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ORIGINAL RESEARCH

# Causal Links Between Brain Functional Networks and Endometriosis: A Large-Scale Genetic-Driven Observational Study

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**Introduction:** Endometriosis is a chronic gynecological disorder that significantly impacts women of reproductive age. Recent evidence suggests a bidirectional link between endometriosis and brain functional networks, though the causal mechanisms remain unclear. This study aims to explore these relationships using Mendelian Randomization (MR) analysis.

**Methods:** Data from 191 resting-state functional MRI (rsfMRI) phenotypes and endometriosis genetic datasets were analyzed using both forward and reverse MR approaches. Genetic Instrument Selection was performed to identify valid instrumental variables, ensuring their independence from confounders and strong association with the exposure. Sensitivity analyses were conducted to ensure the robustness of the findings.

**Results:** Forward MR analysis identified three brain networks (Pheno20, Pheno38, Pheno44) significantly associated with endometriosis risk ($P_{FDR} < 0.05$). Notably, Pheno38 activity was inversely associated with fallopian tube endometriosis, whereas Pheno20 and Pheno44 were positively linked to adenomyosis. Reverse MR analysis revealed that endometriosis of the ovary was inversely associated with functional connectivity in Pheno932, a network involved in cognitive and attention processes. Sensitivity analyses confirmed the reliability of these results.

**Discussion:** This study highlights a complex bidirectional relationship between brain functional networks and endometriosis. Increased activity in specific networks may protect against or predispose individuals to certain subtypes of endometriosis. Conversely, endometriosis also can influence brain connectivity, potentially contributing to cognitive and emotional symptoms.

**Keywords:** endometriosis, brain functional networks, resting-state fMRI, Mendelian randomization

## Introduction

Endometriosis is a chronic, multifactorial gynecological disorder affecting approximately 10% of women of reproductive age worldwide, leading to debilitating symptoms such as chronic pelvic pain, dysmenorrhea, dyspareunia, and infertility.[1,2] Despite its high prevalence, the pathogenesis of endometriosis remains poorly understood, contributing to significant delays in diagnosis and variability in treatment outcomes.[3,4] Increasingly, research has suggested a critical role for the central nervous system (CNS) in both the onset and progression of endometriosis.[5] In addition to pelvic pain, many patients with endometriosis report neuropsychological symptoms, indicating that the disorder may affect broader aspects of brain function.[6]

A prominent area of neurobiological research has focused on brain functional networks—large-scale systems of interconnected brain regions that operate in synchrony to support cognition, emotion regulation, and sensory processing.[7] Key networks, including the default mode network (DMN), central executive network (CEN), and salience network (SN), play essential roles in cognitive and emotional regulation.[8] These networks demonstrate dynamic adaptability, adjusting in response to physiological states and external demands. Functional connectivity, or the synchronized neural activity

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between regions, is central to understanding how these networks function both in health and in disease,[9] particularly in conditions involving endometriosis. In endometriosis, chronic pain is thought to be amplified by central sensitization, a phenomenon in which the CNS intensifies pain signals, increasing sensitivity to both disease-specific and non-specific pain stimuli.[5] Neuroimaging studies have observed changes in brain regions associated with pain and emotional processing, such as the anterior cingulate cortex and prefrontal cortex,[10,11] in individuals with chronic pelvic pain, suggesting that endometriosis may disrupt functional connectivity within these key networks.

The human brain remains active even in the absence of specific tasks, exhibiting complex spontaneous neural activity. Organized in a high-dimensional manner, resting-state brain networks are closely associated with various higher-order cognitive functions, including attention, emotion regulation, memory, and self-awareness.[12,13] These resting-state networks (RSNs) can be measured using resting-state functional MRI (rsfMRI), which captures functional connectivity between distinct brain regions. Hence, this study aims to investigate the causal relationships between brain functional networks and endometriosis using Mendelian Randomization (MR) analysis. By leveraging large-scale genetic data, we will examine the bidirectional effects between rsfMRI phenotypes and various types of endometriosis. MR analysis allows us to use genetic variants as instrumental variables, minimizing the influence of confounding factors and reverse causation to infer causality. We hypothesize that specific brain functional networks are not only affected by endometriosis but may also influence the risk of developing the disease. This approach offers a novel perspective on the neurobiological mechanisms underlying endometriosis, with the potential to identify therapeutic targets for both pain management and broader disease modulation.

# Materials and Methods

## Study Design

The analytical framework for this study is illustrated in Figure 1. This study adheres to the Strengthening the Reporting of Genetic Association Studies (STREGA) guidelines to ensure methodological rigor and transparency in reporting genetic association analyses.[14]

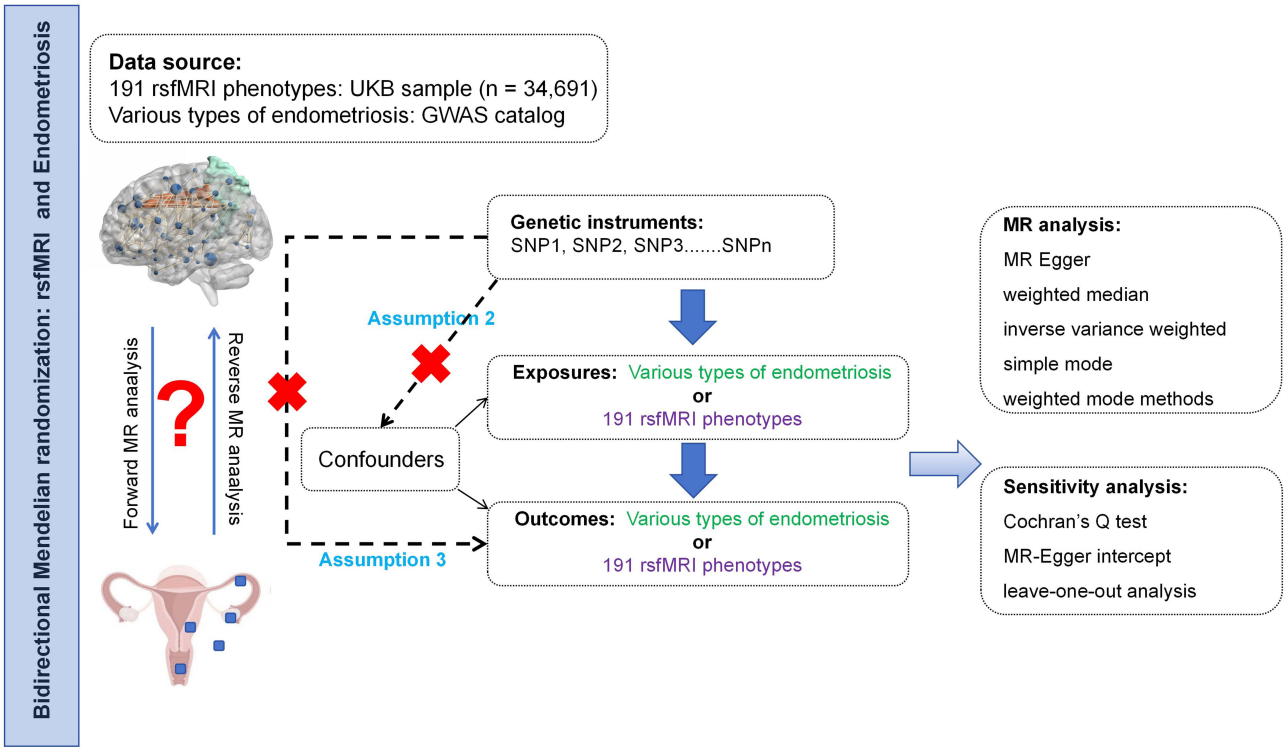


**Figure 1** Workflow of our study.

## Genetic Instrument Selection and Data Sources

For this study, we leveraged data from the UK Biobank (UKB) to identify suitable genetic instruments related to resting-state functional MRI (rsfMRI) phenotypes.[13] The rsfMRI dataset includes 1777 brain activity phenotypes and 9,026,427 common genetic variants, based on UKB participants (n = 34,691). As in previous studies,[15] endometriosis-related genetic data were obtained from the FinnGen cohort and accessed through the OPEN GWAS platform. Diagnoses of endometriosis were made in accordance with the International Classification of Diseases, 10th Edition (ICD-10), covering various anatomical locations where endometriosis manifests. The dataset includes cases identified in the uterus (n = 2372), ovaries (n = 3231), fallopian tubes (n = 116), pelvic peritoneum (n = 2953), vagina and rectovaginal septum (n = 1360), and intestines (n = 177).

## Instrumental Variable Selection

To identify suitable instrumental variables (IVs) for MR analysis, we followed a rigorous selection process. First, we selected single nucleotide polymorphisms (SNPs) that were significantly associated with the exposure of interest, applying a genome-wide significance threshold of $P < 5E\text{-}08$. Next, to ensure independence among selected SNPs, we conducted linkage disequilibrium (LD) pruning using the European population reference from the 1000 Genomes Project. Specifically, we set an LD threshold of $r^2 < 0.001$ and used a clumping window of 1000 kb, effectively removing SNPs in high LD. Further steps were taken to reduce potential confounding. We excluded SNPs known to be associated with confounding variables, which could bias the MR results. To limit the impact of weak instruments, we calculated the F statistic for each SNP, retaining only those with an F statistic > 10, a threshold that minimizes weak instrument bias. Additionally, we harmonized the exposure and outcome datasets to remove palindromic SNPs with minor allele frequencies close to 0.5, as these could lead to ambiguity in effect allele alignment. After implementing these quality control criteria, the remaining SNPs were retained as eligible IVs for analysis. To ensure reliable MR estimates, we required a minimum of three independent SNPs for inclusion in the IV set.

This screening narrowed down the selection to 191 rsfMRI traits for further GWAS analysis. These selected traits include 75 phenotypes representing the amplitude of spontaneous neural activity in specific brain regions (referred to as nodes), 111 phenotypes capturing functional connectivity between brain regions (referred to as edges), and 5 phenotypes representing global functional connectivity metrics. Additional details on these selected rsfMRI phenotypes and their descriptions are provided in Supplementary Table 1.

## MR Analysis

The primary analysis of causal relationships was conducted using the inverse-variance weighted (IVW) method with random effects, which serves as the main approach due to its robustness under the assumption that all SNPs are valid, independent instruments. The IVW method combines the ratio estimates from each SNP, providing a weighted estimate of the causal effect. To enhance the robustness of our findings, we employed additional MR methods—MR-Egger, weighted mode, weighted median, and simple mode. These methods offer complementary assumptions and can improve reliability, particularly in the presence of horizontal pleiotropy or other violations of IV assumptions. Sensitivity analyses were also performed to assess the validity of our IVs. Heterogeneity among the IVs was tested using Cochran's Q test, with a $P < 0.05$ indicating significant heterogeneity. We used the MR-Egger intercept test to detect horizontal pleiotropy, with a non-zero intercept suggesting pleiotropic effects. Finally, a leave-one-out analysis was conducted to examine the influence of individual SNPs on the overall results. By removing each SNP one at a time, this test identifies whether any single SNP has a disproportionate effect on the causal estimate, thereby ensuring that the observed association is not driven by a single instrumental variable.

## Statistical Analysis

To control for potential false positives arising from multiple comparisons, we applied a false discovery rate (FDR) correction to adjust the significance thresholds. Causal associations were considered statistically significant if they met an FDR-adjusted P-value threshold of < 0.05. Effect sizes for the identified causal relationships were expressed as odds

ratios (ORs) with corresponding 95% confidence intervals (CIs), providing a clear measure of the strength and direction of the associations. All statistical analyses were conducted using TwoSampleMR and MR-PRESSO in R software (version 4.2.1).

# Results

## Overview of the Study

Figure 1 illustrates the overall design and workflow of our study. In our study, we employed MR to investigate the bidirectional relationships between rsfMRI phenotypes and various types of endometriosis, including ovarian, pelvic peritoneal, intestinal, vaginal, fallopian tube, and adenomyosis. This design integrated both forward and reverse MR analyses, allowing us to explore potential causal relationships in both directions between brain functional connectivity and endometriosis. Our data sources included 191 rsfMRI phenotypes from the UK Biobank (UKB), encompassing 34,691 participants, and various types of endometriosis from the FINNGEN database. Our approach was grounded in the core assumptions of Mendelian Randomization: 1. Genetic Instruments: Genetic instruments (eg, SNPs like SNP1, SNP2, SNP3, etc.) must be associated with the exposure, in this case, either endometriosis or one of the 191 rsfMRI phenotypes. 2. Independence from Confounders: Genetic instruments should be independent of confounding factors to prevent spurious associations between the exposure and outcome. 3. Exposure-Specific Effects: Genetic instruments should affect the outcome only through the exposure and not via alternative pathways.

In our forward MR analyses, we examined different types of endometriosis as the outcomes, while in the reverse MR analyses, we used the 191 rsfMRI phenotypes as outcomes. To assess causality, we applied a range of MR methods, including MR-Egger regression, weighted median, inverse variance weighting (IVW), and simple or weighted mode approaches. Additionally, we conducted sensitivity analyses to ensure the robustness of our findings. These analyses included Cochran's Q test for heterogeneity, MR-Egger intercept to evaluate pleiotropy, and leave-one-out analyses to assess the stability of our results.

## Casual Effects of rsfMRI Traits on Endometriosis

Forward MR analysis establishes a causal relationship where specific brain functional networks act as the cause and endometriosis as the effect. Our analysis identified three distinct brain functional networks (Pheno20, Pheno38, Pheno44) that may be causally related to endometriosis ($P_{FDR} < 0.05$). Supplementary Table 2 provides detailed information on instrumental variables (IVs) for all significant exposure-outcome pairs in the positive MR analyses, while Supplementary Table 3 summarizes the key results of these analyses. Specifically, increased activity in Pheno38 may have a protective effect against endometriosis of the fallopian tube, whereas Pheno20 and Pheno44 appear to be positively associated with an increased risk of adenomyosis (Figure 2).

Pheno38 is located in the cerebellum and is part of the Subcortical-Cerebellum network. Increased activity in the cerebellum, particularly within the Subcortical-Cerebellum network, is associated with a reduced risk of endometriosis in the fallopian tube (Pheno38: IVW OR = 0.031, $P_{FDR} < 0.05$). Similar to Pheno38, Pheno44 is also located in the cerebellum and is part of the Subcortical-Cerebellum network. However, unlike Pheno38, the functional connectivity within this network is positively associated with an increased risk of adenomyosis (Pheno44: IVW OR = 2.100, $P_{FDR} < 0.05$). This indicates that the cerebellum's role in motor and cognitive-emotional functions may have differential effects on the development of endometriosis depending on the specific anatomical location affected.

Pheno20 involves the precuneus and cingulate regions, which are key components of the Default Mode Network (DMN) and the Central Executive Network (CEN). The DMN is active during rest and introspective activities, including self-referential thinking and memory retrieval, while the CEN is crucial for goal-directed activities and executive functions.[7,16] Pheno20 is positively associated with an increased risk of adenomyosis (Pheno20: IVW OR = 1.563, $P_{FDR} < 0.05$). The high involvement of these brain regions, which are essential for cognitive control and introspective processes, may influence the pathophysiology of endometriosis. This association might be linked to the roles of stress, emotion regulation, and cognitive load in exacerbating or modulating the disease.

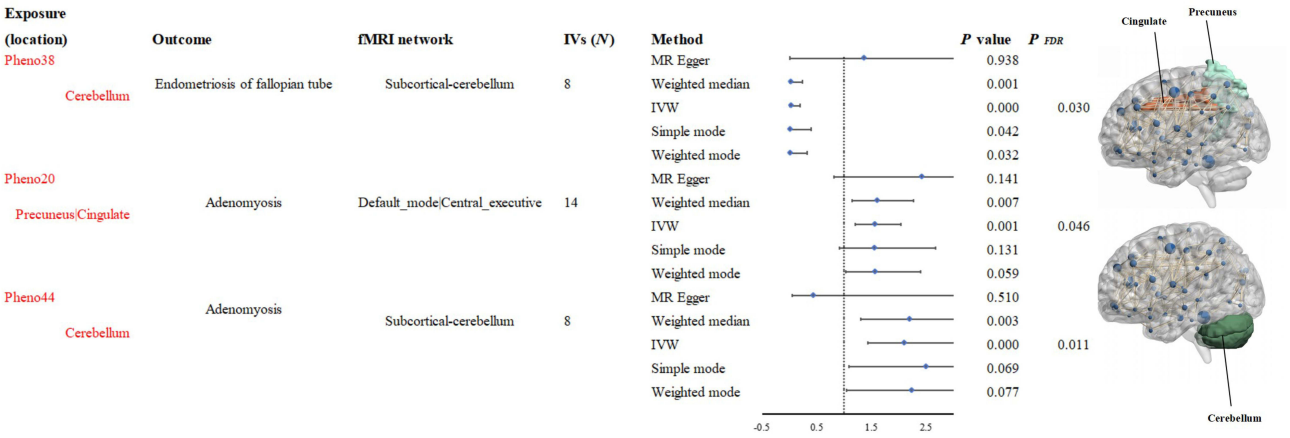


**Figure 2** The causality between various subtypes of endometriosis and brain functional networks in the forward MR.

## Casual Effects of Endometriosis on rsfMRI Traits

To explore the causal impact of endometriosis on brain functional networks, we performed a reverse MR analysis between different types of endometriosis and rsfMRI phenotypes. Supplementary Table 4 provides detailed information on IVs for all significant exposure-outcome pairs in the reverse MR analyses, while Supplementary Table 5 summarizes the key findings.

Pheno932 represents functional connectivity between regions including the precuneus, cuneus, cingulate, and superior parietal cortex, which are integral to the DMN, CEN, and Attention Network (Figure 3). These networks are involved in cognitive processing, self-referential thought, and executive functions. The IVW method (Pheno932: IVW OR = 0.947, $P_{FDR} < 0.05$) provided statistically significant evidence for an inverse relationship. These results suggest that greater connectivity in Pheno932 is significantly associated with a decreased risk of developing endometriosis of ovary, with the IVW method demonstrating particularly robust findings.

## Sensitivity Analysis

To assess the consistency and reliability of our findings, we conducted heterogeneity analyses for both forward and reverse MR analyses. The Cochrane Q test, used to evaluate potential heterogeneity among the studies (Supplementary Table 6), indicated no significant variation or unaccounted factors across the included exposures and outcomes (Cochran's Q statistic, $P > 0.05$). This suggests that the associations observed are stable and not influenced by underlying differences or additional variables. Additionally, our pleiotropy assessment, which examined the presence of pleiotropic effects, found no evidence of such effects in the results (MR-Egger intercept < 0.01, $P > 0.05$). This indicates that the observed associations are unlikely to be distorted by the influence of confounding genetic variants or other unknown factors. To further validate the reliability of our findings, we performed a leave-one-out sensitivity analysis. This approach, which involves systematically excluding each individual study to test the stability of the results, yielded

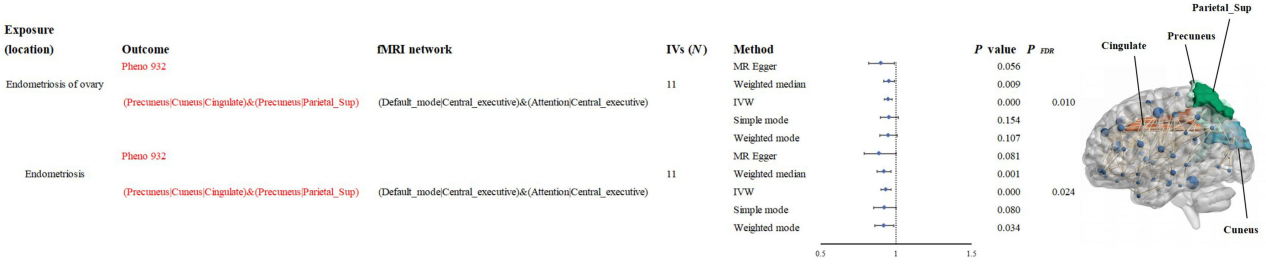


**Figure 3** The causality between various subtypes of endometriosis and brain functional networks in the reverse MR.


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consistent findings throughout. This reinforces the credibility of our conclusions and supports the overall robustness of the study's results (Supplementary Figures 1 and 2).

## Discussion

This study presents novel insights into the bidirectional causal relationships between brain functional networks and the risk of various subtypes of endometriosis, underscoring the importance of neural connectivity in both risk-enhancing and protective pathways. Our Mendelian Randomization (MR) analyses identified three distinct brain functional networks (Pheno20, Pheno38, and Pheno44) significantly associated with endometriosis risk, highlighting the complex interaction between brain function and endometriosis.

In the forward MR analysis, we found that increased activity within Pheno38, part of the Subcortical-Cerebellum network, was associated with a decreased risk of fallopian tube endometriosis. Traditionally, the cerebellum has been known for its role in motor coordination, but emerging evidence shows that it also plays an essential part in emotional and cognitive regulation.[17] Our findings align with studies indicating that increased cerebellar activity may protect against chronic pain by modulating the perception of pain stimuli and reducing stress response, a function particularly relevant in endometriosis, where pain processing is central to disease pathology.

Conversely, increased functional connectivity in Pheno20 and Pheno44, associated with the Default Mode Network (DMN) and Central Executive Network (CEN), was linked to a higher risk of adenomyosis. Both DMN and CEN are integral to higher-order cognitive functions such as memory retrieval, self-referential processing, and executive function.[7,16] Studies suggest that hyperactivity in these networks is often correlated with chronic pain and emotional dysregulation.[18,19] This hyperactivity may exacerbate disease symptoms, as psychological stress and emotional burden are known contributors to the severity and persistence of endometriosis.[6]

Our reverse MR analysis provided evidence that endometriosis, particularly endometriosis of ovary, influences neural connectivity within networks such as the DMN, CEN, and Attention Network. Notably, Pheno932 emerged as a key network in this context, suggesting an inverse relationship between endometriosis and cognitive or attentional processes. This finding is consistent with clinical observations where patients with chronic pelvic pain, such as those with endometriosis, often report cognitive impairments or "brain fog"[20] Glial activation in the CNS, as seen in mouse models of endometriosis, supports the notion of a neurological impact of the disease, potentially contributing to the observed cognitive symptoms.[21]

Further research is needed to develop clinical algorithms that can effectively integrate these neurobiological findings into diagnostic and therapeutic strategies for endometriosis. Understanding how alterations in brain functional networks influence disease progression could lead to targeted interventions that improve patient outcomes. Despite the strengths of our study, several limitations should be acknowledged. First, MR analyses are contingent upon the quality and validity of genetic instruments, which may vary across populations. Thus, further validation in diverse populations is necessary. Additionally, our analyses focused solely on resting-state brain activity; future research should incorporate task-based functional MRI studies to understand if similar associations hold during active cognitive or emotional tasks. The interplay between genetic, environmental, and lifestyle factors in endometriosis warrants more comprehensive exploration, as these factors are likely to influence both the disease process and brain connectivity.

## Conclusion

Our study offers compelling evidence of a bidirectional relationship between brain functional networks and the risk of various subtypes of endometriosis. The findings underscore the role of specific neural circuits in modulating disease processes, suggesting potential avenues for neurobiological interventions aimed at endometriosis management. By understanding how specific brain networks contribute to, or are altered by, endometriosis, additional research is needed to explore the clinical implications of these findings and to develop novel therapeutic strategies targeting the neurobiological underpinnings of this debilitating condition. This approach may ultimately lead to improved outcomes for patients.

## Data Sharing Statement

The datasets analyzed during the current study are available in the FinnGen repository, https://r8.finngen.fi/. The datasets for the brain rsfMRI can be obtained via Zenodo at https://zenodo.org/record/5775047. All analyses were conducted using TwoSampleMR and MR-PRESSO in R software (version 4.2.1).

## Ethics Approval and Consent to Participate

Since all the data utilized in this study are publicly accessible, the research was exempted by the Ethics Committee of the Fourth Affiliated Hospital of Soochow University. According to item 1 and 2 of Article 32 of the Measures for Ethical Review of Life Science and Medical Research Involving Human Subjects (dated February 18, 2023, China), which state that certain types of research using publicly accessible data are exempt from ethics review under specific circumstances, our study meets the exemption criteria. We confirm that the research was conducted in accordance with the principles outlined in the Declaration of Helsinki.

## Consent for Publication

All authors had final approval of the submitted versions.

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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 report no conflicts of interest in this work.

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