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ORIGINAL RESEARCH

Dissecting Causal Relationships Between Immune Cells, Plasma Metabolites, and PCOS: Evidence From Mediating Mendelian Randomization Analysis

Xia-li Wang[1,2,*], Yi-fang He[2,*], Shi-kun Chen[3], Jing Cheng[4], Xiu-ming Wu[5]

[1]Department of Clinical Medicine, Quanzhou Medical College, Quanzhou, 362000, People's Republic of China; [2]Department of Ultrasound, Second Affiliated Hospital of Fujian Medical University, Quanzhou, 362000, People's Republic of China; [3]Department of Clinical Laboratory, Quanzhou Taiwan Investment Zone Disease Prevention and Control Center, Quanzhou, 362000, People's Republic of China; [4]Quanzhou Science and Technology Center, Quanzhou Medical College, Quanzhou, 362000, People's Republic of China; [5]Department of Ultrasound, Quanzhou First Hospital Affiliated to Fujian Medical University, Quanzhou, 362000, People's Republic of China

*These authors contributed equally to this work

Correspondence: Xiu-ming Wu, Department of Ultrasound, Quanzhou First Hospital Affiliated to Fujian Medical University, No. 1028 Anji South Road, Quanzhou, Fujian Province, People's Republic of China, Tel +86 13615916725, Email wxm6725@126.com

Background: The relationship between Polycystic ovary syndrome (PCOS) and immune dysregulation, along with metabolic disturbances, remains unclear. This study used Mendelian Randomization (MR) to investigate causal relationships between immune cells, PCOS, and possible metabolite mediators.

Methods: We explored the genetic-level relationship between immune cells and PCOS, focusing on metabolites as potential mediators. Data from genome-wide association studies (GWAS) included 731 immune cell types (n=3757), 1400 plasma metabolites (n=8299), and PCOS cases (n=797) versus controls (n=140,558). Bidirectional MR analysis examined immune-PCOS relationships, while two-step MR and mediation analysis identified metabolites as potential mediators. The inverse variance-weighted (IVW) method was used for primary causal assessment, with sensitivity analysis validating results.

Results: We identified a total of 33 immune cells that were associated with increased or decreased risk of PCOS ($P < 0.05$), and these immune cells were also associated with alterations in certain metabolite levels ($P < 0.05$). Among them, 12 immune cells were found to influence the occurrence of PCOS through the mediation of 17 metabolites. Notably, the effects of Myeloid DC %DC, NKT AC, CD20 on CD20- CD38-, CD25 on memory B cell, and HLA DR on CD33dim HLA DR+ CD11b+ were mediated by multiple metabolites on PCOS development. Similarly, histidine betaine (hercynine) levels and alpha-ketoglutarate to ornithine ratio mediated the association of more than one immune cell with PCOS.

Conclusion: This study highlights 12 immune cells impacting PCOS through 17 metabolites, advancing the understanding of immune mechanisms in PCOS risk and suggesting potential therapeutic approaches targeting immune modulation.

Keywords: PCOS, immunity, metabolites, mediating role, MR analysis

Introduction

Polycystic ovary syndrome (PCOS) is an endocrine disorder commonly seen in women of reproductive age, characterized by reproductive, metabolic and psychological dysfunction that affects all aspects of life.[1,2] The global prevalence of PCOS is estimated to be between 5% and 18%.[3,4] Women with PCOS typically exhibit menstrual irregularities, higher androgen levels, enlarged ovaries, and metabolic disturbances associated with obesity and insulin resistance.[4] While much has been explored regarding the clinical manifestations and long-term complications, such as type 2 diabetes, cardiovascular disease, infertility, and immune disorders the pathophysiological mechanisms underlying PCOS remain

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poorly understood.[5–9] Immune dysregulation is thought to play a critical role in the pathophysiology of PCOS, yet its precise mechanisms and impact on disease progression are still not fully elucidated.

Recent studies have increasingly concerned the role of inflammation and immune cells in the pathophysiology of PCOS,[10] it has been observed that patients with PCOS were often in a state of chronic low-grade inflammation, which induces oxidative stress in the ovaries and disrupts follicular development and oocyte quality.[11] Studies have shown that chronic low-grade inflammation in patients with PCOS was associated with unusual activation of a variety of immune cells.[12] For example, TLR4 gene polymorphisms in PCOS patients may be associated with their chronic inflammatory state.[13] In addition, macrophages accumulate in the ovary and secrete proinflammatory factors, which may affect the normal function of the ovary.[14] A Mendelian Randomization (MR) study revealed that levels of Memory B cell AC, CD39+ CD4+ %CD4+, CD20 on CD20- CD38-, and HLA DR on CD14- CD16+ monocyte were significantly associated with PCOS risk.[15] However, the precise mechanisms by which immune cells influence the development of PCOS require further investigation.

In recent years, progress in metabolomics technology allows us to explore the role of metabolites in disease more comprehensively. Metabolites are products of cellular metabolic processes that reflect the physiological and pathological state of the organism and can affect the process of disease and be targeted for therapeutic intervention.[16,17] Plasma metabolite abnormalities have been shown to be frequently associated with disturbances in their hormone metabolism and abnormal ovarian morphology in patients with PCOS, and some of the abnormal metabolites were associated with chronic inflammatory states.[18,19] We hypothesized a causal relationship between immune cells, metabolites and PCOS, and immune cells play an important role in the pathogenesis of PCOS by possibly through these metabolites.

To deeply investigate this hypothesis, this study proposed the MR method. MR analysis has become a useful tool for causal inference using single nucleotide polymorphisms (SNP) as instrumental variables (IVs) to effectively control mixtures in traditional observational studies and avoid bias from causality.[20,21] The MR analysis minimizes residual confusion due to the random organization of genetic material during in vitro fertilization, independent of environmental and lifestyle factors. We conducted an MR study and two mediation analyses using summary data from immune cells, blood metabolites, and genome-wide association statistics (GWAS) for PCOS to explore the mediating role of immune cells through blood metabolites, offering new insights for its prevention and treatment.

Materials and Methods

Study Design

We first screened for PCOS-associated immune cells primarily by inverse variance weighting (IVW) using 731 immune cells as the exposure factor and PCOS as the outcome factor. Next, we used 1400 plasma metabolites as mediators and proceeded to employ two-step RM (TSMR) and multivariate MR (MVMR) methods further. Our study attempted to elucidate the important mediating role that plasma metabolites may play in the cause-effect pathway between immune cells and PCOS, providing ideas for mechanisms of disease onset and therapeutic targets (Figure 1).

Data Sources

Genetic information related to PCOS was obtained from the GWAS summary database (https://gwas.mrcieu.ac.uk/) with the dataset ebi-a-GCST90044902, which includes information on genetic variants from 797 PCOS cases and 140,558 controls, totaling 22,981,890 SNPs. The PCOS cases were identified from national registers by ICD codes (ICD-10 E28.2, ICD-9256.4, or ICD-8256.90), and all remaining women were considered controls. These criteria require the presence of at least two of the following three criteria: chronic anovulation, hyperandrogenism, and polycystic ovaries visualized by ultrasonography.[22]

The 731 immune cell characteristics were categorized into four main types, including absolute count (AC) (118), median fluorescence intensities (MFI) (389), morphological parameters (MP) (32) and relative cell (RC) (n=192). Among them, MP mainly included CDC and TBNK panels, while AC, MFI and RC mainly included B cells, CDC, maturation stages of T cells, myeloid cells, monocytes, TBNK and Treg panels (Table S1). GWAS summary data for immunity cells traits were available from the GWAS catalog (accession numbers from ebi-a-GCST90001391 to ebi-a-GCST90002121), which includes 3575 Sardinians, 22 million SNPs.[23] The 1400 plasma metabolites consisted primarily of 1091 metabolites and 309 metabolite ratios, and the data used in this study were derived from a series

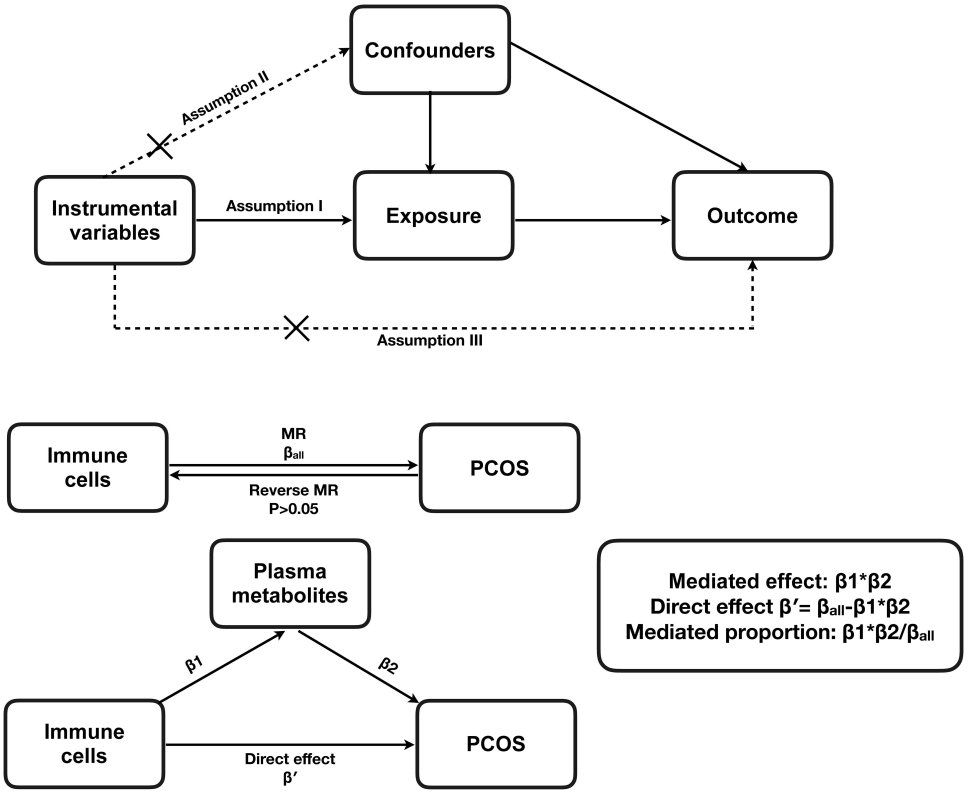


Figure 1 Schematic diagrams illustrating the study design.

of large GWASs by Chen et al that included 8299 individuals from the Canadian Longitudinal Study of Aging (CLSA) cohort,[17] which is available from the GWAS catalog (https://www.ebi.ac.uk/gwas, accession numbers from GCST90199621 to GCST90201020) (Table S2).

Instrumental Variable Selection

In MR analysis, the selection of IVs had to fulfill three assumptions: 1. IVs should have a high correlation with the exposure factor of interest; 2. IVs must not be associated with any confounding factors that could affect the results; 3. IVs affect the results only through the exposure factor and do not have any direct effect on the results.[24] We used *P*-values less than 1×10^{-5} to filter 731 immune cells and 1400 metabolites strongly associated SNPs as IVs.[25,26] We then excluded SNPs in linkage disequilibrium (LD) using $r^2 = 0.001$ and kb = 10,000 as criteria. Then, weak IV bias was also evaluated by calculating the *F*-statistic and weak IVs are excluded by selecting SNPs with an *F*-test value exceeding 10 for further analysis.[27] Finally, to control for the impact of confounding factors on instrumental variables, this study employed the Phenoscanner and R software packages, setting a $P < 1 \times 10^{-5}$ to eliminate palindromic SNPs and incompatible SNPs. This process excluded factors closely associated with the occurrence of PCOS, such as BMI, smoking, diabetes, depression, anxiety, and other related conditions,[28] ensuring that the resulting instrumental variables were appropriate for subsequent analysis.

Sensitivity Analysis

Significant heterogeneity was detected using Cochran's Q test, with a *P*-value less than 0.05 indicating statistical significance. Pleiotropy was evaluated using the MR pleiotropy residual sum and outlier (MR-PRESSO) test and the MR-Egger regression intercept, with *P*-values greater than 0.05 suggesting the absence of pleiotropy. The robustness of our findings was further validated by a leave-one-out analysis, which examined the influence of individual SNPs on the MR results.

Statistical Analysis

Multiple MIR methods, including MR Egger,[29] weighted median,[30] IVW, simple mode, and weighted mode, were used to estimate causal effects, with IVW selected as the primary analytical method, with a $P < 0.05$ considered to be causal.[31] To visualize the MR results, we plotted funnel plots and forest plots, where the funnel facilitates the assessment of potential publication bias, and the forest plots show the causal estimates and their confidence intervals, demonstrating a more comprehensive MR result.

We calculated the total effect (βall) of immune cells on PCOS, the effect of immune cells on metabolites β1, and the effect of metabolites on PCOS β2 by TSMR analysis, which led to the calculation of the indirect effect (β1*β2), the direct effect (β′) as βall - β1*β2, and the proportion of mediating effects as β1*β2/βall.[32] All analyses were performed with R (version 4.3.0) and version 0.5.8 of the TwoSampleMR package.

Results

Causal Effect of Immune Cells and PCOS

Through correlation analysis, LD removal, and calculation of *F*-value, we screened a total of 18,621 SNPs as IVs of immune cells (Table S3). Analyzed by five MR methods, we mainly focused on the IVW method, and based on its *P* value of less than 0.05. Furthermore, the odds ratios (ORs) for the remaining four methods were consistent with the direction of the initial analysis (Supplementary Figure S1A). We obtained a total of 33 immune cells that were causally associated with PCOS ($P<0.05$). Among them, 20 immune cells were risk factors for PCOS, including Myeloid DC % DC, CD4 Treg %T cell, Lymphocyte AC, DN (CD4-CD8-) AC, BAFF-R on CD24+ CD27+, BAFF-R on IgD+ CD24+, BAFF-R on IgD+ CD38- unsw mem, BAFF-R on IgD- CD24-, BAFF-R on IgD- CD27-, BAFF-R on IgD- CD38-, BAFF-R on IgD- CD38br, BAFF-R on memory B cell, BAFF-R on sw mem, CD19 on IgD+ CD38- unsw mem, CD20 on CD20- CD38-, CD20 on IgD- CD38dim, CD24 on IgD+ CD38-, CD25 on memory B cell, CD28 on CD39+ resting Treg, and SSC-A on HLA DR+ T cell, while 13 immune cells were protective factors for PCOS, including CD24+ CD27 + AC, CD11c+ monocyte %monocyte, CD25hi CD45RA+ CD4 not Treg %T cell, CM DN (CD4-CD8-) AC, CD8dim % T cell, NKT AC, CD27 on CD24+ CD27+, CD27 on IgD+ CD38- unsw mem, CD38 on naive-mature B cell, CD16 on CD14- CD16+ monocyte, CD64 on CD14- CD16+ monocyte, CD14 on Mo MDSC, and HLA DR on CD33dim HLA DR+ CD11b+ (Figure 2 and Table 1). In addition, heterogeneity and pleiotropy tests indicated no significant issues ($P > 0.05$, Table 1). The leave-one-out analysis further assessed the stability of the results (Supplementary Figure S1B), and in addition, the results of the funnel plot demonstrated that our results were not biased (Supplementary Figure S1C). Then we performed reverse MR analysis with PCOS as the exposure factor and these 33 immune cells as the outcome factor, we found that there was no causal relationship between PCOS and these immune cells ($P>0.05$, Table 1).

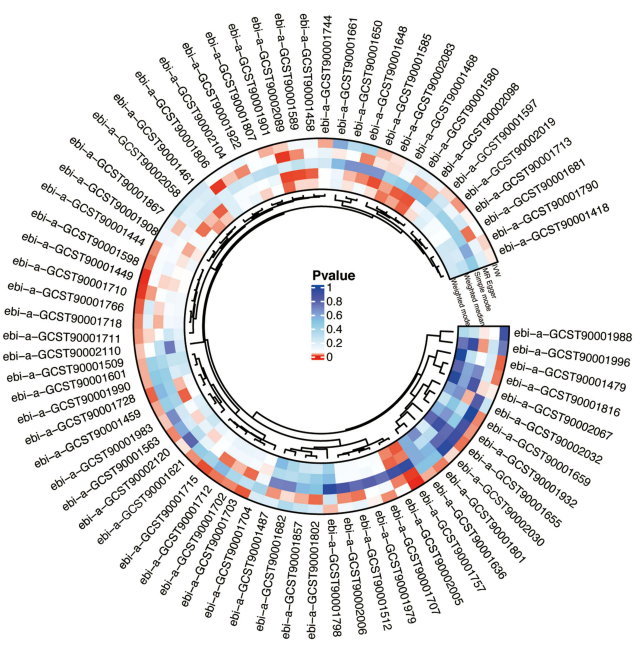
Causal Effect of Metabolites and PCOS

By correlation analysis, LD removal, and *F*-value screening calculations, we identified 34,843 SNPs as plasma metabolites of IVs (Table S4). Analyzed by five MR methods, we mainly focused on the IVW method, and based on its *P* value of less than 0.05. Furthermore, the ORs for the remaining four methods were consistent with the direction of the initial analysis (Supplementary Figure S2A). Consequently, we identified 51 metabolites (including two unknown metabolites) that have a causal relationship with PCOS ($P<0.05$). Of these, 28 metabolites increased the risk of PCOS, including methionine sulfoxide levels, hexanoylcarnitine levels (Biocrates platform) and epiandrosterone sulfate levels. In contrast, 23 metabolites decreased the risk of PCOS, such as N-acetylglycine levels, EDTA levels, and tauro-beta-muricholate levels (Figure 3 and Table 2). Furthermore, there was no heterogeneity or pleiotropy ($P>0.05$, Table 2), and the stability of the results was illustrated by leave-one-out analysis (Supplementary Figure S2B) and funnel plots (Supplementary Figure S2C).

Causal Effect of Immune Cells and Metabolites

Based on the prior identification of immune cells and plasma metabolites, we used a TSMR method to extend our exploration of their mediating role. Firstly, we performed MR analysis from immune cell phenotypes to plasma

A



B

exposure	Panel	nsnp	method	pval	OR(95% CI)
CD24+ CD27+ AC	B cell	23	IVW	0.045	0.958 (0.918 to 0.999)
BAFF–R on CD24+ CD27+	B cell	17	IVW	0.021	1.051 (1.008 to 1.097)
BAFF–R on IgD+ CD24+	B cell	15	IVW	0.009	1.058 (1.014 to 1.103)
BAFF–R on IgD+ CD38– unsw mem	B cell	18	IVW	0.022	1.064 (1.009 to 1.122)
BAFF–R on IgD– CD24–	B cell	21	IVW	0.001	1.080 (1.030 to 1.132)
BAFF–R on IgD– CD27–	B cell	18	IVW	0.020	1.054 (1.008 to 1.102)
BAFF–R on IgD– CD38–	B cell	16	IVW	0.016	1.055 (1.010 to 1.103)
BAFF–R on IgD– CD38br	B cell	15	IVW	0.048	1.093 (1.001 to 1.194)
BAFF–R on memory B cell	B cell	13	IVW	0.007	1.063 (1.017 to 1.112)
BAFF–R on sw mem	B cell	18	IVW	0.011	1.058 (1.013 to 1.105)
CD19 on IgD+ CD38– unsw mem	B cell	18	IVW	0.012	1.054 (1.012 to 1.099)
CD20 on CD20– CD38–	B cell	10	IVW	0.028	1.109 (1.011 to 1.218)
CD20 on IgD– CD38dim	B cell	28	IVW	0.001	1.037 (1.015 to 1.060)
CD24 on IgD+ CD38–	B cell	17	IVW	0.003	1.079 (1.026 to 1.133)
CD25 on memory B cell	B cell	21	IVW	0.039	1.061 (1.003 to 1.123)
CD27 on CD24+ CD27+	B cell	25	IVW	0.034	0.943 (0.893 to 0.995)
CD27 on IgD+ CD38– unsw mem	B cell	24	IVW	0.018	0.948 (0.908 to 0.991)
CD38 on naive–mature B cell	B cell	21	IVW	0.017	0.923 (0.864 to 0.986)
CD11c+ monocyte %monocyte	cDC	17	IVW	0.016	0.906 (0.836 to 0.982)
Myeloid DC %DC	cDC	5	IVW	0.029	1.230 (1.021 to 1.481)
CM DN (CD4–CD8–) AC	Maturation stages of T cell	3	IVW	0.020	0.922 (0.861 to 0.987)
CD16 on CD14– CD16+ monocyte	Monocyte	19	IVW	0.047	0.955 (0.913 to 0.999)
CD64 on CD14– CD16+ monocyte	Monocyte	19	IVW	0.030	0.903 (0.823 to 0.990)
CD14 on Mo MDSC	Myeloid cell	24	IVW	0.024	0.957 (0.922 to 0.994)
HLA DR on CD33dim HLA DR+ CD11b+	Myeloid cell	20	IVW	0.024	0.954 (0.915 to 0.994)
CD8dim %T cell	TBNK	17	IVW	0.041	0.921 (0.851 to 0.997)
DN (CD4–CD8–) AC	TBNK	20	IVW	0.022	1.119 (1.016 to 1.232)
Lymphocyte AC	TBNK	5	IVW	0.030	1.197 (1.018 to 1.409)
NKT AC	TBNK	11	IVW	0.022	0.866 (0.766 to 0.980)
SSC–A on HLA DR+ T cell	TBNK	20	IVW	0.043	1.084 (1.002 to 1.172)
CD4 Treg %T cell	Treg	13	IVW	0.012	1.102 (1.022 to 1.188)
CD25hi CD45RA+ CD4 not Treg %T cell	Treg	21	IVW	0.036	0.963 (0.930 to 0.997)
CD28 on CD39+ resting Treg	Treg	18	IVW	0.030	1.034 (1.003 to 1.065)

1

Figure 2 Mendelian randomized analysis of immune cells and PCOS. (**A**) Circle plot of five MR methods; (**B**) Forest plot of causal relationship between 33 immune cells and PCOS.

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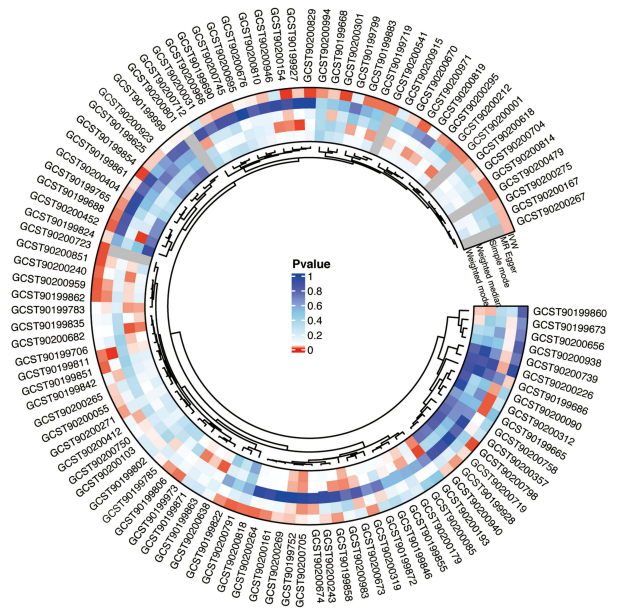
Table 1 MR Analysis of Immune Cells and PCOS

Exposure	Method	Nsnp	Beta	Se	P-val	Rev-Pvale	Pleiotropy	Heterogeneity
CD24+ CD27+ AC	IVW	23	−0.043	0.022	0.045	0.204	0.568	0.793
CD11c+ monocyte %monocyte	IVW	17	−0.099	0.041	0.016	0.446	0.592	0.97
Myeloid DC %DC	IVW	5	0.207	0.095	0.029	0.875	0.825	0.057
CD4 Treg %T cell	IVW	13	0.097	0.038	0.012	0.794	0.589	0.593
CD25hi CD45RA+ CD4 not Treg %T cell	IVW	21	−0.038	0.018	0.036	0.257	0.54	0.273
CM DN (CD4-CD8-) AC	IVW	3	−0.081	0.035	0.02	0.393	0.778	0.71
CD8dim %T cell	IVW	17	−0.082	0.04	0.041	0.632	0.921	0.543
Lymphocyte AC	IVW	5	0.18	0.083	0.03	0.546	0.745	0.313
NKT AC	IVW	11	−0.144	0.063	0.022	0.863	0.784	0.421
DN (CD4-CD8-) AC	IVW	20	0.113	0.049	0.022	0.749	0.332	0.275
BAFF-R on CD24+ CD27+	IVW	17	0.05	0.022	0.021	0.647	0.231	0.73
BAFF-R on IgD+ CD24+	IVW	15	0.056	0.021	0.009	0.691	0.226	0.667
BAFF-R on IgD+ CD38- unsw mem	IVW	18	0.062	0.027	0.022	0.517	0.809	0.091
BAFF-R on IgD- CD24-	IVW	21	0.077	0.024	0.001	0.442	0.333	0.754
BAFF-R on IgD- CD27-	IVW	18	0.053	0.023	0.02	0.675	0.624	0.863
BAFF-R on IgD- CD38-	IVW	16	0.054	0.022	0.016	0.627	0.113	0.364
BAFF-R on IgD- CD38br	IVW	15	0.089	0.045	0.048	0.612	0.827	0.606
BAFF-R on memory B cell	IVW	13	0.061	0.023	0.007	0.706	0.102	0.615
BAFF-R on sw mem	IVW	18	0.057	0.022	0.011	0.688	0.424	0.38
CD19 on IgD+ CD38- unsw mem	IVW	18	0.053	0.021	0.012	0.42	0.231	0.446
CD20 on CD20- CD38-	IVW	10	0.104	0.047	0.028	0.751	0.823	0.375
CD20 on IgD- CD38dim	IVW	28	0.037	0.011	0.001	0.976	0.608	0.908
CD24 on IgD+ CD38-	IVW	17	0.076	0.025	0.003	0.209	0.67	0.901
CD25 on memory B cell	IVW	21	0.059	0.029	0.039	0.257	0.887	0.292
CD27 on CD24+ CD27+	IVW	25	−0.059	0.028	0.034	0.493	0.876	0.618
CD27 on IgD+ CD38- unsw mem	IVW	24	−0.053	0.022	0.018	0.636	0.458	0.947
CD38 on naive-mature B cell	IVW	21	−0.08	0.034	0.017	0.668	0.766	0.747
CD28 on CD39+ resting Treg	IVW	18	0.033	0.015	0.03	0.058	0.963	0.433
CD16 on CD14- CD16+ monocyte	IVW	19	−0.046	0.023	0.047	0.937	0.432	0.948
CD64 on CD14- CD16+ monocyte	IVW	19	−0.102	0.047	0.03	0.128	0.744	0.319
CD14 on Mo MDSC	IVW	24	−0.044	0.019	0.024	0.531	0.985	0.437
SSC-A on HLA DR+ T cell	IVW	20	0.081	0.04	0.043	0.944	0.23	0.145
HLA DR on CD33dim HLA DR+ CD11b+	IVW	20	−0.047	0.021	0.024	0.494	0.385	0.253

Abbreviations: MR, Mendelian randomization; PCOS, polycystic ovary syndrome; Nsnp, number of SNPs; Beta, causal effect size; Se, standard error of the Beta estimate; Rev-Pval, P-value from reverse MR analysis; IVW, inverse-variance weighted method.

metabolites using 33 immune cells as exposure factors and 51 plasma metabolites as outcome indicators. The results showed a causal relationship between 20 immune cells and 32 plasma metabolites, which was further calculated to derive an effect value β1 from immune cells to metabolites. Interestingly, we found that an immune cell phenotype may be causally associated with multiple metabolites. For example, CM DN (CD4-CD8-) AC, CD25 on memory B cell and HLA DR on CD33dim HLA DR+ CD11b+ were causally associated with more than four metabolites. Among them, HLA DR on CD33dim HLA DR+ CD11b+ was positively associated with EDTA levels, tauro-beta-muricholate levels, tetradecanedioate (C14-DC) levels and 2-hydroxyarachidate levels but negatively correlated with Alpha-ketoglutarate to ornithine ratio (Tables 3 and Figure 4). Subsequently, we performed MR analysis and MR-PRESSO tests ($P > 0.05$) with 31 plasma metabolites as exposure factors and PCOS as an outcome, which showed no pleiotropic and unbiased SNPs (Tables 4). With these tests, we determined an effect value of β2 from metabolites to PCOS and calculated an overall effect β_{all} from immune cells to PCOS.

A



B

exposure	nsnp	method	pval	OR(95% CI)
Methionine sulfoxide levels	22	IVW	**0.038**	1.167 (1.008 to 1.351)
Hexanoylcarnitine levels (Biocrates platform)	20	IVW	**0.037**	1.107 (1.006 to 1.218)
N−acetylglycine levels	18	IVW	**0.018**	0.798 (0.661 to 0.962)
EDTA levels	14	IVW	**0.029**	0.808 (0.667 to 0.979)
Tauro−beta−muricholate levels	3	IVW	**0.016**	0.879 (0.792 to 0.976)
Epiandrosterone sulfate levels	20	IVW	**0.037**	1.076 (1.005 to 1.152)
4−hydroxyhippurate levels	16	IVW	**0.024**	1.208 (1.026 to 1.424)
Isovalerylglycine levels	20	IVW	**0.048**	1.138 (1.001 to 1.294)
Tetradecanedioate (C14−DC) levels	5	IVW	**0.025**	1.323 (1.036 to 1.691)
1−oleoyl−GPI (18:1) levels	25	IVW	**0.024**	1.169 (1.021 to 1.338)
4−vinylphenol sulfate levels	21	IVW	**0.041**	0.863 (0.749 to 0.994)
Androstenediol (3beta,17beta) disulfate (2) levels	21	IVW	**0.012**	0.870 (0.780 to 0.970)
5alpha−pregnan−3beta,20alpha−diol monosulfate (2) levels	27	IVW	**0.005**	0.854 (0.766 to 0.953)
Pregnenediol sulfate (C21H34O5S) levels	5	IVW	**0.044**	0.801 (0.645 to 0.994)
4−hydroxycoumarin levels	19	IVW	**0.010**	0.843 (0.740 to 0.961)
Cysteinylglycine disulfide levels	16	IVW	**0.015**	1.153 (1.028 to 1.293)
Histidine betaine (hercynine) levels	15	IVW	**0.037**	1.173 (1.010 to 1.363)
2−stearoyl−GPE (18:0) levels	23	IVW	**0.025**	0.851 (0.739 to 0.980)
Methyl glucopyranoside (alpha + beta) levels	23	IVW	**0.015**	0.913 (0.848 to 0.982)
4−methoxyphenol sulfate levels	17	IVW	0.050	1.174 (1.000 to 1.377)
Lactosyl−N−palmitoyl−sphingosine (d18:1/16:0) levels	17	IVW	**0.037**	0.854 (0.736 to 0.991)
2−hydroxyarachidate levels	22	IVW	**0.020**	1.100 (1.015 to 1.191)
Glucuronide of piperine metabolite C17H21NO3 (5) levels	19	IVW	**0.020**	0.859 (0.756 to 0.976)
Hydroxy−N6,N6,N6−trimethyllysine levels	23	IVW	**0.013**	1.193 (1.037 to 1.373)
Hydroxypalmitoyl sphingomyelin (d18:1/16:0(OH)) levels	25	IVW	**0.041**	1.123 (1.005 to 1.255)
Bilirubin degradation product, C16H18N2O5 (4) levels	17	IVW	**0.039**	1.090 (1.004 to 1.184)
Bilirubin degradation product, C16H18N2O5 (3) levels	24	IVW	**0.026**	1.102 (1.012 to 1.200)
Bilirubin degradation product, C16H18N2O5 (2) levels	21	IVW	**0.035**	1.090 (1.006 to 1.181)
Gamma−glutamyltyrosine levels	23	IVW	**0.025**	0.862 (0.758 to 0.982)
Gamma−glutamylglutamine levels	25	IVW	**0.005**	0.836 (0.737 to 0.948)
Cys−gly, oxidized levels	20	IVW	**0.023**	1.125 (1.017 to 1.245)
Ornithine levels	5	IVW	**0.036**	0.763 (0.592 to 0.982)
X−23782 levels	5	IVW	**0.032**	1.426 (1.032 to 1.970)
X−26111 levels	13	IVW	**0.038**	0.823 (0.684 to 0.989)
N−acetyl−L−glutamine levels	17	IVW	**0.046**	1.075 (1.001 to 1.153)
2′−o−methyluridine levels	14	IVW	**0.048**	0.933 (0.872 to 0.999)
Bilirubin (z,z) levels	31	IVW	**0.042**	1.074 (1.003 to 1.149)
Adenosine 5′−monophosphate (AMP) to phenylalanine ratio	22	IVW	**0.029**	1.188 (1.018 to 1.387)
Creatine to carnitine ratio	15	IVW	**0.011**	0.763 (0.619 to 0.940)
Dopamine 4−sulfate to dopamine 3−O−sulfate ratio	21	IVW	**0.007**	1.209 (1.054 to 1.387)
5−methylthioadenosine (MTA) to phosphate ratio	23	IVW	**0.047**	1.147 (1.002 to 1.314)
Alpha−ketoglutarate to kynurenine ratio	22	IVW	**0.035**	1.146 (1.010 to 1.299)
Alpha−ketoglutarate to ornithine ratio	16	IVW	**0.017**	1.209 (1.034 to 1.413)
Adenosine 5′−diphosphate (ADP) to glycerate ratio	14	IVW	**0.014**	0.832 (0.717 to 0.964)
Bilirubin (Z,Z) to taurocholate ratio	15	IVW	**0.005**	1.218 (1.062 to 1.397)
Adenosine 5′−monophosphate (AMP) to glycine ratio	19	IVW	**0.007**	1.269 (1.067 to 1.510)
Glycerol to mannitol to sorbitol ratio	21	IVW	**0.038**	0.823 (0.684 to 0.989)
Adenosine 5′−diphosphate (ADP) to EDTA ratio	22	IVW	**0.038**	0.894 (0.804 to 0.994)
Choline phosphate to choline ratio	16	IVW	**0.009**	0.794 (0.668 to 0.945)
Leucine to phosphate ratio	22	IVW	**0.012**	1.231 (1.047 to 1.447)
Glutamine to alanine ratio	28	IVW	**0.023**	0.857 (0.750 to 0.979)

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Figure 3 Mendelian randomized analysis of plasma metabolites and PCOS. (**A**) Circle plot of five MR methods; (**B**) Forest plot of causal relationship between 51 plasma metabolites and PCOS.

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Table 2 MR Analysis of Metabolites and PCOS

Exposure	Method	Nsnp	Beta	Se	P-val	Pleiotropy	Heterogeneity
Methionine sulfoxide levels	IVW	22	0.155	0.075	0.038	0.741	0.295
Hexanoylcarnitine levels (Biocrates platform)	IVW	20	0.102	0.049	0.037	0.583	0.71
N-acetylglycine levels	IVW	18	−0.226	0.095	0.018	0.452	0.122
EDTA levels	IVW	14	−0.213	0.098	0.029	0.394	0.651
Tauro-beta-muricholate levels	IVW	3	−0.129	0.053	0.016	0.951	0.551
Epiandrosterone sulfate levels	IVW	20	0.073	0.035	0.037	0.43	0.808
4-hydroxyhippurate levels	IVW	16	0.189	0.084	0.024	0.3	0.528
Isovalerylglycine levels	IVW	20	0.13	0.065	0.048	0.728	0.827
Tetradecanedioate (C14-DC) levels	IVW	5	0.28	0.125	0.025	0.341	0.536
1-oleoyl-GPI (18:1) levels	IVW	25	0.156	0.069	0.024	0.256	0.733
4-vinylphenol sulfate levels	IVW	21	−0.147	0.072	0.041	0.519	0.331
Androstenediol (3beta, 17beta) disulfate (2) levels	IVW	21	−0.14	0.056	0.012	0.947	0.659
5alpha-pregnan-3beta, 20alpha-diol monosulfate (2) levels	IVW	27	−0.157	0.056	0.005	0.192	0.582
Pregnenediol sulfate (C21H34O5S) levels	IVW	5	−0.222	0.11	0.044	0.437	0.72
4-hydroxycoumarin levels	IVW	19	−0.17	0.067	0.01	0.202	0.411
Cysteinylglycine disulfide levels	IVW	16	0.142	0.059	0.015	0.359	0.598
Histidine betaine (hercynine) levels	IVW	15	0.16	0.077	0.037	0.362	0.675
2-stearoyl-GPE (18:0) levels	IVW	23	−0.161	0.072	0.025	0.595	0.327
Methyl glucopyranoside (alpha + beta) levels	IVW	23	−0.091	0.037	0.015	0.83	0.723
4-methoxyphenol sulfate levels	IVW	17	0.16	0.082	0.05	0.55	0.966
Lactosyl-N-palmitoyl-sphingosine (d18:1/16:0) levels	IVW	17	−0.158	0.076	0.037	0.497	0.847
2-hydroxyarachidate levels	IVW	22	0.095	0.041	0.02	0.796	0.9
Glucuronide of piperine metabolite C17H21NO3 (5) levels	IVW	19	−0.152	0.065	0.02	0.719	0.979
Hydroxy-N6,N6,N6-trimethyllysine levels	IVW	23	0.177	0.071	0.013	0.484	0.824
Hydroxypalmitoyl sphingomyelin (d18:1/16:0(OH)) levels	IVW	25	0.116	0.057	0.041	0.706	0.892
Bilirubin degradation product, C16H18N2O5 (4) levels	IVW	17	0.087	0.042	0.039	0.836	0.832
Bilirubin degradation product, C16H18N2O5 (3) levels	IVW	24	0.097	0.044	0.026	0.85	0.167
Bilirubin degradation product, C16H18N2O5 (2) levels	IVW	21	0.086	0.041	0.035	0.462	0.884
Gamma-glutamyltyrosine levels	IVW	23	−0.148	0.066	0.025	0.747	0.551
Gamma-glutamylglutamine levels	IVW	25	−0.179	0.064	0.005	0.477	0.344
Cys-gly, oxidized levels	IVW	20	0.118	0.052	0.023	0.643	0.329
Ornithine levels	IVW	5	−0.271	0.129	0.036	0.207	0.572
X-23782 levels	IVW	5	0.355	0.165	0.032	0.788	0.677
X-26111 levels	IVW	13	−0.195	0.094	0.038	0.946	0.38
N-acetyl-L-glutamine levels	IVW	17	0.072	0.036	0.046	0.118	0.426
2'-o-methyluridine levels	IVW	14	−0.069	0.035	0.048	0.051	0.455
Bilirubin (z,z) levels	IVW	31	0.071	0.035	0.042	0.515	0.438
Adenosine 5'-monophosphate (AMP) to phenylalanine ratio	IVW	22	0.172	0.079	0.029	0.361	0.873
Creatine to carnitine ratio	IVW	15	−0.271	0.107	0.011	0.555	0.317
Dopamine 4-sulfate to dopamine 3-O-sulfate ratio	IVW	21	0.19	0.07	0.007	0.933	0.414
5-methylthioadenosine (MTA) to phosphate ratio	IVW	23	0.138	0.069	0.047	0.5	0.439
Alpha-ketoglutarate to kynurenine ratio	IVW	22	0.136	0.064	0.035	0.284	0.438
Alpha-ketoglutarate to ornithine ratio	IVW	16	0.19	0.08	0.017	0.71	0.774
Adenosine 5'-diphosphate (ADP) to glycerate ratio	IVW	14	−0.185	0.075	0.014	0.637	0.426
Bilirubin (Z,Z) to taurocholate ratio	IVW	15	0.197	0.07	0.005	0.22	0.81
Adenosine 5'-monophosphate (AMP) to glycine ratio	IVW	19	0.238	0.089	0.007	0.246	0.777
Glycerol to mannitol to sorbitol ratio	IVW	21	−0.195	0.094	0.038	0.705	0.226
Adenosine 5'-diphosphate (ADP) to EDTA ratio	IVW	22	−0.112	0.054	0.038	0.677	0.502
Choline phosphate to choline ratio	IVW	16	−0.23	0.089	0.009	0.178	0.847
Leucine to phosphate ratio	IVW	22	0.208	0.083	0.012	0.519	0.355
Glutamine to alanine ratio	IVW	28	−0.154	0.068	0.023	0.917	0.367

Abbreviations: MR, Mendelian randomization; PCOS, polycystic ovary syndrome; Nsnp, number of SNPs; Beta, causal effect size; Se, standard error of the Beta estimate; IVW, inverse-variance weighted method.

Table 3 MR Analysis of Immune Cells and Metabolites

Exposure	Outcome	Method	Nsnp	Beta	Se	P-val	Pleiotropy	Heterogeneity
CD11c+ monocyte %monocyte	Glucuronide of piperine metabolite C17H21NO3 (5) levels	IVW	21	0.072	0.026	0.005	0.102	0.84
CD11c+ monocyte %monocyte	Gamma-glutamyltyrosine levels	IVW	21	−0.053	0.023	0.02	0.213	0.652
CD11c+ monocyte %monocyte	2'-o-methyluridine levels	IVW	21	−0.065	0.024	0.006	0.699	0.783
Myeloid DC %DC	Gamma-glutamylglutamine levels	IVW	27	−0.05	0.019	0.009	0.206	0.54
Myeloid DC %DC	Choline phosphate to choline ratio	IVW	27	−0.041	0.018	0.023	0.891	0.765
CD4 Treg %T cell	Epiandrosterone sulfate levels	IVW	16	−0.046	0.02	0.019	0.59	0.401
CD4 Treg %T cell	Histidine betaine (hercynine) levels	IVW	16	−0.043	0.022	0.048	0.623	0.668
CD25hi CD45RA+ CD4 not Treg %T cell	Hydroxy-N6,N6,N6-trimethyllysine levels	IVW	26	0.026	0.009	0.003	0.993	0.234
CD25hi CD45RA+ CD4 not Treg %T cell	N-acetyl-L-glutamine levels	IVW	26	0.017	0.008	0.034	0.575	0.844
CD25hi CD45RA+ CD4 not Treg %T cell	Creatine to carnitine ratio	IVW	26	0.023	0.007	0.002	0.571	0.884
CM DN (CD4-CD8-) AC	Bilirubin degradation product, C16H18N2O5 (4) levels	IVW	4	−0.069	0.029	0.017	0.929	0.682
CM DN (CD4-CD8-) AC	Bilirubin degradation product, C16H18N2O5 (3) levels	IVW	4	−0.061	0.029	0.037	0.899	0.416
CM DN (CD4-CD8-) AC	Bilirubin degradation product, C16H18N2O5 (2) levels	IVW	4	−0.075	0.03	0.013	0.862	0.345
CM DN (CD4-CD8-) AC	Leucine to phosphate ratio	IVW	4	0.059	0.027	0.028	0.516	0.785
CD8dim %T cell	Isovalerylglycine levels	IVW	18	0.07	0.036	0.05	0.183	0.112
CD8dim %T cell	Pregnenediol sulfate (C21H34O5S) levels	IVW	18	−0.062	0.023	0.008	0.408	0.82
CD8dim %T cell	X-26111 levels	IVW	18	0.061	0.028	0.027	0.691	0.794
DN (CD4-CD8-) AC	Histidine betaine (hercynine) levels	IVW	19	0.08	0.032	0.011	0.861	0.542
NKT AC	Cysteinylglycine disulfide levels	IVW	33	−0.039	0.018	0.027	0.685	0.612
NKT AC	Cys-gly, oxidized levels	IVW	33	−0.045	0.019	0.021	0.905	0.214
BAFF-R on CD24+ CD27+	Histidine betaine (hercynine) levels	IVW	19	0.034	0.016	0.028	0.504	0.235
BAFF-R on IgD+ CD24+	4-hydroxycoumarin levels	IVW	18	0.033	0.015	0.023	0.721	0.59
BAFF-R on IgD+ CD38- unsw mem	2'-o-methyluridine levels	IVW	21	0.028	0.014	0.039	0.284	0.806
BAFF-R on IgD+ CD38- unsw mem	Leucine to phosphate ratio	IVW	21	−0.027	0.012	0.024	0.901	0.97
BAFF-R on IgD- CD38-	4-vinylphenol sulfate levels	IVW	17	0.029	0.013	0.032	0.177	0.561
BAFF-R on sw mem	4-methoxyphenol sulfate levels	IVW	19	0.027	0.014	0.048	0.43	0.696
CD20 on CD20- CD38-	4-hydroxycoumarin levels	IVW	16	0.078	0.033	0.019	0.604	0.871
CD20 on CD20- CD38-	Ornithine levels	IVW	16	−0.084	0.032	0.009	0.498	0.357
CD20 on CD20- CD38-	Alpha-ketoglutarate to ornithine ratio	IVW	16	0.075	0.031	0.016	0.584	0.973
CD25 on memory B cell	Tetradecanedioate (C14-DC) levels	IVW	24	0.043	0.019	0.023	0.059	0.138
CD25 on memory B cell	1-oleoyl-GPI (18:1) levels	IVW	24	0.038	0.017	0.024	0.171	0.903
CD25 on memory B cell	X-23782 levels	IVW	24	0.044	0.016	0.007	0.952	0.844
CD25 on memory B cell	Choline phosphate to choline ratio	IVW	24	−0.033	0.017	0.05	0.429	0.39
CD27 on IgD+ CD38- unsw mem	2-stearoyl-GPE (18:0) levels	IVW	23	0.032	0.015	0.029	0.996	0.97
CD38 on naive-mature B cell	Isovalerylglycine levels	IVW	26	−0.042	0.021	0.041	0.335	0.488
CD64 on CD14- CD16+ monocyte	Hydroxy-N6,N6,N6-trimethyllysine levels	IVW	19	−0.05	0.025	0.045	0.337	0.592
CD64 on CD14- CD16+ monocyte	Dopamine 4-sulfate to dopamine 3-O-sulfate ratio	IVW	19	0.079	0.029	0.007	0.881	0.426
SSC-A on HLA DR+ T cell	Tetradecanedioate (C14-DC) levels	IVW	22	−0.053	0.024	0.031	0.426	0.247
SSC-A on HLA DR+ T cell	X-23782 levels	IVW	22	−0.049	0.022	0.027	0.894	0.97
HLA DR on CD33dim HLA DR+ CD11b+	EDTA levels	IVW	21	0.039	0.011	0.001	0.999	0.382

(*Continued*)

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Table 3 (Continued).

Exposure	Outcome	Method	Nsnp	Beta	Se	P-val	Pleiotropy	Heterogeneity
HLA DR on CD33dim HLA DR+ CD11b+	Tauro-beta-muricholate levels	IVW	21	0.032	0.016	0.037	0.421	0.588
HLA DR on CD33dim HLA DR+ CD11b+	Tetradecanedioate (C14-DC) levels	IVW	21	0.027	0.011	0.015	0.761	0.677
HLA DR on CD33dim HLA DR+ CD11b+	2-hydroxyarachidate levels	IVW	21	0.026	0.012	0.026	0.427	0.487
HLA DR on CD33dim HLA DR+ CD11b+	Alpha-ketoglutarate to ornithine ratio	IVW	21	−0.024	0.012	0.036	0.595	0.423

Abbreviations: MR, Mendelian randomization; PCOS, polycystic ovary syndrome; Nsnp, number of SNPs; Beta, causal effect size; Se, standard error of the Beta estimate; IVW, inverse-variance weighted method.

Mediation Analysis

To illuminate plasma metabolites mediating the causal relationship between immune cells and PCOS through mediation, we performed a mediation analysis. We identified 17 plasma metabolites that mediated the relationship between 12 immune cells and PCOS ($P < 0.05$). Notably, immune cells do not necessarily exert their effects on PCOS through a single plasma metabolite. Specifically, the effects of CD25 on memory B cells and HLA DR on CD33dim HLA DR+ CD11b+ on PCOS were mediated by three distinct plasma metabolites. Similarly, the effects of Myeloid DC %DC, NKT AC, and CD20 on CD20- CD38- on PCOS were mediated by two different plasma metabolites.

Additionally, both metabolites, Histidine betaine (hercynine) levels and Alpha-ketoglutarate to ornithine ratio, mediated more than one type of causal relationship between immune cells and PCOS. Moreover, 17 plasma metabolites played a mediating role, and the top five plasma metabolites with the highest mediating ratios were as follows: X-23782 levels had the highest mediating ratio of 26.4% (P=0.007), followed by ornithine levels with a mediating ratio of 22.0% (P=0.013),

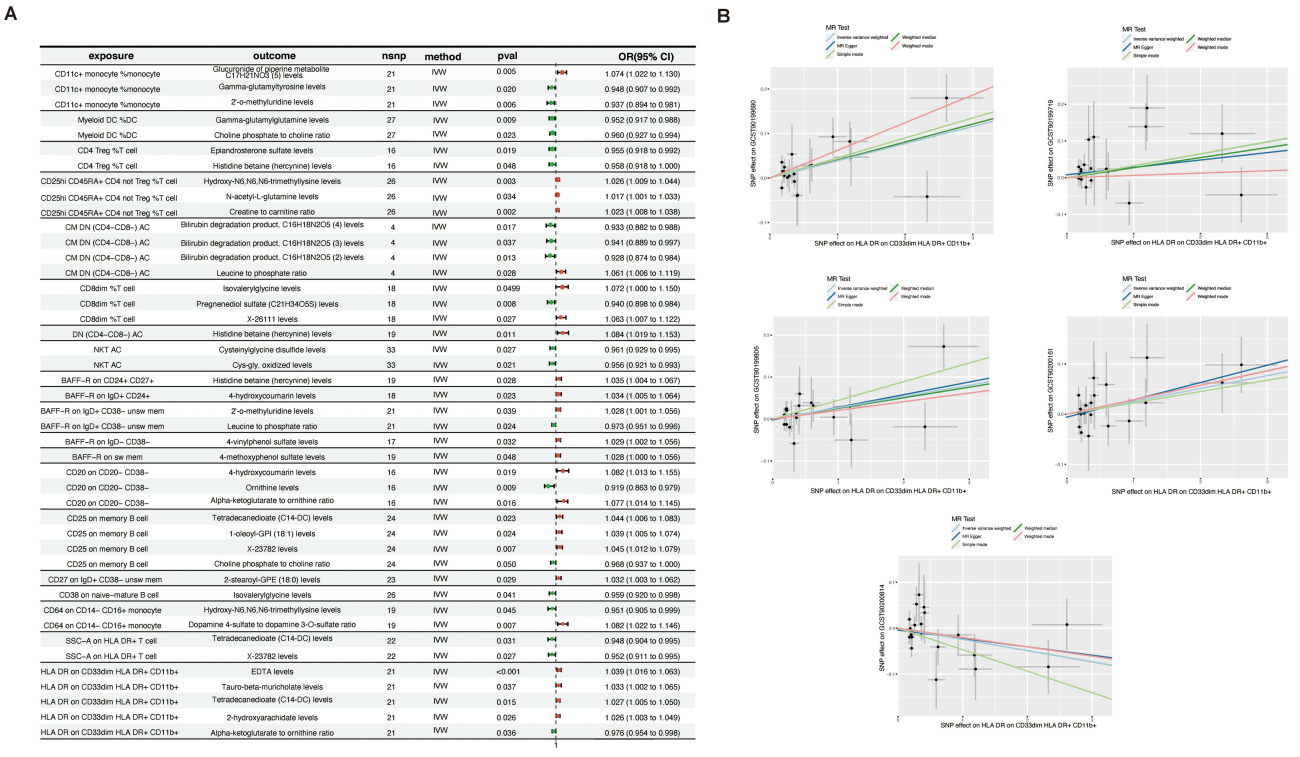

Figure 4 Mendelian randomized analysis of immune cells and plasma metabolites. (**A**) Forest plot of causal relationship between 20 immune cells and 32 plasma metabolites; (**B**) Scatter plots showed that HLA DR on CD33dim HLA DR+ CD11b+ was positively associated with EDTA levels, Tauro-beta-muricholate levels, Tetradecanedioate (C14-DC) levels and 2-hydroxyarachidate levels, and negatively correlated with Alpha-ketoglutarate to ornithine ratio.

Table 4 MR-PRESSO Tests of Metabolites and PCOS

Exposure	Outcome	RSSobs	Pvalue
EDTA levels	PCOS	11.957	0.702
Epiandrosterone sulfate levels	PCOS	15.826	0.839
Isovalerylglycine levels	PCOS	14.459	0.822
Tetradecanedioate (C14-DC) levels	PCOS	5.237	0.581
1-oleoyl-GPI (18:1) levels	PCOS	20.789	0.753
Pregnenediol sulfate (C21H34O5S) levels	PCOS	5.706	0.6
4-hydroxycoumarin levels	PCOS	20.46	0.419
Cysteinylglycine disulfide levels	PCOS	13.902	0.663
Histidine betaine (hercynine) levels	PCOS	13.11	0.652
2-stearoyl-GPE (18:0) levels	PCOS	26.613	0.343
4-methoxyphenol sulfate levels	PCOS	8.357	0.965
2-hydroxyarachidate levels	PCOS	14.229	0.924
Glucuronide of piperine metabolite C17H21NO3 (5) levels	PCOS	8.894	0.978
Hydroxy-N6,N6,N6-trimethyllysine levels	PCOS	17.201	0.832
Bilirubin degradation product, C16H18N2O5 (4) levels	PCOS	13.11	0.681
Bilirubin degradation product, C16H18N2O5 (3) levels	PCOS	34.089	0.296
Bilirubin degradation product, C16H18N2O5 (2) levels	PCOS	14.203	0.909
Gamma-glutamyltyrosine levels	PCOS	22.162	0.605
Gamma-glutamylglutamine levels	PCOS	28.026	0.381
Cys-gly, oxidized levels	PCOS	21.95	0.444
Ornithine levels	PCOS	5.112	0.569
X-23782 levels	PCOS	3.425	0.705
X-26111 levels	PCOS	14.883	0.394
N-acetyl-L-glutamine levels	PCOS	21.784	0.482
2'-o-methyluridine levels	PCOS	44.175	0.451
Creatine to carnitine ratio	PCOS	18.056	0.367
Dopamine 4-sulfate to dopamine 3-O-sulfate ratio	PCOS	23.205	0.442
Alpha-ketoglutarate to ornithine ratio	PCOS	12.04	0.786
Choline phosphate to choline ratio	PCOS	10.916	0.851
Leucine to phosphate ratio	PCOS	24.82	0.388

Abbreviations: MR-PRESSO, Mendelian Randomization Pleiotropy RESidual Sum and Outlier; RSSobs, Residual Sum of Squares observed; PCOS, polycystic ovary syndrome.

tetradecanedioate (C14-DC) levels with a mediating ratio of 20.2% (P=0.025), EDTA levels with a mediating ratio of 17.3% (P=0.001), and the creatine-to-carnitine ratio with a mediating ratio of 16.3% (P=0.002) (Figure 5 and Table 5).

Discussion

To investigate the relationship between immune cells, plasma metabolites, and PCOS, we conducted the first multiple bidirectional two-sample MR studies and mediation studies to investigate the causal relationship between immune cells and PCOS via plasma metabolites. Our study highlights 12 immune cells impacting PCOS through 17 metabolites, advancing the understanding of immune mechanisms in PCOS risk and suggesting potential therapeutic approaches targeting immune modulation. To our knowledge, this is the first large-scale genetic correlation analysis of immune cells, plasma metabolites, and PCOS. Because we used GWAS genetic data, the results are not susceptible to environmental confounders.

Our MR analysis identified a total of 33 immune cells causally associated with PCOS, with more than half belonging to the B cell panel, including 10 protective factors and 8 risk factors. The importance of B cells in the progression of PCOS has been emphasized in recent studies and our finding not only highlights the important role of B cells in the development of PCOS but also underscores the complexity of their involvement in PCOS pathogenesis.[33] CD20, a specific antigen located on the surface of B lymphocytes, is likely crucial for regulating B cell proliferation, differentiation, and signal transduction processes.[34] Based on this, a recent MR analysis suggested that CD20- CD38- B cells may act as protective factors for

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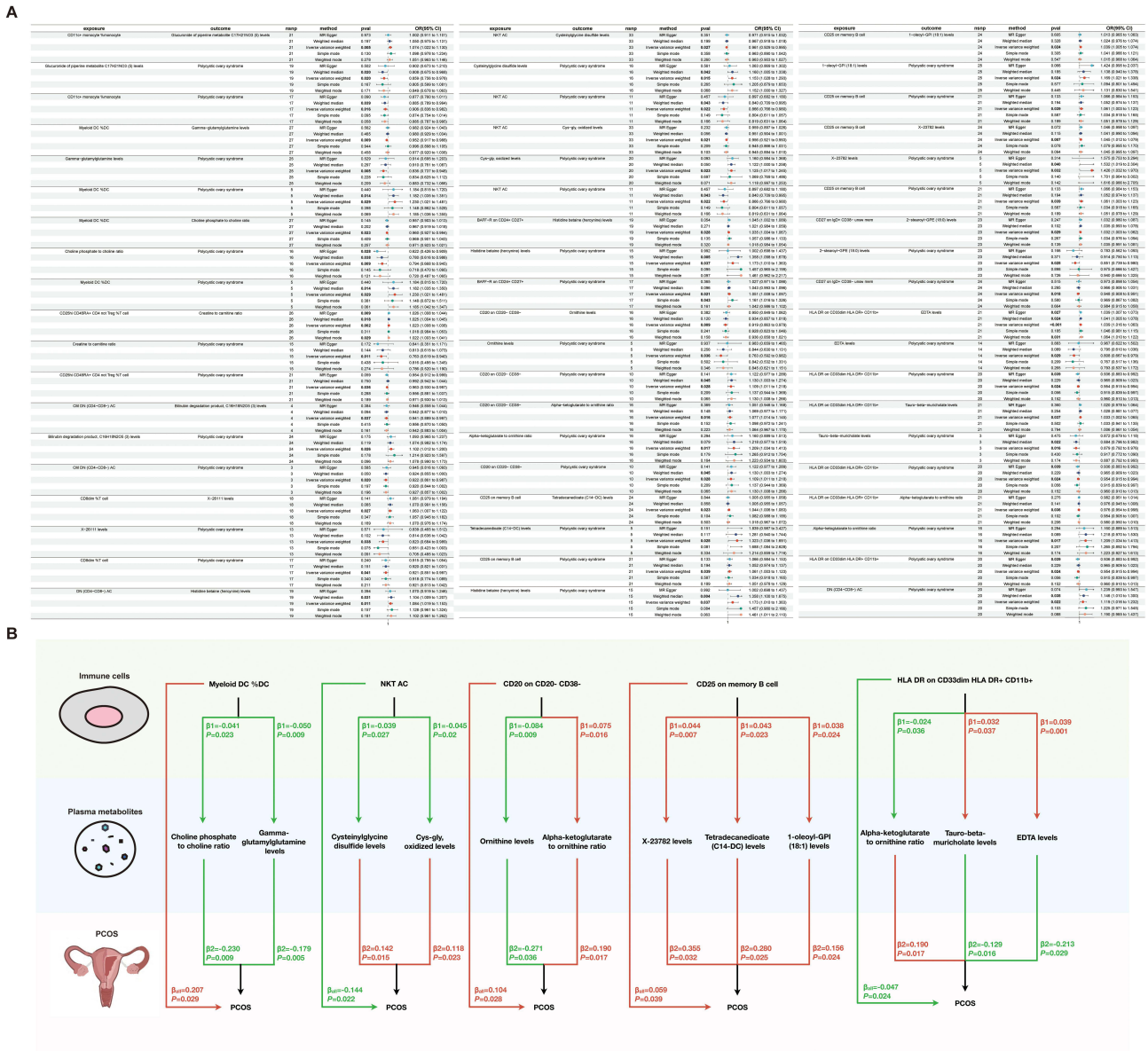

Figure 5 Mendelian randomized analysis of the causal effects between immune cells, plasma metabolites and PCOS. (**A**) Forest plots of immune cells, plasma metabolites, and PCOS; (**B**) Plasma metabolites mediated the causal relationship between immune cells and PCOS (risk factors in red, protective factors in green).

PCOS, potentially by safeguarding normal ovarian morphology through the preservation of normal immune system pathways.[35] However, our study found that CD20 on CD20- CD38- and CD20 on IgD- CD38dim B cells were associated with an increased risk of PCOS. This suggests that greater attention should be given to the characteristic of memory B cells rich in autoantibodies, as this trait is related to chronic infections, autoimmune diseases, and disease accumulation.[35] Additionally, observational studies have consistently confirmed the dysregulation of immune cell levels in PCOS, which aligns with our observation of the risk factors for PCOS involve distinct immune cell phenotypes, including Myeloid DC %DC, CD4 Treg %T cells, Lymphocyte AC, DN (CD4-CD8-) AC, and various B and T cell subsets.[36] These findings support the notion that immune dysfunction, particularly an overactive immune response, plays a significant role in PCOS.[37] Such overactivation could lead to chronic inflammation, which may further promote the development of insulin resistance and hormonal imbalances-hallmarks of PCOS pathogenesis.[38]

On the other hand, 13 immune cells were identified as protective factors for PCOS. These immune cells, such as CD24+ CD27+ AC, CD11c+ monocyte %monocyte, CD25hi CD45RA+ CD4 not Treg %T cell, and various other

Table 5 MR Analysis of the Causal Effects Between Immune Cells, Plasma Metabolites and PCOS

Immune Cell	Metabolite	Outcome	Mediated Effect	Direct Effect	Mediated Proportion	P-value
CD11c+ monocyte %monocyte	Glucuronide of piperine metabolite C17H21NO3 (5) levels	PCOS	−0.0109	−0.088	11%	0.012
Myeloid DC %DC	Choline phosphate to choline ratio	PCOS	0.00943	0.197	4.56%	0.025
Myeloid DC %DC	Gamma-glutamylglutamine levels	PCOS	0.00889	0.198	4.30%	0.012
CD25hi CD45RA+ CD4 not Treg %T cell	Creatine to carnitine ratio	PCOS	−0.00615	−0.032	16.30%	0.002
CM DN (CD4-CD8-) AC	Bilirubin degradation product, C16H18N2O5 (3) levels	PCOS	−0.00588	−0.075	7.25%	0.078
CD8dim %T cell	X-26111 levels	PCOS	−0.0119	−0.07	14.50%	0.035
DN (CD4-CD8-) AC	Histidine betaine (hercynine) levels	PCOS	0.0129	0.1	11.40%	0.023
NKT AC	Cysteinylglycine disulfide levels	PCOS	−0.00559	−0.138	3.89%	0.033
NKT AC	Cys-gly, oxidized levels	PCOS	−0.00529	−0.138	3.68%	0.031
BAFF-R on CD24+ CD27+	Histidine betaine (hercynine) levels	PCOS	0.00547	0.044	11%	0.032
CD20 on CD20- CD38-	Ornithine levels	PCOS	0.0228	0.081	22%	0.013
CD20 on CD20- CD38-	Alpha-ketoglutarate to ornithine ratio	PCOS	0.0141	0.09	13.60%	0.025
CD25 on memory B cell	X-23782 levels	PCOS	0.0157	0.044	26.40%	0.007
CD25 on memory B cell	Tetradecanedioate (C14-DC) levels	PCOS	0.012	0.047	20.20%	0.025
CD25 on memory B cell	1-oleoyl-GPI (18:1) levels	PCOS	0.00595	0.054	10%	0.028
CD27 on IgD+ CD38- unsw mem	2-stearoyl-GPE (18:0) levels	PCOS	−0.00512	−0.048	9.68%	0.032
HLA DR on CD33dim HLA DR+ CD11b+	Alpha-ketoglutarate to ornithine ratio	PCOS	−0.00462	−0.043	9.73%	0.038
HLA DR on CD33dim HLA DR+ CD11b+	Tauro-beta-muricholate levels	PCOS	−0.00415	−0.043	8.76%	0.043
HLA DR on CD33dim HLA DR+ CD11b+	EDTA levels	PCOS	−0.00821	−0.039	17.30%	0.001

Abbreviations: MR, Mendelian randomization; PCOS, polycystic ovary syndrome.

immune cell types like NKT AC, CD27 on CD24+ CD27+, and HLA DR on CD33dim HLA DR+ CD11b+, were found to be associated with a decreased risk of developing PCOS. We hypothesize that these immune cells, particularly those involved in the maturation stages of T cells, monocytes, and myeloid cells, may play a role in modulating the inflammatory state through their corresponding immune functions. By maintaining a balanced immune response, these protective immune cells could potentially reduce the risk of PCOS. This may involve suppression of excessive inflammation, modulation of insulin sensitivity, or regulation of hormonal pathways, ultimately contributing to a protective effect against the disease.[39,40] Overall, these findings further support the hypothesis that immune cell-mediated inflammation plays a crucial role in PCOS, with certain immune cell types acting as risk factors and others serving as protective factors. Understanding the roles of these immune cells in PCOS pathogenesis could offer novel therapeutic targets for managing or preventing the disease.

Metabolites have a vital role in recognizing high-risk populations at an early stage and in preventing disease, with potential clinical applications.[41] An MR analysis identified metabolites that may be causally involved in the development of PCOS, which was also identified in our study as having impact on the risk of PCOS.[42] An increasing number of studies highlight the role of both innate and adaptive immunity in responding to changes in metabolic conditions.[43] The emerging field of immunometabolism is built upon evidence of immune-derived signals being activated in metabolically significant tissues, and it explores how immune cells assist tissues in adapting to environmental challenges.[44] Adipose tissue is one of the most explored tissues in the field, and the expression and activation of various immune cell types and anti-inflammatory cytokines have been studied in both lean and obese states.[45] In addition, the immune profile of adipose tissue, generating chronic low-grade inflammation, gradually becomes systemic and leads to insulin resistance and metabolic diseases.[46] The results of this study also confirmed causal relationships between 20 immune cell types and 32 plasma metabolites. Therefore, although the specific molecular mechanisms underlying the interactions between immune cells and metabolites during the pathogenesis of

PCOS remain unclear, it could be speculated that research from the perspectives of immunometabolism may provide new insights for the comprehensive understanding, diagnosis, and treatment of PCOS.

We found a causal relationship between CD25 on memory B cell and PCOS through three mediators, X-23782 levels, tetradecanedioate (C14-DC) levels, and 1-oleoyl-GPI (18:1) levels. CD25 on memory B cell is positively correlated with these three metabolites, which in turn are positively correlated with PCOS. Tetradecanedioate (C14-DC) is considered a biomarker of transporter function, a metabolite of fatty acid ω-oxidation.[47,48] As it turns out, its role in the metabolism associated with pregnancy is significant, and we hypothesize that it may indirectly affect ovarian function and reproductive health.[49] 1-oleoyl-GPI (18:1) is a phospholipid compound that has been shown to be upregulated in activated platelets, but its association with ovarian disease requires further study.[50]

In addition, we found that HLA DR on CD33dim HLA DR+ CD11b reduced the risk of PCOS through three mediators, Alpha-ketoglutarate to ornithine ratio, tauro-beta-muricholate levels, and EDTA levels. HLA DR on CD33dim HLA DR+ CD11b+ was negatively correlated with Alpha-ketoglutarate to ornithine ratio, while Alpha-ketoglutarate to ornithine ratio was positively correlated with PCOS. HLA DR on CD33dim HLA DR+ CD11b+ was positively correlated with both tauro-beta-muricholate levels and EDTA levels, while both two were negatively correlated with PCOS. Alpha-ketoglutarate is a key intermediate in the Krebs cycle, shown to promote oocyte meiosis and embryonic development and reduce oxidative stress.[51,52] Ornithine is an amino acid metabolite mainly involved in the urea cycle. Macrophages metabolize arginine through different pathways to produce ornithine, which affects the immune response and inflammatory processes.[53] When Alpha-ketoglutarate combines with ornithine to form ornithine-alpha-ketoglutarate (OKG), some studies have found that it may be capable of enhancing immune function and reducing oxidative stress and inflammation in patients with severe illness.[54,55] These effects on immune and reproductive function Alpha-ketoglutarate to ornithine ratio affect PCOS and provide indirect evidence. Interestingly, we found that Ornithine levels and Alpha-ketoglutarate to ornithine ratio again play a mediating role in CD20 on CD20- CD38- increased PCOS risk. CD20 on CD20- CD38- was negatively correlated with Ornithine levels, which were negatively correlated with PCOS, while CD20 on CD20- CD38- was positively correlated with Alpha-ketoglutarate to ornithine ratio.

Our study also discovered that Myeloid DC %DC may affect PCOS through two mediators, Choline phosphate to choline ratio and Gamma-glutamylglutamine levels. Choline metabolism has been shown to have a role in ovarian function, and one study found that choline supplementation promoted ovarian follicular development and increased the number of corpus luteum in the ovary.[56] Gamma-glutamylglutamine is a metabolite produced through the action of gamma-glutamyltransferase (GGT), and it has been shown that GGT levels correlate with the prognosis of ovarian cancer.[57] Therefore, we believe that these two metabolites are relevant to PCOS. In addition, our results suggested that NKT AC affected the development of PCOS through two mediators, cysteinylglycine disulfide levels and cys-gly, oxidized levels. These two are both products of glutathione metabolism, which are related to oxidative stress and can reflect the state of intracellular redox balance,[58] and oxidative stress is a factor in PCOS. Notably, histidine betaine (hercynine) levels acted as a mediator not only in DN (CD4-CD8-) AC with PCOS, but also in BAFF-R on CD24+ CD27 + and PCOS. Histidine betaine (hercynine) is a metabolite derived from histidine, and as Histamine has been reported to induce ovulation in rats,[59] which may be indirect evidence for its regulation of PCOS.

In recent years, there has been growing attention on the identification of immune cells and plasma metabolites, as diagnostic biomarkers for polycystic ovary syndrome. The decreased transcript levels of HDDC3 and SDC2 were validated in granulosa cells from women with PCOS and indicated that HDDC3 and SDC2 might serve as candidate biomarkers for PCOS in clinical practice.[10] As for plasma metabolites, Murri et al published a valuable review where they compared few studies based on the analyses of plasma obtained from PCOS women and healthy controls and as can be observed metabolites connected with lipid, amino acid, as well as energy metabolism such as citric acid cycle seem to as the most characteristic for PCOS.[60,61] Our findings open new avenues for understanding the pathogenesis of PCOS and highlight the key role of blood metabolites in mediating the relationship between immune cells and PCOS, rather than focusing solely on one or the other. This not only deepens our understanding of the pathological processes of PCOS but also lays the theoretical foundation for the development of new immune therapies and metabolite-targeted interventions.

Our Mendelian mediator analysis provides new insights into how immune cells affect PCOS via plasma metabolites. The relationship between the three is illustrated from a genetic perspective, highlighting potential biomarkers and

therapeutic targets that can be used in clinical practice. While the use of a large amount of GWAS pooled data increased the robustness and reliability of our findings, this study still has some limitations. First, MR is not a substitute for objective clinical trials, as it is only a method to analyze the causal relationship between exposure and outcome. Causality is elucidated from a genetic perspective and cannot be determined from environmental factors. Second, the data in this study were primarily from a European population, which may limit its generalizability to other ethnic groups. Future studies should focus on validating these findings in different populations, and functional studies are needed to elucidate the specific biological mechanisms of how the identified immune cells and metabolites affect PCOS. Investigating the therapeutic potential of these immunometabolic pathways may open new avenues for the treatment of PCOS.

Conclusions

In this study, we systematically evaluated the causal relationship between immune cells and plasma metabolites and PCOS and found that 12 immune cells were causally linked to PCOS through 17 metabolites. The results reveal the important roles of immune cells and metabolites in the pathogenesis of PCOS and advance PCOS treatment strategies, emphasizing the necessity of further research to explore these complex biological interactions and their potential therapeutic application.

Data Sharing Statement

The datasets presented in the study are available in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Materials.

Ethics Approval and Informed Consent

The study was approved by the Medical Ethical Review Board of Quanzhou Medical College [Ethical Review No. (2025003), Quanzhou Medical College]. In addition, all participants were exempted from providing informed consent, as the databases used in this study all report obtaining informed consent from the patients.

Consent for Publication

We confirm that we have reviewed the article and consent to the publication of any details, images, videos, recordings, or other materials included within it. We understand that this content will be made publicly available as part of the publication process. We further confirm that I have been provided with the final version of the article and am fully aware of the materials being published.

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Author Contributions

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

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Disclosure

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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