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ORIGINAL RESEARCH

UDP-Glucose Ceramide Glucosyltransferase Inhibition, Immune Cell Mediation, and Endometriosis Risk: A Mendelian Randomization Study

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Purpose: Previous studies have reported a link between UDP-glucose ceramide glucosyltransferase (UGCG), immune regulation, and endometriosis (EM). We hypothesized that UGCG inhibitors might exert therapeutic effects on EM. Therefore, in this study, we performed a two-sample, two-step Mendelian randomization (MR) analysis modeling genetic variation of UGCG inhibitors to investigate the causal relationship between genetically determined *UGCG* inhibition, EM, and mediation of immune cells.

Methods: Two-sample MR was conducted using genome-wide association study data on *UGCG* inhibition and EM. Furthermore, we adopted strict instrumental variable selection criteria to ensure the robustness of our results and primarily employed the inverse-variance weighted method, along with the weighted median, MR-Egger, and MR-PRESSO methods for sensitivity analyses. Additionally, a two-step MR analysis was performed to investigate the potential role of immune cells in mediating the relationship between *UGCG* inhibition and EM.

Results: *UGCG* inhibition was associated with a decreased EM risk, with an odds ratio of 0.915, 95% confidence interval of 0.859–0.975, and $P = 0.006$, demonstrating the robustness of our results and potential clinical significance of our study. Among 731 types of immune cells analyzed, 12 were significantly associated with *UGCG* inhibition and EM, 8 of which acted as mediators. Terminally differentiated CD4+ T cells (from the maturation stages of the T-cell panel) accounted for the highest proportion of mediating cells (26.727%), whereas immunoglobulin D (IgD) expression on IgD+ $CD38^{dim}$ B cells (from the B-cell panel) accounted for the lowest proportion (7.816%).

Conclusion: Inhibiting UGCG activity may reduce the risk of EM, and immune cells may mediate this effect. Our findings provide novel insights for the development of potential therapeutic strategies for EM treatment.

Keywords: UDP-glucose ceramide glucosyltransferase inhibition, endometriosis, immune cells, mediating effect, Mendelian randomization

Introduction

Endometriosis (EM) is a chronic inflammatory gynecological disorder that affects approximately 190 million women worldwide.[1,2] Current treatments for EM include medical therapies (eg, hormone treatments) and surgical interventions (eg, hysterectomy);[3–5] however, EM symptoms often persist or recur.[1,6] The pathogenesis of the disease remains incompletely understood, and it is believed to involve the interplay of multiple factors, including genetic susceptibility, immune dysfunction, hormonal imbalances, and environmental influences.[1,2,5,6] Several etiological hypotheses proposed by researchers suggest that immune system abnormalities may be a key factor in the growth of ectopic endometrial tissue. Immune dysfunction may impair the normal immune surveillance mechanisms, preventing the clearance of these ectopic

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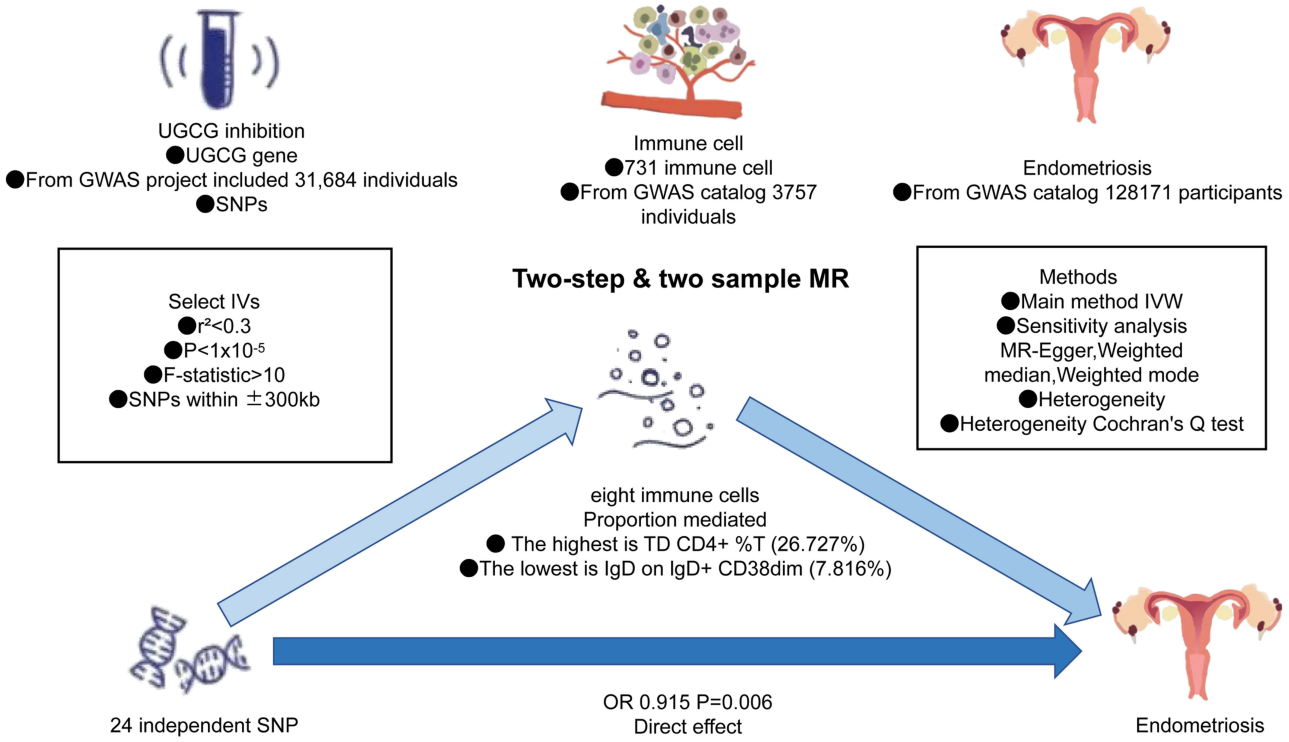
Graphical Abstract



tissues. Additionally, hormonal imbalances, particularly abnormal estrogen levels, may promote the proliferation and survival of ectopic endometrial cells. Furthermore, genetic susceptibility and environmental factors may also play a synergistic role in the onset of the disease, further complicating the pathological process of EM. Understanding the pathogenesis of EM and subsequent development of effective treatments are essential for improving therapeutic outcomes.[1]

Previous research focusing on identifying diagnostic biomarkers for EM has highlighted various proteins and genes with potential as diagnostic, therapeutic, and prognostic markers.[3,7,8] Elevated expression of UDP-glucose ceramide glucosyltransferase (*UGCG*) has emerged as a promising therapeutic target for EM.[9] Notably, dysregulated immune cell activities, compositions, and regulatory functions in patients with EM may contribute to disease progression. The immune alterations include reduced cytotoxicity of $CD8^+$ T cells, distinct macrophage profiles from those in normal endometrial tissues, and impaired natural killer cell function.[10–12] UGCG, which plays a critical role in cancer pathogenesis, affects immune cell functions, and inhibition of *UGCG* expression reportedly enhances $CD8^+$ T cell immunogenicity and potentially inhibits tumor growth.[13–15] Although UGCG inhibitors, such as eliglustat and migalastat, are approved by the United States Food and Drug Administration (FDA) for other conditions, their application in EM remains unexplored.[13,16,17] Moreover, existing research on EM predominantly relies on observational studies, limiting causal inference.[18] Considering the potential of UGCG inhibitors, such as eliglustat and migalastat, in modulating immune responses and their approval for other conditions by the FDA, investigating their role as drug targets in EM is warranted. Therefore, robust research approaches are needed to investigate the causal relationships between *UGCG* inhibition, immune cells, and EM onset.

Mendelian randomization (MR) is a powerful tool for addressing complex questions in the fields of human biology and epidemiology. MR analytical methods adopt statistical techniques from economics, allowing researchers to analyze

the effects of the environment, drug treatments, and other factors on human biology and disease.[19] Drug target MR offers a promising approach to assess the causal effects of *UGCG* inhibition on immune cell functions and the onset of EM. MR utilizes natural genetic variations to establish causal relationships, minimizing the effects of confounding factors and enhancing the validity of research findings.[20,21] This method enables the exploration of the long-term effects of pharmacological interventions on EM risk, akin to simulated clinical trials targeting the action mechanisms of various drugs.[22,23] Davies et al[24] highlighted that advancements in genome-wide association studies (GWASs) and molecular studies have strengthened the basis for MR research.[24]

In the present study, we aimed to investigate the causal relationships between *UGCG* inhibition, immune cells, and EM, particularly, the mediating role of immune cells, using two-sample and two-step MR analyses. The findings of this study may deepen our understanding of the pathological mechanisms linking *UGCG* inhibition to EM development.

Methods

Study Design

A two-sample, two-step MR design was employed (Figure 1a–c). Drug target MR uses single nucleotide polymorphisms (SNPs) restricted to the gene locus encoding the drug target as indicators for a specific drug.[25] To ensure the validity of potential causal effects in the MR analysis of drug targets, the following key assumptions should be met: (i) Correlation assumption: the instrumental variables should exhibit a strong association with *UGCG* inhibition, with a significance level of $P < 1 \times 10^{-5}$ indicating a robust correlation. Instrumental variables with an F-statistic ≤ 10 were therefore excluded from further analysis; (ii) Independence assumption: the selected instrumental variables must not be influenced by confounding factors; (iii) Exclusion restriction assumption: the instrumental variables should influence EM solely via *UGCG* inhibition and do not exhibit direct association with EM, ensuring the absence of genetic pleiotropy, and non-significant differences ($P > 0.05$) in the intercept term of the MR-Egger regression analysis indicating the absence of genetic pleiotropy; (iv) Target gene relevance: instrumental variables should fall within the cis-regulatory range of the target gene (kb = 300).[26] This study adhered to the principles specified in the Strengthening the Reporting of Observational Studies in Epidemiology guidelines for MR (Supplementary Material 1).[27]

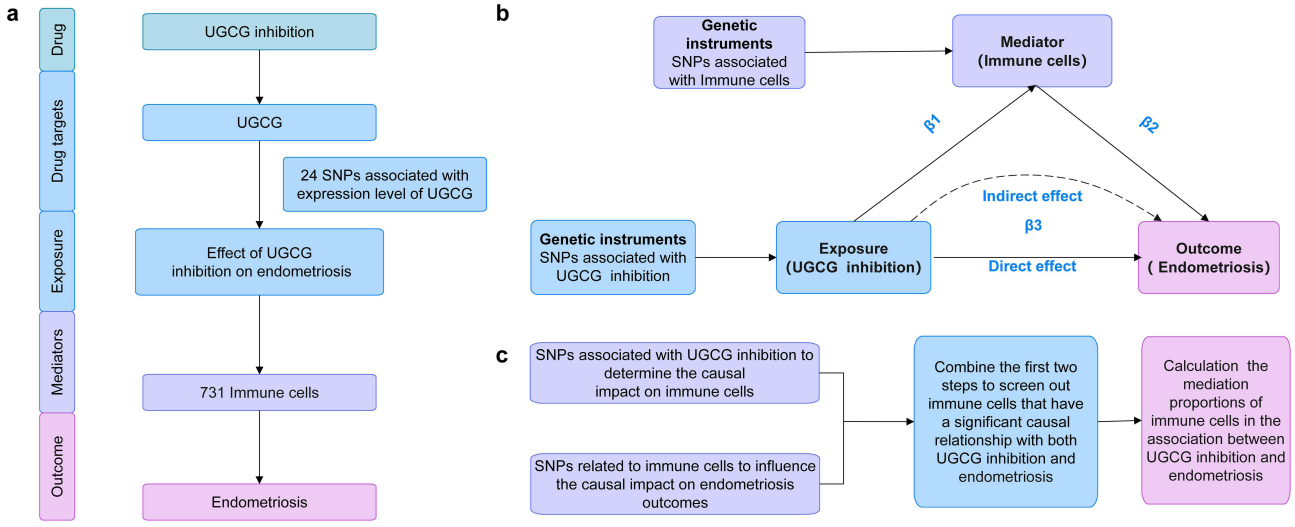


Figure 1 Outline of the study structure. (**a**) Flowchart depicting the assessment of the role of immune cells in mediating the influence of *UGCG* inhibition on EM. (**b**) In the two-step MR analysis, β1 represents the effect of *UGCG* inhibition on immune cells, β2 represents the effect of immune cells on EM, and β3 represents the direct effect of *UGCG* inhibition on EM. (**c**) The flow diagram of conducting the two-step MR step by step.
Abbreviations: *UGCG*, UDP-glucose ceramide glucosyltransferase; EM, endometriosis; MR, Mendelian Randomization.

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Table 1 Summary of the eQTL and GWAS Databases Used in MR Analyses

Data Source	Phenotype	Sample Size	Cases	Population	Adjustment
IEU Open GWAS project (eqtl-a-ENSG00000148154)	UGCG	31,684	-	European	Sex
IEU Open GWAS project	Immune cells	3757	-	European	-
FinnGen (N14_ENDOMETRIOSIS)	EM	128,171	16,588	European	-

Abbreviations: eQTL, expression quantitative trait loci; GWAS, genome-wide association study; MR, Mendelian randomization; UGCG, UDP-glucose ceramide glucosyltransferase; EM, endometriosis.

Data Sources

Expression quantitative trait loci (eQTLs) associated with the target gene *UGCG* were obtained from the IEU OpenGWAS platform (https://gwas.mrcieu.ac.uk/;accessionnumber:ENSG00000148154). This study used 731 immune cell trait data from the GWAS catalog (numbered from GCST0001391 to GCST0002121).[28] The immune cell characteristics of this dataset can be divided into seven panels: B cells; conventional dendritic cells (cDC); maturation stages of T cells; monocytes; myeloid cells; B cells, natural killer cells, T cells (TBNK); and T regulatory cells (Tregs). GWAS data for EM (N14_ENDOMETRIOSIS) were sourced from the FinnGen project website (https://www.finngen.fi/en), accessed on March 13, 2024 (Table 1). By using distinct datasets from separate sources, we minimized the risk of sample overlap and enhanced the robustness of our findings.

Selection of eQTL Instrumental Variables for UGCG Inhibition Targets

Genetic variants involved in drug target genes were identified through the following four steps (Figure 1a): (i) SNPs were selected based on $P < 1 \times 10^{-5}$, linkage disequilibrium coefficient (r^2) < 0.3, region width = 100 kb, and minor allele frequency (MAF) > 0.01. Recent studies widely endorse these as standard parameters, promoting SNP independence and reducing the influence of linkage disequilibrium.[29–31] (ii) Confounding SNPs were removed using PhenoScanner (http://www.phenoscanner.medschl.cam.ac.uk/), which identifies and excludes SNPs linked to confounding factors and outcomes. (iii) eQTL data were used to extract instrumental variables within 300 kb of the cis-regulatory region associated with drug target genes. (iv) For data filtering, relevant SNPs from GWAS data of 731 types of immune cells and EM (Table 1) were selected, excluding SNPs with palindromic structures and MAF > 0.42. Outlier SNPs were removed using MR-PRESSO.

Selection of Immune Cell Instrumental Variables

The use of at least 10 independent SNPs as instrumental variables ensures sufficient statistical efficiency in MR analyses; therefore, a threshold of $P < 1 \times 10^{-5}$ was used based on previous findings.[29,32] (i) To ensure SNP independence and minimize the effect of linkage disequilibrium, a linkage disequilibrium coefficient of $r^2 < 0.001$ and region width of 10,000 kb were established. (ii) PhenoScanner was used to exclude SNPs linked to confounding factors and outcomes. (iii) For data extraction and filtering, SNPs that met the above criteria were extracted from the summary GWAS data of EM (Table 1). SNPs with palindromic structures and MAF > 0.42 were excluded. (4) To remove outliers, abnormal SNPs were eliminated using MR-PRESSO.[33]

Statistical Analysis

MR Analysis Assessing the Effect of UGCG Inhibition on EM

Five regression models were used in this study: MR-Egger regression,[34] the inverse-variance weighted (IVW) method,[35,36] weighted median estimator,[37] weighted mode,[38] and a simple model. We performed a two-sample MR analysis, utilizing eQTLs as instrumental variables, to examine the possible causal association between *UGCG* inhibition and EM risk. The selected models were designed to enhance both the reliability and robustness of the findings by mitigating the effects of various biases and offering complementary perspectives on the potential causal relationship. There are various ways to interpret results in drug target MR analysis. Notably, when UGCG is used as an indicator for MR analysis of drug targets, the OR is > 1, indicating elevated UGCG as a risk factor for the outcome. However, the

UGCG inhibitor is the exposure factor, which acts on the drug target and inhibits the increase in UGCG. Therefore, the result should be interpreted in reverse, that is, the actual effect of the UGCG inhibitor on the EM outcome is the inverse of the original OR value $\frac{1}{OR}$. $\frac{1}{OR}$<1. This suggests that the use of UGCG inhibitor drugs may reduce the risk of adverse outcomes.

The IVW method was primarily used for causal estimation because of its precision and efficiency. The IVW results were interpreted as the main causal estimates. MR-Egger regression was additionally employed to identify and correct pleiotropy, ensuring that the causal estimates were not biased by pleiotropic effects of the instrumental variables. The Wald ratio method was used to evaluate the effects of individual SNPs on the outcome when there were three or fewer SNPs, whereas the fixed-effect IVW method was applied for the other analyses. The random-effects IVW method was used for analyses involving more than three SNPs. This approach calculates causal effects by weighting each SNP's estimate with its inverse variance (R^2) before summing the weighted estimates to determine the final causal influence. MR-Egger estimates the causal effect using a weaker assumption, termed the InSIDE assumption, based on IVW. Incorporating a regression intercept helps identify and adjust for bias caused by the pleiotropic effects of instrumental variables, facilitating the estimation of the causal relation between exposure and outcomes. MR-Egger results are particularly valuable when horizontal pleiotropy is present.[34,39] The causal effect estimates for *UGCG* inhibition on EM were evaluated using an online efficacy calculator (https://shiny.cnsgenomics.com/mRnd/), with the threshold for statistical power set at 0.8.[40]

Two-Step MR Analysis Linking UGCG Inhibition to EM via Immune Cells

Given the key role of immune cells in EM development, we utilized a two-step MR analysis to explore their role in mediating between *UGCG* inhibition and EM risk (Figure 1b). First, we used SNPs associated with *UGCG* inhibition to determine their causal effects on immune cells. Second, we employed SNPs related to immune cells to evaluate their causal effects on EM outcomes. Subsequently, we examined how *UGCG* inhibition directly influences EM development and the indirect role of immune cells in modulating this effect. The mediation ratio was calculated to quantify this relationship (Figure 1c). Notably, β1 represents the influence of *UGCG* inhibition on immune cell behavior, whereas β2 reflects the role immune cells play in EM. The indirect effect of *UGCG* inhibition on EM, or the mediation effect, was calculated as the product of β1 and β2 (β1 × β2). β3 indicates the total causal effect of *UGCG* inhibition on EM. The mediation effect (β1 × β2) was divided by the total effect (β3) to calculate the proportion of the indirect effect (E%). When the total, direct, and indirect effects were consistent in direction and the mediation proportion surpassed 5%, immune cells were identified as likely mediators, indicating a notable contribution of the indirect effect.

Sensitivity Analysis

We assessed SNP heterogeneity using Cochran's Q test and the I-squared (I^2) statistic.[36] Cochran's Q test indicated heterogeneity when $P < 0.05$, whereas $I^2 > 50\%$ indicated notable variability in the IVW findings. The formula for I^2 is $I^2 = \frac{Q-Q\_df}{Q} \times 100\%$. We performed heterogeneity analysis utilizing the intercept from MR-Egger regression alongside the MR-PRESSO test. Sensitivity analysis was conducted using the leave-one-out approach, where each SNP was individually removed, and the remaining data were reanalyzed to evaluate the effect of excluding that SNP on the overall results. An intercept close to zero in the MR-Egger regression with a non-significant *P*-value ($P > 0.05$), along with an MR-PRESSO $P > 0.05$, suggested the absence of pleiotropy among the selected SNPs. All analyses were performed using the TwoSampleMR package v0.5.10 in R v4.1.0, with a significance threshold of $\alpha = 0.05$.

Results

Causal Relationships Between UGCG Inhibition and EM

We identified 24 SNPs linked to EM from the *UGCG* eQTL data, all exhibiting an F-statistic value of >21 (Supplementary Table 1). The statistical power of the study was 1.0, indicating a very high probability that the study correctly rejected the null hypothesis if there was a true effect (Supplementary Table 2). MR analysis revealed that *UGCG* inhibition was associated with a decreased risk of EM, as indicated by the IVW results (odds ratio [OR] = 0.915,

Table 2 Causal Association Analysis Between UGCG Inhibition and EM

Exposure	Outcome	Method	Nsnp	MR		Heterogeneity			Horizontal Pleiotropy			MR-PRESSO
				OR (95% CI)	*P*	I^2 (%)	Cochran's Q	*P*	Egger Intercept	SE	*P*	*P*
UGCG inhibition	EM	IVW	24	0.915 (0.859–0.975)	0.006	20	28.798	0.187				0.236
		MR-Egger	24	0.930 (0.822–1.053)	0.264	23	28.687	0.154	0.002	0.008	0.773	
		Weighted median	24	0.934 (0.858–1.016)	0.111							
		Simple mode	24	0.946 (0.816–1.097)	0.472							
		Weighted mode	24	0.921 (0.820–1.034)	0.178							

Abbreviations: MR, Mendelian randomization; UGCG, UDP-glucose ceramide glucosyltransferase; EM, endometriosis; Nsnp, number of single-nucleotide polymorphisms; OR, odds ratio; CI, confidence interval; SE, Standard Error of β; P, P-value.

95% confidence interval [CI]: 0.859–0.975, $P = 0.006$). The IVW results revealed no heterogeneity among the eQTLs associated with *UGCG* inhibition and EM ($I^2 = 20\%$, Cochran's Q = 28.798, $P = 0.187$). The MR-Egger results indicated that the intercept term and deviation from zero were not significant ($P = 0.773$), suggesting the absence of horizontal pleiotropy. Additionally, MR-PRESSO did not detect substantial horizontal pleiotropy ($P = 0.236$), corroborating the reliability of the MR findings (Table 2). Sensitivity analyses further validated the results.

The leave-one-out analysis showed that the calculated effect sizes did not significantly differ after excluding individual SNPs, indicating the stability of the results. A funnel plot suggested no apparent bias, as the points on both sides of the symmetry axis were approximately balanced. A scatter plot indicated that the risk of developing EM increased with increasing *UGCG* expression, implying a positive association between *UGCG* activation and the risk of EM. A forest plot showed that the 95% CI for the overall effect size was situated to the right of 0, confirming that *UGCG* activation was positively associated with an increased risk of developing EM (Figure 2a–d).

Two-Step MR Analysis Linking UGCG Inhibition with EM via Immune Cells

We obtained 15,033 SNPs associated with 731 immune cells from the eQTL data for the UGCG inhibition drug target gene (Supplementary Table 3). Using eQTLs linked to UGCG suppression to assess their causal impact on immune cells, the IVW method demonstrated a statistically significant causal relationship between UGCG suppression and 156 immune cells. *UGCG* inhibition exerted dual effects: it acted as a protective factor for 84 immune cells (OR < 1, $P < 0.05$) and as a risk factor for 72 immune cells (OR > 1, $P < 0.05$) (Supplementary Table 4).

The IVW results showed no significant heterogeneity between *UGCG* inhibition and most immune cell-associated SNPs. MR-Egger analysis validated the MR findings, indicating no considerable heterogeneity or horizontal pleiotropy. However, IVW analysis revealed significant heterogeneity for T/B cells ($P = 0.035$) and B cell %lymphocytes ($P = 0.043$). Nonetheless, the MR-Egger analysis confirmed the robustness of the results without significant horizontal pleiotropy, and MR-PRESSO analysis revealed no significant levels of horizontal pleiotropy.

In the second step, 10,496 EM-associated SNPs were identified in 731 types of immune cells (Supplementary Table 5). The IVW analysis revealed significant causal relationships between 41 immune cells and EM. These immune cells had dual effects on EM, with 21 immune cells acting as protective factors (OR < 1, $P < 0.05$) and 20 immune cells acting as risk factors (OR > 1, $P < 0.05$) (Supplementary Table 6) Consistent with the results obtained in the first step, the IVW findings showed no evidence of heterogeneity between the SNPs associated with these immune cell types and EM. MR-Egger analysis confirmed the stability of the MR findings, with no significant heterogeneity or horizontal pleiotropy.

Mediating Role of Immune Cells

Combining the results of the first two steps, 12 immune cell types exhibited significant causal relationships with both *UGCG* inhibition and EM (Figure 3 and Table 3). The final 12 immune cells obtained from the intersection included: B cell panel: IgD+ CD24+ %B cell, CD20 on CD20- CD38-, CD24 on IgD+ CD38-, IgD on IgD+ $CD38^{dim}$, B-cell activating factor receptor (BAFF-R) on CD20-; Maturation stages of T cell panel: terminal differentiation (TD) CD4+ % T cell, CD4 on naive CD4+; Myeloid cell panel: HLA DR on $CD33^{br}$ HLA DR+ $CD14^{dim}$; TBNK panel: HLA DR++

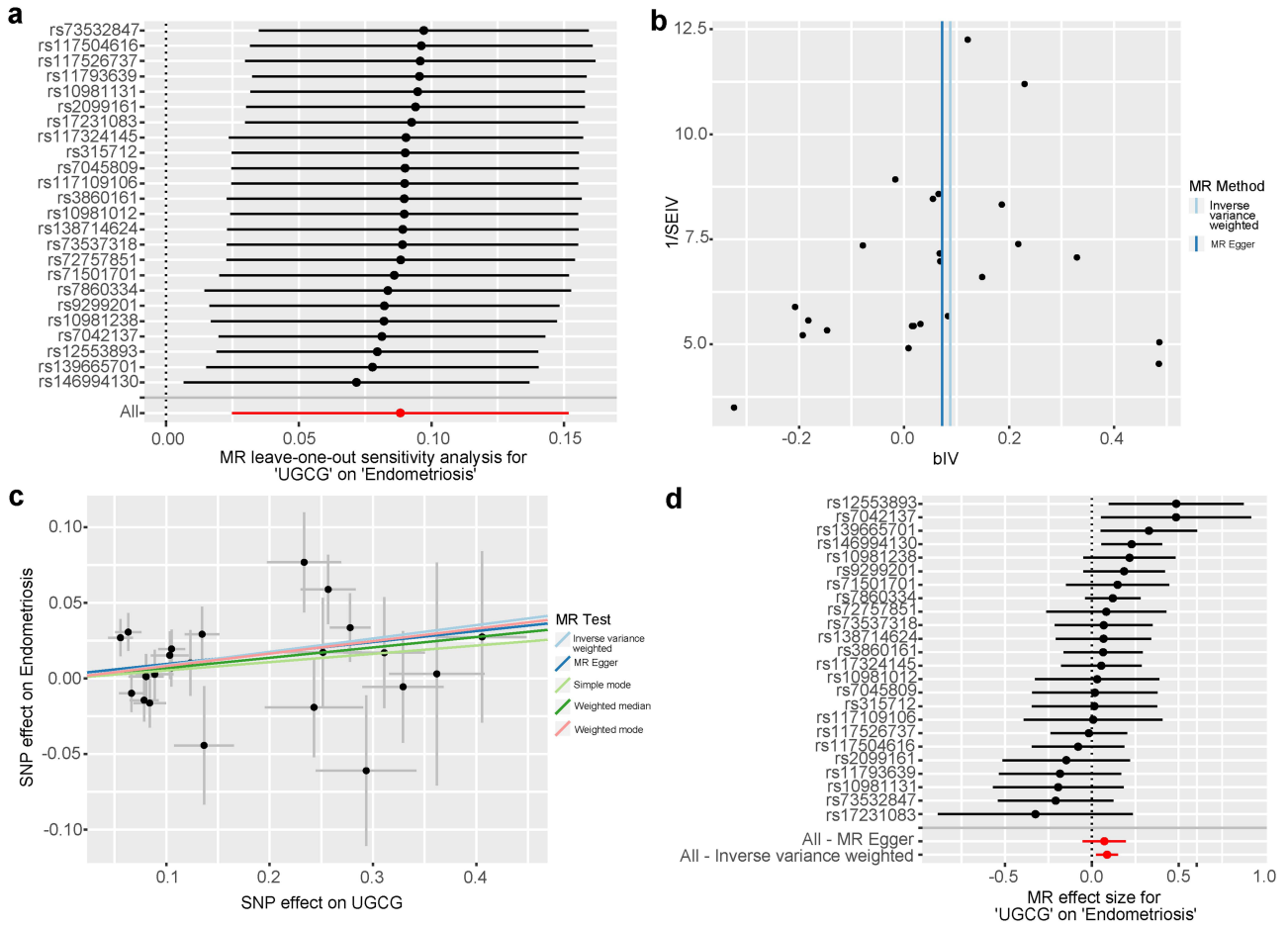

Figure 2 Sensitivity analysis. (**a**) Sensitivity analysis using a leave-one-out approach for *UGCG* and EM. (**b**) Funnel plot for *UGCG* and EM. (**c**) Scatter plot for *UGCG* and EM. (**d**) Forest plot for *UGCG* and EM.
Abbreviations: *UGCG*, UDP-glucose ceramide glucosyltransferase; EM, endometriosis.

monocyte %leukocyte, CD4+ %T cell, CD45 on B cell; Treg panel: CD127- CD8^{br} %T cell. Notably, eight of these immune cells could serve as potential mediators of *UGCG* inhibition and EM. The mediators were as follows: B cell panel: IgD+ CD24+ %B (15.499%), CD24 on IgD+ CD38- (16.627%), IgD on IgD+ CD38^{dim} (7.816%), BAFF-R on CD20- (14.379%); Maturation stages of T cell panel: TD CD4+ %T cell (26.727%); Myeloid cell panel: HLA DR on CD33^{br} HLA DR+ CD14^{dim} (13.946%); TBNK panel: CD45 on B cell (26.081%); Treg panel: CD127- CD8^{br} %T cell (14.005%) (details of the leave-one-out sensitivity analysis are illustrated in Supplementary Figures 1 and 2). TD CD4+ %T cells exhibited the highest mediation proportion, at 26.727% (95% CI: 25.198–28.255%). This indicates that *UGCG* inhibition indirectly reduces the risk of EM by impacting TD CD4+ T cells. IgD on IgD+ CD38^{dim} cells accounted for the lowest proportion of mediating cells at 7.816% (95% CI: 6.33–9.303%). The mediating effects of these eight immune cell types were negative, indicating that *UGCG* inhibition indirectly decreased the risk of EM by affecting these immune cells (Figure 4a–h).

Discussion

We examined the causal relationship between *UGCG* inhibition and EM. Our findings suggested that *UGCG* inhibition may help prevent or reduce EM. Additionally, we investigated the role of immune cells in mediating this relationship. Our findings indicated that immune cells regulate the effects of *UGCG* inhibition on EM. The eight mediator immune

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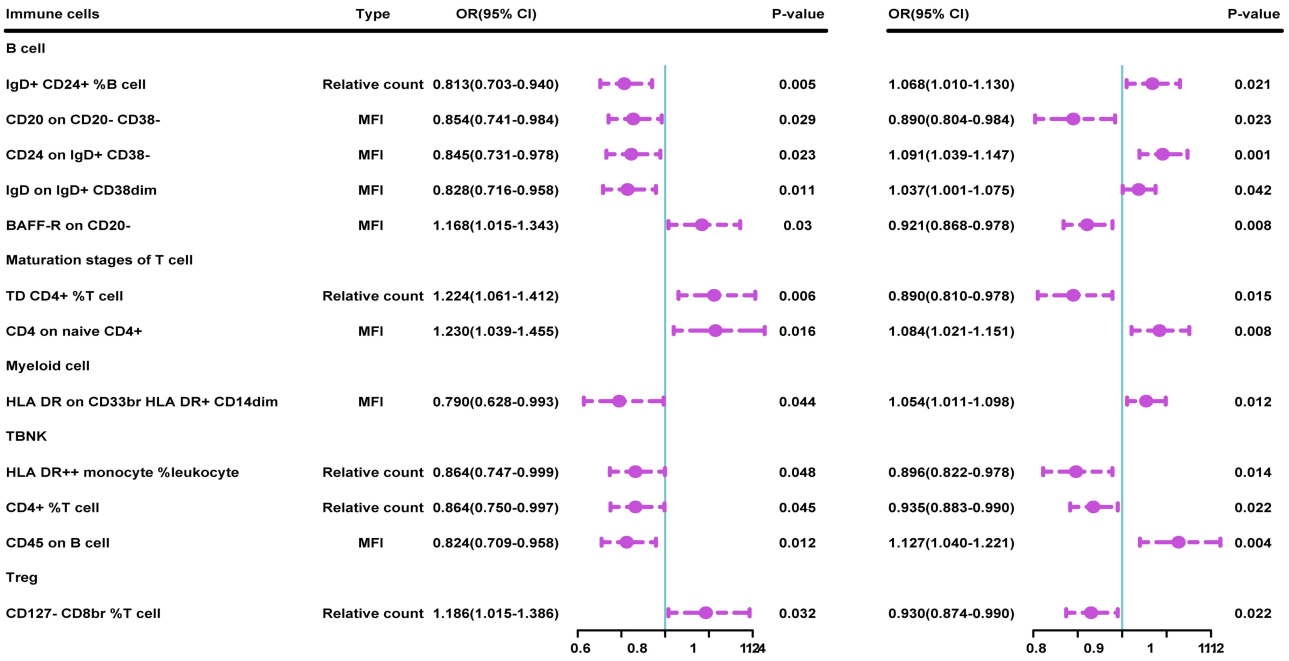

Figure 3 Forest plot depicting the effects of inhibiting UGCG activity on immune cells and the influence of immune cells on EM.
Abbreviations: *UGCG*, UDP-glucose ceramide glucosyltransferase; EM, endometriosis.

cell phenotypes identified included four B cell-related phenotypes, one TBNK-related phenotype, one phenotype associated with mature T cells, one Treg-related phenotype, and one myeloid cell-related phenotype, revealing the biological basis of EM, which may potentially aid in the development of new therapeutic targets and personalized treatments.

UGCG expression is elevated in patients with EM.[9,41] This upregulation can cause the accumulation of glycosphingolipids, including glucosylceramide, in the endometrial tissues of individuals with EM. Glucosylceramide is essential for immune regulation, and its overexpression may result in immune dysfunction, influencing the EM pathogenesis and progression.[41] The immune microenvironment plays a central role in EM pathogenesis. The peritoneal immune microenvironment, including innate and adaptive immunity, has a significant effect on the vascularization and fibrosis of EM lesions. Immune cells, such as macrophages, natural killer cells, dendritic cells, neutrophils, T cells, and B cells, as well as related cytokines and inflammatory mediators, may accelerate the implantation and development of

Table 3 Immune Cells as Mediators of the Effects of UGCG Inhibition on EM

Mediator	β1	β2	Mediating Effect	Direct Effect	Total Effect	Proportion Mediated (%)
IgD^{+} $CD24^{+}$ %B cells	–0.207	0.066	-0.014	–0.075	–0.088	15.499 (13.938–17.06)
HLA DR^{++} monocyte %leukocytes	–0.147	–0.109	0.016	–0.104	–0.088	–18.138 (–19.467–16.809)
TD $CD4^{+}$ %T cells	0.202	–0.117	–0.024	–0.065	–0.088	26.727 (25.198–28.255)
$CD4^{+}$ %T cells	–0.146	–0.067	0.01	–0.098	–0.088	–11.056 (–12.363–9.749)
$CD127^{-}$ $CD8^{br}$ %T cells	0.17	–0.073	–0.012	–0.076	–0.088	14.005 (12.533–15.476)
CD20 on $CD20^{-}$ $CD38^{-}$ cells	–0.158	–0.117	0.018	–0.107	–0.088	–20.945 (–22.295–19.595)
CD24 on IgD^{+} $CD38^{-}$ cells	–0.168	0.087	–0.015	–0.074	–0.088	16.627 (15.223–18.031)
IgD on IgD^{+} $CD38^{dim}$ cells	–0.188	0.037	–0.007	–0.081	–0.088	7.816 (6.33–9.303)
BAFF-R on $CD20^{-}$ cells	0.155	–0.082	–0.013	–0.076	–0.088	14.379 (13.05–15.708)
CD45 on B cells	–0.193	0.119	–0.023	–0.065	–0.088	26.081 (24.553–27.609)
CD4 on naive $CD4^{+}$	0.207	0.081	0.017	–0.105	–0.088	–18.87 (–20.545–17.195)
HLA DR on $CD33^{br}$ HLA DR^{+} $CD14^{dim}$	–0.236	0.052	–0.012	–0.076	–0.088	13.946 (11.835–16.057)

Abbreviations: UGCG, UDP-glucose ceramide glucosyltransferase; EM, endometriosis; β1, represents the effect of UGCG inhibition on immune cells; β2, represents the effect of immune cells on EM.

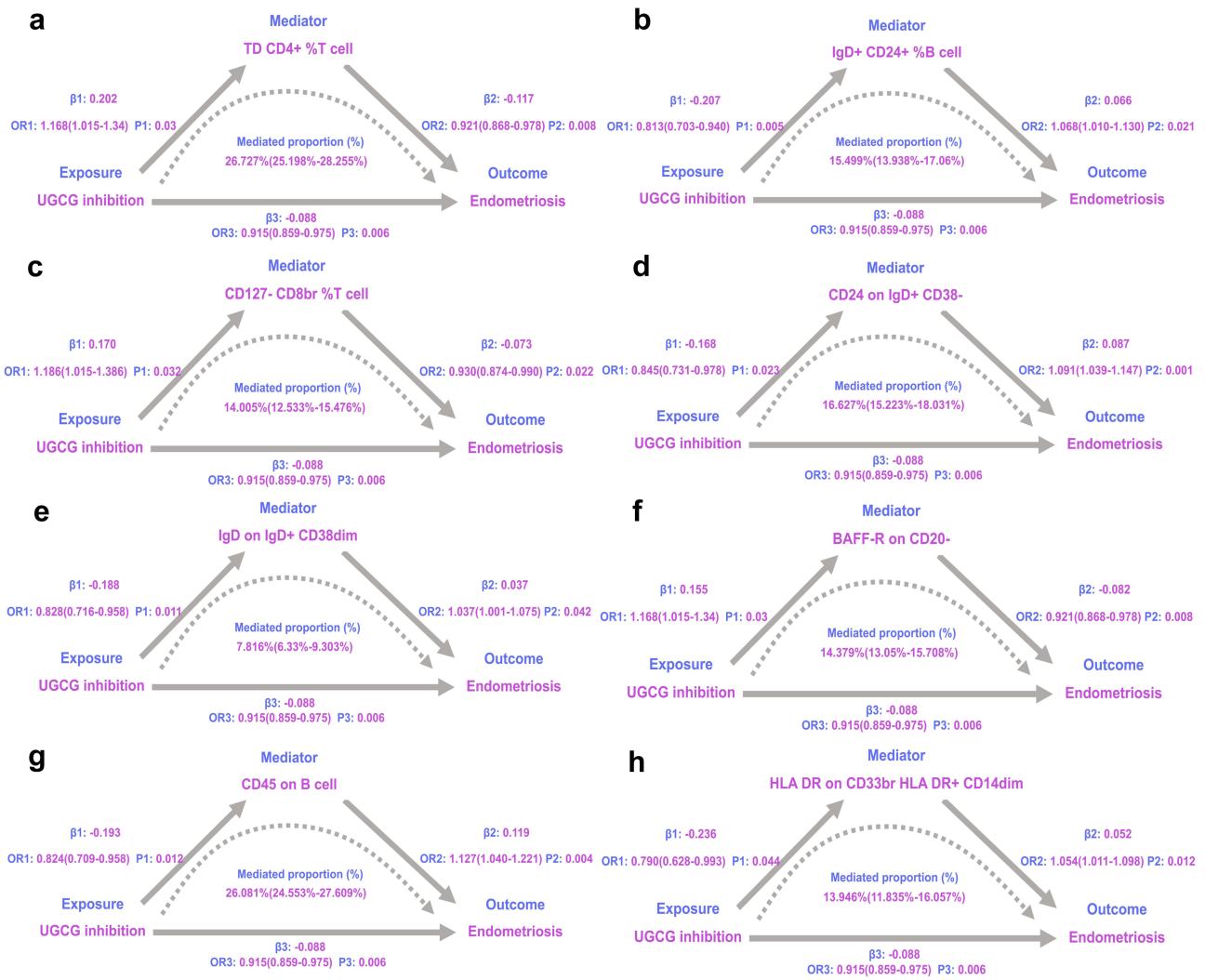

Figure 4 Mediation diagrams. Mediation effects of inhibiting *UGCG* activity on EM exerted via various immune cell populations: (**a**) TD $CD4^+$ %T cell, (**b**) IgD^+ $CD24^+$ %B cell, (**c**) CD127- $CD8^{br}$ %T cell, (**d**) CD24 on IgD^+ CD38-, (**e**) IgD on IgD^+ $CD38^{dim}$, (**f**) BAFF-R on $CD20^-$, (**g**) CD45 on B cell, and (**h**) HLA DR on $CD33^{br}$ HLA DR^+ $CD14^{dim}$.
Abbreviations: *UGCG*, UDP-glucose ceramide glucosyltransferase; EM, endometriosis.

ectopic lesions by promoting the angiogenesis and fibrosis of lesions.[42] Immune cells play pivotal roles in EM pathogenesis. For instance, Chen et al[42] demonstrated that dysfunctional macrophages can disrupt the immune microenvironment and promote the development and exacerbation of EM. A study by Mobarak et al[43] indicated that *UGCG* inhibition reduced the glycosphingolipid content in cell membranes, weakening the binding of Toll-like receptor 4 to the intracellular signaling protein Mal, thus inhibiting the immune response to pathogens. This suggests that *UGCG* inhibition holds significant potential for improving immune cell function, regulating the immune microenvironment, and controlling disease progression in patients with EM. B cell receptor engagement is reportedly involved in the conversion of ceramide into glucosylceramide by UGCG.[44] Therefore, UGCG plays a key role in B cell glycolipid metabolism. UGCG activity is essential for the maintenance of glycolipid expression on the B cell membrane, and UGCG inhibitors may weaken the proinflammatory activity of B cells by reducing the glycolipid content on the B cell membrane, thereby interfering with the immune evasion and chronic inflammatory processes of EM lesions. UGCG expression in plasmacytoid dendritic cells is crucial for their cytokine production and antiviral immune response. The regulation of plasmacytoid dendritic cell glucose and lipid metabolism by UGCG inhibitors may reduce the release of proinflammatory factors and the inflammatory state of the local immune microenvironment, thereby inhibiting the angiogenesis and fibrosis of EM lesions, thus hindering the implantation and development of EM lesions.[45]

Ma et al[10] demonstrated that decreased T cell activation and impaired natural killer cell function lead to reduced cell clearance and increased EM lesion growth. TD $CD4^+$ T cells, products of T cell activation and differentiation, have essential immunomodulatory functions. Our study revealed that 26.727% of the effect of *UGCG* inhibition on the risk of developing EM is mediated by TD $CD4^+$ T cells. These findings align with previous research findings, confirming a causal association between immune cells and *UGCG*. Furthermore, *UGCG* inhibition was causally associated with immune cells. Previous research has suggested a causal relationship between immune cells and EM, with a high frequency of $CD33^{br}$ HLA-DR^+ $CD14^-$ cells being positively correlated with EM risk.[46] Our study revealed that the negative regulatory effect of UGCG inhibitors on $CD33^{br}$ HLA-DR^+ $CD14^{dim}$ cells may lower EM risk. UGCG inhibitors may reduce EM risk by regulating different immune subsets in the $CD33^{high}$ and HLA-DR^+ compartments.

Glycosphingolipids are limiting metabolites required for cancer immune escape, and UGCG plays an important role in this process as a key enzyme, catalyzing ceramide to glycosphingolipid conversion.[47] Inhibition of glycosphingolipid synthesis using the UGCG inhibitor eliglustat increases the exposure of major histocompatibility complex and tumor antigens, thereby enhancing the antitumor response of $CD8^+$ T cells.[48] Furthermore, UGCG inhibition reduces the glycolipid content on the immune cell membrane, affecting antigen presentation and cell–cell interactions, thereby weakening the immune escape ability of tumor cells and enhancing the recognition by and killing effects of the immune system. UGCG inhibitors may work through similar mechanisms in EM treatment. Specifically, UGCG inhibitors may inhibit the immune evasion of EM lesion cells, boost immune recognition, and decrease inflammatory responses by regulating glycolipid expression on the surfaces of immune cells. Subsequently, this may enhance the functionality of antitumor immune cells, inhibit immunosuppressive cells, reduce the release of proinflammatory factors, and promote immune cell infiltration, thereby exerting a therapeutic effect.

Combination therapy studies have demonstrated the potential synergistic effects of UGCG inhibitors. The FDA-approved UGCG inhibitor eliglustat, when used in combination with a lysosomal autophagy inhibitor, significantly inhibited tumor growth and improved the survival rate in drug-resistant patients.[13] Similarly, eliglustat in combination with checkpoint blockade therapy significantly reduces tumor burden in mice.[47] This combination therapy is not only effective in tumor treatment but also has broad metabolic and immune-regulatory potential, providing a multilevel treatment option for various immune-related diseases such as EM.

Mediation analysis separates the effects of an exposure on an outcome into direct effects and those exerted through a mediating variable by estimating the causal effects among the three categories of variables, including exposure, mediating variables, and outcome. This method retains the advantages of using genetic tools for causal inference, avoiding bias due to confounding, while estimating the different effects required for mediation analysis.[19] The present study used MR analysis methods to reduce the influence of confounding factors such as basic research and observational studies in EM research, as well as the possibility of reverse causality. In addition, MR studies reduce the bias due to subjective measurement errors such as self-reporting and improve the credibility of causal inference. Compared with non-instrumental variable mediation methods, instrumental variable mediation methods can improve causal inference in mediation analysis.[49] We verified the potential therapeutic targets for EM through MR research, which can guide drug development.[50–52]

Despite the promising results, our study has a few limitations. First, genetic variations mimicking the effects of UGCG inhibitors may reflect the lifelong effects of UGCG inhibitors, but not accurately represent their short-term effects. Second, MR analysis focuses on determining potential causal directions rather than quantifying their magnitude. Therefore, further research is required to determine the generalizability of the study findings to populations of European descent. Future research should explore the potential mechanisms of UGCG inhibitors in treating EM, as well as their effects on immune regulation and apoptosis. Additionally, investigating novel UGCG inhibitors or developing therapeutic strategies that target immune cells may help establish more effective EM treatments.

Conclusions

The present study confirmed the association among genetically predicted *UGCG* inhibition, immune cells, and EM and demonstrated that eight immune cell types mediate the protective effects of *UGCG* inhibition on EM. The findings provide genetic insights into how *UGCG* inhibition reduces the risk of EM.

Abbreviations

BAFF-R, B-cell activating factor receptor; CI, Confidence interval; eQTL, Expression quantitative trait loci; EM, Endometriosis; FDA, Food and Drug Administration; GWAS, Genome-wide association study; HLA, Human leukocyte antigen; IVW, Inverse-variance weighted; MAF, Minor allele frequency; MR, Mendelian randomization; OR, Odds ratio; SNP, Single nucleotide polymorphism; TD, Terminal differentiation; Treg, T regulatory cell; UGCG, UDP-glucose ceramide glucosyltransferase.

Data Sharing Statement

The data used in the present study were sourced from publicly available archives and previous studies, as detailed in the Methods section.

Institutional Review Board Statement

This study was approved by the Traditional Chinese Medicine Ethics Committee (2024-063) of Jinjiang Hospital of Traditional Chinese Medicine. This committee waived approval for studies using publicly available data.

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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 no competing interests for this work.

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