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Case Study

Deciphering the genetic interplay between depression and dysmenorrhea: a Mendelian randomization study

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Abstract

Background: This study aims to explore the link between depression and dysmenorrhea by using an integrated and innovative approach that combines genomic, transcriptomic, and protein interaction data/information from various resources.
Methods: A two-sample, bidirectional, and multivariate Mendelian randomization (MR) approach was applied to determine causality between dysmenorrhea and depression. Genome-wide association study (GWAS) data were used to identify genetic variants associated with both dysmenorrhea and depression, followed by colocalization analysis of shared genetic influences. Expression quantitative trait locus (eQTL) data were analyzed from public databases to pinpoint target genes in relevant tissues. Additionally, a protein–protein interaction (PPI) network was constructed using the STRING database to analyze interactions among identified proteins.
Results: MR analysis confirmed a significant causal effect of depression on dysmenorrhea ['odds ratio' (95% confidence interval) = 1.51 (1.19, 1.91), $P = 7.26 \times 10^{-4}$]. Conversely, no evidence was found to support a causal effect of dysmenorrhea on depression ($P = .74$). Genetic analysis, using GWAS and eQTL data, identified single-nucleotide polymorphisms in several genes, including *GRK4*, *TRAIP*, and *RNF123*, indicating that depression may impact reproductive function through these genetic pathways, with a detailed picture presented by way of analysis in the PPI network. Colocalization analysis highlighted rs34341246(*RBMS3*) as a potential shared causal variant.
Conclusions: This study suggests that depression significantly affects dysmenorrhea and identifies key genes and proteins involved in this interaction. The findings underline the need for integrated clinical and public health approaches that screen for depression among women presenting with dysmenorrhea and suggest new targeted preventive strategies.

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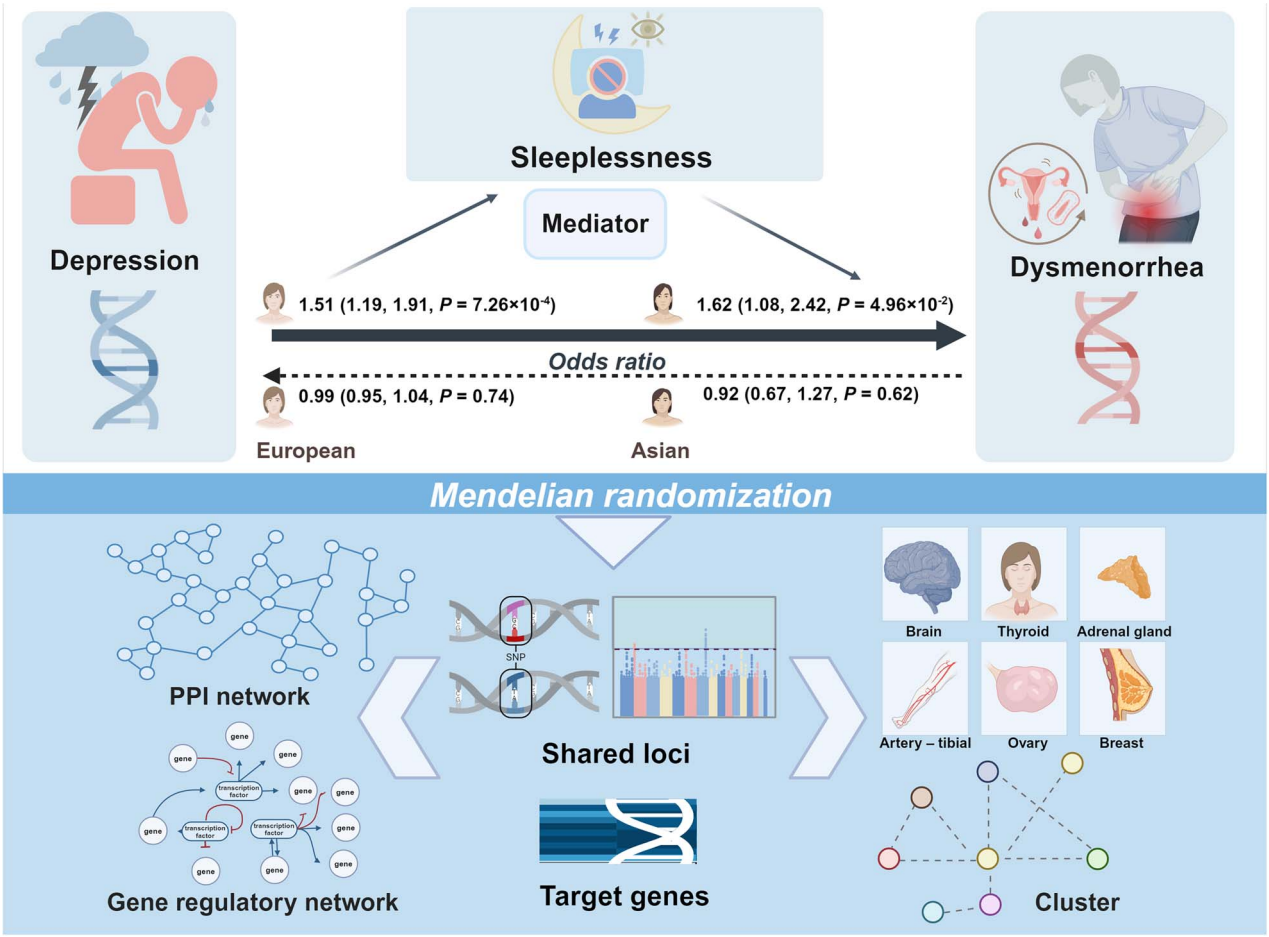
Graphical Abstract



Keywords: depression; dysmenorrhea; genetics; genomics; Mendelian randomization; mental health

Introduction

Major depressive disorder (MDD) exhibits considerable gender differences, affecting women significantly more than men [1]. The lifetime prevalence of MDD is ~21.3% in women and 12.7% in men [2].Therefore, women are twice as likely as men to suffer from depression, which ranks as the second most common cause of health-related issues among females [3]. These gender differences are particularly evident during reproductive years, encompassing phases such as the premenstrual, pregnancy, and perimenopausal periods [4]. Gender-specific depressive disorders, such as premenstrual dysphoric disorder and postpartum depression, further highlight these differences [5, 6]. It has been postulated that the variation in brain functions between men and women, including differences in sensitivity to neurotransmitters and hormones, contributes to these observed differences [7–9].

Women with MDD often experience more severe physical MDD symptoms and greater functional impairment, with specific manifestations including more frequent sleep disturbances, changes in appetite, and signs of fatigue [10–16]. Additionally, women with MDD experience significant impairments in work, social, and family functioning [17, 18]. Importantly, while many studies have highlighted the association between menstrual cycle irregularities—such as dysmenorrhea, menorrhagia, and premenstrual symptoms—and depression, the causal mechanisms and genetic pathways underlying these comorbidities remain under-recognized and poorly understood [19–22].

Previous research has consistently highlighted a strong association between dysmenorrhea and increased rates of depression [23–25]. Alateeq *et al.* observed that female students with severe dysmenorrhea face a heightened risk of depression compared to their peers [26]. This correlation is also evident among adolescent girls, who are particularly prone to depression and anxiety when experiencing dysmenorrhea, leading to considerable psychological distress [27, 28]. Despite these associations, establishing a causal relationship has been challenging due to the inherent difficulties in controlling for the many confounding factors in observational studies [29]. To address these limitations, researchers have turned to Mendelian randomization (MR), which uses genetic variants as instrumental variables to infer causality [30]. MR leverages the law of independent assortment, mimicking randomization in controlled trials and isolating the genetic influence on the disease [31, 32]. An extension of this approach is the multivariable Mendelian randomization (MVMR), which enables researchers to examine the independent or combined effects of multiple exposures on an outcome using genetic variants associated with these exposures [33]. In MR studies, genetic variants

are associated with an exposure (e.g. depression) and influence the outcome (e.g. dysmenorrhea) only through that exposure, providing a robust framework for examining these links. While both conditions are influenced by genetic and environmental factors, MR focuses on the genetic component, assuming no confounding by environmental factors.

MR studies have implicated early menarche, first birth, and young age at initial sexual intercourse as risk factors for MDD [34, 35]. They have established that early menarche correlates causally with depressive symptoms by the age of 14 years old [36]. Furthermore, a genetic predisposition to depression or dysthymia increases the risk of various female reproductive disorders [37]. Despite these insights, to date no MR study has investigated the potential causal relationship between dysmenorrhea and depression. Additionally, while Alateeq *et al.* [26] have highlighted sleeplessness as a contributing factor in the dysmenorrhea–depression nexus, the indirect effects mediated by sleep disturbances remain unquantified in these relationships.

Previous genome-wide association studies (GWASs) have identified genome-wide significant loci associated with dysmenorrhea and depression but did so independently [38–40]. For dysmenorrhea, a significant locus near the nerve growth factor gene has been consistently reported in multiple studies [38, 41]. Additionally, variants functioning as expression quantitative trait loci (eQTLs) for relevant protein-coding or long noncoding RNAs have also been identified [42]. For depression, GWAS studies have identified multiple genetic loci associated with MDD including *TRAF3*, *NEGR1*, *DRD2*, and *HACE1* [40, 43]. However, these studies typically analyze each disease in isolation, neglecting the potential genetic correlations and heritability shared between these related phenotypes.

This study, uses MR to analyze summary statistics from prior GWASs, aiming to investigate the causal relationships between depression [44] and dysmenorrhea [38]. By integrating these findings with eQTL data from genotype-tissue expression (GTEx), it seeks to establish a comprehensive theoretical foundation for understanding the etiology and pathogenesis of these conditions. Additionally, by constructing protein–protein interaction (PPI) networks and exploring transcriptional regulatory networks, it aims to identify potential biomarkers, therapeutic targets, and novel pathways that contribute to the pathogenesis of both dysmenorrhea and depression.

To further validate genetic findings, we performed a colocalization analysis [45]. This analysis provided evidence that the genetic associations observed for both depression and dysmenorrhea share common causal variants, enhancing the robustness of the identified genes for downstream functional outcome analyses. By integrating MR, eQTL, PPI, and colocalization findings, this study provides deeper insight into the molecular mechanisms linking these conditions.

Methods

Study design and data sources

A two-sample bidirectional MR analysis was conducted using GWAS summary statistics to investigate the potential causal effect of depression on dysmenorrhea. GWAS summary statistics are the aggregated results from genome-wide association studies, typically including information such as single-nucleotide polymorphism (SNP) identifiers, effect sizes, standard errors, *P*-values, and sometimes additional data like effect alleles and their frequencies. These summary statistics provide a comprehensive overview of genetic associations across the genome without requiring access to individual-level data. The data, sourced from publicly available GWAS databases, included ethically approved studies with informed consent (Table 1). To prevent sample overlap and minimize bias, distinct databases for exposure, outcome, and mediator variables were selected. By using different databases, this study enhances the robustness and reliability of its results [51].

Next, a two-sample MR analysis was performed to determine the genetic causal relationship between depression and dysmenorrhea. A two-step MR analysis was also used to investigate the role of potential mediators [57]. To further support these findings, MVMR was conducted to assess the independent or combined effects of multiple mediators. Sensitivity analyses, including assessments of pleiotropy and heterogeneity, were performed to ensure robustness and accuracy [58]. The odds ratio (OR) was used as the effect measure, representing the change in odds of the outcome per unit increase in the exposure or vice versa in the reverse analysis. The ORs were reported with 95% confidence interval (CI). Statistical significance was defined as $P < .05$. Data processing and visualization were carried out by using the TwoSampleMR (version 0.6.2), along with the MendelianRandomization (version 4.3.3) and MVMR R packages (version 0.4) [59].

The GTEx project database, version 8 (https://gtexportal.org) was used to identify target genes associated with depression and dysmenorrhea. Using the SNPs identified from the MR analysis as input, the GTEx database was queried to examine the associations between these SNPs and gene expression levels across various tissues. This approach helped identify potential regulatory relationships. The STRING 12.0 database [60] was employed to construct a PPI network of these genes. Additionally, KnockTF 2.0 (https://bio.liclab.net/KnockTFv2/) was used to build a transcription factor (TF) and a differentially expressed gene network, identifying key regulatory relationships for genes linked to depression and dysmenorrhea. In addition, a colocalization analysis was conducted by using the coloc R package (version 5.2.3) to confirm shared genetic variants between depression and dysmenorrhea. By identifying overlapped genetic regions, the colocalization analysis revealed common causal factors and further strengthened the findings from the MR analysis.

Selecting single-nucleotide polymorphisms and statistical analysis in two sample bidirectional Mendelian randomization

SNPs with inverse variance weighting (IVW) *P*-value $<5 \times 10^{-7}$ in both forward and reverse MR studies were selected. For MR-Egger and weighted median (WM) analysis, the threshold was relaxed to $P < 5 \times 10^{-6}$ to retain enough SNPs after linkage disequilibrium (LD) pruning, as previously performed in similar studies [37, 61–64]. To minimize LD effects, we excluded SNPs with an R^2 >0.001 within a 10 000 KB range through clumping, enhancing the independence of instrumental variables (IVs) [65]. R^2 represents the proportion of variance explained by the IVs related to exposures, calculated as follows:

$$R^2 = \left(\frac{\beta}{\mathrm{SD}}\right)^2 \times \mathrm{EAF} \times (1 - \mathrm{EAF}) \quad (1)$$

The effect allele frequency (EAF) represents the proportion of times a particular allele occurs in the population, β is the estimated effect, and SD is the standard error. We evaluated the robustness of each SNP using *F* statistics, calculated as follows:

$$F = \frac{K(N-K-1)}{K} \times \frac{R^2}{1-R^2} \quad (2)$$

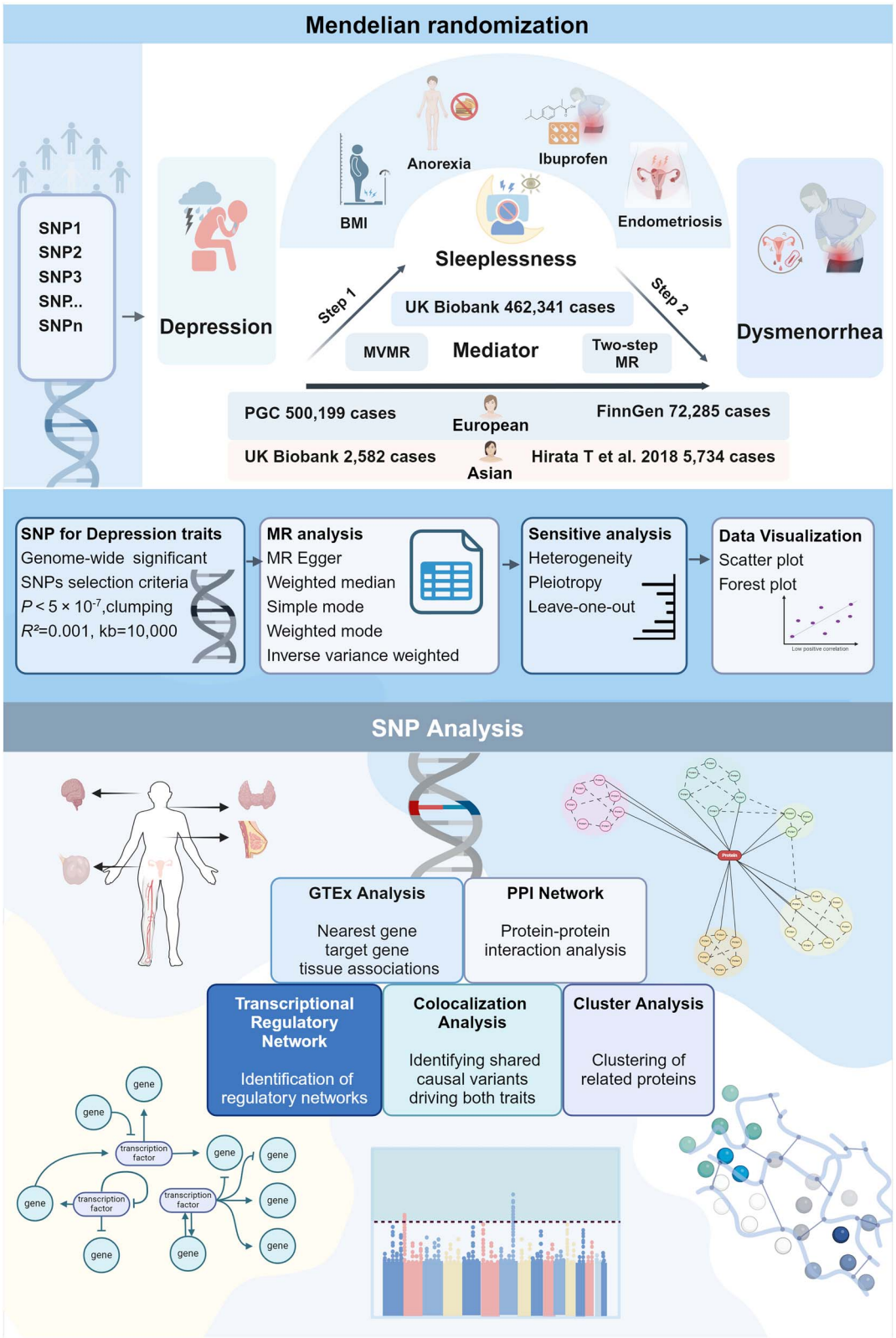


Figure 1. Overview of the study methodology. MR analysis was conducted in both European and Asian populations to investigate the causal relationship between depression and dysmenorrhea. Potential mediators, including sleeplessness [46], anorexia [47], BMI [48], endometriosis [49], and ibuprofen use [50] were analyzed for their role in the depression–dysmenorrhea link through a two-step MR process and MVMR. A comprehensive genetic and tissue analysis was performed, focusing on SNPs, target genes, and relevant tissues to identify key genetic factors. Additionally, key genes were identified through a PPI network analysis with results integrated across various network thresholds. Finally, validation was conducted using the STRING database during the PPI network analysis to confirm the functionality and biological mechanisms by cross-referencing with existing literature. Colocalization analysis was used to determine whether shared causal variants were driving both depression and dysmenorrhea.

Table 1. Summary of the GWAS datasets used in the MR analyses [39, 44, 52–56].

	Ancestry	Consortium	Author	Year of publication	PMID	Participants		Web source
						Cases (*n*)	Controls (*n*)	
Exposure								
Major depression	European	PGC [52]	Howard *et al.*	2019	30718901 [44]	170 756	329 443	https://gwas.mrcieu.ac.uk/datasets/ieu-b-102/
Seen doctor (GP) for nerves, anxiety, tension, or depression	East Asian	UK Biobank [53]	Pan-UKB team	2020	N/A	430	2152	https://gwas.mrcieu.ac.uk/datasets/ukb-e-2090_EAS/
Outcome								
Pain and other conditions associated with female genital organs and menstrual cycle	European	FinnGen [54]	N/A	2021	N/A	3316	68 969	https://gwas.mrcieu.ac.uk/datasets/finn-b-N14_FEMGENPAIN/
Pain medicine use during menstruation	East Asian	N/A	Hirata *et al.*	2018	29855537 [39]	1813	3921	doi: 10.1038/s41598-018-25065-9.
Mediator								
Sleeplessness/insomnia	European	UK Biobank	Elsworth *et al.*	2018	N/A	N/A	N/A	https://gwas.mrcieu.ac.uk/datasets/ukb-b-3957/
Medication for pain relief, constipation, and heartburn: ibuprofen (e.g. Nurofen)	European	MRC-IEU [55]	Elsworth *et al.*	2018	N/A	68 195	389 352	https://gwas.mrcieu.ac.uk/datasets/ukb-b-8888/
BMI	European	MRC-IEU	Elsworth *et al.*	2018	N/A	N/A	N/A	https://gwas.mrcieu.ac.uk/datasets/ukb-b-19,953/
Anorexia nervosa	European	GCAN [56]	Boraska *et al.*	2014	24514567 [56]	2907	14 860	https://gwas.mrcieu.ac.uk/datasets/ieu-a-45/
Diagnoses—main ICD10: N80 Endometriosis	European	UK Biobank	Neale *et al.*	2018	N/A	1496	359 698	https://gwas.mrcieu.ac.uk/datasets/ukb-d-N80/

Abbreviation: PGC, Psychiatric Genomics Consortium; NA, Not Applicable.

Here, N is the sample size, K represents the number of candidate SNPs, and R^2 is the proportion of variance explained by the instrumental variables. This method ensures the reliability and validity of the selected SNPs for the analysis (Supplementary Tables S1, S2, S6, S7, S11, S12, S15, S16, S20, S21, S25, S26, S30, S31, S35, and S36) [29].

To identify the causal links between depression and dysmenorrhea, IVW was used and complemented by MR-Egger and WM methods for sensitivity analyses. IVW estimates the weighted regression slope of the SNP outcome on SNP exposure, providing unbiased results assuming validity. WM is an unbiased estimate when at least 50% of the SNPs meet the validity assumption. MR-Egger introduces an intercept term to account for pleiotropy but may result in wider CIs with reduced statistical power [66]. Beta values in this study were converted to ORs. and 95% CI were calculated.

Following this, heterogeneity among causal estimates as suggested by IVs was examined using Cochran's Q analysis to check for potential violations of MR assumptions [67]. MR-Egger regression was used to assess potential pleiotropic effects [68]. A leave-one-out analysis was performed to evaluate the influence of individual IVs by sequentially removing each SNP and reassessing the remaining set. The impact of each SNP was illustrated using forest plots.

Two-step Mendelian randomization analysis via potential mediators

To explore the genetic mechanisms linking depression and dysmenorrhea, MR analyses were performed with depression as the exposure and various potential mediators as outcomes (Supplementary Tables S21, S22, S26, and S27). These mediators included sleeplessness, anorexia, body mass index (BMI), endometriosis, and ibuprofen. GWAS summary statistics for these mediators were obtained from the MRC Integrative Epidemiology Unit (IEU) OpenGWAS database (https://gwas.mrcieu.ac.uk/) with detailed information provided in Table 1. A two-step MR approach was conducted: first, calculating the causal impact of depression on the mediators and then estimating the causal effect of these mediators on dysmenorrhea. This analysis assessed causal effects at each step, exploring how depression influences the risk of dysmenorrhea through mediating variables. Primary results were evaluated using estimates from the IVW method with statistical significance set at $P < .05$.

Multivariable Mendelian randomization analysis via potential mediators

Next, we conducted an MVMR analysis to further explore the causal relationships between depression, dysmenorrhea, and selected mediators. MVMR simultaneously evaluates multiple mediators, controlling for pleiotropy and confounders, and assesses each mediator's independent effect. We used the IVW method as the primary approach to assessing variable significance in MVMR.

Functional annotation of regulatory pathways

Expression quantitative trait locus analyses

To investigate target genes at identified loci, eQTL data were extracted from the GTEx Portal for the SNPs identified in the MR results. These SNPs were considered to have a causal relationship with the outcome variable if the associations reached statistical significance (typically P-value <.05). The analysis, combining methods such as eQTL analysis, RNA-Seq, and statistical association tests, focused on tissues relevant to dysmenorrhea and depression: muscle, thyroid, putamen (basal ganglia), hippocampus, substantia nigra, frontal cortex, amygdala, and anterior cingulate cortex.

Protein–protein interaction analysis

The STRING database was used to build a PPI network. This PPI network was then annotated with information to reflect human phenotype items. The network incorporates both physical interactions and functional associations between proteins. The PPI network was visualized using Cytoscape (version 3.8.0) [69]. Markov Clustering (MCL) was applied with different colors to identify natural clusters based on stochastic flow [70].

Analysis of the regulatory network of key genes

KnockTF 2.0 was used to construct transcription factor (TF)–differentially expressed gene networks and perform network analyses for specific genes. KnockTF 2.0 is a comprehensive database of TF knockdown/knockout experiments across various tissues and cell types. This study analyzed 18 key genes, which were identified from the target genes and nearest genes of SNPs as derived from the MR analysis. These SNPs were considered to have a causal relationship with the outcome variable if the associations reached statistical significance (typically P-value <.05). The regulation networks of these 18 genes were then examined, revealing their interactions with numerous transcription factors.

Colocalization analyses between the two traits

Subsequently, we performed Bayesian colocalization analyses to determine if SNPs linked to both depression and dysmenorrhea share the same causal variant under IVW $P < 5 \times 10^{-6}$ threshold. This method calculates posterior probabilities for five hypotheses about shared causality using approximate Bayes factors. We focused on the posterior probability of hypothesis four (PPH4), which found shared causal SNPs between the two traits.

Results

Causal association between depression and dysmenorrhea identified through Mendelian randomization

Two-sample bidirectional Mendelian randomization in the European population

MR analysis demonstrated a consistent causal association between depression and dysmenorrhea in the European population, applying both IVW $P < 5 \times 10^{-6}$ and IVW $P < 5 \times 10^{-7}$ thresholds. Using the $P < 5 \times 10^{-7}$ threshold, the IVW method found a statistically significant causal effect of depression on dysmenorrhea with an OR of 1.51 (95% CI 1.19, 1.91, $P = 7.26 \times 10^{-4}$), −log10(P) values were included in the results (Fig. 2A and B, Supplementary Table S3, Supplementary Fig. S1). Similarly, using the $P < 5 \times 10^{-6}$ threshold, the IVW method also demonstrated a statistically significant causal effect of depression on dysmenorrhea with an OR of 1.48 (95% CI 1.23, 1.77, $P = 3.07 \times 10^{-5}$) (Fig. 2C and D, Supplementary Table S8, Supplementary Fig. S4).

Next, we performed MR-Egger intercept tests for horizontal pleiotropy using a $P < 5 \times 10^{-7}$ threshold. The intercept was 0.03 (SE = 0.02, $P = .05$), indicating minimal pleiotropy and supporting the causal inferences from IVW and WM methods (Supplementary Table S4). Cochran's Q tests showed no significant heterogeneity for both MR-Egger (Q = 87.17, $P = .30$) and IVW (Q = 91.41, $P = .22$) (Supplementary Table S5). Leave-one-out analyses revealed no outliers, confirming the robustness

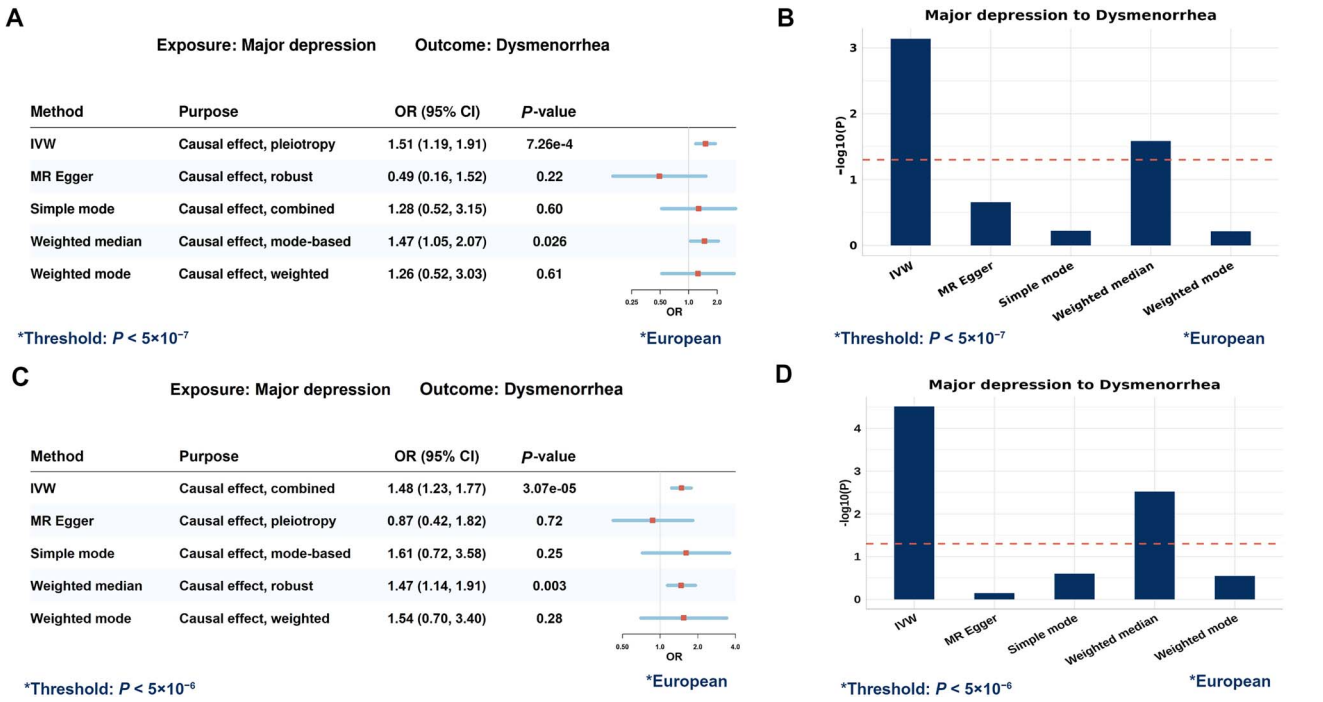


Figure 2. Forest plots and bar plots illustrating the MR analysis results examining the causal relationship between dysmenorrhea and depression in the European population under two significance thresholds $P < 5 \times 10^{-7}$ and $P < 5 \times 10^{-6}$.

of the associations (Supplementary Fig. S2). At a relaxed threshold of $P < 5 \times 10^{-6}$, MR-Egger and IVW also detected no pleiotropy or heterogeneity (Supplementary Tables S9 and S10), and leave-one-out analyses remained robust without outliers (Supplementary Fig. S5).

In Fig. 2, relevant information was prepared and presented in the following manner: (A) MR analysis results with major depression as the exposure and dysmenorrhea as the outcome, using different MR methods (IVW, MR-Egger, Simple mode, Weighted median, and Weighted mode), SNP threshold: $P < 5 \times 10^{-7}$. The OR (95% CI) and *P*-values are presented. (B) The bar plot shows the −log10(*P*) values for the MR analysis with major depression as the exposure and dysmenorrhea as the outcome across various MR methods, SNP threshold: $P < 5 \times 10^{-7}$. The red dashed line represents the significance threshold ($P = .05$). (C) MR analysis results with major depression as the exposure and dysmenorrhea as the outcome, using different MR methods (IVW, MR Egger, Simple mode, Weighted median, and Weighted mode), SNP threshold: $P < 5 \times 10^{-6}$. The OR (95% CI) and *P*-values are presented. (D) The bar plot showing the −log10(*P*) values for the MR analysis with major depression as the exposure and dysmenorrhea as the outcome across various MR methods, SNP threshold: $P < 5 \times 10^{-6}$. The red dashed line represents the significance threshold ($P = .05$).

There was no evidence of reverse causality between dysmenorrhea and depression in the European population. In the reverse MR analysis, using both SNP thresholds of $P < 5 \times 10^{-7}$ and $P < 5 \times 10^{-6}$, the IVW method did not indicate any significant causal association. Specifically, the IVW analysis with the $P < 5 \times 10^{-7}$ threshold showed no significant results (OR of 0.99, 95% CI: 0.95–1.04, $P = .74$) (Supplementary Table S13, Supplementary Fig. S3). Similarly, using the $P < 5 \times 10^{-6}$ threshold, the IVW method again showed no significant association (OR of 1.00, 95% CI: 0.98–1.03, $P = .76$) (Supplementary Table S17, Supplementary Fig. S6).

Both the MR-Egger intercept test and the Cochran Q statistic results were nonsignificant at these thresholds, indicating a lack of substantial pleiotropy and heterogeneity among the genetic instruments (Supplementary Tables S14, S18, and S19). No extreme outliers were detected in the leave-one-out analysis (Supplementary Fig. S7).

Two-sample bidirectional Mendelian randomization in the Asian population

MR analysis was used to also investigate the causal association between depression and dysmenorrhea in the Asian population. The MR-Egger method suggested a significant causal effect of depression on dysmenorrhea with an OR of 1.62 (95% CI 1.08, 2.42, $P = 4.87 \times 10^{-2}$) (Fig. 3A, Supplementary Fig. S8, Supplementary Table S21). However, the WM method did not show significant results (OR of 1.00, 95% CI 0.93, 1.08, $P = .99$). The IVW method also failed to demonstrate a significant association (OR of 0.95, 95% CI 0.87, 1.03, $P = .23$). To provide a more comprehensive understanding and better context of the significance levels, −log10(*P*) values were included in the results (Fig. 3B).

The MR-Egger intercept test was performed to assess horizontal pleiotropy. The intercept was found to be −0.24 (SE = 0.09, $P = .03$), indicating that while there may be some pleiotropic effects, they are not strongly significant (Supplementary Table S22). The Cochran Q statistic for heterogeneity was significant for the IVW method ($Q = 24.37$, $P = 3.75 \times 10^{-3}$), suggesting the presence of significant heterogeneity among the SNPs. For the MR Egger method, the Cochran Q statistic was 13.12 with a *P*-value of .11, indicating no significant heterogeneity (Supplementary Table S23). Leave-one-out analysis revealed no extreme outliers (Supplementary Fig. S9).

Additionally, there was no evidence of reverse causality between dysmenorrhea and depression in both primary and sensitivity analyses (OR of 0.92, 95% CI 0.67–1.27, $P = .62$).

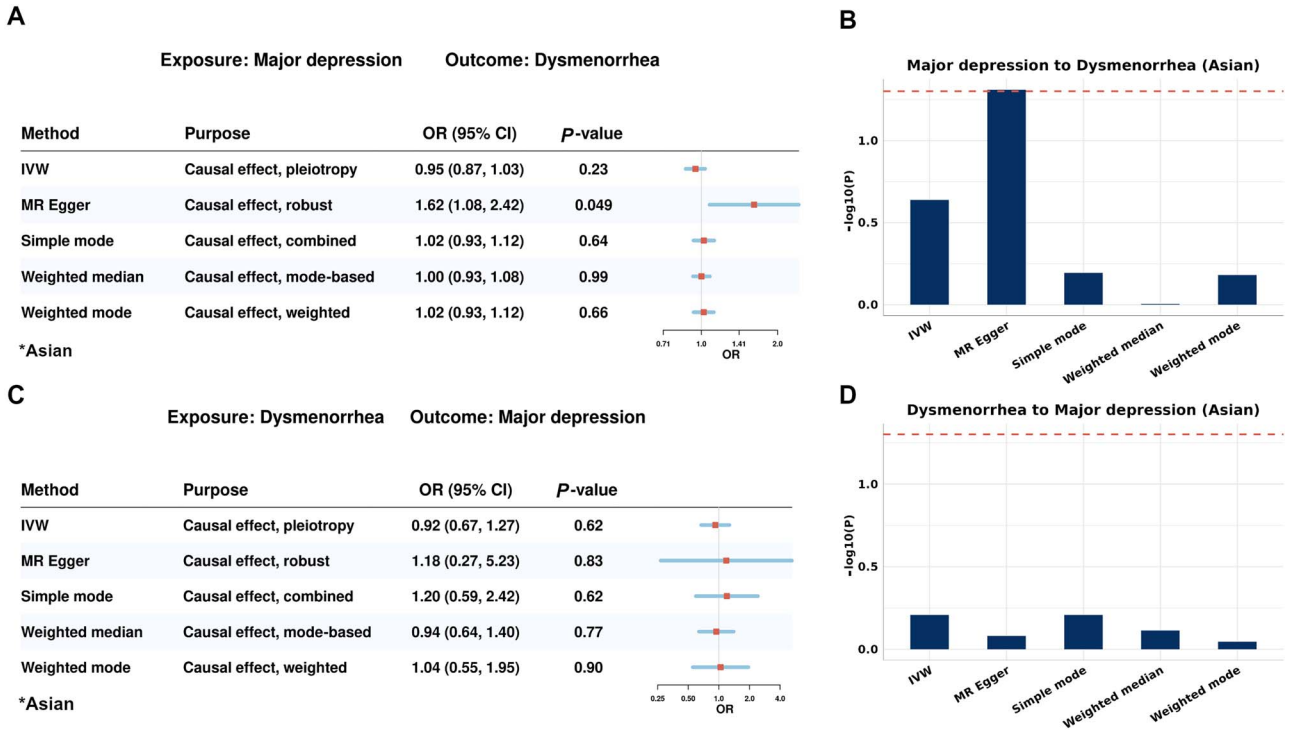


Figure 3. Forest plots and bar plots illustrating the MR analysis results examining the causal relationship between dysmenorrhea and depression in the Asian population.

The other four methods yielded similar nonsignificant results (Fig. 3C, 3D, Supplementary Fig. S10, Supplementary Table S26).

The MR-Egger intercept test was performed to assess horizontal pleiotropy. The intercept was found to be −0.04 (SE = 0.12, $P = .74$), indicating that there are no significant pleiotropic effects (Supplementary Table S27). The Cochran Q statistic for heterogeneity was also not significant for both the MR Egger method ($Q = 27.35$, $P = .05$) and the IVW method ($Q = 27.53$, $P = .07$), suggesting the absence of significant heterogeneity among the SNPs (Supplementary Table S28). Leave-one-out analysis revealed no extreme outliers (Supplementary Fig. S11).

In Fig. 3, relevant information was prepared and presented in the following manner: (A) MR analysis results with major depression as the exposure and dysmenorrhea as the outcome, using different MR methods (IVW, MR-Egger, Simple mode, Weighted median, and Weighted mode). The OR (95% CI) and *P*-values are presented. (B) Bar plot showing the −log10(*P*) values for the MR analysis with major depression as the exposure and dysmenorrhea as the outcome across various MR methods. The red dashed line represents the significance threshold ($P = .05$). (C) MR analysis results with dysmenorrhea as the exposure and major depression as the outcome were assessed using different MR methods (IVW, MR Egger, Simple mode, Weighted median, and Weighted mode). The OR (95% CI) and *P* -values are presented. (D) Bar plot showing the −log10(*P*) values for the MR analysis with dysmenorrhea as the exposure and major depression as the outcome across various MR methods. The red dashed line represents the significance threshold ($P = .05$).

Two-step Mendelian randomization analysis via potential mediators

MR analysis provided evidence of a significant causal effect of depression on sleeplessness. The IVW method showed an OR of 1.10 (95% CI 1.07, 1.12, $P = 1.21 \times 10^{-14}$), indicating a strong association (Fig. 4A–C, Supplementary Fig. S12, Supplementary Table S31). This was further supported by the WM method with an OR of 1.10 (95% CI 1.07, 1.12, $P = 4.09 \times 10^{-15}$). However, the MR-Egger method did not show significant results (OR of 1.05, 95% CI 0.94, 1.18, $P = .39$).

The MR-Egger intercept test was performed to assess horizontal pleiotropy. The intercept was found to be 1.3×10^{-3} ($SE = 1.7\times 10^{-3}$, $P = .43$), indicating that there were no significant pleiotropic effects (Supplementary Table S32). The Cochran Q statistic for heterogeneity was significant for both the MR-Egger and IVW methods ($Q = 268.33$, $P = 5.42 \times 10^{-23}$ for MR-Egger; $Q = 270.53$, $P = 4.61 \times 10^{-23}$ for IVW), suggesting the presence of significant heterogeneity among the SNPs (Supplementary Table S33). Leave-one-out analysis revealed no extreme outliers (Supplementary Fig. S13).

MR analysis provided evidence of an indirect effect of major depression on dysmenorrhea via sleeplessness, with an OR of 3.32 (95% CI 1.74 to 6.31, $P = 2.50 \times 10^{-4}$). This suggests that sleeplessness may mediate the causal relationship between depression and dysmenorrhea (Fig. 4A). The IVW method also demonstrated a significant causal effect of sleeplessness on dysmenorrhea with an OR of 3.01 (95% CI 1.58, 5.73, $P = 7.67 \times 10^{-4}$) (Fig. 4D and E, Supplementary Fig. S14, Supplementary Table S36). The MR-Egger method did not show significant results for the sleeplessness to dysmenorrhea relationship (OR of 1.02, 95% CI 0.60, 1.75, $P = .50$). Similarly, the WM method did not show significant results (OR of 1.58, 95% CI 0.60, 4.19, $P = .36$).

The MR-Egger intercept test was performed to assess horizontal pleiotropy. The intercept was found to be 0.02 (SE = 0.01, $P = .06$), indicating that there were no strongly significant pleiotropic effects (Supplementary Table S37). The Cochran Q statistic for heterogeneity was not significant for both the MR-Egger method ($Q = 64.29$, $P = .73$) and the IVW method ($Q = 67.87$, $P = .65$), suggesting the absence of significant heterogeneity among the

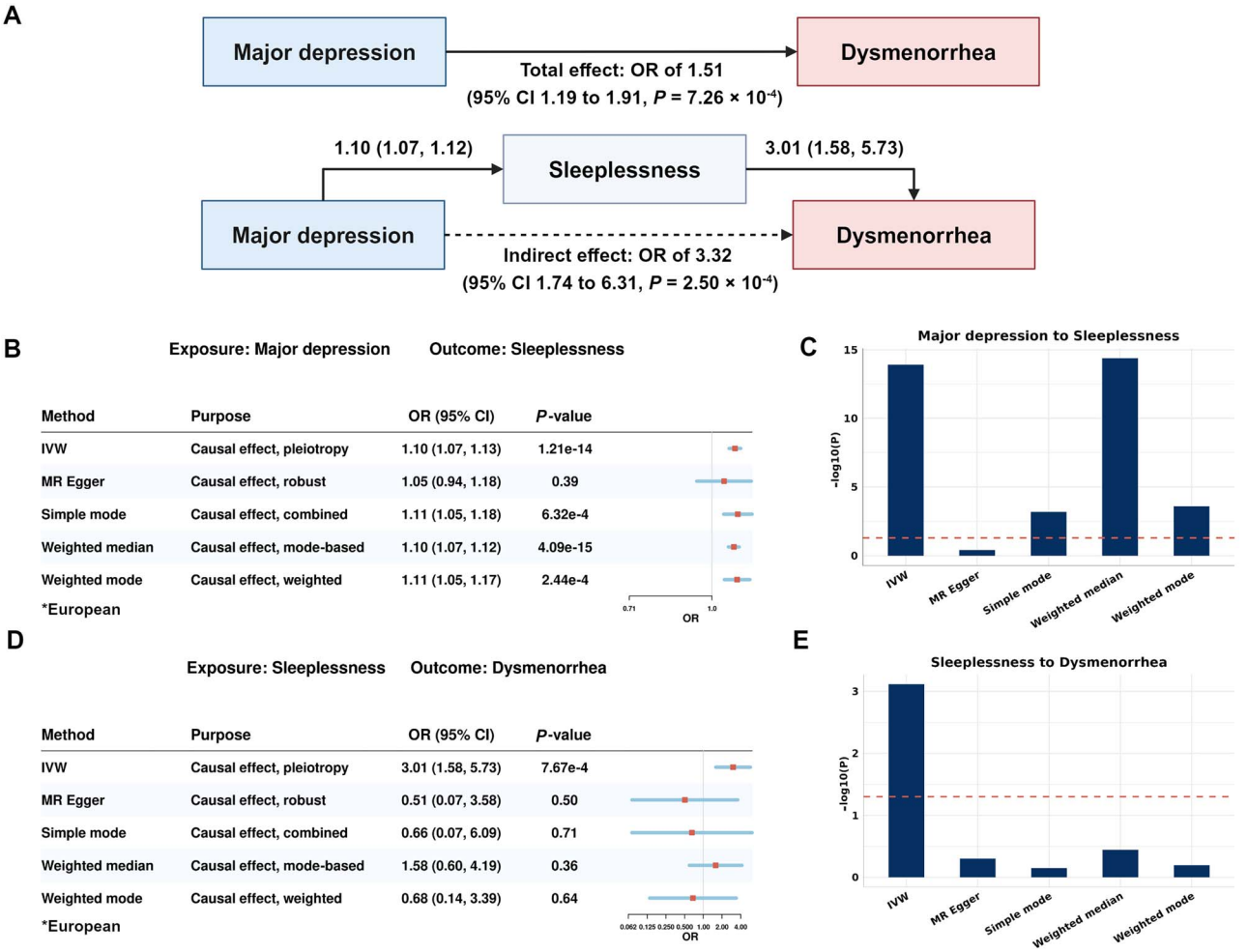


Figure 4. Mediation analysis of the effect of depression on dysmenorrhea using two-step MR.

SNPs (Supplementary Table S38). Leave-one-out analysis revealed no extreme outliers (Supplementary Fig. S15).

This study did not explore mediation via sleeplessness from dysmenorrhea to depression, as no significant total causal effect was observed in this direction.

To further explore potential mediators, additional analyses were conducted on factors such as ibuprofen, endometriosis, anorexia nervosa, and BMI. However, these analyses revealed no significant indirect effects in either direction. This indicates a lack of evidence for these mediators in the relationship between depression and dysmenorrhea using two-step MR (Table 2).

In Fig. 4, relevant information was prepared and presented in the following manner: (A) Mediation analysis via sleeplessness in European populations. (B) MR analysis results with depression as the exposure and sleeplessness as the outcome. (C) The additional −log10(P) values enhance the visualization of statistical significance across different methods from depression to sleeplessness. (D) MR analysis results with sleeplessness as the exposure and dysmenorrhea as the outcome. (E) The additional −log10(P) values enhance the visualization of statistical significance across different methods from sleeplessness to dysmenorrhea.

Multivariable Mendelian randomization analysis via potential mediators

In the MVMR analysis, we investigated the causal relationship between depression and dysmenorrhea, adjusting for potential mediators including sleeplessness, anorexia, BMI, endometriosis, and ibuprofen use (Fig. 5, Table 3). After adjusting for sleeplessness, the effect size was slightly attenuated, with an OR of 1.39 (95% CI: 1.08, 1.80, $P = 1.02 \times 10^{-2}$), suggesting that sleeplessness mediates the relationship between depression and dysmenorrhea. The anorexia-adjusted analysis yielded an OR of 1.46 (95% CI: 1.10, 1.93, $P = 8.35 \times 10^{-3}$). Adjusting for BMI and endometriosis resulted in an OR of 1.45 (95% CI: 1.11, 1.91, $P = 6.81 \times 10^{-3}$) and OR 1.55 (95% CI: 1.23, 1.96, $P = 1.86 \times 10^{-4}$), respectively. Finally, ibuprofen use–adjusted analysis resulted in an OR 1.49 (95% CI: 1.17, 1.89, $P = 1.16 \times 10^{-3}$). No significant heterogeneity or horizontal pleiotropy was detected across the models, suggesting the robustness of the causal estimates (Table 3).

In the MVMR analysis, we further investigated the causal relationship between sleeplessness and dysmenorrhea, adjusting for potential mediators such as anorexia, BMI, endometriosis, and ibuprofen use (Fig. 5). When unadjusted, the OR effect of sleeplessness on dysmenorrhea was 3.01 (95% CI 1.58, 5.73, $P = 7.67 \times 10^{-4}$). After adjusting for anorexia, the OR slightly increased to 3.72 (95% CI 1.48, 9.35, $P = 5.19 \times 10^{-3}$). Adjusting for BMI resulted in an OR of 2.90 (95% CI 1.30, 6.44, $P = 9.04 \times 10^{-3}$). After adjusting for endometriosis, the OR was 2.77 (95% CI 1.50, 5.11, $P = 1.15 \times 10^{-3}$). Finally, adjusting for ibuprofen use yielded an OR of 2.75 (95% CI 1.43, 5.28, $P = 2.41 \times 10^{-3}$). No significant heterogeneity or horizontal pleiotropy was detected, supporting the robustness of the causal estimates across the models. The

Table 2. MR analysis of potential mediators between depression and dysmenorrhea in European populations using two-step MR.

Step 1						Step 2				
Exposure	**Method**	**No. of SNPs**	**OR**	***P*-value**	**Mediator**	**Method**	**No. of SNPs**	**OR**	***P*-value**	**Outcome**
Major depression	MR-Egger	297	0.10 (0.98, 1.01)	0.58	Ibuprofen	MR-Egger	156	0.30 (0.01, 9.23)	0.49	Dysmenorrhea
	Weighted median	297	1.02 (1.01, 1.02)	1.59e-7		Weighted median	156	0.94 (0.14, 6.21)	0.95	
	IVW	297	1.02 (1.01, 1.02)	1.17e-12		IVW	156	1.71 (0.45, 6.43)	0.43	
	Simple mode	297	1.03 (1.00, 1.05)	0.02		Simple mode	156	0.10 (4.27e-4, 24.95)	0.42	
	Weighted mode	297	1.02 (1.00, 1.05)	0.03		Weighted mode	156	0.36 (4.11e-3, 31.35)	0.65	
	MR-Egger	319	1.00 (1.00, 1.00)	0.74	Endometriosis	MR-Egger	95	22.00 (1.60e-6, 3.02e+8)	0.71	
	Weighted median	319	1.00 (1.00, 1.00)	0.001		Weighted median	95	0.96 (9.23e-6, 1.01e+05)	0.10	
	IVW	319	1.00 (1.00, 1.00)	3.94e-4		IVW	95	5.45 (0.002, 1.63e+04)	0.68	
	Simple mode	319	1.00 (1.00, 1.01)	0.17		Simple mode	95	0.03 (1.95e-14, 5.87e+10)	0.81	
	Weighted mode	319	1.00 (1.00, 1.01)	0.14		Weighted mode	95	38.00 (1.38e-09, 1.05e+12)	0.77	
	MR-Egger	73	2.02 (0.37,10.96)	0.42	Anorexia nervosa	MR-Egger	40	1.03 (0.99, 1.07)	0.42	
	Weighted median	73	0.88 (0.53,1.45)	0.62		Weighted median	40	1.03 (1.00, 1.05)	0.62	
	IVW	73	0.91 (0.62,1.32)	0.61		IVW	40	1.02 (0.99, 1.05)	0.61	
	Simple mode	73	0.57 (0.16,2.06)	0.39		Simple mode	40	0.90 (0.76, 1.06)	0.39	
	Weighted mode	73	0.52 (0.13,2.13)	0.37		Weighted mode	40	1.03 (1.00, 1.06)	0.37	
	MR-Egger	297	0.98 (0.88, 1.08)	0.65	BMI	MR-Egger	811	0.76 (0.54, 1.09)	0.13	
	Weighted median	297	1.01 (1.01, 1.01)	1.28e-4		Weighted median	811	0.93 (0.72, 1.19)	0.56	
	IVW	297	1.06 (1.03, 1.09)	5.21e-5		IVW	811	1.11 (0.97, 1.28)	0.14	
	Simple mode	297	1.09 (1.00, 1.19)	4.94e-2		Simple mode	811	0.94 (0.38, 2.32)	0.90	
	Weighted mode	297	1.07 (0.99, 1.15)	0.11		Weighted mode	811	0.55 (0.34, 0.88)	0.01	

results showed that sleeplessness retained a significant effect on dysmenorrhea across all adjusted models, indicating that the relationship between sleeplessness and dysmenorrhea was not influenced by these factors.

Expression quantitative trait locus analyses associated with target genes and relevant tissues

MR analysis identified seven SNPs shared between dysmenorrhea and depression in a European population: rs10913112 (*COP1*), rs12619197 (intergenic), rs3905238 (*HTT*), rs4653218 (intergenic), rs754287 (*TRMT61A*), rs9529218 (*B3GLCT*), and rs9831648 (*KLHDC8B*) (Supplementary Table S39). To determine if these identified SNPs had downstream functional impacts, eQTL data were extracted from the GTEx Portal. Five of these SNPs showed significant eQTL associations with 72 genes across relevant tissues, suggesting these genes as potential functional targets. Figure 6A visualizes these associations across various tissue types.

Among the target genes, 30 showed significant associations with the expression of nearby genes across various tissues, including the central nervous system (e.g. cortex, cerebellum), reproductive organs (e.g. ovary, uterus), and metabolic tissues (e.g. liver, pancreas), with the level of this significance being $P < .05$. For example, the rs3905238-A risk allele was associated with

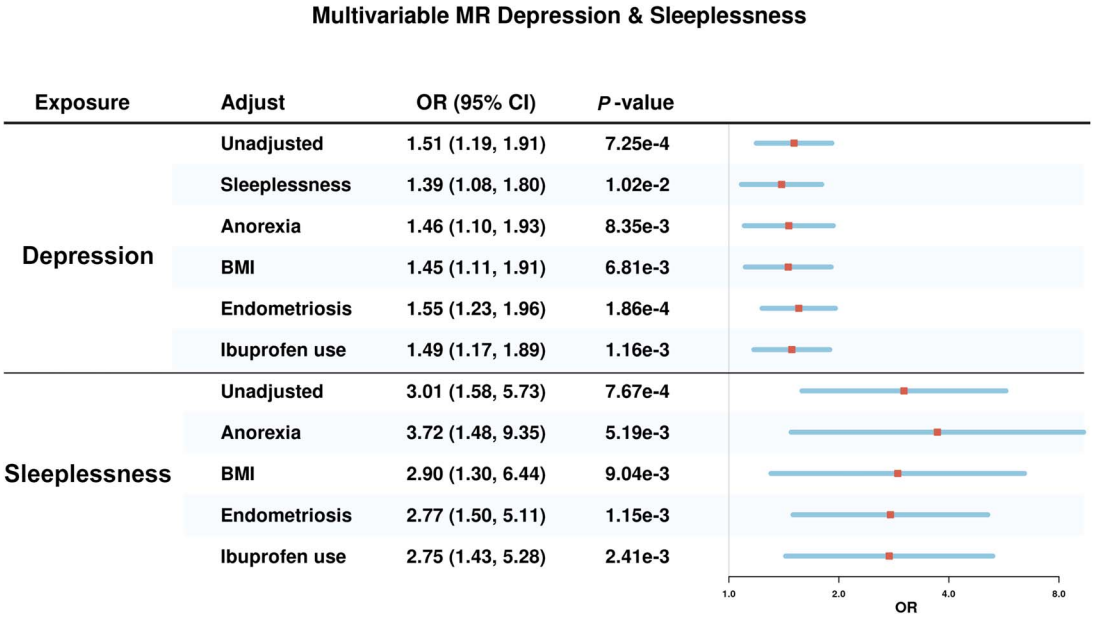


Figure 5. MVMR results for depression and sleeplessness on dysmenorrhea, adjusted for potential mediators.

Table 3. MVMR results for depression and sleeplessness on dysmenorrhea, including heterogeneity and pleiotropy tests.

Exposure		NSNPs	OR	P-value	Heterogeneity test		Pleiotropy test		
					Q statistic	P-value	Egger intercept	SE	P-value
Depression	Unadjusted	83	1.51 (1.19, 1.91)	7.26e-4	91.41	0.22	0.03	1.65e-2	0.05
	Sleeplessness	41	1.39 (1.08, 1.80)	0.01	126.76	0.46	1e-3	6e-3	0.80
	Anorexia	34	1.46 (1.10, 1.93)	8.35e-3	69.12	0.17	-5e-3	4e-3	0.23
	BMI	9	1.45 (1.11, 1.91)	6.80e-3	595.71	0.29	1e-3	2e-3	0.53
	Endometriosis	48	1.55 (1.23, 1.96)	1.86e-4	99.35	0.21	0.03	0.01	0.05
	Ibuprofen use	48	1.49 (1.17, 1.89)	1.16e-3	108.97	0.19	7e-3	0.01	0.55
Sleeplessness	Unadjusted	74	3.01 (1.58, 5.73)	7.67e-4	67.87	0.65	0.02	0.01	0.06
	Anorexia	23	3.72 (1.48, 9.35)	5.19e-3	40.75	0.39	8e-3	5e-3	0.11
	BMI	4	2.90 (1.30, 6.44)	9.04e-3	596.63	0.27	0.01	3e-3	≈0
	Endometriosis	39	2.77 (1.50, 5.11)	1.15e-3	66.77	0.74	0.02	0.01	0.07
	Ibuprofen use	38	2.75 (1.43, 5.28)	2.41e-3	82.51	0.53	0.02	0.01	0.09

decreased expression of *GRK4* in tissues such as the thyroid, skin, colon (transverse), cultured fibroblasts, aorta, visceral adipose tissue (omentum), whole blood, subcutaneous adipose tissue, lung, and adrenal gland. The rs9831648-G risk allele was linked to decreased expression of *RNF123* in subcutaneous adipose tissue and the gastroesophageal junction of the esophagus. This allele was also associated with increased expression of *NCKIPSD* in multiple tissues, including the uterus, tibial nerve, cultured fibroblasts, thyroid, tibial artery, lung, skin, sigmoid colon, esophagus, caudate (basal ganglia), cerebellar hemisphere, skeletal muscle, putamen (basal ganglia), cortex, pituitary, nucleus accumbens (basal ganglia), hippocampus, mammary tissue, hypothalamus, adrenal gland, whole blood, frontal cortex (BA9), and cerebellum. Additionally, the rs754287-T risk allele was associated with decreased expression of *MARK3* in the thyroid, skin, ovary, mammary tissue, tibial artery, and pituitary. Overall, the data demonstrate a functional impact of these loci across several related tissues.

In Fig. 6, relevant information was prepared and presented in the following manner: (A) heatmap depicting the −log10(*P*-value) of eQTL associations. The input for this heatmap consists of the target genes and nearest genes identified from the significant SNPs in the MR analysis. The output is a visual representation of the eQTL associations between these genes and their expression across different tissues, with *P*-values larger than 1×10^{-8}. Darker red indicates significant positive associations, while darker blue indicates significant negative associations. (B) PPI network graph. The target genes and nearest genes identified from the significant SNPs in the MR analysis were loaded into the STRING database for network creation, and the resultant files were imported into Cytoscape for visualization. (C) Regulatory network. The target genes and nearest genes identified from the significant SNPs in the MR analysis were used to construct a regulatory network. (D) MCL-cluster. The genes identified from the significant SNPs in the MR analysis were clustered using the MCL algorithm. (E) Identified terms. The target genes and nearest genes identified from the significant SNPs in the MR analysis were analyzed to identify associated terms. Each bubble represents the gene count, with its position along the *x*-axis reflecting the strength of the association. The color intensity of each bubble indicates the false discovery rate (−log10 FDR), with darker shades representing more significant associations.

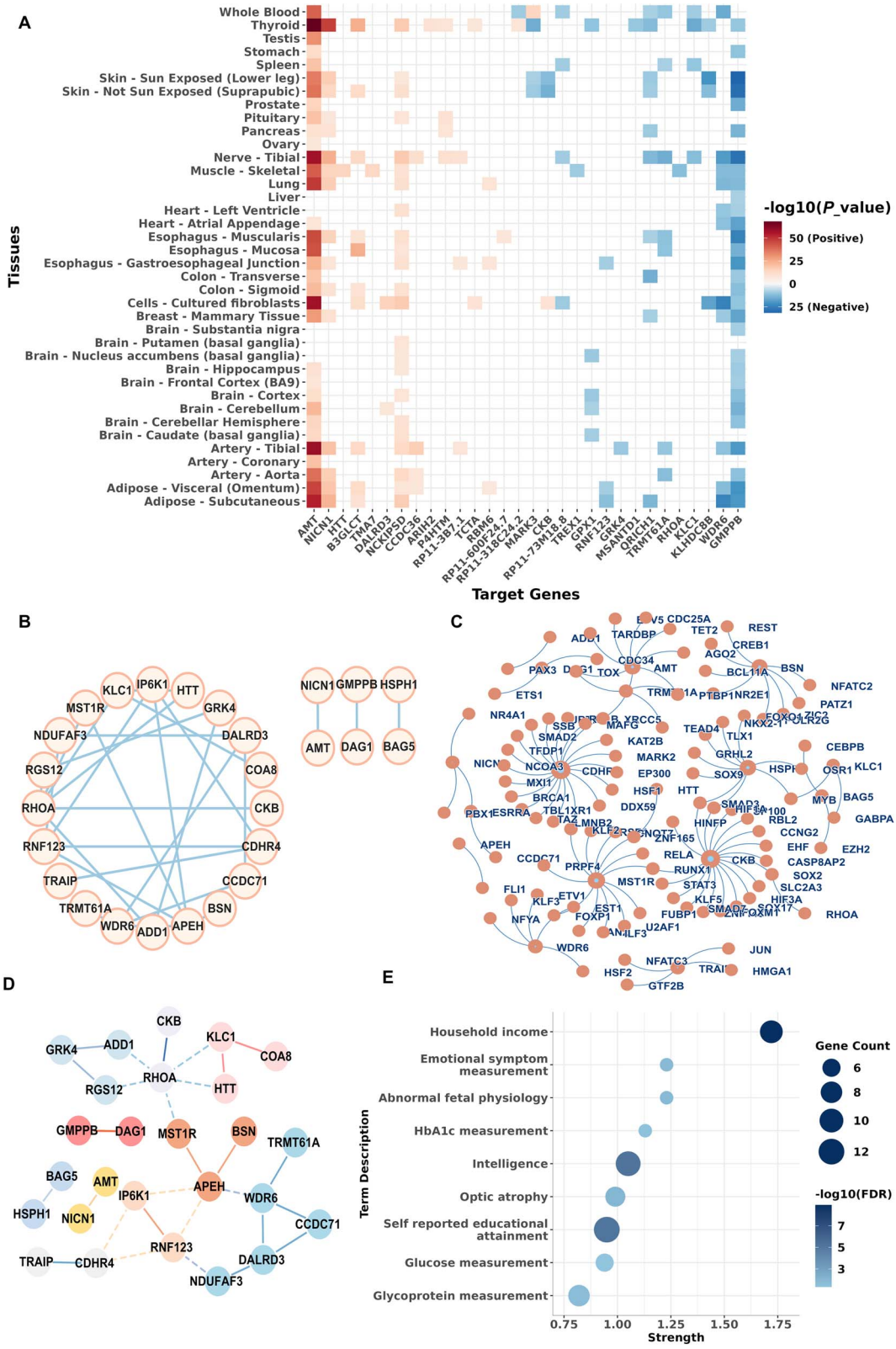


Figure 6. Shared genetic factors between dysmenorrhea and depression.

Significant associations between identified terms and protein–protein interaction network

PPI analysis was conducted using the STRING database with an interaction score set to 0.400 (Fig. 6B). Analysis revealed a highly interconnected network of proteins, indicating substantial interactions and suggesting their involvement in shared biological pathways. The network includes several clusters, demonstrating functional associations among proteins (Fig. 6D, Supplementary Table S41). Key nodes in the network, such as GRK4, TRAIP, and RNF123, link different functional groups. These proteins are

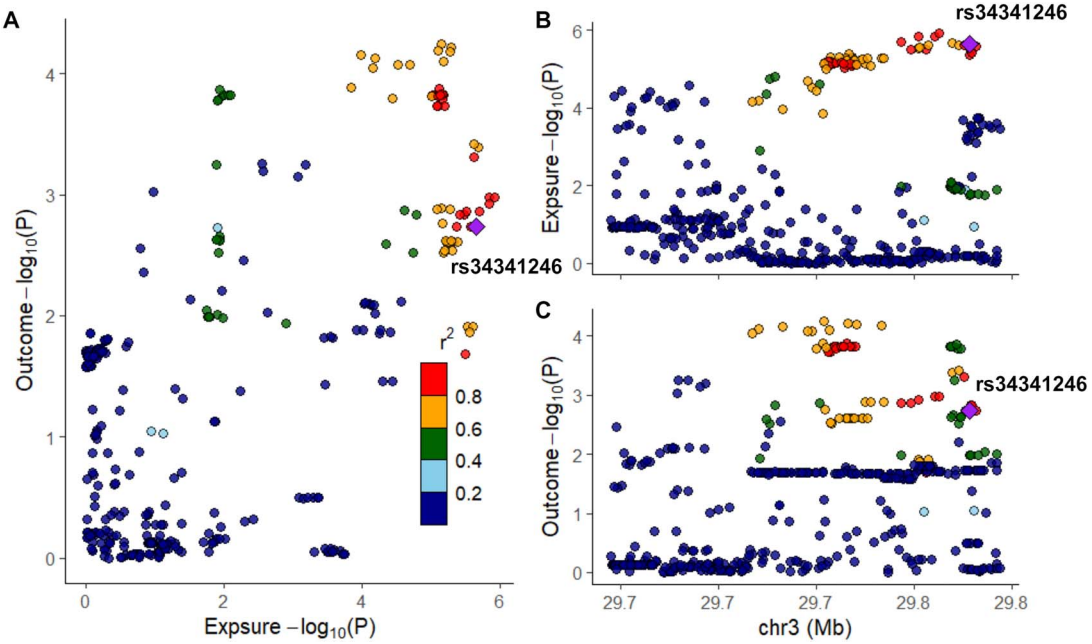


Figure 7. Colocalization plot for rs34341246, the most significant SNP linking depression (exposure) and dysmenorrhea (outcome). (A) The relationship between the exposure and outcome for each SNP. (B) Association between depression and rs34341246 across chromosome 3. (C) Association between dysmenorrhea and rs34341246 across chromosome 3. The color scale represents linkage disequilibrium (r^2) values. Higher r^2 values mean the SNPs are more strongly correlated and likely to be inherited together.

involved in a wide range of biological processes, including signal transduction, metabolic processes, structural organization, and transcriptional regulation. They play critical roles in pathways associated with neurodegenerative diseases, metabolic disorders, and cell growth regulation (Supplementary Table S41).

In Fig. 6E, the identified terms represent a diverse range of phenotypes and measurements as derived from STRING's functional enrichment analysis. The bubble chart visualizes the strength and significance of associations between these terms and the gene set, with each bubble representing a specific term. The strength of associations and low FDRs across these phenotypes indicate statistically significant relationships. These terms focus on medical conditions, biomarkers, metabolic markers, cognitive traits, and socioeconomic factors, with household income showing particularly strong significance (Supplementary Table S40).

Analysis of the regulatory network of key genes

The transcriptional regulatory network includes several key transcription factors and regulatory proteins, such as SMAD2, SMAD3, RUNX1, FOXO1, and STAT3. Each of these factors plays a significant role in regulating various cellular processes, highlighting their pivotal roles in transcriptional regulation within the network (Fig. 6C, Supplementary Table S42).

Colocalization analysis identifies a potential shared causal variant between depression and dysmenorrhea

In the colocalization analysis, rs34341246 (*RBMS3*) was identified as the most significant variant linked to the causal relationship between depression and dysmenorrhea, with a 74% probability of sharing a causal variant (PPH4 = 0.74). The result indicates that rs34341246 contributes to the shared genetic basis of depression and dysmenorrhea (Fig. 7).

Discussion

This study utilizes large-scale GWAS data from the UK Biobank, Psychiatric Genomics Consortium (PGC), and Finngen to perform comprehensive MR analyses by thoroughly investigating the potential causal relationship between depression and dysmenorrhea. The findings provide preliminary evidence to suggest that depression may act as a causal factor rather than a consequence of dysmenorrhea. This conclusion is reinforced by the analyses of data from both the European and East Asian populations, which revealed a consistent causal direction.

These findings align well with previous MR studies, which examined the causal relationship between gynecological diseases and depression [35, 37] [49]. Wang *et al.* used MR to demonstrate a causal relationship between female reproductive factors and the incidence of MDD throughout life, further supporting our findings [35]. Additionally, Ling *et al.* provided compelling evidence that women with depression or dysthymia have a significantly higher risk of developing conditions such as polycystic ovary syndrome, ovarian cysts, abnormal uterine bleeding, and endometriosis [37]. Furthermore, Zhang *et al.*'s MR analysis presented genetic data indicating that depression and neuroticism are risk factors for endometriosis, but no evidence was found for a causal connection between anxiety and the development of endometriosis [49].

Dysmenorrhea is often a symptom of these gynecological conditions [71]. The association between these gynecological diseases and depression further supports the findings of our MR study, highlighting the interconnectedness of reproductive health and mental health. However, these studies focused on diseases rather than symptoms. Our study is the first of its kind to use a two-sample MR analysis to investigate the causal relationship between dysmenorrhea and MDD.

In a systematic review and meta-analysis, Zhao *et al.* found a positive relationship between primary dysmenorrhea and depression, suggesting a potential link between mental health and menstrual pain [72]. Similarly, Adib-Rad *et al.* reported in a

cross-sectional study that dysmenorrhea is associated with psychological distress, further supporting our conclusions [28]. However, these studies did not provide evidence of causality or genetic mechanisms.

In our study, the European population showed a significant causal effect of depression on dysmenorrhea using the IVW and WM methods, while the MR-Egger method did not show significant results, suggesting potential pleiotropy. The MR-Egger intercept and absence of significant heterogeneity reinforce our causal inference. No evidence was found for reverse causality in the European population. Furthermore, by using both the $P < 5 \times 10^{-6}$ and $P < 5 \times 10^{-7}$ thresholds in the forward and reverse analysis, we strengthened our conclusions, showing consistency in our findings across these thresholds.

In the Asian population, the MR-Egger method suggested a significant causal effect of depression on dysmenorrhea, while the IVW and WM methods did not, likely due to population-specific factors or limitations in genetic instruments [73]. Significant heterogeneity observed in the IVW method underscores the need for further research among ethnically diverse populations [74]. When combining findings from both populations, there was no evidence to support reverse causality, strengthening the conclusion that dysmenorrhea is unlikely to cause depression.

Our analysis revealed evidence for a causal effect of depression on sleeplessness, consistent with clinical observations and MR findings that genetic predisposition to depression increases the risk of sleep disturbances [75–77]. The consistency across IVW and WM methods, and the absence of significant pleiotropy, support this causal relationship despite MR-Egger's sensitivity to pleiotropic effects. Significant heterogeneity suggests that the genetic instruments used may have varying effects on sleeplessness due to several factors, such as independent pathways influencing sleeplessness or gene–environment interactions [78]. Our subsequent mediation analysis utilized the MVMR method, and the results showed no evidence of horizontal pleiotropy or heterogeneity. This further supports the robustness of our findings, indicating that the genetic instruments used in the analysis specifically target the pathways involved in sleeplessness without being influenced by pleiotropic effects or heterogeneity.

The relationship between genetics, environment, and complex diseases such as depression and dysmenorrhea is multifaceted and warrants careful consideration [79]. The IVW method also showed a significant causal effect of sleeplessness on dysmenorrhea, suggesting that sleep disturbances may exacerbate dysmenorrhea. Addressing sleep issues could be crucial in managing both conditions. Mediation analysis by Li *et al.* supports our results, showing an association between dysmenorrhea and depression, with sleep disturbances as a mediating factor [24]. This finding is consistent with our two-step MR mediation analysis.

The risk factor analyses suggest that conditions such as anorexia [80], BMI [81], endometriosis [82], and ibuprofen use [83] may influence the susceptibility to dysmenorrhea associated with depression. However, our two-step mediation analysis indicates that the exact mechanisms and inferences remain uncertain. These conditions may share common biological pathways affecting the risk of both depression and reproductive health issues. Further research is needed to clarify the underlying biological mechanisms and confirm causality.

Various mechanisms have been suggested to explain the impact of depression on female reproductive health including through the hypothalamic–pituitary–adrenal (HPA) axis and the hypothalamic–pituitary–ovarian axis. The corticotropin-releasing hormone (CRH), produced by the hypothalamus, plays a crucial role in reproductive functions such as follicular development, ovulation, and luteolysis within the ovarian CRH system [84].

In this study, analysis of loci, target genes, and tissues indicates the involvement of the thyroid, nervous system, brain (particularly the hypothalamus and pituitary), adrenal glands, and ovaries. Protein-related analyses identified key proteins that act as crucial nodes connecting different functional groups in the network, including GRK4, TRAIP, and RNF123. GWAS has linked the RNF123 locus to chronic widespread musculoskeletal pain, implicating RNF123 in chronic pain pathways [85]. There is also evidence supporting the involvement of GRK4 in chronic pain mechanisms [84].

Moreover, TRAIP has been linked to substance abuse, antisocial behavior, and attention-deficit hyperactivity disorder [86]. The phenotypes identified, including household income [87], intelligence [35], self-reported educational attainment [88], optic atrophy [89], emotional symptom measurement [90], abnormal fetal physiology [91], glycoprotein measurement [92], HbA1c measurement [93], and glucose measurement [94] are connected to certain biological processes or conditions through the PPI network. Studies have shown that newborns of depressed mothers exhibit biochemical and physiological profiles that mimic their mothers' prenatal status [95]. However, no significant differences in optic atrophy were observed between patients with MDD and healthy controls [89].

Further research suggests that changes in serum glycoprotein levels before and after treatment with fluoxetine may correlate with the clinical status of MDD [96]. SLIT, a secretory glycoprotein, and its derivatives have shown increased immunoreactivity in ectopic endometrium, correlating positively with the severity of dysmenorrhea. This finding indicates that SLIT may serve as a significant predictor of dysmenorrhea severity in women with adenomyosis [97].

In our PPI network analysis, we identified terms related to HbA1c, which may further elucidate the genetic factors involved in the causal relationship between depressive symptoms and dysmenorrhea. Depressive symptoms are prevalent among young adults with early-onset type 1 diabetes, with HbA1c levels showing different associations with depressive symptoms in men and women [93]. Studies of women with dysmenorrhea have observed increased regional glucose metabolism in the thalamic, orbitofrontal, and prefrontal areas during menstrual pain, in contrast to decreased metabolism in the lateral somatic sensorimotor regions during the pain-free phase of their menstrual cycle. These findings suggest that glucose metabolism changes may play a role in the experience of menstrual pain and its association with depressive symptoms [94].

Among other identified proteins, HSPH1 (Heat Shock Protein H1) has been suggested as a marker for depression. Sun *et al.* developed an electrochemical immunosensor designed for the early screening of depression markers, focusing particularly on heat shock protein 70 (HSP70) [98]. Depression is a prevalent neuropsychiatric symptom of Huntington's disease (HD) and can present decades before the onset of motor symptoms. HD is caused by the expansion of cytosine-adenine-guanine (CAG) trinucleotide repeats in exon 1 of the *HTT* (Huntingtin gene), resulting in a mutant HTT (Huntingtin protein) [99]. Additionally, overactivity of the HPA axis has also been observed in HD, further linking these pathways to depression [99].

The transcriptional regulatory network includes several key transcription factors and regulatory proteins, such as SMAD2, SMAD3, RUNX1, FOXO1, and STAT3. These proteins demonstrate high connectivity, underscoring their pivotal role in

transcriptional regulation within the network. Specifically, FoxO, SMA and MAD-related (SMAD) TFs have been implicated in modulating muscle growth, potentially enhancing the atrophic response through the activation of myostatin expression [100]. Previous investigations have highlighted the significance of RUNX1 in ovarian cancer, where it exhibits markedly elevated expression levels and governs various tumor cell functions. These insights underscore the critical involvement of RUNX1 in the regulatory mechanisms underlying ovarian cancer pathogenesis [101]. While previous MR analyses have not established a significant causal link between depression and ovarian cancer [37], observational studies indicate a higher prevalence of depression among ovarian cancer patients [102].

These observations raise intriguing questions regarding the interplay between depression and female reproductive health. Discrepancies between MR analyses and observational studies, along with controversies within the latter, may arise from inherent confounding factors and biases. MR emerges as a powerful tool for delineating causal relationships due to its reduced susceptibility to confounding influences [103]. Collectively, these findings point toward complex interactions between depression and female reproductive health with multiple genetic and molecular pathways contributing to the shared pathophysiology.

Our colocalization analysis identified an SNP and a gene distinctly associated with the causal relationship between these conditions. The most significant SNP, rs34341246, was identified in the causal analysis of both conditions. This SNP is located within the *RBMS3* gene (RNA Binding Motif Single-Stranded Interacting Protein 3). This gene has been reported to be associated with various traits, including vaginal microbiome composition [104], cleft lip with or without cleft palate (related to maternal periconceptional alcohol intake interaction) [105], breast size [106], and preeclampsia in nulliparas [107]. These results are useful in strengthening the conclusions of this study by revealing the shared genetic contributions to both depression and female reproductive health.

This study showcases several strengths:

1. It utilizes data from both European and East Asian populations, which enhances the generalizability of our findings.

2. The adoption of a two-sample bidirectional MR with nonoverlapping datasets minimizes the influence of confounding variables, strengthening the validity of our results.

3. Implementing a two-step MR analysis enables the exploration of intermediary factors, such as sleeplessness, in the causal pathway from depression to dysmenorrhea.

4. It incorporates MVMR to help address the limitations of horizontal pleiotropy and heterogeneity that can obscure causal relationships. MVMR simultaneously investigates multiple mediators and confounders, clarifying their roles in causal pathways and exploring the moderating effects of other variables. This approach provides a more comprehensive understanding of the genetic and molecular mechanisms involved. By elucidating potential mediating mechanisms, we gain new insights into the complex interplay between these conditions, paving the way for targeted interventions and personalized treatment strategies.

However, it is important to recognize the inherent limitations of our study that necessitate a cautious interpretation of its outcomes. First, the mediation analysis was constrained by the lack of individual-level data, relying instead on summary-level GWAS data. This approach limited the calculation of the proportion of mediation effects, which could have provided a more nuanced understanding of the pathways involved. Furthermore, the reliance on summary statistics rather than raw data restricted our ability to conduct subgroup analyses, potentially obscuring important variations within the population.

Second, while examining the East Asian population is valuable, the heterogeneity in the phenotype definition and the limited power of the GWAS data necessitate a cautious interpretation of these findings. This limitation highlights the need for further research with more robust and homogenous datasets to validate our findings in this population.

Third, we employed a relaxed *P* threshold ($P < 5 \times 10^{-5}$) for the instrument-exposure association to include more SNPs, particularly for traits with limited availability. While this approach expanded the inclusion of genetic instruments, it may have also introduced additional noise or potential false positives into the analysis [73]. Using a small number of SNPs suggests that further studies with a large number of genetic instruments is warranted, when available. In the European population, we also applied thresholds of $P < 5 \times 10^{-6}$ and $P < 5 \times 10^{-7}$ to support our conclusions. However, the implementation of the $P < 5 \times 10^{-7}$ threshold resulted in too few SNPs for reverse causality analysis, leaving us with only IVW-based MR results and a Q-test, but lacking more robust sensitivity tests such as leave one out and MR-Egger. This limitation suggests that future studies with larger genetic datasets may be necessary to validate the robustness of the reverse causality of our findings.

In addition, we examined sleeplessness as a mediator. In the two-step MR analysis, sleeplessness significantly influenced dysmenorrhea (OR = 3.01, 95% CI [1.58, 5.73], $P = 7.67 \times 10^{-4}$). In the MVMR analysis, adjusting for sleeplessness attenuated the effect of depression on dysmenorrhea, though it remained significant (OR = 1.39, 95% CI [1.08, 1.80], $P = 1.02 \times 10^{-2}$). This suggests sleeplessness not only may be a primary mediator but also reveals the complexity of potential multivariable mediation. Hence, it emphasizes the need to conduct comprehensive multifactor analyses in diverse populations to better understand mediation mechanisms.

Subsequently, the MVMR results showed that the causal effect of sleeplessness on dysmenorrhea remained significant after adjusting for other mediators. This indicates that SNPs primarily influence dysmenorrhea through sleeplessness rather than anorexia (OR = 3.72, 95% CI [1.48, 9.35], $P = 5.19 \times 10^{-3}$), BMI (OR = 2.90, 95% CI [1.30, 6.44], $P = 9.04 \times 10^{-3}$), or endometriosis (OR = 2.77, 95% CI [1.50, 5.11], $P = 1.15 \times 10^{-3}$). The analysis confirmed that sleeplessness independently mediates the effect on dysmenorrhea, demonstrating its key role in the multivariable model. Future studies should conduct subgroup analyses to explore how these mediating effects vary across different populations.

Fourth, while the SNPs included in our study were adequate for identifying causal relationships, they were not sufficient for conducting comprehensive enrichment analyses. This limitation primarily stems from the scope of the SNP dataset, which focused on identifying target genes based solely on proximity to these SNPs. This approach inherently limits the diversity of genes analyzed and impacts downstream bioinformatic analyses. Given the inherent limitations of the PGC, Finngen, and UK Biobank datasets, it is crucial for future studies to validate causal associations and explore underlying mechanisms using individual patient data. Such investigations are necessary for generating meaningful clinical recommendations that accurately guide medical practice, contribute to precision medicine, and optimize treatment outcomes for patients.

Finally, despite its limitations our study provides valuable insights into the complex genetic relationship between depression

and dysmenorrhea. It highlights the need to conduct future research studies with more robust datasets, homogenous phenotypic definitions, and advanced analytical methods to further validate these findings and uncover the details of the underlying biological mechanisms. Such efforts will be critical in helping translate genetic research into meaningful clinical applications and improve patient outcomes.

Conclusions

This innovative study used extensive genetic data to investigate the relationship between depression and dysmenorrhea, providing evidence for a causal link with depression as a potential causal factor. Sleeplessness was also shown as a mediator in this relationship. Shared genetic factors, including *GRK4, TRAIP,* and *RNF123,* as well as transcription factors like SMAD2, SMAD3, RUNX1, FOXO1, and STAT3, and significant colocalization signals for rs34341246 on *RBMS3,* suggest common biological pathways. These findings highlight the importance of mental health in managing female reproductive symptoms and lay the foundation for understanding the genetic and molecular interplay between these conditions. Further validation by conducting larger studies and biological experiments is needed to fully elucidate this causal association, paving the way for improved prevention and new treatment strategies.

Key Points

- We provide evidence that depression may act as a causal factor, rather than a consequence, of dysmenorrhea.
- We identify sleeplessness as a mediator between depression and dysmenorrhea.
- We identify related genes (*GRK4*, *TRAIP*, and *RNF123*) and transcription factors (SMAD2, SMAD3, RUNX1, FOXO1, and STAT3), significant colocalization signals rs34341246 on *RBMS3* that link the biological and genetic aspects of depression and dysmenorrhea.

Supplementary data

Supplementary data are available at *Briefings in Bioinformatics* online.

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Author contributions

Shuhe Liu (Writing—original draft, Formal analysis), Daniel F. Carr (Review & Editing), Zhen Wei (Project administration, Funding acquisition, Conceptualization, Supervision, Review & Editing), and John Moraros (Project administration, Funding acquisition, Conceptualization, Supervision, Review & Editing). All authors read and approved the final manuscript.

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Data availability

The data underlying this article are available in the article and in its online supplementary materials.

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