Letter to the Editor

Genetically Predicted Plasma Levels of Amino Acids and Endometriosis: A Mendelian Randomization Study*

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Endometriosis (EMS) is one of the most common disorders among women of reproductive age and affects approximately 6%–10% of the world total population[1]. Women with EMS often experience symptoms such as dysmenorrhea, infertility, or difficulties in conceiving. Recent studies focusing on amino acids (AAs) metabolism have shed light on the mechanisms underlying the development and progression of EMS[2]. However, causal relationships remain unclear owing to the inherent limitations of observational studies. Mendelian randomization (MR) utilizes genetic variations identified in genome-wide association studies (GWASs) as instrumental variables (IVs) to ascertain the causal effects of risk factors (or exposures) on outcomes, thereby considerably reducing the biases arising from confounding factors and reverse causation[3]. Based on the exposed, this study aims to investigate the causal association between 20 AAs and EMS using MR analysis. Furthermore, considering that changes in AAs metabolism might be a consequence rather than a cause of EMS, we conducted a reverse MR analysis after EMS exposure.

Alanine, glutamine, glycine, histidine, isoleucine, leucine, phenylalanine, tyrosine, and valine levels were analyzed using the Nightingale Health metabolic biomarker platform based on high-throughput nuclear magnetic resonance. This analysis was conducted using approximately 118,000 EDTA plasma samples and 5,000 repeated assessments from baseline recruitment in the UK Biobank[4]. Data for the remaining 11 AAs were derived from an analysis conducted by Shin et al. using an ultra-high-performance liquid chromatography-tandem mass spectrometry platform involving approximately 7,800 participants[5]. EMS data were obtained from a GWAS meta-analysis of 24 cohorts encompassing 21,779 cases and 449,087 controls[6] (Table 1).

Single-nucleotide polymorphisms (SNPs) were used as IVs in the MR analysis with genome-wide significant associations ($P < 0.001$) and strict linkage disequilibrium parameter sets ($r^2 < 0.001$, clumping distance = 10,000 kb). Owing to the absence of genome-wide significant SNPs for the 11 AAs from Shin et al.'s GWAS study, a more lenient threshold of 5×10^{-6} was adopted. Weak IVs were assessed using the F-statistic ($F = \text{beta}^2/\text{se}^2$), which exceeded 10. In the absence of an SNP in the resulting dataset, an alternative SNP strongly linked to disequilibrium ($r^2 > 0.8$) with the primary SNP was selected. The MR-Steiger filter was applied to eliminate variations that were more strongly correlated with the outcome than with the exposure. The MR study methods employed the inverse-variance weighted approach[7], with Cochrane's Q test to check for heterogeneity, and MR-Egger and MR pleiotropy residual sum and outlier (MR-PRESSO) tests for pleiotropy. Considering multiple comparisons, Bonferroni correction was applied, setting a significance threshold for causal association at $P < 0.002$ (0.05/20). Associations with *P*-values between 0.002 and 0.05 were considered suggestive of causal evidence.

Bidirectional MR analyses (Figure 1) were conducted using IVs ranging from 2 to 44. All IVs passed the MR-Steiger filter and outliers identified by MR-PRESSO were excluded from the final analyses. The average F-statistics of the IVs varied from 22 to 522, indicating a significant reduction in bias due to weak IVs. In the forward MR analysis, a significant causal association was found; specifically, genetically predicted higher circulating levels of tyrosine were associated with an increased

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risk of EMS (odds ratio [*OR*] = 1.171, 95% confidence interval [*CI*] = 1.073–1.279, *P* < 0.001). Additionally, two suggestive pieces of evidence were identified: a protective causal effect of leucine on EMS (*OR* = 0.836, 95% *CI* = 0.704–0.993, *P* = 0.04) and a harmful causal effect of aspartic acid on EMS (*OR* = 2.011, 95% *CI* = 1.152–3.509, *P* = 0.01). No causal evidence was found for other AAs in the EMS. In the reverse MR analysis, two suggestive pieces of evidence were found: genetically predicted EMS, per one standard deviation increase, was associated with higher circulating levels of phenylalanine (β = 0.027, 95% *CI* = 0.005–0.049, *P* = 0.01) and tyrosine (β = 0.027, 95% *CI* = 0.004–0.051, *P* = 0.02). No causal evidence was found for the effect of EMS on the other AAs. The sensitivity analyses further corroborated the robustness of the results (Figure 1).

We performed an extensive MR analysis, revealing a bidirectional causal relationship between tyrosine levels and EMS risk, along with other suggestive evidence. Tyrosine, a precursor of key neurotransmitters, including dopamine, norepinephrine, and thyroid hormones, may affect neuroendocrine system function when in high levels[8], thus impacting hormonal balance and the physiological state of the endometrium. In addition, tyrosine may exacerbate oxidative stress and inflammatory responses, which have key roles in the pathogenesis[9]. EMS can also indirectly elevate tyrosine levels by affecting metabolic pathways such as chronic inflammation, liver function, and hormonal balance[10]. Therefore, regulating tyrosine levels or the associated metabolic pathways may help control EMS development. Overall, this MR study suggests a role for AA metabolism in EMS and could guide future research and treatment strategies. Further validation of the suggestive causal evidence is required in future studies.

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Table 1. Detailed information of data sources

Phenotype	Ref	Ieu id	Consortium	Ancestry	Participants, number
Alanine	36402876	met-d-Ala	UKB	EUR	115,074
Glutamine	36402876	met-d-Gln	UKB	EUR	114,750
Glycine	36402876	met-d-Gly	UKB	EUR	114,972
Histidine	36402876	met-d-His	UKB	EUR	114,895
Isoleucine	36402876	met-d-Ile	UKB	EUR	115,075
Leucine	36402876	met-d-Leu	UKB	EUR	115,074
Phenylalanine	36402876	met-d-Phe	UKB	EUR	115,025
Tyrosine	36402876	met-d-Tyr	UKB	EUR	114,911
Valine	36402876	met-d-Val	UKB	EUR	115,048
Arginine	24816252	met-a-347	MRC-IEU	EUR	7,528
Asparagine	24816252	met-a-638	MRC-IEU	EUR	7,761
Aspartic acid	24816252	met-a-388	MRC-IEU	EUR	7,721
Cysteine	24816252	met-a-455	MRC-IEU	EUR	7,692
Glutamic acid	24816252	met-a-466	MRC-IEU	EUR	7804
Lysine	24816252	met-a-326	MRC-IEU	EUR	7,812
Methionine	24816252	met-a-327	MRC-IEU	EUR	7,795
Proline	24816252	met-a-355	MRC-IEU	EUR	7,816
Serine	24816252	met-a-464	MRC-IEU	EUR	7,796
Threonine	24816252	met-a-324	MRC-IEU	EUR	6,020
Tryptophan	24816252	met-a-304	MRC-IEU	EUR	7,804
EMS	36914876	NA	Rahmioglu N	EUR	21,779

Note. EUR, European; MRC-IEU, MRC integrative epidemiology unit; UKB, UK biobank; EMS, endometriosis; Ref, reference (pubmed id); Ieu id, integrative epidemiology unit identification; NA, not applicable.

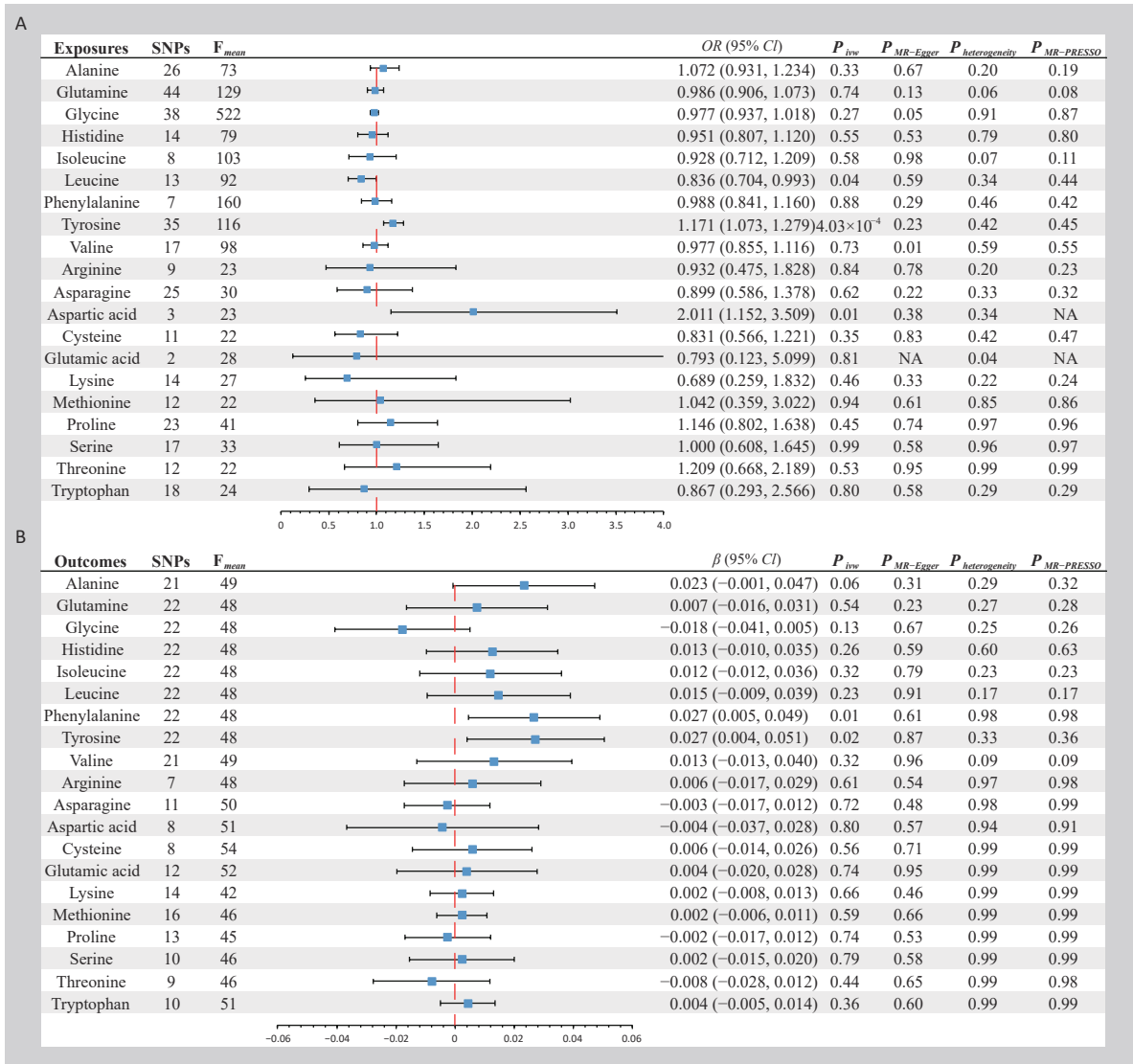

Figure 1. Bidirectional Mendelian randomization analysis of plasma amino acid levels and endometriosis. (A) Plasma amino acid levels as exposure phenotype and endometriosis as outcome. (B) Endometriosis as exposure phenotype and plasma amino acid levels as outcome phenotype. *OR*, Odds ratio; *CI*, Confidence interval; IVW, Inverse variance weighted; SNPs, Single nucleotide polymorphisms; MR-PRESSO, Mendelian randomization -pleiotropy residual sum and outlier.

investigators for sharing the GWAS summary statistics used in this study.

Conflict of Interest All authors declare no conflicts of interest.

Data Availability Statement All data used in this study were derived from publicly accessible GWAS. The datasets were fully accessible and obtained from the referenced articles cited in this document.

Ethics Statement This study was based solely on publicly accessible, de-identified data from published studies and summary databases. No ethical approval was required for this study.

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