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Novel insights into the complex interplay of immune dysregulation and inflammatory biomarkers in preeclampsia and fetal growth restriction: A two-step Mendelian randomization analysis

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ABSTRACT

Background: The relationship between genetic immune dysregulation and the occurrence of preeclampsia (PE) or PE with fetal growth restriction (PE with FGR) has yielded inconsistent findings, and the underlying mediators of this association remain elusive. We aimed to explore the causal impact of genetic immune dysregulation on the risk of PE or PE with FGR and to elucidate the role of specific transcriptomes in mediating this relationship.
Methods: A two-step Mendelian randomization (MR) analysis was performed to explore the link between immune dysregulation and PE or PE with FGR, as well as to identify potential inflammatory biomarkers that act as mediators. GWAS summary data for outcomes were obtained from the FinnGen dataset. The analyses encompassed five systemic immune-associated diseases, four chronic genital inflammatory diseases, and thirty-one inflammatory biomarkers. Summary-data-based MR (SMR) and HEIDI analysis were conducted to test whether the effect size of single nucleotide polymorphisms (SNPs) on outcomes was mediated by the expression of immune-associated genes.
Results: The primary univariable analysis revealed a significant positive correlation between systemic lupus erythematosus (SLE), type 1 diabetes (T1D), type 2 diabetes (T2D), and rheumatoid arthritis (RA) with the risk of PE or PE with FGR. Surprisingly, a counterintuitive finding showed a significant negative association between endometriosis of pelvic peritoneum (EMoP) and the risk of PE with FGR. None of the inflammatory factors had a causal relationship with PE or PE with FGR. However, there was a significant association between lymphocyte count and the risk of PE with FGR. Within the lymphocyte subset, both the proportion of Natural Killer (NK) cells and absolute counts of naïve $CD4^+$ T cells demonstrated significant effects on the risk of PE with FGR. Two-step MR analysis underscored the genetically predicted lymphocyte count as a significant mediator between T1D and PE with FGR. Additionally, SMR analysis indicated the potential involvement of SH2B3 in the occurrence of PE with FGR.
Conclusions: Our findings provided substantial evidence of the underlying causal relationship between immune dysregulation and PE or PE with FGR and some of these diseases proved to accelerate immune cells disorders and then contribute to the risk of incident PE or PE with FGR.

1. Introduction

Preeclampsia (PE) is a life-threatening pregnancy complication traditionally defined clinically by new-onset or worsening hypertension during pregnancy >20 weeks, along with accompanying proteinuria or other signs of organ system involvement. Its incidence is estimated at 2 %–4 % worldwide [1]. PE can be classified as early-onset preeclampsia (EOPE) or late-onset preeclampsia (LOPE), depending on whether if develops before or at 34 weeks of gestation [2]. While the presenting features of the two conditions are similar, they have different maternal and fetal outcomes, heritability, biochemical markers, and clinical features [3]. EOPE is more often associated with fetal growth restriction (FGR), adverse perinatal outcomes, and high perinatal mortality. The immune system plays a pivotal role in the normal progression of

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physiological pregnancy and fetal development. However, dysregulated maternal systemic immune responses are major contributors to the pathogenesis of EOPE [4]. Some systemic diseases associated with immune disorders, such as systemic lupus erythematosus (SLE) [5], type 1 and type 2 diabetes (T1D, T2D) [6], chronic kidney disease (CKD) [7], and rheumatoid arthritis (RA) [8], have been shown to increase the risk of EOPE. Immune cells and multiple inflammatory factors actively participate in maternal immune tolerance, trophoblast invasion, and uterine spiral artery remodeling. Imbalances in these components are closely linked to the pathogenesis of EOPE [3,4].

Despite the wealth of evidence from previous observational studies, establishing a causal relationship between immune dysregulation and the onset of PE remains challenging. The inherent limitations of observational studies hinder a comprehensive understanding of the association between immune dysregulation and PE. This warrants further investigations from different perspectives. Mendelian randomization (MR) analysis, which leverages genetic information, offers a valuable complement to observational studies [9]. It has been widely used to explore causal links between exposures and diseases [10–13]. However, only a few MR analyses have assessed the association between immune system and PE [14,15]. Moreover, these previous MR studies considered only a limited set of diseases or inflammatory factors. To address these limitations, our current study employs a large two-step MR approach to investigate the genetic associations of five systemic immune-associated diseases, fourteen immune cell types, and seventeen inflammatory factors with the risk of PE. Research on the association between innate local tissue inflammation and the risk of PE is limited [16]. Given that chronic genital inflammation can lead to immune microenvironment dysregulation, we investigated endometriosis and upper reproductive inflammation to elucidate the role of genetically predicted genital inflammation in PE. Considering that a significant portion of EOPE cases often involve FGR and in the absence of direct EOPE data, we included data from PE with FGR in our MR analyses to explore shared and distinct genetic risk factors between these two diagnoses. These findings aim to complement previous epidemiological studies and provide novel insights into the pathogenesis of PE or PE with FGR.

2. Materials and methods

2.1. Data source

2.1.1. GWAS data of exposures and mediators

In this study, we included five systemic diseases, namely SLE [17], T1D [18], T2D [19], CKD [20], and RA [21]. We also included four types of genital inflammation obtained from FinnGen. Additionally, we obtained GWAS summary data for fourteen immune cell types, including leukocyte count, eosinophil count, basophil count, neutrophil count, lymphocyte count, monocyte count [22]. This data covered both the proportion of specific lymphocytes and their absolute count in blood [23]. For inflammatory factors known to be associated with PE based on prior research, we collected the GWAS summary data from multiple sources, including the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Inflammation Working Group (CIWG) [24], a plasma proteome GWAS study that involved 4907 aptamers in 35,559 Icelanders [25], the KORA F4 study of 3080 subjects living in southern Germany [26], the INTERVAL study that involved 3301 individuals of European descent [27], and 21,758 participants from 13 cohorts of European ancestry [28]. Additional details about the selected GWAS data for the exposures and mediators are provided in Tables S1–S2.

2.1.2. GWAS data of outcomes

International guidelines define PE as the presence of new-onset hypertension, proteinuria, or end-organ dysfunction in the later stages of pregnancy. The diagnosis in this study was based on the International Classification of Disease (ICD-10: O36.5). FGR is defined as abnormal fetal growth in utero, often indicated by birth weight below the 10th percentile for gestational age or other signs of insufficient growth during pregnancy. Its ICD codes are ICD-10: P05.1 and ICD-10: P05.2. Summary data for PE and PE with FGR were extracted from FinnGen, which included a total of 10,297 cases and 200,573 controls.

2.2. Mendelian randomization analysis

2.2.1. Selection of instrumental variables

The genetic instrumental variables (IVs) were chosen through a multistep process. First, SNPs associated with the exposures or mediators were selected based on the conventional GWAS threshold ($p < 5 \times 10-8$). Then, a linkage disequilibrium (LD) threshold of $r2 < 0.001$ and a distance of 10,000 kb between the genetic instruments were applied to ensure that the SNPs were independent of each other. All LD estimates presented in this study were calculated using individuals of European ancestry from the 1000 genomes reference panel (released in Oct 2012). Next, the beta coefficients and standard errors of the selected SNPs from the GWAS of PE were extracted. Ambiguous SNPs with inconsistent alleles and palindromic SNPs with ambiguous strands were either corrected or excluded. To ensure the reliability of IVs, the strength of the relationship between IVs and the phenotype was assessed using the F-statistics. The F-statistic of the chosen IVs was ensured to be greater than 10, as calculated using the following equation [29].

2.2.2. Two-sample Mendelian randomization analysis

In this study, two-sample MR analyses were employed to investigate the relationship between immune dysregulation and the risk of PE or PE with FGR. This analysis was based on three key assumptions: (i) genetic variants were associated with the exposures (or mediators); (ii) genetic variants were independent of any confounding factors affecting the exposure/mediator-outcome association; and (iii) genetic variants were independent of the outcome and only associated with the outcome through the gene expression of the exposures (or mediators). To account for potential confounding factors, the PhenoScanner website (http://www.phenoscanner.medschl.cam.ac.uk) was utilized to identify and remove any confounders. The primary MR method was IVW, either IVW-FE or IVW-RE, was selected based on Cochrane's Q heterogeneity test. Other two MR methods, namely MR-Egger, and weighted median, were applied to estimate the causality between exposures or mediators and the outcome. Furthermore, MR-Pleiotropy Residual Sum and Outlier (MR-PRESSO) approaches [30] was applied to correct any outliers in the study. The results were presented as odds ratios (ORs) with their corresponding 95 % confidence intervals (CIs).

2.2.3. Two-step and multivariable Mendelian randomization analysis

The significance of the mediation effect of specific inflammatory biomarkers was assessed using a stepwise testing approach. In the first stage regression, SNPs associated with immune dysregulation were utilized to estimate the causal effect of the risk factors on the specific inflammatory biomarkers, quantified as βXZ, through the standard two-sample MR approach. In the second stage regression, we used MVMR analysis to estimate the direct effect, βXY1 and the effect of the specific inflammatory biomarkers on PE or PE with FGR, represented as βZY. The mediation effect was then calculated as the product of βXZ and βZY. The total effect, denoted as βXY, represents the overall association between the immune dysregulation with PE or PE with FGR. The mediation effect was then calculated as the product of βXZ and βZY. The total effect, denoted as βXY, represents the overall association between immune dysregulation and PE or PE with FGR. We calculated the proportion of the indirect effect of immune dysregulation on PE/PE with FGR that was mediated by the inflammatory biomarkers by dividing the mediation effect by the total effect. If either βXZ or βZY is not statistically significant, the mediation is considered to be interrupted. If βXY1 is statistically significant, it indicates incomplete mediation, whereas nonsignificance suggests complete mediation.

2.2.4. SMR & HEIDI analysis

The SMR & HEIDI analyses can be interpreted as analyses to test if the effect size of a SNP on the phenotype is mediated by gene expression [30]. In our study, we utilized Blood eQTL summary statistics obtained from eQTLGen, which includes genetic data on blood gene expression from 31,684 individuals across 37 datasets [31], as the exposures. For the outcomes, we selected GWAS data of PE, PE with FGR, T1D, T2D, and RA. Our criteria for significant results were as follows: SMR FDR<0.05, HEIDI P > 0.05, *cis*-eQTLs, and GWAS P < 1 × 10−5 [32].

2.2.5. Sensitivity analysis

MR-Egger, MR-Egger intercept, and MR-PRESSO analyses were performed to estimate whether pleiotropy, which might affect the results of the MR analysis, was present. Scatter and funnel plots were visually examined, and genetic variations with outlier estimates were ruled out in the presence of excessive heterogeneity. Additionally, leave-one-out analysis was performed by eliminating a single SNP one by one.

2.2.6. Statistical software

We carried out the two-step, two-sample MR analyses by utilizing R version 4.2.2 (2022-10-31) (R Foundation for Statistical Computing, Vienna, Austria, 2008) and R studio version 2022.07.2 Build 576 (Boston, MA, United States). MR analyses were performed in R with R packages "VariantAnnotation", "gwasglue", "data.table", "dplyr", "TwoSampleMR", "MRPRESSO", "MendelianRandomization". The P value was two-sided and the statistical significance was set at <0.05.

3. Results

3.1. Study design

A large two-step, two-sample MR analysis was conducted to explore the relationships between systemic diseases (SLE, T1D, T2D, CKD, RA) and genital inflammation (endometriosis of uterus (EMoU), endometriosis of pelvic peritoneum (EMoP), uterus inflammation (UI), and pelvic inflammation (PI)) with genetically predicted risk of PE or PE with FGR. Excluding HLA (human leukocyte antigen)-related SNPs is a common practice in genetic studies, especially those related to autoimmune diseases. We used PhenoScanner to identify and remove these SNPs, reducing potential confounding effects and enhancing clarity in genetic associations outside the HLA region in autoimmune diseases. The multivariable Mendelian randomization (MVMR) analysis was employed to investigate whether these associations could be mediated by circulating inflammatory biomarkers, including immune cells and inflammatory factors. Then, summary-data-based (SMR) & HEIDI analyses were employed to identify the shared target genes and risk loci for the mediators and outcomes. Fig. 1 provides a schematic diagram illustrating our study design.

3.2. Association between immune dysregulation and PE or PE with FGR

The genetic association between immune dysregulation and PE or PE with FGR was analyzed through MR, as depicted in Fig. 2. Given the absence of heterogeneity among these systemic diseases, except for T2D, we utilized IVW (inverse-variance-weighted method)-FE (fixed-effect) to calculate their effects on outcomes, while employing IVW-RE (random-effect) for T2D. Additionally, no horizontal pleiotropy was detected in any of the diseases (Tables S3 and S4). In the two-sample MR analysis, a significant positive correlation of exposures on PE was observed among SLE (OR 1.03, 95 % CI 1.01 to 1.06 for IVW-FE, p 0.02), T1D (OR 1.03, 95 % CI 1.01 to 1.05 for IVW-FE, p 0.01), and T2D (OR 1.22, 95 % CI 1.08 to 1.37 for IVW-RE, p 0.001). Moreover, when examining the relationship between these diseases and PE with FGR, T1D (OR 1.03, 95 % CI 1.01 to 1.04 for IVW-FE, p 0.0003), T2D (OR 1.09, 95 % CI 1.01 to 1.17 for IVW-RE, p 0.001), and RA (OR 1.06, 95 % CI 1.02 to 1.10 for IVW-FE, p 0.01) were significantly associated with a

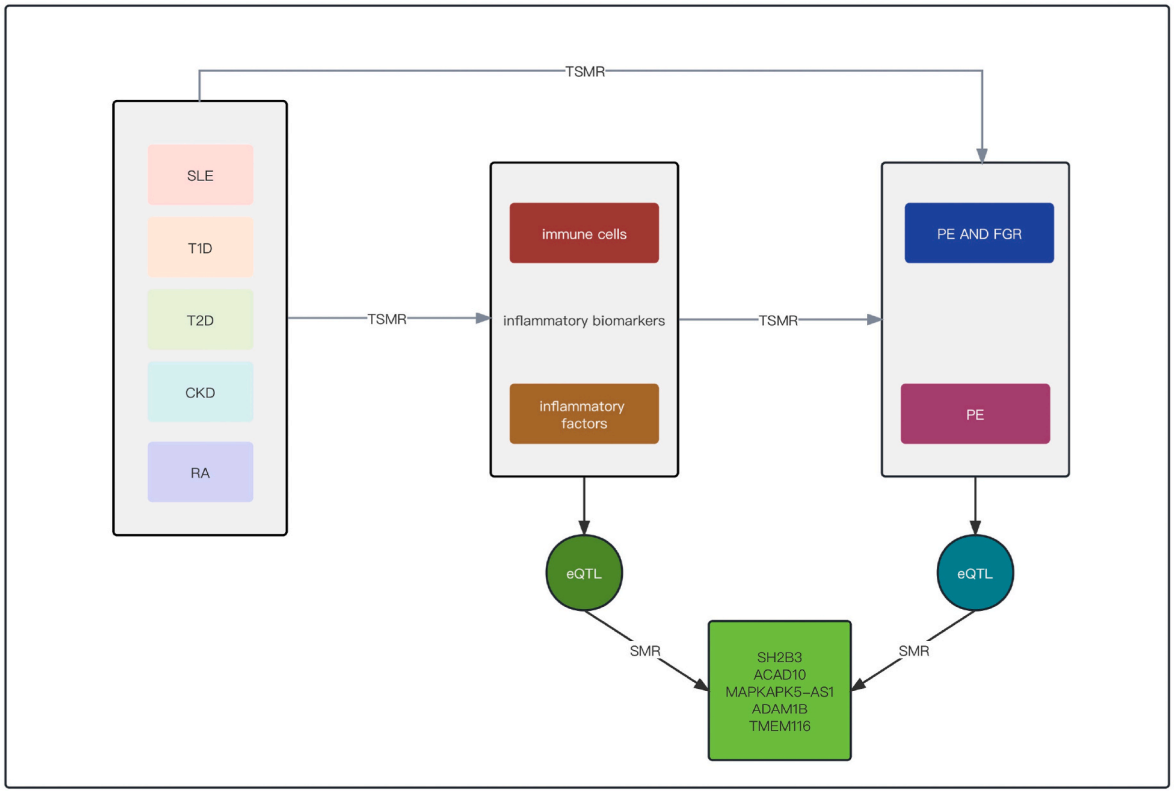

Fig. 1. Conceptual schematic of the two-step Mendelian randomization for the proposed causal relationships between immune dysregulation, inflammatory markers, and PE or PE with FGR.

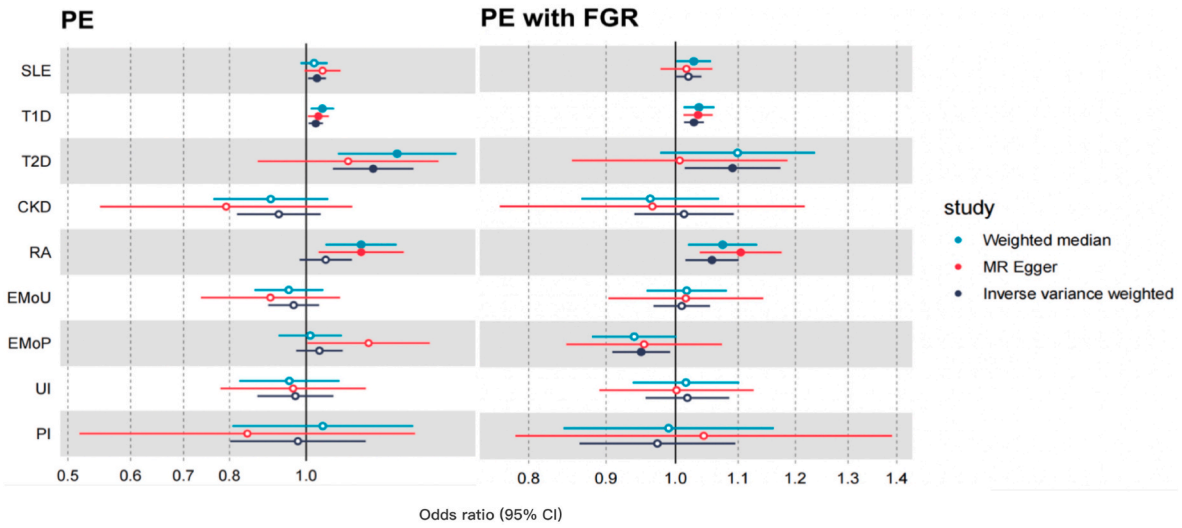

Fig. 2. The genetic association between immune dysregulation and PE or PE with FGR according to two-sample MR analysis.

higher risk of PE with FGR. A surprising and counterintuitive finding was the significant negative association between EMoP and PE with FGR (OR 0.95, 95 % CI 0.91 to 0.99 for IVW-FE, p 0.020), indicating a protective role of EMoP in PE with FGR.

3.3. Association between inflammatory biomarkers and PE or PE with FGR

Significant between-SNP heterogeneity was detected for most of the immune cells. No evidence of horizontal pleiotropy was found for any of the biomarkers except for the absolute count of naïve $CD4^+$ T cells (Pintercept 0.02) in PE, monocyte count (Pintercept 0.049) and Natural Killer (NK) cell count (Pintercept 0.021) in PE with FGR (Tables S5–6).

In the two-sample MR analysis, no association was observed between inflammatory factors and PE or PE with FGR (Table S7), and no significant relationship between immune cells and PE (Table S5). However, a positive relationship was found between lymphocyte count (OR 1.10, 95 % CI 1.01 to 1.21 for IVW-RE, p 0.04) and PE with FGR (Table S6).

To further investigate the impact of lymphocytes on PE with FGR, additional specific subsets of lymphocytes, such as the proportion of NK cells, $CD4^+$ T cells, $CD8^+$ T cells, and absolute counts of various lymphocytes, including naïve $CD4^+$ T cells, terminally differentiated $CD4^+$ T cells, memory $CD4^+$ T cells, effector $CD4^+$ T cells, CD4 regulatory T cells, and NK cells, were included. Among these, significant effects were observed for the proportion of NK cells (OR 0.95, 95 % CI 0.92 to 0.99 for IVW-FE, p 0.01) and absolute count of naïve $CD4^+$ T cells (OR 1.10, 95 % CI 1.02 to 1.20 for IVW-RE, p = 0.02) on PE with FGR (Table S7, Fig. 3). These findings suggest that lymphocyte count, the effect of which was mainly regulated by naïve $CD4^+$ T cells, were associated with a higher risk of PE with FGR. Meanwhile, the proportion of NK cells was considered a protective factor against PE with FGR.

3.4. Mediation of inflammatory biomarkers between immune dysregulation and PE with FGR

To investigate the mechanistic pathways underlying the causal relationships between immune dysregulation and PE with FGR, a two-step MR analysis was conducted with inflammatory biomarkers as mediator

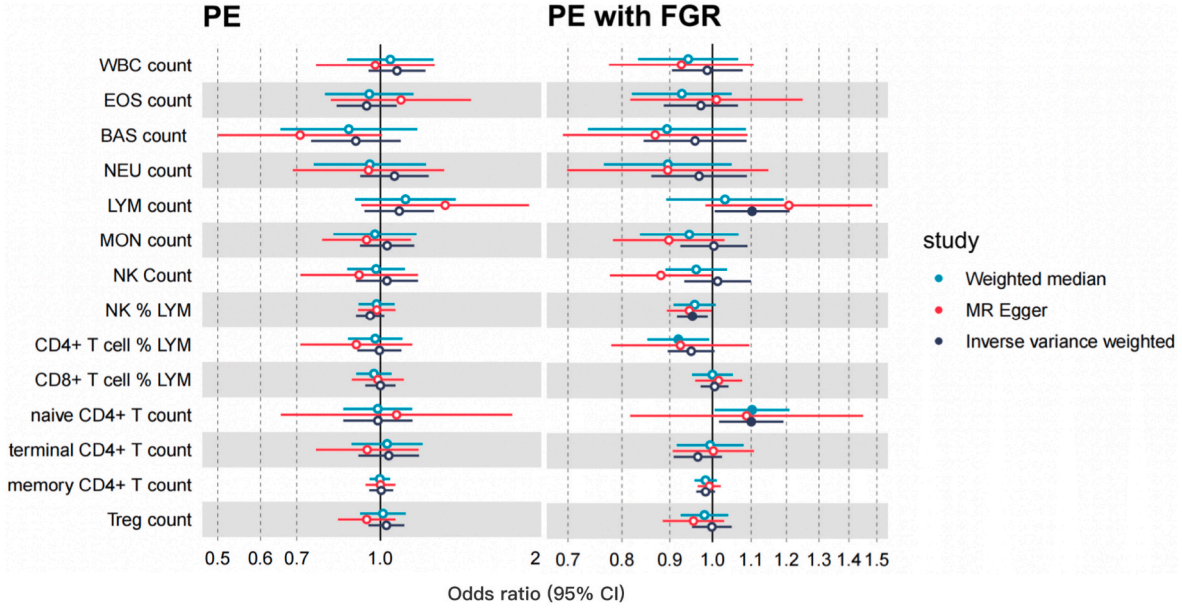

Fig. 3. The genetic association between immune cells and PE or PE with FGR according to two-sample MR analysis.

variables. When utilizing GWAS data for T1D as the exposure of interest and GWAS data for PE with FGR as the outcome, lymphocyte count was identified as a significant intermediate variable linking T1D with PE with FGR (Table 1). In more detail, there was a negative causal effect of T1D on lymphocyte count ($p < 0.05$; $\beta < 0$), as well as a positive causal effect of lymphocyte count on PE with FGR ($p < 0.05$; $\beta > 0$). Importantly, these causal relationships remained significant even after applying MVMR correction. Similarly, two-step MR analysis indicated that naive $CD4^+$ T cells acted as an intermediate variable linking RA with PE with FGR. However, after performing MVMR correction, these causal relationships between naïve $CD4^+$ T cells and PE with FGR became non-significant (Table 1). Furthermore, there were no significant effects of other inflammatory biomarkers as intermediaries in the relationship between immune dysregulation and PE with FGR. In summary, two-step and MVMR analyses highlight a substantial and positive causal effect of T1D on PE with FGR, with lymphocyte count playing an incomplete mediating role in this relationship.

3.5. Identification of target genes and risk loci for PE with FGR by SMR analysis

Expression quantitative trait loci (eQTL) refer to genetic variants associated with gene expression phenotypes [32]. In our analyses utilizing whole blood eQTL data and GWAS data for lymphocyte count, PE, and PE with FGR for SMR analysis, we identified specific genes that exhibited significant positive associations with risk loci for both lymphocyte count and PE with FGR (Table 2). These genes include SH2B3, ACAD10, MAPKAPK5-AS1, ADAM1B, and TMEM116. It suggested that reduced expression of SH2B3 represents a shared risk factor for both lymphocyte count and PE with FGR, while other gene variants are protective factors. However, SH2B3 exhibits pleiotropy in the context of lymphocyte count (HEIDI $p < 0.05$), whereas it does not exhibit pleiotropy in relation to PE with FGR, indicating that the lower expression of SH2B3 is associated with the pathogenesis of preeclampsia combined with fetal growth restriction, possibly mediated through their effects on lymphocyte count.

4. Discussion

In this study, we conducted a comprehensive analyses of the genetic associations between a variety of innate immune dysregulated diseases with the risk of PE or PE with FGR and identified mediators. Our findings revealed that SLE, T1D, and T2D were associated with the occurrence of PE, and T1D, T2D, RA, and surprisingly EMoP were associated with the occurrence of PE with FGR. An intriguing phenomenon emerged from our study, indicating a positive causal association between the count of lymphocytes and PE with FGR, with a particularly notable effect observed for naïve $CD4^+$ T cells. They also served as the mediators between T1D and PE with FGR. Conversely, the proportion of NK cells was found to be negatively associated with PE with FGR, while the absolute count of NK cells did not significantly affect PE with FGR. These novel findings offer valuable insights into the complex interplay between the immune system and PE risk.

Numerous hypotheses for the etiologies of PE have been extensively investigated, including placental syncytiotrophoblast stress [33], diminished trophoblast differentiation [34], metabolomic dysfunction [35], and a significant focus on innate immune dysregulation [36,37]. Conventional observational studies, including prospective cohort studies, have inherent limitations in elucidating etiology, such as potential confounders and information bias. Moreover, the absence of a well-established animal model for PE presents challenges in validating these hypotheses. Usually, choriocarcinoma cell lines or immortalized trophoblast cell lines, which still exhibit significant differences from the human placental formation process, are commonly used for simulating trophoblast invasion or syncytialization. In comparison, MR analysis has been chosen in this study to investigate the causal relationship between innate immune dysregulation and PE, with several strengths: it is less susceptible to confounders since genotypes are allocated during meiosis, less susceptible to information bias since genotype information is accurately obtained through sequencing, and easy to perform as it only requires GWAS summary data, not individual data [38].

Previous population-based studies have investigated the association between certain inflammatory traits and PE or EOPE. For instance, SLE was associated with a significantly increased risk of PE (RR 7.8, 95 % CI 4.8 to 12.9) [5]. T1D raised the risk of EOPE with an OR of 4.47 (95 % CI 3.77 to 5.31) [39], and T2D also increased the risk of PE, with consistent findings even when women were matched for BMI [40,41]. Epidemiological observational studies have investigated the relationship between RA and EOPE [8]. Moreover, a MR study also suggested a strong causal relationship between RA and PE (OR 1.05, 95%CI 1.01 to 1.09, $p < 0.05$) [42]. However, most studies did not specifically distinguish between EOPE and LOPE. This lack of differentiation was also reflected in the available GWAS databases, which typically did not classify EOPE separately. Therefore, our investigation focused on examining the relationships between PE or PE with FGR in relation to various systemic immune disorders. The results revealed a positive causal relationship between SLE, T1D, T2D, and PE. Additionally, T1D, T2D, and RA exhibited a strong positive causal relationship with PE with FGR. These findings suggested that systemic immune dysregulation may play distinct roles in the development of PE and PE with FGR.

Endometriosis and obstetric complications may share common pathophysiologic mechanisms, involving abnormal activation of inflammation [43]. However, the relationship between endometriosis and PE remains contentious. A meta-analysis reported no differences between endometriosis and gestational hypertension or PE [44], while another study indicated that endometriosis was associated with an increased risk of PE (RR 1.47, 95 % CI 1.13 to 1.89, $p = 0.003$) [45]. These discrepancies could be attributed to the lack of subdivision based on endometriosis locations, small sample sizes, and significant heterogeneity among studies. In our study, endometriosis of pelvic peritoneum showed a significant negative association with PE with FGR. This unexpected observation raises questions as it contradicts what is typically observed in clinical settings. We suspect that ectopic endometrial glandular cells may secrete various pro-angiogenic factors, promoting

Table 1
Two-step MR results of immune cells as a mediator variable for immune dysregulation and PE with FGR.

	Method	nsnp	beta	se	pval	OR	95%CI	Ph	Egger intercept	Pintercept
T1D to LC	MR Egger	139	−0.01	0.01	3.21E-02	0.99	[0.97, 0.99]	0.00	0.00	0.34
	Weighted median	139	−0.01	0.00	3.67E-08	0.99	[0.98, 0.99]			
	Inverse variance weighted	139	−0.01	0.00	3.11E-02	0.99	[0.98, 0.99]	0.00		
T1D to PE with FGR	MVMR	14	0.05	0.02	2.40E-03	1.06	[1.02, 1.09]			
LYM to PE with FGR	MVMR	228	0.11	0.05	2.92E-02	1.12	[1.01, 1.23]			
RA to naive T	MR Egger	58	−0.20	0.07	3.66E-03	0.82	[0.72, 0.93]	0.05	0.02	0.09
	Weighted median	58	−0.20	0.05	1.62E-04	0.82	[0.74, 0.91]			
	Inverse variance weighted	58	−0.11	0.04	7.16E-03	0.90	[0.83, 0.97]	0.03		
RA to PE with FGR	MVMR	0	−0.14	0.10	1.36E-01	0.87	[0.72, 1.05]			
naive T to PE with FGR	MVMR	19	0.04	0.03	1.16E-01	1.05	[0.99, 1.11]			

Table 2
Results by SMR analysis of eQTL data for blood and GWAS data for PE, PE with FGR, and lymphocyte count.

Gene	Chr	Probe_bp	topSNP	A1	A2	Freq	b_GWAS	p_GWAS	b_eQTL	p_eQTL	b_SMR	p_SMR	p_HEIDI	FDR
PE														
SH2B3	12	111,866,589	rs587914	C	T	0.20	−0.10	1.07E-05	−0.23	5.82E-126	0.44	1.50E-05	1.70E-01	4.86E-02
ACAD10	12	112,159,380	rs10774634	G	A	0.17	−0.11	2.62E-06	0.10	1.05E-23	−1.07	2.09E-05	5.73E-02	4.86E-02
MAPKAPK5-AS1	12	112,279,138	rs16941759	A	G	0.17	−0.12	2.48E-06	0.45	0.00E+00	−0.26	2.96E-06	2.39E-01	2.29E-02
ADAM1B	12	112,365,821	rs7134084	A	G	0.17	−0.12	2.48E-06	0.41	2.21E-54	−0.28	6.57E-06	4.14E-01	2.97E-02
TMEM116	12	112,410,027	rs4767068	G	A	0.17	−0.11	3.04E-06	0.48	0.00E+00	−0.24	3.37E-06	1.67E-01	2.29E-02
ERP29	12	112,456,187	rs11066119	G	A	0.09	−0.12	9.83E-06	−0.22	1.93E-53	0.56	2.15E-05	3.67E-03	4.86E-02
PE with FGR														
SH2B3	12	111,866,589	rs587914	C	T	0.20	−0.09	3.08E-07	−0.23	5.82E-126	0.41	5.59E-07	3.42E-01	7.58E-03
ACAD10	12	112,159,380	rs10774634	G	A	0.17	−0.10	2.18E-07	0.10	1.05E-23	−0.96	4.12E-06	9.04E-02	1.23E-02
MAPKAPK5-AS1	12	112,279,138	rs16941759	A	G	0.17	−0.10	1.60E-06	0.45	0.00E+00	−0.21	1.94E-06	4.30E-02	8.77E-03
ADAM1B	12	112,365,821	rs7134084	A	G	0.17	−0.10	1.60E-06	0.41	2.21E-54	−0.23	4.55E-06	1.17E-01	1.23E-02
TMEM116	12	112,410,027	rs4767068	G	A	0.17	−0.10	1.42E-06	0.48	0.00E+00	−0.20	1.60E-06	1.30E-01	8.77E-03
LYM														
SH2B3	12	111,866,589	rs587914	C	T	0.20	−0.05	1.59E-31	−0.23	5.82E-126	0.23	9.34E-26	8.59E-11	5.29E-23
ACAD10	12	112,159,380	rs10774634	G	A	0.17	−0.05	2.52E-23	0.10	1.05E-23	−0.45	1.59E-12	1.24E-06	2.45E-10
MAPKAPK5-AS1	12	112,279,138	rs16941759	A	G	0.17	−0.05	1.59E-23	0.45	0.00E+00	−0.11	4.29E-22	1.15E-15	1.77E-19
ADAM1B	12	112,365,821	rs7134084	A	G	0.17	−0.05	6.06E-24	0.41	2.21E-54	−0.12	2.64E-17	8.57E-09	7.80E-15
TMEM116	12	112,410,027	rs4767068	G	A	0.17	−0.05	7.35E-24	0.48	0.00E+00	−0.10	6.05E-23	1.87E-02	2.83E-20

the remodeling of uterine spiral arteries during placental formation, thereby reducing the occurrence of PE with FGR. Certainly, this intriguing discovery warrants further investigation into its mechanisms, as well as clinical research involving clinical data from real-world cases. Due to the lack of typical clinical symptoms and difficulties in obtaining samples for chronic pelvic and uterus inflammation, studies investigating the relationship between innate upper reproductive tract inflammation and PE were scarce, with only one report mentioning that septic pelvic thrombophlebitis was commonly associated with PE (45 %) [46]. Similarly, our study also revealed that inflammation in the upper reproductive tract inflammation had no direct causal relationship with PE or PE with FGR.

The association between inflammatory biomarkers and the development of PE has indeed yielded conflicting and even contradictory findings in various studies [47,48], leading to uncertainties and complexities surrounding the role of these factors in the pathogenesis of PE. Such discrepancies could be attributed to a variety of factors, such as the distinct pathogenic mechanisms between EOPE and LOPE, the multifaceted nature of immune cell functions and inflammatory responses, and the intricate nature of causal relationships among these factors. In light of this, our study employed a two-sample MR analysis to provide specific insights into the genetic-level associations of certain immune cells and inflammatory factors with PE or PE with FGR. None of the genetic variations in inflammatory factors directly led to the onset of PE or PE with FGR. However, a genetic causal relationship was observed between lymphocyte counts and PE with FGR. Further subtyping of lymphocytes revealed that the risk effect may be attributed to the count of naïve $CD4^+$ T cells. An established model transfers naïve $CD4^+$ T cells into immunodeficient mice, leading to inflammatory bowel diseases after four weeks [49], suggesting the pro-inflammatory potential of naïve $CD4^+$ T cells. However, their role in PE was not well-explored. Naïve $CD4^+$ T cells can differentiate into distinct mature subsets, like Th1, Th2, Th17, and regulatory T cells (Treg cells), upon proper stimulation. An imbalance between Tregs and effector T cells (Teffs) is increasingly linked to PE [50–52]. Further research is needed to determine if an increase in naïve $CD4^+$ T cells alone triggers PE or if dysregulation of differentiated T cell subsets is involved. In contrast, the proportion of NK cells was found to have a negative causal relationship with PE rather than the absolute number of NK cells. As the most abundant lymphocytes during pregnancy, NK cells are recruited and activated by ovarian hormones and play pivotal roles throughout pregnancy [53], being fundamental to achieving embryo implantation and successful pregnancy [54]. The phenotypic transformation of peripheral blood NK cells upon entering the maternal-fetal interface might be one of the reasons why the absolute number of NK cell subtypes was not causally related to PE, while the proportion of NK cells did show a significant association.

Our study also revealed the expression of some specific immune-associated genes mediated the occurrence of PE with FGR. SH2B3, also known as Lnk (Lymphocyte adaptor protein), is known to negatively regulate JAK-STAT signaling, a pathway essential for the development and functioning of immune cells. Mutations or dysregulation of SH2B3 have been associated with various autoimmune diseases and hematological disorders, making it an important player in the field of immunology and hematology research [55]. However, its role in the pathogenesis of PE has not been extensively studied. Research has shown that mice with a rs3184504 missense mutation created using CRISPR-Cas9 technology did not exhibit hypertension on their own. Still, during chronic Ang II infusion, they manifested a significant increase in systolic blood pressure, approximately 10 mmHg, along with associated renal and vascular dysfunction [55]. Pregnancy naturally involves an endogenous increase in Ang II, which might potentially

exacerbate hypertension related to SH2B3 polymorphisms, thereby contributing to the development of PE. The specific mechanisms underlying this association warrant further investigation. Furthermore, SMR results suggested that SNP variants influencing lymphocyte count were consistent with those contributing to PE with FGR, indicating that SH2B3 may be involved in the pathogenesis of PE through its impact on lymphocyte participation.

Compared to previous studies [56–58], this study has both strengths and limitations. The strengths include a thorough examination of the relationships between various autoimmune diseases and PE, specifically PE with FGR. Moreover, the exclusion of SNPs associated with HLA confounding factors provides a clearer depiction of key pathways. The focus on lymphocytes has facilitated the identification of pathogenic SNPs associated with expression levels. However, limitations include the restriction of the analysis to the European population due to database constraints as well as the absence of another cohort to validate the conclusions.

5. Conclusions

In summary, we conducted a comprehensive MR analysis to explore the genetic associations between systemic immune disorders, chronic genital inflammation, immune cells, and inflammatory factors with PE risk. Significant associations were observed between PE occurrence and SLE, T1D, and T2D, while T1D, T2D, RA, and EMoP had a strong causal relationship with PE with FGR. Notably, lymphocyte count could be a mediator between T1D and PE with FGR. Potential therapeutic targets and preventive strategies for PE may be focusing on SH2B3.

Credit author statement

CM and ZL contributed to the conception and design of the study. CM performed the MR analysis and wrote the first draft of the manuscript. JT provided technical guidance throughout the study and critical revisions to the manuscript. HY assisted in organizing the database. All authors have read and agreed to the published version of the manuscript.

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Institutional review board statement

Our study was a secondary analysis of publicly available data. Informed consent was obtained from all participants as per the original GWAS protocols, and all ethical approvals for the GWAS were obtained by the original GWAS authors.

CRediT authorship contribution statement

Chumei Zeng: Conceptualization, Data curation, Formal analysis, Writing - original draft. **Huiying Liu:** Data curation, Project administration. **Zilian Wang:** Conceptualization, Funding acquisition, Supervision. **Jingting Li:** Funding acquisition, Resources, Supervision, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at .https://doi.org/10.1016/j.jtauto.2023.100226

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