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Article

Causal Relationship Between Inflammation and Preeclampsia: Genetic Evidence from a Mendelian Randomization Study

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Abstract

Preeclampsia (PE) is a hypertensive disorder of pregnancy. PE patients were reported to have higher serum levels of C-reactive protein (CRP), interleukin-6 (IL-6) and tumor necrosis factor α (TNF-α) than those in healthy controls. However, whether the expressions of these inflammation biomarkers have a causal relationship with PE is unspecified. We applied the Mendelian randomization method to infer the causal relationship between inflammation biomarkers (e.g., CRP, IL-6, interleukin 1 receptor antagonist [IL-1ra] and TNF-α) and PE. Single nucleotide polymorphisms (SNPs) strongly related to inflammation biomarkers were used as instrumental variables. CRP, IL-1ra and IL-6 levels showed no significant effect on PE progression, while the genetic predicted higher level of TNF-α significantly increased the risk of PE (*OR* per 1-*SD* increase in TNF-α: 4.33; 95% CI [1.99, 9.39]; $p = .00021$). The findings suggest that pro-inflammatory activity of TNF-α could be a determinant for PE progression. More antenatal care should be given to those pregnant women with higher level of inflammation biomarkers, especially TNF-α.

Keywords: Preeclampsia; Mendelian randomization method; inflammation; TNF-α

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Preeclampsia (PE) and eclampsia are the most severe hypertensive disorders of pregnancy, characterized by the occurrence of new-onset hypertension after 20 weeks' gestation (Khedagi & Bello, 2021). About 3–8% pregnant women in the United States are affected by PE, and this disease is also responsible for more than 60,000 maternal deaths and over 500,000 fetal deaths worldwide every year (Ma'ayeh & Costantine, 2020). The complications of PE includes proteinuria, acute kidney injury, hepatic dysfunction, hemolysis and thrombocytopenia (Lambert et al., 2014). Unfortunately, PE is also the major risk factor for preterm birth and low birth weight, both of which are considered as the negative predictors of the child's future health and cardiovascular risk (Ardissino et al., 2022).

Nowadays, although the advancement in therapies seems promising, delivery of the fetus in advance is the only definitive treatment strategy. Irrespective of the gestational age, severe PE patients with a gestational week ≥34 weeks or with unstable maternal or fetal conditions are recommended to commence delivery as soon as possible once the maternal condition is stable (Roberts et al., 2013), while for severe PE patients with a gestational age of less than 34 weeks and with stable conditions, corticosteroids treatment are recommended to avoid fetal lung immaturity and to receive intensive care at a facility to maintain (Roberts et al., 2013).

Early diagnosis and adequate human care are essential for PE patients. More importantly, more attention should be paid to fetal intrauterine status by daily monitoring of fetal movement and fetal heart changes. Despite this, there is an urgent need to develop effective drugs against the complications of PE.

Previous studies indicated that inflammatory status at the maternal-fetal interface throughout the pregnancy period is associated with PE progression (Perucci et al., 2017; Raguema et al., 2018; Subha et al., 2016), suggesting the potential of inflammation-related genes serving as genetic biomarkers and drug targets for PE (Wang et al., 2022). The serum levels of inflammation markers were found to associate with PE (Guven et al., 2009). The study also found a significant difference in levels of high sensitivity C-reactive protein (CRP), interleukin-6 (IL-6) and tumor necrosis factor α (TNF-α) among uncomplicated pregnancies, and mild and severe PE patients (Guven et al., 2009). Nevertheless, it was difficult to infer the causal relationship on the basis of observational evidence alone. Additionally, observational epidemiological studies are susceptible to confounding and reverse causation (Zheng et al., 2017).

Mendelian randomization (MR) is an effective, potent and efficient method for determining the causal relationship between two correlated phenotypes (Greenland, 1990). MR, utilizing the summary statistics from genomewide association studies (GWAS), is a commonly used causal inference method to distinguish whether the exposure phenotype (e.g., inflammation) has a causal effect on the outcome phenotype (e.g., PE; Burgess et al., 2016; Dalbeth et al., 2015) by using the genetic variants as instrumental variables (IVs).

In this study, single nucleotide polymorphisms (SNPs) strongly associated with inflammation biomarkers (e.g., CRP, IL-6, interleukin 1 receptor antagonist and TNF-α) were used as the IVs to infer the causal relationship between inflammation and PE. Since it was difficult to obtain the exposure and outcome data from

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the same person in a large cohort, a two-sample MR method was performed using the effect of IVs on the exposure and outcome phenotypes from two independent studies (Pierce & Burgess, 2013).

Materials and Methods

GWAS Summary Data

PE heritability is estimated at 50−55%, with maternal genetic contribution risk of 30−35% and fetal genetic contribution risk of 20% (Gray et al., 2018). The genetic association of PE was extracted from the analysis of the seventh release of the FinnGen consortium data (https://finngen.gitbook.io/documentation). The FinnGen consortium performed a large GWAS to identify the genetic variants associated with PE in 5265 cases and 160,670 controls of Finnish ancestry. The summary statistics identified 16,382,829 variants.

Based on the available GWAS summary data of inflammation biomarkers, the following inflammation biomarkers were selected as exposures: CRP, IL-1 receptor antagonist (IL-1ra), IL-6 and TNF-α. The genetic association estimates for the circulating levels of CRP were collected from a recent large meta-analysis of 88 GWAS studies performed on 204,402 European participants (Ligthart et al., 2018). For IL-1ra, a GWAS of 90 circulating cardiovascular-related proteins conducted on 30,931 European individuals across 14 studies (Folkersen et al., 2020) was used in the current study. The genetic association estimates for circulating serum IL-6 levels were extracted from a recent GWAS meta-analysis on 67,428 European participants across 47 cohorts (Ahluwalia et al., 2021). TNF-α association results were extracted from a meta-analysis of GWAS from 25 cohorts of 30,912 European participants (Prins, 2016).

MR Analyses

The MR analysis has been well reported in a previous study (Davey Smith & Hemani, 2014). To investigate the relationship between inflammation markers and PE, a two-sample MR method was used. The independent variants that are strongly associated with inflammation markers were selected as IVs ($p < 5\times10^{-8}$ and linkage disequilibrium (LD) $r^2 < .01$). Inverse-variance weighted method, weighted median and MR Egger (bootstrap) were used in this research. While the estimate for MR-Egger regression slope provides the pleiotropy-corrected causal effect, a published study confirmed that the weighted median approach affords some distinct superiorities over MR-Egger, such as its improved power of causal effect detection and lower type I error (Bowden et al., 2016). In this research, to ensure more robust MR estimates, we also applied the weighted median method to complement the MR-Egger regression. The weighted median method may generate correct estimates even if up to 50% of SNPs are invalid IVs (Bowden et al., 2016).

Sensitivity Analysis

Cochran's Q test, a heterogeneity test, was performed to identify whether there was a higher heterogeneity among causal effects estimated using each variant individually rather than being expected by chance (Burgess et al., 2016).

To assess the possibility of other horizontal pleiotropic effects on how IVs affect inflammation biomarkers via other biological pathways, we performed a MR-Egger regression (Bowden et al., 2015). The intercept that deviated from the origin may provide evidence for potential pleiotropic effects across the IVs. All data analyses were conducted using R software (4.0.4 version).

Results

After harmonizing the exposure and outcome data and excluding the correlated SNPs, 25 SNPs for CRP, 4 SNPs for IL-1ra, 2 SNPs for IL-6 and 3 SNPs for TNF-α were used as IVs (Table 1, Supplementary Tables 1–3).

By applying the inverse-variance weighted method with fixed effects, CRP was found to have no significant association with increased PE risk (*OR* per 1-*SD* increase in CRP: 0.9, 95% CI [0.82, 1.04], $p = .21$). The risk of PE was not increased in patients with higher genetic predicted IL-1ra (*OR* per 1-*SD* increase in IL-1ra: 1.03, 95% CI [0.89, 1.20], $p = .67$). Patients with higher genetic predicted IL-6 had no association with increased PE risk (*OR* per 1-*SD* increase in IL-6: 0.86, 95% CI [0.55, 1.32], $p = .48$). However, the genetic predicted higher level of TNF-α was found to significantly increase the risk of PE (*OR* per 1-*SD* increase in TNF-α: 4.34, 95% CI [1.99, 9.45], $p = .00022$; Figure 1). The analysis using the weighted median method showed similar results with that using the MR Egger (bootstrap) method (Table 2).

A heterogeneity test was applied to identify the possible effect of a higher heterogeneity among the estimated causal effects using the variants individually than being expected by chance. The result suggested that there was no heterogeneity ($I^2 = 41.4\%$, 95% CI [0.0%, 82.2], $p = .18$). The slope estimation of TNF-α level on PE using MR-Egger also showed no significant difference (beta = 0.14, 95% CI [-0.026, 0.31], $p = .35$).

Discussion

In this study, MR analysis was performed to detect whether inflammatory markers (CRP, IL-1ra, IL-6, TNF-α) were causally associated with PE. The results showed that the genetically elevated TNF-α level had putatively causal effect on the increased risk of PE and no causal relationship was identified between other inflammatory markers (i.e., CRP, IL-1ra and IL-6) and PE.

Delivery generally resolves symptomology, suggesting that the placenta plays a major role in the pathophysiology of PE (Rosser & Katz, 2013). TNF-α overproduced by the placenta in response to local ischemia and hypoxia contributed to the increased TNF-α level in the plasma (Conrad & Benyo, 1997). Previous studies suggested that TNF-α released by the placenta to maternal circulation could elevate the expressions of proinflammatory cytokines secreted by endothelial cells, like IL-6, IL-8 and MCP-1 (Shaw et al., 2016). Meanwhile, the activation and dysfunction of the endothelium is the main cause for PE (Roberts et al., 1989). Elevated expression of TNF-α can induce the systemic acute phase response, which consequently stimulates the liver to synthesize CRP (Hansson, 2005). However, in this study, we only identified TNF-α causally increased the risk of PE, but not CRP and IL-6.

Maternal adipokines (i.e., IL-6, TNF-α, leptin and adiponectin) is responsible for the linkage of maternal nutritional status and adipose tissue metabolism with placental function (Reslan & Khalil, 2010). A previous study suggested that the common indices of obesity posed a higher genetic risk of reproductive disorders, like PE (Venkatesh et al., 2022). Although TNF-α serum level was higher in PE patients compared with healthy controls, obesity was not associated with serum TNF-α level in both PE and control groups (Founds et al., 2008). This result suggested that adipose tissue did not causally lead to PE via changing TNF-α level in the serum.

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Table 1. Characteristics of SNPs associated with serum TNF-α level

			Serum TNF-α level			Preeclampsia		
SNP	Effect allele	Other allele	Beta	*SE*	*p* value	Beta	*SE*	*p* value
rs10744774	A	C	0.044	0.007	6.94×10^{-11}	0.034	0.027	.21
rs2857602	G	A	0.032	0.006	3.30×10^{-12}	0.077	0.021	.00025
rs7182229	T	G	0.05	0.009	1.07×10^{-09}	0.061	0.042	.14

Note: SNP, single nucleotide polymorphisms; TNF-α, tumor necrosis factor α.

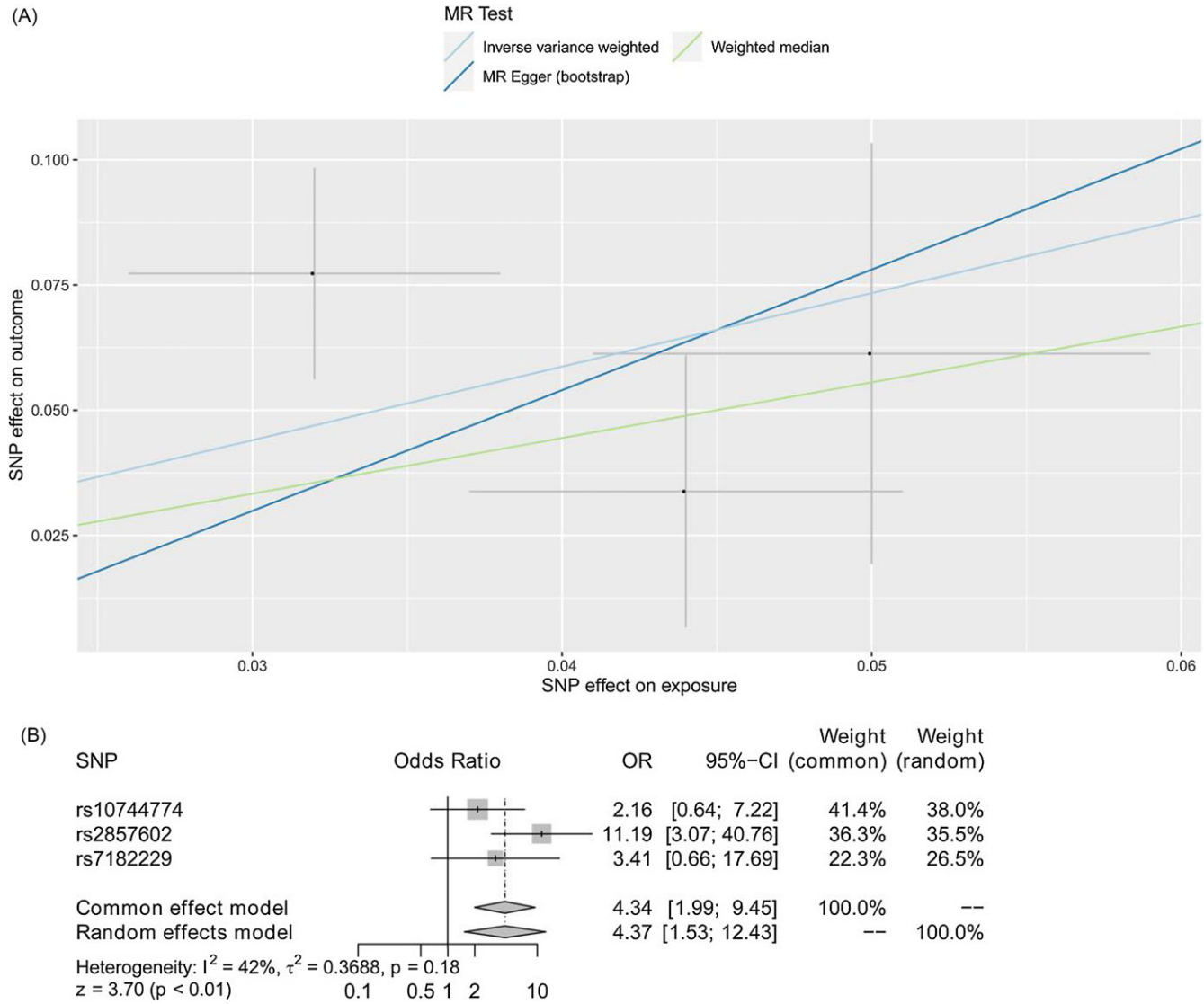

Figure 1. Mendelian randomization estimates for the relationships between TNF-α and preeclampsia. (A) The effects of the selected instrumental variable on TNF-α level and their effects on preeclampsia. (B) The forest plot showed the combined effects of TNF-α levels on preeclampsia.
Note: SNP, single nucleotide polymorphisms; TNF-α, tumor necrosis factor α.

There are some limitations that need to be mentioned. First, only a limited number of SNPs were used as IVs to infer the causal relationship between inflammation biomarkers (e.g., CRP, IL-1ra, IL-6, and TNF-α) and PE. Previous studies indicated that the power for recognizing the causal relationship between complex traits by using genetic variants as IVs was enhanced with the increasing variance explained by these genetic variants (Brion et al., 2013; Freeman et al., 2013). So, when more variants were employed, the power could be increased and the results more credible. Second, the IVs were identified from cohorts of both sexes. However, the GWAS results of PE were generated from females. If sex-specific GWAS data of these inflammation biomarkers were available, the results would be more robust.

In summary, we identified that the genetically elevated TNF-α level had putatively causal effect on the increased risk of PE. This finding provided a therapeutic target for PE treatment, and

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Table 2. Mendelian randomization estimates of inflammation biomarkers on preeclampsia

	IVW			Weight median			MR Egger		
	OR	95% CI	*p* value	*OR*	95% CI	*p* value	*OR*	95% CI	*p* value
CRP	0.93	0.82, 1.04	.21	0.88	0.76, 1.03	.11	0.99	0.86, 1.14	.47
IL-1ra	1.03	0.89, 1.20	.67	1.03	0.89, -1.20	.68	0.97	0.62. 1.51	.43
IL-6	0.86	0.55, 1.32	.48	NA	NA	NA	NA	NA	NA
TNF-α	4.34	1.99, 9.45	.00022	3.04	1.08, 8.55	.035	9.94	0.43. 229.5	.058

Note: IVW, inverse-variance weighted; CRP, C-reactive protein; IL-1ra, interleukin 1 receptor antagonist; IL-6, interleukin-6; TNF-α, tumor necrosis factor α; NA, because there were only two SNPs used in weighted median and MR Egger method, no result was generated.

pregnant women with a higher level of inflammation biomarkers, especially TNF-α, should receive more antenatal care.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/thg.2023.27.

Data availability. All data were uploaded as supplementary materials.

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