: 1) direct association with exposure, 2) lack of association with potential confounding factors, and 3) effect on the outcome solely through exposure.[15] Figure 1 summarized the overall study design. Initially, the IVs for each inflammatory protein were extracted using a stringent threshold (p<5e-7, r2=0.001, kb=10000). Subsequently, we acquired endometriosis GWAS summary statistics from independent sources (UK Biobank and FinnGen) and performed separate MR analyses to explore causal associations. To enhance the robustness of the causal relationships between CXCL5 and endometriosis, we meta-analyzed MR results from both sources. Finally, we assessed the causal relationship between CXCL5 and five subtypes

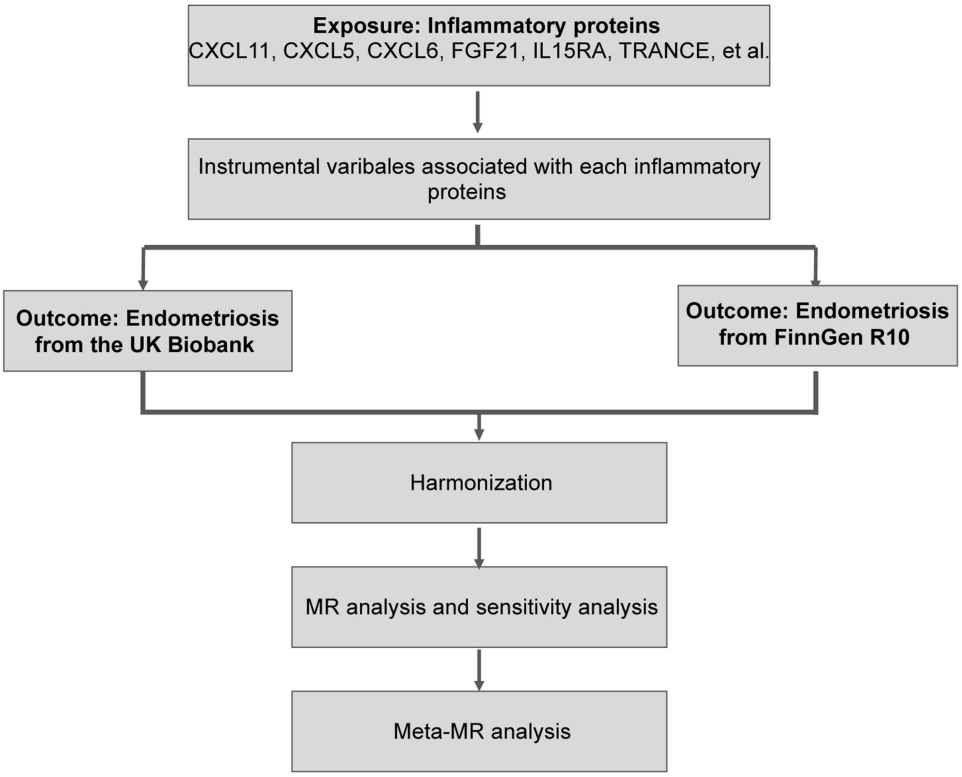

Figure 1 Overall design of this study in the form of a flow chart. The flowchart depicts how the MR analysis was conducted in a step-by-step manner.

of endometriosis: endometriosis of the ovary, intestine, pelvic peritoneum, fallopian tube, rectovaginal septum, and the vagina. This study adhered to STROBE-MR (Strengthening the reporting of observational studies in epidemiology using mendelian randomization) guideline[17,18] (Supplemental Table 1).

Data Sources

Genome-wide protein quantitative trait locus (pQTL) data for circulating inflammatory mediators were sourced from a recent study by Zhao et al, who identified multiple genetic variants influencing protein levels.[19] The GWAS data for endometriosis in European population (ukb-saige-615) was retrieved from Pheweb under the term of "endometriosis", a UK Biobank Single Variant Association Analysis by LeeLab.[20] Accordingly, endometriosis data in the FinnGen were obtained with the ID "finnGen-n14-endometriosis".[21] GWAS data from ukb-saige-615 comprised 4053 cases and 399757 controls, whereas the data from the FinnGen included 16588 cases and 111583 controls. Additionally, we acquired five different subtypes of endometriosis from the FinnGen: endometriosis of the intestine (492 cases and 111583 controls), ovary (6444 cases and 111583 controls), pelvic peritoneum (6237 cases and 111583 controls), fallopian tubes (239 cases and 111583 controls) and the rectovaginal septum and vagina (2687 cases and 111583 controls).

Detailed information on each phenotypic exposure and outcome is presented in Supplementary Table 2.

Selection of Instrumental Variables

At a threshold of p<5e-7, SNPs were selected for further analysis of 91 plasma inflammatory proteins. Linked disequilibrium clumping was performed to verify the independence of each SNPs under the stringent criteria (r2=0.001, window size=10000). Non-concordant and palindromic alleles were excluded during the harmonization of exposure and outcome. The remaining SNPs were considered instrumental variables. The PhenoScanner demonstrated no potential association between these instrumental variables and confounding factors. Furthermore, F-statistics were calculated for these instrumental variables, and none exhibited weak instrumental variable bias.[22,23] Detailed information on the eligible instrumental variables used for the MR analysis was provided in Supplementary Table 3.

Mendelian Randomization and Sensitivity Analysis

Two-sample Mendelian randomization analyses were performed between each inflammatory protein and endometriosis using the UK Biobank (UKB). We employed inverse variance weighting (IVW),[24] weighted median (WM),[25] and MR-Egger methods[26] to calculate the odds ratio, with IVW as the primary approach. Among the factors with P_{IVW} less than 0.05, six inflammatory proteins were identified, and their causal directions were consistent across the different methods (Supplementary Table 4). Statistical power was calculated to assess the robustness of each factor by using an online source (https://sb452.shinyapps). Cochran's Q was used to identify heterogeneity among the instrumental variables of these six factors, with a P-value > 0.05, indicating heterogeneity.[27] To address the potential effect of pleiotropy, MR-Egger was employed with P-value for intercept term below 0.05 suggesting significance. Furthermore, MR-PRESSO was employed to eliminate any outliers.[28] Steiger tests were incorporated to examine causal directions.[29] All MR analyses were performed using RStudio with TwoSampleMR (0.5.7).

Results

Screening of Inflammatory Proteins Associated with Endometriosis

To explore the effects of inflammatory mediators on endometriosis, two-sample MR analyses were performed. Briefly, we extracted SNPs from GWAS summary statistics of each inflammatory protein under a well-recognized threshold (p<5e-7, r2=0.001, kb=10000) and removed palindromic SNPs and those related to confounding factors according to the PhenoScanner. After excluding inflammatory proteins without qualified SNPs, 86 inflammatory proteins were identified as exposures, the instrumental variables of which all showed F-statistics > 10 (Supplementary Table 3). Subsequent two-sample MR analysis of these proteins and endometriosis revealed six inflammatory proteins at a significant P_{IVW} of 0.05, including CXCL11, CXCL5, CXCL6, FGF21, IL15RA and TNF-related activation induced cytokine (TRANCE).

Causal Effects of Inflammatory Mediators on Endometriosis

Based on GWAS summary statistics from the UKB, MR analysis was performed to estimate the effect of the six inflammatory mediators on endometriosis. All three approaches, IVW, MR-Egger, and weighted median, demonstrated the same causal direction, as presented in Figure 2. Surprisingly, the levels of three chemokines, CXCL11, CXCL5, and CXCL6, are associated with a reduced risk of endometriosis. Specifically, genetically elevated levels of CXCL5 (1 SD increase) were casually associated with 11.2% lower odds for the risk of endometriosis (IVW: OR=0.888, 95% CI: 0.797–0.989, P: 3.06e-2; WM: 0.906, 95% CI: 0.801–1.026, P: 1.20e-1; MR-Egger: 0.903, 95% CI: 0.761–1.072, P: 0.298) (Figure 2, and Supplementary Table 5). In addition, three other inflammatory factors were identified to be significantly associated with the risk of endometriosis via the IVW method, with FGF21 and TRANCE exhibiting a positive causal relationship, and IL15RA showing the opposite (Figure 2, and Supplementary Table 5). Among the sensitivity tests, Cochran's Q test revealed no heterogeneity in the risk of endometriosis for all six factors ($P_{heterogeneity}$ >0.05), and the MR-Egger test did not identify any pleiotropy ($P_{pleiotropy}$>0.05) (Supplementary Table 5). Moreover, the leave-one-out analysis demonstrated no potentially influential instrumental variables for CXCL5 (Supplementary Figure 1 and Supplementary Table 6). Through MR Steiger tests, we confirmed the consistency in causal directions of all six factors on endometriosis (All $P_{steiger}$<0.0001) (Supplementary Table 5). The leave-one-out analysis and scatter plots for 6 inflammatory proteins were visualized in Supplementary Figures 1 and 2.

In the replication analysis, GWAS data for endometriosis were acquired using the FinnGen R10. Circulating CXCL5 was confirmed to negatively affect endometriosis (IVW: OR=0.914, 95% CI: 0.862–0.968, P: 2.18e-3; WM: 0.912, 95% CI: 0.855–0.973, P: 5.13e-3; MR-Egger: 0.893, 95% CI: 0.814–0.981, P: 0.064) (Figure 3). No significant associations were found between CXCL11, CXCL6, FGF21, IL-15A, or TRANCE and the risk of endometriosis (Figure 3). We observed no heterogeneity or pleiotropy for CXCL5 and endometriosis using Cochran's Q and MR-Egger tests (Supplementary Table 7). The leave-one-out analysis did not identify any potentially influential SNP (Supplementary Table 8). The directionality of causal estimations was confirmed using the MR-Steiger test (Supplementary Table 7). Leave-one-out forest plots and scatter plots were constructed to illustrate the findings (Supplementary Figures 3 and 4). In conclusion, genetically predicted CXCL5 levels demonstrated a causal association with a reduced incidence of endometriosis, supported by both datasets.

Exposure	No.of SNP	Method	OR(95% CI)	P
CXCL11	9	Inverse variance weighted	0.821 (0.690 to 0.977)	0.026
		MR Egger	1.057 (0.744 to 1.503)	0.765
		Weighted median	0.826 (0.669 to 1.019)	0.075
CXCL5	7	Inverse variance weighted	0.888 (0.797 to 0.989)	0.031
		MR Egger	0.903 (0.761 to 1.072)	0.298
		Weighted median	0.906 (0.801 to 1.026)	0.120
CXCL6	3	Inverse variance weighted	0.880 (0.795 to 0.974)	0.014
		MR Egger	0.804 (0.665 to 0.971)	0.265
		Weighted median	0.877 (0.789 to 0.975)	0.015
FGF21	7	Inverse variance weighted	1.202 (1.018 to 1.420)	0.030
		MR Egger	1.648 (1.059 to 2.563)	0.078
		Weighted median	1.211 (0.978 to 1.498)	0.079
IL15RA	3	Inverse variance weighted	0.898 (0.807 to 0.998)	0.046
		MR Egger	0.885 (0.672 to 1.164)	0.542
		Weighted median	0.898 (0.800 to 1.006)	0.064
TRANCE	11	Inverse variance weighted	1.166 (1.023 to 1.328)	0.021
		MR Egger	1.266 (0.958 to 1.673)	0.132
		Weighted median	1.242 (1.051 to 1.467)	0.011

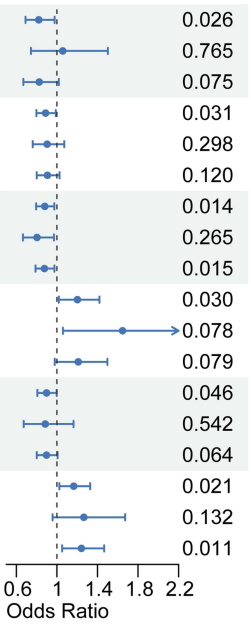

Figure 2 Causal estimates of six inflammatory proteins on endometriosis in the UK Biobank. Forest plots showing causal estimates of CXCL11, CXCL5, CXCL6, FGF21, IL15RA and TRANCE in endometriosis in the UK Biobank of European ancestry. The odds ratio (OR) was estimated using the IVW, MR-Egger, and weighted median methods. Horizontal bars represent 95% confidence intervals (CI). CXCL5 was highlighted in red text.

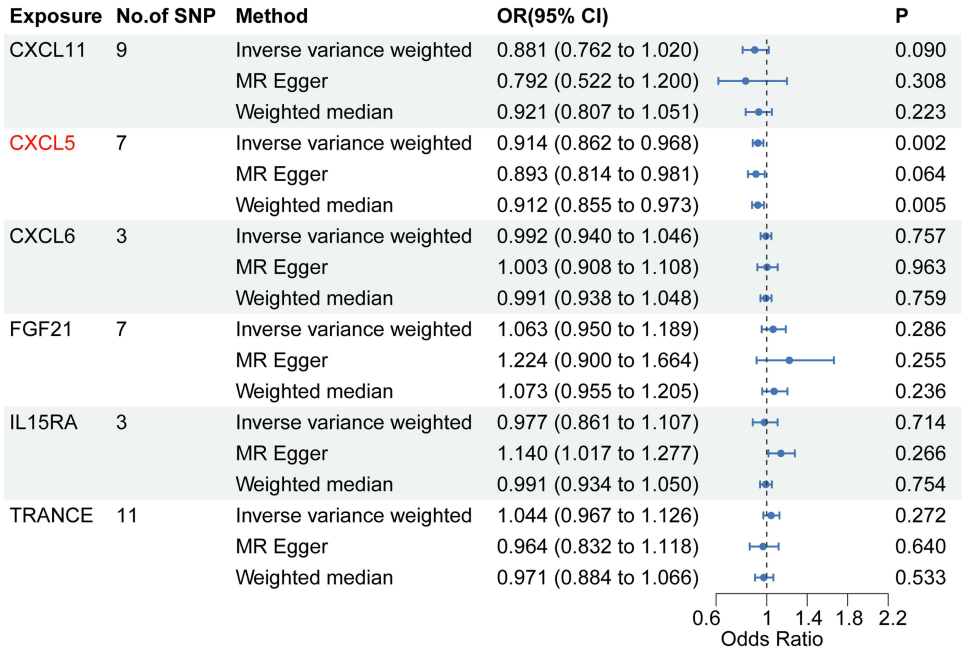

Figure 3 Causal estimates of six inflammatory proteins on endometriosis in the FinnGen. Forest plots showing causal estimates of CXCL11, CXCL5, CXCL6, FGF21, IL15RA and TRANCE in endometriosis in the FinnGen of European ancestry. The OR was estimated using the IVW, MR-Egger, and weighted median approaches. The horizontal bars represent 95% CI. CXCL5 was highlighted in red text.

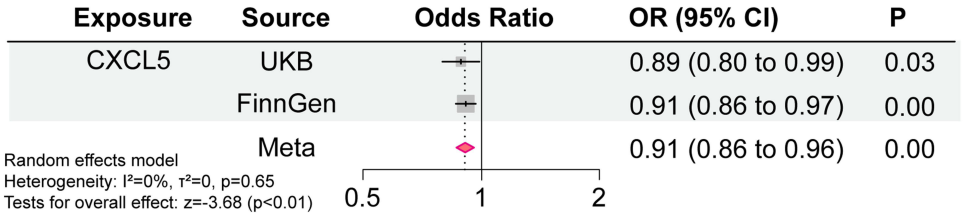

Figure 4 Causal estimates of six inflammatory proteins on endometriosis in a meta-analysis. Forest plots showing causal estimates of CXCL5 in endometriosis in a meta-analysis of the UK Biobank and FinnGen. The OR was estimated using IVW. The horizontal bars represent 95% CI.

To verify our findings, we meta-analyzed the MR results with outcomes from the UKB and FinnGen databases. These results corroborated the significant causal effects of CXCL5 on endometriosis (OR: 0.91, 95% CI: 0.86–0.96, P<0.01) (Figure 4). Based on these results, we concluded that genetically elevated CXCL5 levels are negatively associated with endometriosis.

Causal Effects of CXCL5 on Different Subtypes of Endometriosis

From the FinnGen R10 database, we obtained GWAS summary statistics for the five subtypes of endometriosis. Subsequently, an MR analysis was conducted to investigate the causality of CXCL5 expression in various endometriosis subtypes. The findings demonstrated significant negative effects of CXCL5 on ovarian and pelvic peritoneal endometriosis (OR: 0.879, 95% CI: 0.790–0.977, P: 0.017 for endometriosis of ovary; OR: 0.894, 95% CI: 0.801–0.997, P: 0.045 for endometriosis of pelvic peritoneum), as presented in the forest plot in Figure 5. In the Cochran's Q test, we observed no heterogeneity in the SNPs associated with CXCL5 and endometriosis of both ovary and pelvic peritoneum (Supplementary Table 9).

Discussion

Based on the published large-scale GWAS studies of European ancestry from independent sources, we investigated the causality between inflammatory mediators and endometriosis. Our findings uncovered a notable inverse relationship

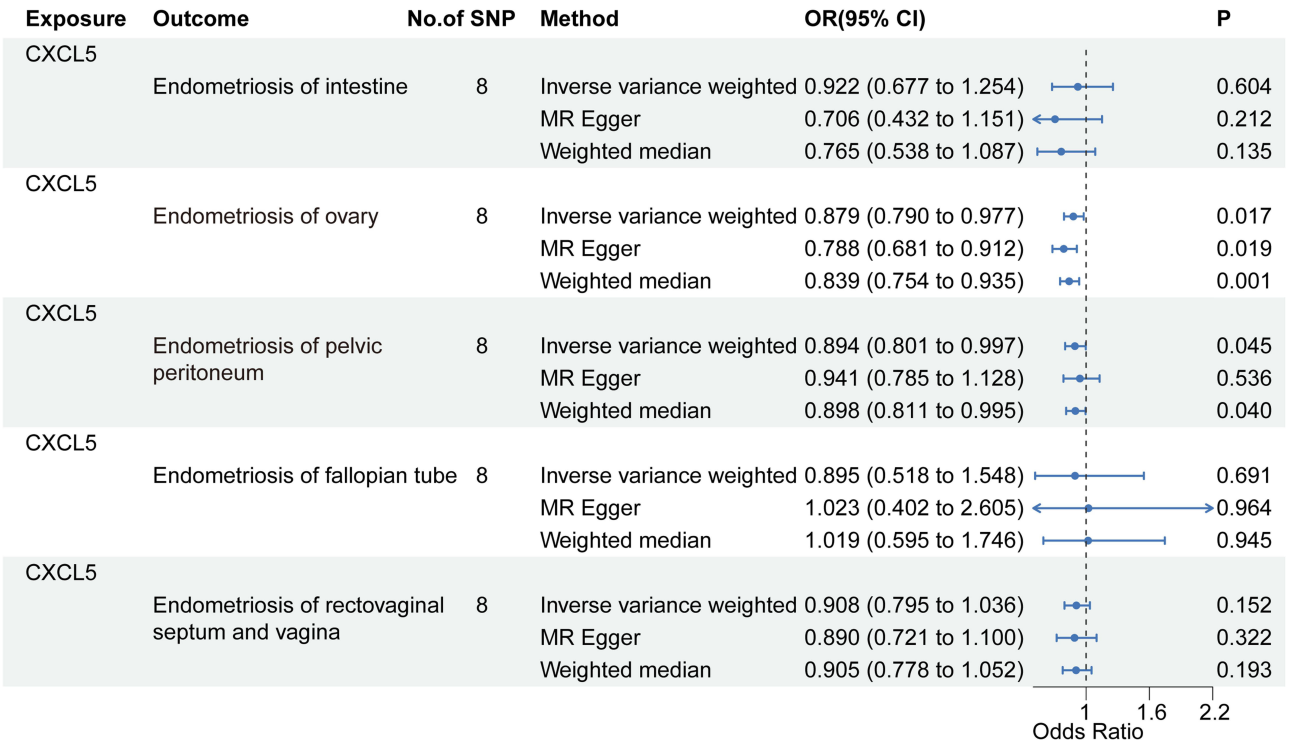

Figure 5 Causal estimates of six inflammatory proteins on endometriosis in a meta-analysis. Forest plots showing causal estimates of CXCL5 in different subtypes of endometriosis in the FinnGen of European ancestry. The OR was estimated using the IVW, MR-Egger, and weighted median approaches. The horizontal bars represent 95% CI.

between circulating CXCL5 levels and endometriosis. The causality was robustly validated through a meta-analysis of two independent MR analyses. Additionally, the investigation on the role of CXCL5 across the five subtypes of endometriosis showed a consistent causal direction, which further confirmed its protective effect. Our findings underscored the significance of modulating CXCL5 levels in the prevention and treatment of endometriosis.

Emerging evidence implies that an imbalanced interplay of immune cells and inflammatory proteins contributes to the formation of blood vessels and fibrous tissues in ectopic lesions, thereby hastening their implantation and progression.[30] Despite contradictory discoveries regarding immune factors in endometriosis, there is consensus that chemokines of the CXC family modulate immune cell recruitment and activation, thereby exerting a pivotal influence on the formation of ectopic lesions. The CXC chemokines encompass 17 types of cytokines, the majority of which have been implicated in endometriosis.[8] For instance, Ruiz et al discovered the involvement of the CXCL12-CXCR4/7 axis in endometriosis, with enhanced proliferation and movement of endometriotic cells.[31] Additionally, CXCL16 was found to be upregulated in women with endometriosis, and blocking the CXCL16/CXCR6 axis was shown to reduce the motility of endometriotic cells.[32] Similarly, an antagonist of the CXCL12 receptor (CXCR4), AMD3100, reduced endometriosis progression both in vitro and in vivo.[31,33] Consequently, an imbalance in inflammatory protein levels may reflect an overall change in the inflammatory status of endometriosis patients. However, the etiology and causal relationships underlying these correlations remain unclear.

This study represents the first comprehensive evaluation of the association between overall alterations in the inflammatory protein profile and endometriosis, using Mendelian randomization. The protective role of CXCL5 in endometriosis has been validated in two independent cohorts. Yet, these findings contradict earlier researches indicating that CXCL5 levels increase in both peritoneal fluid and blood in cases of deep infiltrating endometriosis (DIE).[34] Mice deficient in *Cxcr2*, the receptor for *Cxcl5*, exhibited diminished endometriotic lesions, suggesting a disease-promoting effect of the *Cxcl5/Cxcr2* axis in endometriosis.[35] Interestingly, the contradictory role of CXCL5 was in line with two independent MR studies on ulcerative colitits (UC), in which genetically heightened CXCL5 was correlated with a lower disease risk despite its strong upregulation in serum samples from patients with UC.[19,36] The protective role of CXCL5

extends beyond that in endometriosis and UC. Cui et al revealed CXCL5 as a protective factor in breast cancer.[37] Therefore, it would be intriguing to investigate the underlying mechanism of CXCL5 in the inhibition of multiple diseases characterized by enhanced inflammatory responses. CXCL5, initially known as a neutrophil-activating peptide, is secreted by various cell types including epithelial cells,[38] endothelial cells,[39] macrophages,[40] smooth muscle cells.[41] CXCL5 attracts neutrophils and monocytes through its receptors CXCR1 and CXCR2, and modulates neutrophil homeostasis at mucosal sites.[42] Additionally, CXCL5 is also critical for B cell accumulation in inflamed tissues.[43] The seemingly paradoxical role of CXCL5 could be explained by neutrophils which maintain innate immune surveillance under normal conditions, but contribute to tissue damage once inflammation initiates. In the case of endometriosis, we propose that reduced CXCL5 levels impair neutrophil homeostasis and facilitates immune evasion of endometriotic cells, leading to their subsequent implantation. However, the exact etiology of CXCL5 in endometriosis requires further investigation.

Nonetheless, some limitations have to be addressed for this study. Firstly, the number of instrumental variables used in the analysis ranged from three to nine, which could potentially impact the robustness of the MR findings. However, this is unlikely to mislead the study, as the F-statistics of all instrumental variables exceeded 10, suggesting a minimal risk of weak instrumental bias. Secondly, it is worth mentioning that the GWAS study on inflammatory proteins included both female and male volunteers. Thus, it is important to acknowledge potential bias, given that our two-sample MR study was conducted on a population background that may not be consistent across genders. Finally, our findings are not representative of other populations, as they were based solely on European ancestry. Therefore, cautions should be exercised when extending our conclusions to populations of different ancestral backgrounds.

Conclusions

In conclusion, our study have provided evidence of causality between inflammatory mediators and endometriosis through an extensive two-sample MR analysis, punctuating the intricacy between immune system and endometrium. Specifically, our findings support the causal inference that circulating CXCL5 levels reduce endometriosis risk. Further research is warranted to deepen our understanding of the involvement of CXCL5 in the pathogenesis of endometriosis and its therapeutic implications.

Data Sharing Statement

The GWAS summary statistics for inflammatory proteins can be accessed via the supplementary materials of the article.[19] The GWAS summary statistics for endometriosis was available on Leelab Pheweb (https://pheweb.org/UKB-TOPMed/pheno/615) for the UK Biobank cohort and on the FinnGen R10 (https://r10.risteys.finngen.fi/) for the FinnGen cohort.

Ethics Statement

According to item 1 and 2 of Article 32 of "the Measures for Ethical Review of Life Science and Medical Research Involving Human Subjects", this study is exempt from ethical review and approval, as it utilized summary statistics from public GWAS studies.

Acknowledgments

Yunfang Wei and Xianlei Zhao are co-first authors for this study. We thank the MendelR team for their technical support in this study.

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.

Funding

This study was funded by Key Specialty Construction Project of Pudong Health and Family Planning Commission of Shanghai (Grant No. 2022-14).

Disclosure

The authors declare that they have no competing interest for this work.

References

1. Taylor HS, Kotlyar AM, Flores VA. Endometriosis is a chronic systemic disease: clinical challenges and novel innovations. *Lancet*. 2021;397 (10276):839–852. doi:10.1016/S0140-6736(21)00389-5
2. Girling JE. Harnessing the inflammatory processes in endometriosis. *Nat Rev Endocrinol*. 2024;20(2):69–70. doi:10.1038/s41574-023-00937-x
3. Wang Y, Nicholes K, Shih IM. The origin and pathogenesis of endometriosis. *Annual Rev Pathol*. 2020;15:71–95. doi:10.1146/annurev-pathmechdis-012419-032654
4. Saunders PTK, Horne AW. Endometriosis: etiology, pathobiology, and therapeutic prospects. *Cell*. 2021;184(11):2807–2824. doi:10.1016/j.cell.2021.04.041
5. Zondervan KT, Becker CM, Missmer SA. Endometriosis. *New Engl J Med*. 2020;382(13):1244–1256. doi:10.1056/NEJMra1810764
6. Christodoulakos G, Augoulea A, Lambrinoudaki I, Sioulas V, Creatsas G. Pathogenesis of endometriosis: the role of defective 'immunosurveillance'. *European J Contracep Reprod Health Care*. 2007;12(3):194–202. doi:10.1080/13625180701387266
7. Lamceva J, Uljanovs R, Strumfa I. The main theories on the pathogenesis of endometriosis. *Int J Mol Sci*. 2023;24(5):4254. doi:10.3390/ijms24054254
8. Ullah A, Wang MJ, Wang YX, Shen B. CXC chemokines influence immune surveillance in immunological disorders: polycystic ovary syndrome and endometriosis. *Biochimica Et Biophysica Acta Mol Basis Dis*. 2023;1869(5):166704. doi:10.1016/j.bbadis.2023.166704
9. Carmona F, Chapron C, Martínez-Zamora M, et al. Ovarian endometrioma but not deep infiltrating endometriosis is associated with increased serum levels of interleukin-8 and interleukin-6. *J Reprod Immunol*. 2012;95(1–2):80–86. doi:10.1016/j.jri.2012.06.001
10. Matteo M, Cicinelli E, Neri M, et al. Pro-inflammatory M1/Th1 type immune network and increased expression of TSG-6 in the eutopic endometrium from women with endometriosis. *European J Obstetrics Gynecol Reprod Biol*. 2017;218:99–105. doi:10.1016/j.ejogrb.2017.08.014
11. Malutan AM, Drugan T, Costin N, et al. Pro-inflammatory cytokines for evaluation of inflammatory status in endometriosis. *Central-European j Immunol*. 2015;40(1):96–102. doi:10.5114/ceji.2015.50840
12. Gui C, Wei J, Mo C, et al. Therapeutic implications for localized prostate cancer by multiomics analyses of the ageing microenvironment landscape. *Int J Biol Sci*. 2023;19(12):3951–3969. doi:10.7150/ijbs.85209
13. Cho YJ, Lee SH, Park JW, Han M, Park MJ, Han SJ. Dysfunctional signaling underlying endometriosis: current state of knowledge. *J Mol Endocrinol*. 2018;60(3):R97–r113. doi:10.1530/JME-17-0227
14. Sanderson E, Glymour MM, Holmes MV, et al. Mendelian randomization. *Nat Rev Meth Primers*. 2022;2. doi:10.1038/s43586-021-00092-5.
15. Davies NM, Holmes MV, Davey Smith G. Reading Mendelian randomisation studies: a guide, glossary, and checklist for clinicians. *BMJ*. 2018;362:k601. doi:10.1136/bmj.k601
16. Verduijn M, Siegerink B, Jager KJ, Zoccali C, Dekker FW. Mendelian randomization: use of genetics to enable causal inference in observational studies. *Nephrol Dial Transplant*. 2010;25(5):1394–1398. doi:10.1093/ndt/gfq098
17. Skrivankova VW, Richmond RC, Woolf BAR, et al. Strengthening the reporting of observational studies in epidemiology using Mendelian randomization: the STROBE-MR statement. *JAMA*. 2021;326(16):1614–1621. doi:10.1001/jama.2021.18236
18. Skrivankova VW, Richmond RC, Woolf BAR, et al. Strengthening the reporting of observational studies in epidemiology using mendelian randomisation (STROBE-MR): explanation and elaboration. *BMJ*. 2021;375:n2233. doi:10.1136/bmj.n2233
19. Zhao JH, Stacey D, Eriksson N, et al. Genetics of circulating inflammatory proteins identifies drivers of immune-mediated disease risk and therapeutic targets. *Nature Immunol*. 2023;24(9):1540–1551. doi:10.1038/s41590-023-01588-w
20. Zhou W, Nielsen JB, Fritsche LG, et al. Efficiently controlling for case-control imbalance and sample relatedness in large-scale genetic association studies. *Nature Genet*. 2018;50(9):1335–1341. doi:10.1038/s41588-018-0184-y
21. Elsworth B, Lyon M, Alexander T, et al. The MRC IEU OpenGWAS data infrastructure. BioRxiv. 2020;2020:1.
22. Lawlor DA, Harbord RM, Sterne JA, Timpson N, Davey Smith G. Mendelian randomization: using genes as instruments for making causal inferences in epidemiology. *Stat Med*. 2008;27(8):1133–1163. doi:10.1002/sim.3034
23. Li B, Martin EB. An approximation to the F distribution using the chi-square distribution. *Computat Stat Data Anal*. 2002;40(1):21–26. doi:10.1016/S0167-9473(01)00097-4
24. Burgess S, Small DS, Thompson SG. A review of instrumental variable estimators for Mendelian randomization. *Stat Meth Med Res*. 2017;26 (5):2333–2355. doi:10.1177/0962280215597579
25. Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent estimation in Mendelian randomization with some invalid instruments using a weighted median estimator. *Genetic Epidemiol*. 2016;40(4):304–314. doi:10.1002/gepi.21965
26. Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. *Int J Epidemiol*. 2015;44(2):512–525. doi:10.1093/ije/dyv080
27. Greco MF, Minelli C, Sheehan NA, Thompson JR. Detecting pleiotropy in Mendelian randomisation studies with summary data and a continuous outcome. *Stat Med*. 2015;34(21):2926–2940. doi:10.1002/sim.6522
28. Verbanck M, Chen CY, Neale B, Do R. Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. *Nat Genet*. 2018;50(5):693–698. doi:10.1038/s41588-018-0099-7
29. Hemani G, Tilling K, Davey Smith G. Orienting the causal relationship between imprecisely measured traits using GWAS summary data. *PLoS Gene*. 2017;13(11):e1007081. doi:10.1371/journal.pgen.1007081

30. Li W, Lin A, Qi L, et al. Immunotherapy: a promising novel endometriosis therapy. *Front Immunol*. 2023;14:1128301. doi:10.3389/fimmu.2023.1128301
31. Ruiz A, Ruiz L, Colón-Caraballo M, et al. Pharmacological blockage of the CXCR4-CXCL12 axis in endometriosis leads to contrasting effects in proliferation, migration, and invasion. *Biol Reprod*. 2018;98(1):4–14. doi:10.1093/biolre/iox152
32. Peng Y, Ma J, Lin J. Activation of the CXCL16/CXCR6 axis by TNF-α contributes to ectopic endometrial stromal cells migration and invasion. *Reprod Sci*. 2019;26(3):420–427.
33. Zhang W, Li X, Li H, et al. 17β-estradiol promotes bone marrow mesenchymal stem cell migration mediated by chemokine upregulation. *Biochem Biophys Res Commun*. 2020;530(2):381–388. doi:10.1016/j.bbrc.2020.07.135
34. Perricos A, Husslein H, Kuessel L, et al. Does the use of the "Proseek(®) multiplex inflammation I panel" demonstrate a difference in local and systemic immune responses in endometriosis patients with or without deep-infiltrating lesions? *Int J Mol Sci*. 2023;24(5). doi:10.3390/ijms24055022.
35. Zhang T, Zhou J, Man GCW, et al. MDSCs drive the process of endometriosis by enhancing angiogenesis and are a new potential therapeutic target. *European J Immunol*. 2018;48(6):1059–1073. doi:10.1002/eji.201747417
36. Chen J, Xu F, Ruan X, et al. Therapeutic targets for inflammatory bowel disease: proteome-wide Mendelian randomization and colocalization analyses. *EBioMedicine*. 2023;89:104494. doi:10.1016/j.ebiom.2023.104494
37. Cui K, Song N, Fan Y, et al. A two-sample Mendelian randomization analysis: causal association between chemokines and pan-carcinoma. *Front Gene*. 2023;14:1285274. doi:10.3389/fgene.2023.1285274
38. Walz A, Burgener R, Car B, Baggiolini M, Kunkel SL, Strieter RM. Structure and neutrophil-activating properties of a novel inflammatory peptide (ENA-78) with homology to interleukin 8. *J Exp Med*. 1991;174(6):1355–1362. doi:10.1084/jem.174.6.1355
39. Sun N, Chu B, Choi DH, Lim L, Song H. ETV2 enhances CXCL5 secretion from endothelial cells, leading to the promotion of vascular smooth muscle cell migration. *Int J Mol Sci*. 2023;24(12):9904.
40. Lin D, Kang X, Shen L, et al. Efferocytosis and Its associated cytokines: a light on non-tumor and tumor diseases? *Mol Ther Oncolytics*. 2020;17:394–407. doi:10.1016/j.omto.2020.04.010
41. Chang TT, Liao LY, Chen JW. Inhibition on CXCL5 reduces aortic matrix metalloproteinase 9 expression and protects against acute aortic dissection. *Vasc Pharmacol*. 2021;141:106926. doi:10.1016/j.vph.2021.106926
42. Mei J, Liu Y, Dai N, et al. Cxcr2 and Cxcl5 regulate the IL-17/G-CSF axis and neutrophil homeostasis in mice. *J Clin Invest*. 2012;122(3):974–986. doi:10.1172/JCI60588
43. Guo L, Li N, Yang Z, et al. Role of CXCL5 in regulating chemotaxis of innate and adaptive leukocytes in infected lungs upon pulmonary influenza infection. *Front Immunol*. 2021;12:785457. doi:10.3389/fimmu.2021.785457