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ORIGINAL RESEARCH

Genetic Association and Drug Target Exploration Between Inflammation-Related Proteins and the Risk of Primary Ovarian Insufficiency

Chong Wang[1], Xian-Hua Lin[2], Xiao-Jing Yang[1]

[1]Department of Reproductive Medicine, Hangzhou Women's Hospital, Hangzhou, Zhejiang Province, People's Republic of China; [2]Women and Children's Medical Center, Shenzhen Maternity and Child Healthcare Hospital, Southern Medical University, Shenzhen, Guangdong Province, People's Republic of China

Correspondence: Xian-Hua Lin, Women and Children's Medical Center, Shenzhen Maternity and Child Healthcare Hospital, Southern Medical University, Shenzhen, Guangdong Province, People's Republic of China, Email xl_1290@126.com; Xiao-Jing Yang, Department of Reproductive Medicine, Hangzhou Women's Hospital, Hangzhou, Zhejiang Province, People's Republic of China, Email moving_yang@163.com

Purpose: Primary ovarian insufficiency (POI) exhibits ovarian dysfunction characteristics, which develops from diminished ovarian reserve (DOR). However, the pathogenesis remains unclear. In this study, we aimed to analyze the causal relationship between inflammation-related proteins and the occurrence of POI at the genetic level, and to identify potential druggable gene targets from inflammation-related genes.

Patients and Methods: We conducted a Mendelian randomization (MR) analysis to explore causal association for inflammation-related proteins and POI. Genetic instruments for 91 inflammation-related proteins were derived from the Olink® Target Inflammation panel with totally 14,824 European participants. Summary statistics for 424 POI cases and 118,796 controls were acquired from the FinnGen. Furthermore, by combining the Olink results from DOR patients with MR results, we highlighted five inflammation-related moleculars in ovarian aging. All were validated by Western-blot and RT-PCR in the POI model. Bioinformatics analysis was performed to reveal potential pathways, and potential drug screening was performed by the DGIdb database.

Results: Via inverse-variance weighted (IVW) method, our study identified two proteins, CXCL10 and CX3CL1 might exert protective effects against POI; whereas IL-18R1, IL-18, MCP-1, and CCL28 might increase the risk of POI. Moreover, Wald ratio analyses highlighted additional protective proteins, such as IL-17C, TRANCE, uPA, LAP TGF-β1, and CXCL9; along with risk proteins, including TNFSF14, CD40, IL-24, ARTN, LIF-R, and IL-2RB. Meanwhile, MCP-1/CCL2, TGFB1, ARTN, and LIFR were significantly changed in the POI model, which converged in the oncostatin M signaling pathway. Notably, gene-drug analysis identified CCL2 and TGFB1 as potential therapeutic targets, whereas genistein and melatonin were prioritized as potential drugs for POI treatment.

Conclusion: Our study highlights the causal role of specific inflammation-related proteins in POI, advancing our understanding of its etiology, and further extends the therapeutic options for improving ovarian function and delaying POI onset.

Keywords: primary ovarian insufficiency, inflammation-related proteins, mendelian randomization, gene-drug analysis

Introduction

Primary ovarian insufficiency (POI) is a unique example of isolated organ senescence that manifests as primary or secondary amenorrhoea that occurs before the age of 40.[1] Patients with POI exhibit ovarian dysfunction characteristics such as reduced follicular numbers, which stem from diminished ovarian reserve (DOR).[2] Menstrual irregularities, reduced fertility, hot flushes, night sweats, alongside osteoporosis, significantly threaten both the physiological and psychological health of POI women. However, despite the widespread recognition of the clinical presentation of POI, the underlying mechanisms remain unclear. In addition, the clinical treatment for POI is limited. The main available treatments, including hormone replacement therapy (HRT, estrogen, and progesterone supplementation), fertility intervention programs (assisted reproductive technology with donated oocytes and ovarian tissue freezing), and supportive care (calcium and vitamin D supplementation, lifestyle modification, and psychological counselling), do not provide a good prognosis for patients with POI.[3,4]

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In recent years, studies have shown that inflammatory response plays a critical role in the development of various ovarian diseases, including POI. Inflammatory factors may influence ovarian function by regulating the immune system and cellular signaling pathways, thereby affecting female reproductive health.[5,6] Huang et al[7] further proposed that cytokines regulated by nuclear factor-κB (NF-κB), including IL-1β, IL-2, and IL-6, play a significant role in the pathogenesis of POI. Therefore, investigating the role of inflammation-related factors in POI is important for understanding its pathogenesis, and may provide potential therapeutic targets for clinical treatment.

Traditional observational studies, although helpful in revealing associations between inflammatory factors and POI, are unable to establish causal relationships. To further clarify the causal links between inflammatory factors and POI, Mendelian Randomization (MR) offers a powerful tool. MR utilizes genetic variants associated with exposure (such as inflammatory factors) as instrumental variables (IVs), allowing for causal inference while minimizing confounding bias,[8,9] making it an excellent tool for studying the association between genetic variations and diseases. On this basis, searching for drug targets at the genetic level will provide great assistance for the development of new drug targets for POI and the protection of female fertility.

This study aims to use MR method to explore the causal relationship between inflammation-related factors and POI, and identify potential therapeutic targets by gene-drug analysis. By analyzing data from large-scale genome-wide association studies (GWAS),[10] we derived genetic variants from 91 inflammatory proteins and assessed their potential impact on POI. Additionally, through combined analysis of Olink proteomic results from DOR patients and genetic validation in POI model, we attempted to reveal the key genes/proteins responsible for the altered inflammatory state in ovarian dysfunction and ovarian aging. Finally, through gene-drug analysis and bioinformatics analysis, we gained the prioritized druggable gene targets, potential mechanisms and pathways for novel therapeutic strategies. This study seeks to provide new insights into the biological basis of POI and offer a foundation for identifying potential therapeutic targets.

Materials and Methods

Study Design

The overall structure of the study is shown in Figure 1. For non-interventional studies, ethical approval is not required due to national laws (item 1 and 2 of Article 32 of the Measures for Ethical Review of Life Science and Medical Research Involving Human Subjects dated February 18, 2023, China).

The basic principle of MR research is to use genetic variations associated with exposure and outcome as IVs to determine whether a causal relationship exists between the two. We proposed three indispensable MR assumptions: (1) genetic tools are associated with exposure, (2) genetic variation is independent of any confounding factors, and (3) genetic tools only affect outcomes through risk variables.[9]

Data Source for Inflammation-Related Proteins

The update GWAS data for 91 inflammation-related proteins were acquired from 14,824 European participants of 11 cohorts. By measuring genome-wide genetic data and plasma proteomics data with the Olink® Target Inflammation panel, Zhao et al[10] generated the inflammation-related proteins. A linear regression-based additive genetic association model was applied to each cohort for GWAS analysis. Detailed quality control information are available in the original literature.

Data Source for POI

The FinnGen Consortium (FinnGen) provided GWAS summary statistics on the risk of POI in 424 Finnish adult female cases and 118,796 controls.[11] The diagnosis of POI was determined according to the International Classification of Diseases-10 (ICD-10), which classifies "primary ovarian failure" into subcategory E28.3 of "ovarian dysfunction" (E28).

Selection of Instrumental Variables

To meet the MR assumptions, we identified SNPs associated with inflammation-related proteins that had genome-wide significance at a threshold of P-value$<5\times10^{-8}$. We also applied clustering based on linkage disequilibrium at 10,000 kb

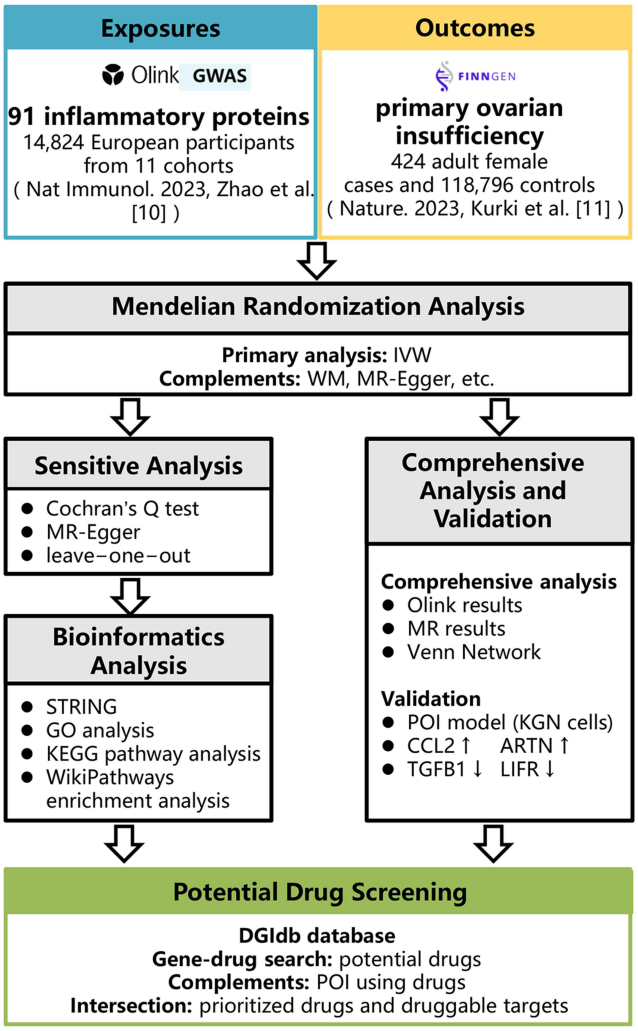

Figure 1 The workflow of MR investigation and potential drug screening for inflammation-related proteins with POI.
Abbreviations: IVW, inverse-variance weighted; WM, weighted median; MR, mendelian randomization; POI, primary ovarian insufficiency.

with R^2<0.001 to ensure IV isolation. All IVs were estimated using the F-statistic to avoid instrumental bias, and SNPs with F-statistics < 10 were excluded.[12]

Statistical Analysis

We explored the causal relationship between inflammation-related proteins and POI by performing two-sample MR analysis. The IVW method delivered consistent estimates of the causal effects for all genetic variants as the primary approach. Wald ratio, weighted median (WM), MR-Egger, simple mode, and weighted mode method were also performed to estimate the causal effects between inflammation-related proteins and POI.

To assess whether independent effects between genetic variables would violate the assumptions of the MR analysis,[13] we also performed sensitivity analyses. Cochran's Q test was implemented to quantify heterogeneity, P>0.05 represents no heterogeneity, P<0.05 indicates the possibility of intergenic heterogeneity. Horizontal pleiotropy detection adopt methods as MR-Egger method (commonly represented by the intercept of the MR-Egger) and MR-PRESSO global test. The intercept of MR-Egger test represents potential bias in effect estimation, and a P value<0.05 indicates horizontal pleiotropy of IVs. The ideal result of the "leave-one-out" (LOO) approach should be that discarding each SNP in turn, the result does not change the result significantly, demonstrating the reliability and robustness of the MR analysis.

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Our specific implementation process is as follows. The MR program and sensitivity analysis were implemented using the "TwoSampleMR" software package in R software. The Bonferroni adjustment was used to calculate the significance thresholds for multiple tests. Inflammation-related proteins with *P*-value less than 1e-04 were defined as significant. For POI complications, the significance threshold for an adjusted *P*-value was 1e-03. In sensitivity analysis tests, a two-tailed *P*-values <0.05 were considered statistically significant.

Comprehensive Analysis and Experimental Validation

The Olink proteomics results of DOR patients were derived from our previous findings,[14] and the proteomics results were crossed with this MR results using Wekemo Bioincloud (https://www.bioincloud.tech).[15]

Human granulosa-like tumor cell lines (KGNs, iCell-h298, icell bioscience Inc, Shanghai, China) were cultured in RPMI 1640 medium and cultivated at 37 °C with 5% CO_2. KGNs cells were treated with 1 mg/mL cyclophosphamide (CTX, F403282; felixbio, Shanghai, China) for 48 h to model POI.[16,17]

Western-blot analysis was used to measure the protein levels as previously described.[18] Some of the primary antibodies were purchased from Proteintech Technology (Wuhan, China), including anti monocyte chemotactic protein 1 (MCP-1) antibody (29547-1-AP, 1:1000), anti leukemia inhibitory factor receptor (LIF-R) antibody (22779-1-AP, 1:500), and anti GAPDH antibody (60004-1-Ig, 1:50,000). Anti growth factor beta-1 (TGF-β1) antibody (bs-0086R, 1:1000), anti tumor necrosis factor ligand superfamily member 14 (TNFSF14) antibody (bs-2462R, 1:500), and anti artemin (ARTN) antibody (bs-22203R, 1:500) were purchased from Bioss Technology (Beijing, China). Secondary antibodies as goat anti-mouse IgG-HRP (A21010, Abbkine, Georgia, USA; 1:10000) and goat anti-rabbit IgG-HRP (A21020, Abbkine, Georgia, USA; 1:10000) were also used. All original gel images were represented in Supplementary Figure 1.

Total RNA was extracted using the TRIzol method and quantified using the Nanodrop 2000 (ThermoFisher Scientific, MA, USA). The SeqHunt® First Strand cDNA Synthesis Kit and 2x Blue universal SYBR qPCR master mix (CA01, AF07; Seq-Hunt Biotechnology) were used to measure mRNA levels of each gene. Table 1 lists the primer sequences used in this study.

Bioinformatics Analysis

We analyzed protein-protein network by STRING software (http://string-db.org/). After gene annotation, cellular components, molecular functions, and biological processes were analyzed using the Gene Ontology (GO) database. Kyoto Encyclopedia of Genes and Genomes (KEGG, http://www.kegg.jp/) pathway analysis was performed using the KEGG database (false discovery rate<5.00%), whereas WikiPathways enrichment analysis (https://www.wikipathways.org/index.php/WikiPathways) was also performed.

Table 1 List of Primer Sequences Used for RT-PCR

Name	Sequence (5'-3')
GAPDH	GGAGCGAGATCCCTCCAAAAT
	GGCTGTTGTCATACTTCTCATGG
TGFB1	GAGAAGCGGTACCTGAACCC
	TGAACCCGTTGATGTCCACT
CCL2	GCCAGATGCAATCAATGCCC
	GGTTTGCTTGTCCAGGTGGT
TNFSF14	GGTCACCAAAGCTGGCTACT
	CTCCAGGTGTACCACACCAC
LIFR	GTTACCACCTGGTCTTGCGA
	AAGAGCACTGCTTCCCTCAC
ARTN	ACACCCGAGCTGCCTCAA
	CTCAGCAGAGCCAGAGCG

Potential Drug Screening

The Drug-Gene Interaction Database (DGIdb, https://dgidb.org) is a publicly accessible resource which brings together gene or gene product, drug, and drug-gene interaction records, and it is widely used in drug target exploration.[19] We searched four validated potential genes that were significant for POI using the DGIdb database for potential drugs. By matching the druggable genes of potential drugs with their targets of using drugs, we prioritized potential drugs. A Sankey plot was created by the SankeyMATIC software to visualize the association between drugs and druggable targets (top 20 druggable targets, https://www.sankeymatic.com/).

Results

Causality of Inflammation-Related Proteins on POI

A total of 99 SNPs corresponding to 91 inflammatory proteins were included in this study. We identified 6 inflammatory proteins underlying a potential causal association with POI in a multiplicative random-effects model of the IVW method (Table 2), and other 11 inflammatory proteins by the Wald ratio method (Table 3) adopting previous findings.[10] Specifically, there was a negative causal relationship between two inflammatory proteins and POI using the IVW method, such as C-X-C motif chemokine 10 (CXCL10) (OR=0.205, 95% CI=0.097–0.043, $P<0.0001$) and fractalkine (CX3CL1) (OR=0.397, 95% CI=0.272–0.578, $P<0.0001$), indicating that CXCL10 and CX3CL1 may have a protective effect on POI. Furthermore, four inflammatory proteins were associated with an increased risk of POI:, including interleukin-18 receptor 1 (IL-18R1) (OR=1.896, 95% CI=1.774–2.025, $P<0.0001$), interleukin-18 (IL-18) (OR=2.924, 95% CI=2.360–3.621, $P<0.0001$), MCP-1 (OR=3.037, 95% CI=2.383–3.871, $P<0.0001$), and C-C motif chemokine 28 (CCL28) (OR=3.797, 95% CI=2.760–5.224, $P<0.0001$). The Wald ratio method was used for exposure to single SNP. Five inflammatory proteins associated with a reduced risk of POI were determined, including interleukin-17C (IL-17C) (OR=0.021, 95% CI=0.002–0.186, $P=0.0005$), TNF-related activation-induced cytokine (TRANCE) (OR=0.044, 95% CI=0.008–0.259, $P=0.0005$), urokinase-type plasminogen activator (uPA) (OR=0.119, 95% CI=0.038–0.375, $P=0.0003$), latency-associated peptide transforming growth factor beta-1 (LAP TGF-β1) (OR=0.138, 95% CI=0.042–0.447, $P=0.0010$), and C-X-C motif chemokine 9 (CXCL9) (OR=0.213, 95% CI=0.088–0.519, $P=0.0007$). In contrast, six other inflammatory proteins were found to be associated with an elevated risk of POI, including TNFSF14 (OR=2.008, 95% CI=1.352–2.983, $P=0.0005$), CD40L receptor (CD40) (OR=2.401, 95% CI=1.485–3.880, $P=0.0003$), interleukin-24 (IL-24) (OR=3.966, 95% CI=1.848–8.510, $P=0.0004$), ARTN (OR=5.628, 95% CI=2.024–15.646, $P=0.0009$), LIF-R (OR=8.300, 95% CI=2.363–29.159, $P=0.0010$), and interleukin-2 receptor subunit beta (IL-2RB) (OR=9.589, 95% CI=2.525–36.407, $P=0.0009$).

Sensitivity Analysis

We further assessed the robustness of the IVW results through sensitivity analyses. For 6 inflammation-related proteins excavated by IVW, no clear evidence of horizontal pleiotropy was found by MR-Egger intercept test ($P>0.05$; Table 4). And Cochran's Q-test also showed no heterogeneity among the IVs ($P>0.05$; Table 4), indicating that the estimated results were robust. For CXCL10, MCP-1, and IL-18R1, the direction of the SNP effect for the 5 MR method was consistent (Figure 2A–C) in the scatter plots, whereas for IL-18 and CCL28, the SNP effect direction of the MR Egger method was different (Figure 2D and E). Since there was only one CX3CL1 SNP in our study, only the IVW method was used for sensitivity analysis (Figure 2F). Because of the insignificant results of the MR method (Table 2), we explored the SNP effects by the IVW method. LOO analysis was also taken for the impact of individual SNPs on the association between inflammatory regulators and POI risk (Figure 3). By excluding any one SNP, the causality between CXCL10, IL-18R1, IL-18, MCP-1, CCL28, and POI was unlikely to be influenced by certain SNPs.

Comprehensive Analysis and Experimental Validation

Gleicher et al suggested[20] POI and DOR represent a continuum in phenotypical expression of different etiologies of premature ovarian senescence. To better understand the sustained role of inflammation-related proteins/genes in ovarian aging, we subsequently compared the differentially expressed proteins of DOR patients in follicular fluid using Olink results and POI causality-related proteins by MR results, via a Venn network (Figure 4A). MCP-1, LAP TGF-β1,

Table 2 Causal Associations Between Inflammation-Related Proteins and POI Based on Five Mendelian Randomization Method

Exposure (Gene Name)	Protein Name	nSNPs	Method	OR (95% CI)	*P* value
CXCL10	C-X-C motif chemokine 10 (CXCL10)	3	MR Egger	0.328 (0.112–0.961)	0.2910
			Weighted median	0.211 (0.076–0.586)	0.0028
			IVW	**0.205 (0.097–0.043)**	**<0.0001**
			Simple mode	0.109 (0.027–0.443)	0.0901
			Weighted mode	0.263 (0.108–0.641)	0.0989
CX3CL1	Fractalkine (CX3CL1)	2	**IVW**	**0.397 (0.272–0.578)**	**<0.0001**
IL18R1	Interleukin-18 receptor 1 (IL-18R1)	28	MR Egger	1.216 (0.002–773.691)	0.9531
			Weighted median	1.892 (1.732–2.067)	<0.0001
			IVW	**1.896 (1.774–2.025)**	**<0.0001**
			Simple mode	1.888 (1.611–2.213)	<0.0001
			Weighted mode	1.888 (1.607–2.218)	<0.0001
IL18	Interleukin-18 (IL-18)	8	MR Egger	0.010 (0.000–4.55e+30)	0.9090
			Weighted median	2.846 (2.026–3.997)	<0.0001
			IVW	**2.924 (2.360–3.621)**	**<0.0001**
			Simple mode	2.839 (1.801–4.475)	0.0028
			Weighted mode	2.838 (1.743–4.621)	0.0041
CCL2	Monocyte chemotactic protein 1 (MCP-1)	7	MR Egger	3.055 (0.318–29.347)	0.3778
			Weighted median	3.069 (2.059–4.576)	<0.0001
			IVW	**3.037 (2.383–3.871)**	**<0.0001**
			Simple mode	3.073 (1.831–5.156)	0.0054
			Weighted mode	3.073 (1.849–5.108)	0.0049
CCL28	C-C motif chemokine 28 (CCL28)	6	MR Egger	0.921 (1.000–1,136,320.915)	0.9914
			Weighted median	3.846 (2.358–6.274)	<0.0001
			IVW	**3.797 (2.760–5.224)**	**<0.0001**
			Simple mode	3.897 (2.075–7.317)	0.0082
			Weighted mode	3.897 (2.051–7.403)	0.0089

Notes: Bold text: Among multiple methods, the IVW method was selected as the primary approach to assess the correlation between inflammation-related proteins and the risk of POI.
Abbreviations: SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval; MR, mendelian randomization; IVW, inverse-variance weighted.

Table 3 Mendelian Randomization Associations Between Inflammation-Related Proteins and POI Based on Wald Ratio Method

Exposure (Gene Name)	Protein Name	nSNPs	Method	OR (95% CI)	*P* value
IL17C	Interleukin-17C (IL-17C)	1	Wald ratio	0.021 (0.002–0.186)	0.0005
TNFSF11	TNF-related activation-induced cytokine (TRANCE)	1	Wald ratio	0.044 (0.008–0.259)	0.0005
PLAU	Urokinase-type plasminogen activator (uPA)	1	Wald ratio	0.119 (0.038–0.375)	0.0003
TGFB1	Latency-associated peptide transforming growth factor beta-1 (LAP TGF-β1)	1	Wald ratio	0.138 (0.042–0.447)	0.0010
CXCL9	C-X-C motif chemokine 9 (CXCL9)	1	Wald ratio	0.213 (0.088–0.519)	0.0007
TNFSF14	Tumor necrosis factor ligand superfamily member 14 (TNFSF14)	1	Wald ratio	2.008 (1.352–2.983)	0.0005
CD40	CD40L receptor (CD40)	1	Wald ratio	2.401 (1.485–3.880)	0.0003
IL24	Interleukin-24 (IL-24)	1	Wald ratio	3.966 (1.848–8.510)	0.0004
ARTN	Artemin (ARTN)	1	Wald ratio	5.628 (2.024–15.646)	0.0009
LIFR	Leukemia inhibitory factor receptor (LIF-R)	1	Wald ratio	8.300 (2.363–29.159)	0.0010
IL2RB	Interleukin-2 receptor subunit beta (IL-2RB)	1	Wald ratio	9.589 (2.525–36.407)	0.0009

Abbreviations: SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval.

Table 4 Sensitivity Analysis of the Causal Relationship Between Inflammation-Related Proteins and POI

Exposure	Pleiotropy		Heterogeneity			
	MR Egger		MR Egger		IVW	
	Intercept	*P* value	Q	*P* value	Q	*P* value
CXCL10	−0.118	0.4631	1.582	0.2084	3.578	0.1671
CX3CL1	–	–	–	–	0.000	0.9942
IL-18R1	0.160	0.8938	0.045	1.0000	0.063	1.0000
IL-18	1.094	0.8878	0.136	0.9999	0.158	1.0000
MCP-1	−0.001	0.9962	0.028	1.0000	0.028	1.0000
CCL28	1.060	0.8527	0.000	1.0000	0.039	1.0000

Abbreviations: MR, mendelian randomization; IVW, inverse-variance weighted.

TNFSF14, ARTN, and LIF-R are the intersection proteins and may be the key proteins that alter the inflammatory state during ovarian aging. MCP-1 and ARTN were significantly increased in POI model (P=0.0028; P<0.0001), whereas TGF-β1 and LIF-R were significantly decreased in POI (P=0.0015; P=0.0067). In addition, there was no significant difference in the expression of TNFSF14 levels (Figure 4B).

To further explore potential druggable gene targets, we measured differential expression of the five genes (CCL2, TGFB1, TNFSF14, ARTN, and LIFR) in the POI model. Corresponding to protein levels, there was also no significant differential expression of the TNFSF14 gene in the POI model (Figure 4C). the mRNA levels of CCL2 and ARTN were

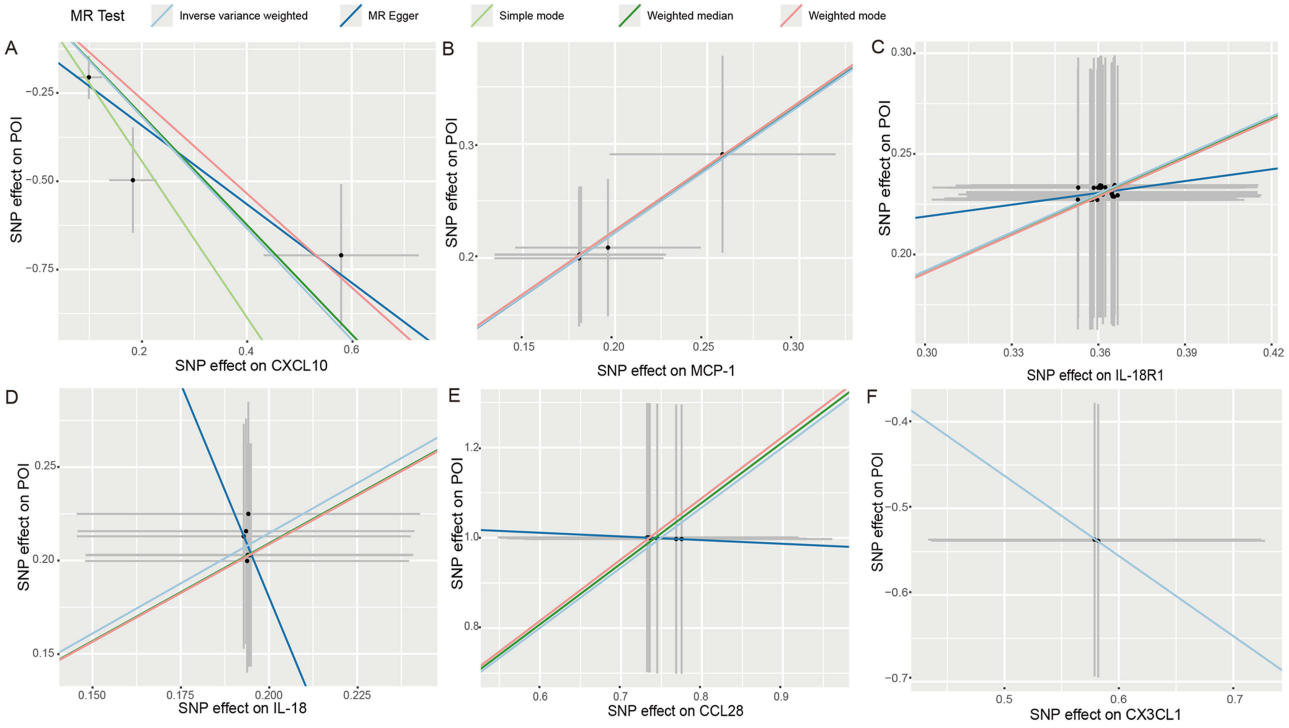

Figure 2 Scatter plots of significant causality of the inflammation-related regulators and POI. The direction of the SNP effect for 5 MR method is consistent in (**A**) CXCL10, (**B**) MCP-1, and (**C**) IL-18R1, while the direction of the SNP effect for MR Egger method is different in (**D**) IL-18 and (**E**) CCL28. Due to the fact that there are only 2 SNPs in (**F**) CX3CL1, only IVW method was used for analysis.
Abbreviations: MR, mendelian randomization; POI, primary ovarian insufficiency; SNP, single nucleotide polymorphism.

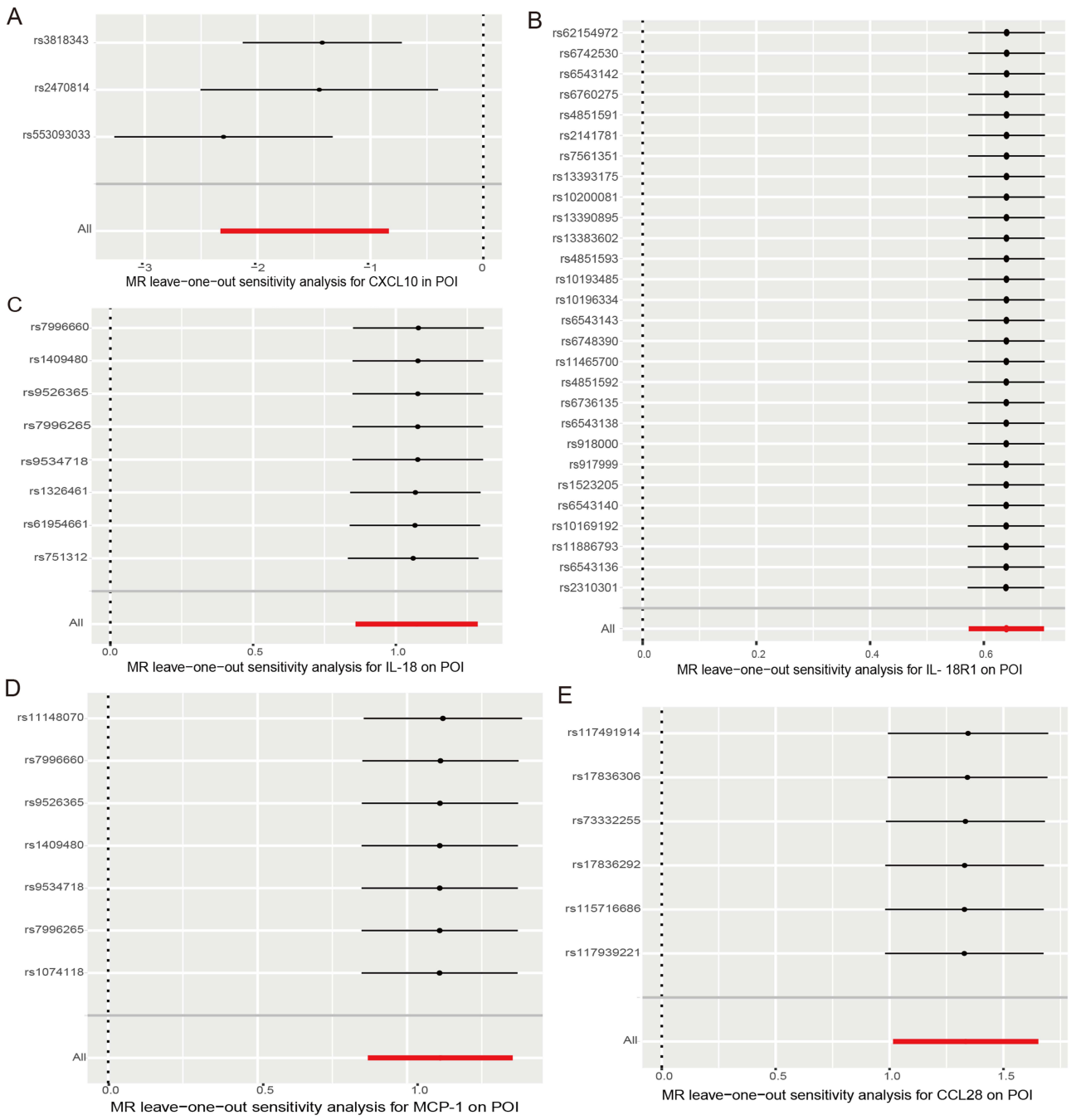

Figure 3 Leave-one-out analysis for the impact of individual SNPs on the association between inflammation-related regulators and POI risk. The causality between (**A**) CXCL10, (**B**) IL-18R1, (**C**) IL-18, (**D**) MCP-1, (**E**) CCL28 and POI is unlikely to be influenced by certain SNPs.
Abbreviations: MR, mendelian randomization; POI, primary ovarian insufficiency.

significantly increased in POI (P=0.0016; P=0.0003; Figure 4D and E), whereas TGFB1 and LIFR were significantly decreased in POI (P<0.0001; P<0.0001; Figure 4F and G).

Bioinformatics Analysis

There are protein-protein interactions among the inflammatory protective proteins (Figure 5A) and risk proteins (Figure 5B). GO analysis showed that most of the protective genes were involved in the cell surface receptor signaling

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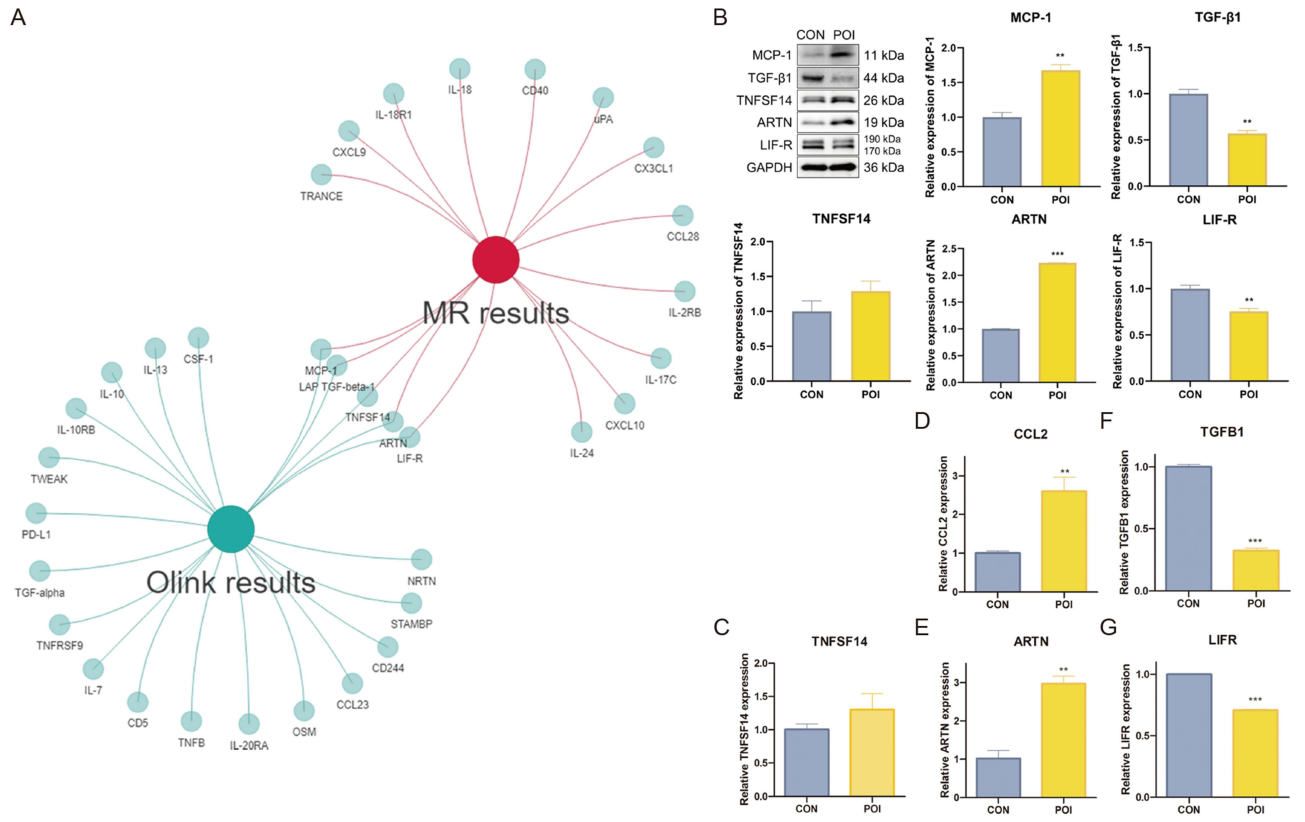

Figure 4 Comprehensive analysis and experimental validation for inflammation-related genes during ovarian aging. (**A**) A Venn network of differentially expressed proteins of DOR patients in follicular fluid by Olink results (green nodes) and POI causality related proteins by MR results (red nodes) was constructed. MCP-1, LAP TGF-β1, TNFSF14, ARTN, and LIF-R are the intersection proteins, and (**B**) the expression levels of these proteins have been validated in the POI model. To further explore potential druggable gene targets, the gene expression levels of (**C**) TNFSF14, (**D**) CCL2, (**E**) ARTN, (**F**) TGFB1, and (**G**) LIFR were also validated. ***P*<0.01 and ****P*<0.0001.
Abbreviations: MR, mendelian randomization; POI, primary ovarian insufficiency; CON, control.

pathway (7 genes) and response to stress (7 genes), while the risk genes were involved in response to chemical (10 genes), signal transduction (10 genes), and positive regulation of biological process (10 genes) (Figure 5C). The NF-kappa B signaling pathway was enriched in both protective and risk genes; however, the IL-17 signaling pathway (two genes; IL17C, CXCL10) was enriched in protective genes and the JAK-STAT signaling pathway (three genes; IL2RB, IL24, and LIFR) was enriched in risk genes (Figure 5D). WikiPathways analysis highlighted the IL-18 signaling pathway (three genes; CCL2, IL18, IL18R1) and oncostatin M signaling pathway (two genes; CCL2, LIFR) in POI risk genes (Figure 5E). Since CCL2 and LIFR have been validated at the genetic level, we are more certain of the role of the oncostatin M signaling pathway in ovarian aging.

Potential Drug Screening

We searched the 4 validated potential genes (CCL2, TGFB1, ARTN, LIFR) significant for POI through DGIdb, and a total of 66 potential drugs were obtained, among which the ARTN gene does not have any potential drugs. Sankey plot for druggable targets and drugs highlighted the prioritized potential drugs and targets (Figure 6), focusing on potential genes such as CCL2 and TGFB1. The potential drug for LIFR, emilermin, acts only on the LIFR gene and does not have the same target as the using POI drugs. The potential drug for CCL2 targets CYP enzyme family genes, which may treat POI by inhibiting ferroptosis. The potential drug for TGFB1 could target several known drug targets for POI (BDNF, VDR, ESR1, ESR2, etc) and the protective gene for POI in our MR results (PLAU), making it a prioritized therapeutic target.

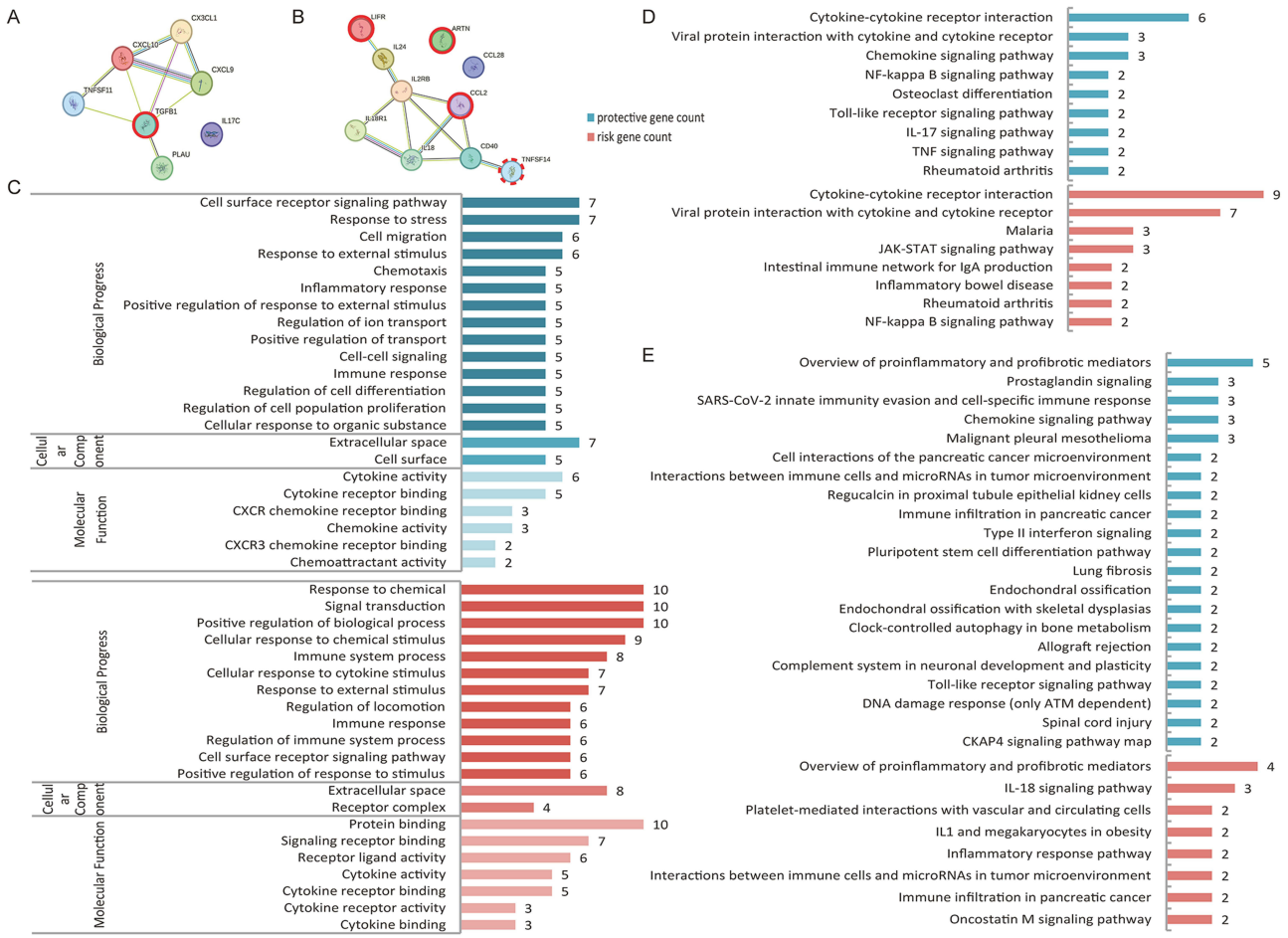

Figure 5 Data mining of the set of inflammation-related protective gene versus risk gene for POI. STRING analysis revealed that (**A**) protective proteins and (**B**) risk proteins both formed a good interaction network. Proteins with red circle are the intersection proteins for comprehensive analysis (solid line: significant difference; dashed line: no significant difference). (**C**) GO, (**D**) KEGG, and (**E**) WikiPathways enrichment analysis showed different enriched terms for protective and risk genes.

Discussion

Our study explored the causal associations between inflammation-related proteins and POI using a bidirectional MR framework. These findings reveal that specific inflammatory proteins might have protective or detrimental effects on the risk of POI. These results offer novel insights into the pathophysiology of POI, and provide potential targets for therapeutic interventions.

We identified six inflammatory proteins with significant causal associations with POI via the IVW method, including CXCL10 and CX3CL1, which demonstrated protective effects, and IL-18R1, IL-18, MCP-1, and CCL28, which were associated with an increased risk of POI. Moreover, Wald ratio analyses highlighted additional protective proteins, such as IL-17C, TRANCE, uPA, LAP TGF-β1, and CXCL9, along with risk proteins, including TNFSF14, CD40, IL-24, ARTN, LIF-R, and IL-2RB.

CXCL10 and CX3CL1 demonstrated strong protective effects against POI. Both are known for their roles in modulating immune responses, particularly in the recruitment and activation of immune cells. Wang et al[21] demonstrated that the concentration of CXCL10 is negatively associated with ovarian reserve function, both in the serum and follicular fluid. CXCL10 might act by promoting collagen synthesis and causing ovarian fibrosis. CX3CL1 is the only CX3C chemokine that can drive a variety of diseases by activating the specific receptor CX3CR1 to form the CX3CL1/CX3CR1 axis. Raei et al[22] found that insufficient CX3CL1 expression is related with elevated apoptosis and inflammation in granulosa cells. Moreover, significantly elevated CX3CL1 levels were also found in the serum of advanced ovarian

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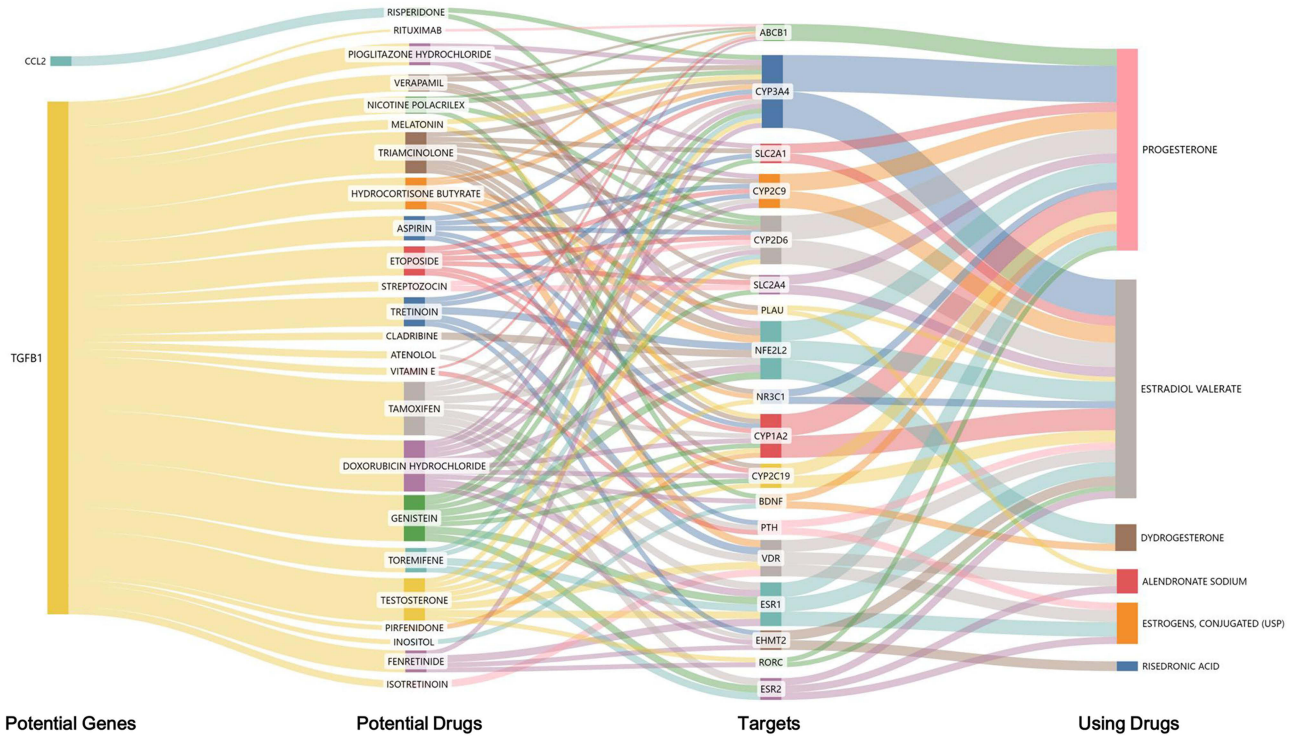

Figure 6 Interaction between prioritized druggable gene targets and current POI drugs.

cancer patients.[23] Additionally, proteins such as IL-17C,[24] TRANCE,[25] and uPA,[26] identified through the Wald ratio, are involved in promoting tissue repair and modulating inflammatory responses, which may contribute to ovarian repair function. Meanwhile, ovarian surface epithelial cells in preovulatory ovine follicles could secrete uPA to participate in ovarian ovulation.[27] These proteins may exert a protective effect by inhibiting inflammatory signaling pathways, such as the NF-κB signaling pathway (TRANCE, uPA) and the IL-17 signaling pathway (IL-17C, CXCL10) (Figure 5D).

In addition to protective proteins, risk proteins were also identified in this study. Inflammatory proteins associated with increased POI risk, such as IL-18, IL-18R1, and MCP-1, are well-known pro-inflammatory mediators involved in chronic inflammation. For example, IL-18 in CTX-induced POI rats induced granulosa cell pyroptosis and impaired ovarian reserve.[28] Similarly, MCP-1, also known as chemokine ligand 2 (CCL2), a potent monocyte chemoattractant, may contribute to damage to oocyte quality through prolonged inflammation in obese mice.[29] IL-2RB,[30] IL-24,[31] and LIF-R[32] have been identified using the Wald ratio method, and have been shown to drive dysregulated inflammatory responses and exacerbate ovarian insufficiency. WikiPathways analysis highlighted the IL-18 signaling pathway (IL-18, IL-18R1, and MCP-1) and the oncostatin M signaling pathway (MCP-1, LIF-R) (Figure 5E), whereas the JAK-STAT signaling pathway (IL-2RB, IL-24, and LIF-R) was enriched in the KEGG analysis (Figure 5D). Since CCL2 and LIFR have been validated at the genetic level, we are more certain about the role of the oncostatin M signaling pathway in ovarian aging, consistent with our previous research.[14]

BDNF,[33] VDR,[34] ESR1, and ESR2[35] are known drug targets for now using POI drugs by gene-drug analysis. By cross-referencing the drug targets and preliminary pharmacological screening, we are more inclined to believe that drugs such as genistein, tretinoin, and isotretinoin are potential drugs for treating POI. Since tretinoin and isotretinoin are contraindicated in pregnant women, we recommend genistein as the preferred choice for POI patients. Interestingly, NFE2L2/NRF2 is considered a key regulatory factor of ferroptosis, and the CYP enzyme family genes can also drive ferroptosis. Research has demonstrated that it is possible to treat POI by targeting ferroptosis using genistein,[36] tretinoin[37] and melatonin[38] medication. Further pharmacology experiment is necessary to confirm the efficacy and safety of these therapeutic strategies.

Our findings underscore the important and dual role of inflammation in ovarian function, emphasizing the need to maintain a balanced immune response during ovarian aging. The identification of potentially druggable genes for POI also provides a new insights into the understanding of POI pathogenesis and the establishment of new drug therapies.

A major strength of this study lies in the robust MR design, which minimizes confounding and reverse causation biases, thereby enabling more reliable causal inferences. Additionally, sensitivity analyses, including LOO and MR-Egger tests, validated the robustness of our findings. Nevertheless, several limitations should be noted. First, it focused on European populations, thus limiting the generalizability of the results to other ethnic groups. Second, the FinnGen statistics for POI were derived from a relatively small sample size, which may have reduced the power to detect weaker associations. And, while MR reduces bias from confounding factors, it cannot account for all potential sources of bias, such as pleiotropic effects that are not captured by sensitivity analyses. Finally, the prioritized drugs for POI treatment obtained in this study still require further investigation in cell lines and animal models prior to clinical application.

Conclusions

Our study highlights the causal role of specific inflammation-related proteins in POI, advancing our understanding of its etiology. And, by experimental validation and gene-drug screening, we further extends the therapeutic options for improving ovarian function and delaying POI onset.

Data Sharing Statement

The datasets generated and analysed during the current study are available in the FinnGen repository (https://www.finngen.fi/en), and GWAS repository (https://gwas.mrcieu.ac.uk). Data supporting the results of this study are available from the corresponding author upon request.

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

The authors declare that this study was conducted without any business or financial relationships that could be perceived as a potential conflict of interest.

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