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Association Between Gallstones and Depressive Symptoms: Results from NHANES and Mendelian Randomization Study

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Purpose: Prior research has suggested a correlation between gallstones and depressive symptoms, yet the specifics of this relationship remain unclear. This study aims to explore the association between gallstones and depressive symptoms among adults.

Patients and Methods: Initially, we conducted a cross-sectional study using data from the National Health and Nutrition Examination Survey (NHANES) 2017 – March 2020. After propensity score matching (PSM) for participants with gallstones and those without gallstones, multivariate logistic regression analysis was used to explore the potential association between gallstones and depressive symptoms. This was followed by Mendelian randomization (MR) analysis to further elucidate the causal relationship between them. Using the genome-wide association study database, we extracted instrumental variables and performed bidirectional univariate and multivariate MR analyses.

Results: In the cross-sectional study of NHANES 2017 – March 2020, 835 pairs of participants with comparable characteristics, both with and without gallstones, were identified after PSM. The multivariate adjusted logistic regression analyses revealed a significant association between gallstones and depressive symptoms [fully adjusted model: OR=1.821 (95% CI, 1.181–2.808), *P*=0.007]. Subsequent MR analyses further clarified the causal relationship, indicating that genetically determined gallstones significantly increase the risk of developing depressive symptoms [forward univariate MR analysis: OR=1.04 (95% CI, 1.01–1.06), *P*=0.002; multivariate MR analysis: OR=1.03 (95% CI, 1.01–1.05), *P*=0.009], with no evidence of reverse causation [inverse univariate MR analysis: OR=1.28 (95% CI, 0.90–1.83), *P*=0.17].

Conclusion: Gallstones are a risk factor for depressive symptoms among adults. Hence, we recommend timely depression screening for patients diagnosed with gallstones, facilitating early detection and effective treatment of depressive symptoms, thus alleviating its impact on both individuals and society.

Keywords: depressive symptoms, gallstones, risk factor, causality, genetic analysis, cross-sectional study

Introduction

Depressive disorder is a mental disorder characterized by persistent sadness or an inability to experience pleasure, accompanied by deficits in daily functioning.[1] It is increasingly common in clinical settings. In the United States (US), the prevalence of depressive disorder ranges from 5% to 10%, and in certain primary care or specialty settings, it can reach as high as 40% to 50%.[2] Meanwhile, depressive symptoms are also prevalent among Chinese primary care patients. Research has found that 30.6% of elderly primary care patients in China exhibit depressive symptoms.[3] Depressive disorder not only limits the psychosocial functioning of sufferers, significantly reducing their quality of life, but also imposes a severe socioeconomic burden. It is projected that by 2030, the global economic cost associated with depressive disorder will nearly double.[4] Alongside the high prevalence and severe burden of depressive disorder, there is a low recognition rate. Studies have shown that among elderly individuals in China receiving primary care, the recognition rate

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for depressive disorder is only 1.3%.[5] In healthcare, particularly within primary care settings, there is an urgent need for timely screening, detection, and treatment of depressive symptoms to promote mental health. Thus, identifying risk factors for depressive symptoms is crucial for effective screening and prevention. Studies have found that people with depressive disorder have a significantly higher incidence of digestive diseases compared to the general population. Chronic liver disease, functional dyspepsia, and inflammatory bowel disease play a crucial role in the onset and progression of depressive disorder.[6,7] Research has also suggested a bidirectional relationship between peptic ulcers, gastroesophageal reflux disease, and depressive disorder.[8,9] Gallstones, primarily cholesterol or mixed stones, are among the most common digestive system diseases. Statistics show that up to 20% of adults have gallstones, yet only 20% of these individuals exhibit symptoms or complications.[10] As a significant digestive disease, the causal relationship between gallstones and depressive symptoms remains unclear. Some studies have suggested that gallstones may influence personality formation and the occurrence of depressive symptoms,[11] while others propose that depressive disorder could increase the risk of developing gallstones.[12] A cohort study[13] from the United Kingdom discovered that depressive disorder may be a risk factor for gallstones in the population, while a cross-sectional study[14] from Spain also indicated that the incidence of gallstones is significantly higher among patients with depressive disorder over the age of 60. However, a German study[15] revealed that patients with gallstones have a higher risk of developing depressive disorder, and a cross-sectional study[16] from China showed that individuals with gallstones exhibit poorer mental health. Overall, the causal relationship between gallstones and depressive symptoms remains highly contentious. Furthermore, current research is mainly observational, often limited by small sample sizes and the inevitable presence of confounding factors and reverse causality.

The National Health and Nutrition Examination Survey (NHANES) is a research program designed and managed by the National Center for Health Statistics in the US, aimed at assessing the health and nutritional status of American adults and children. This project annually surveys a nationally representative sample of approximately 5,000 individuals from across the US, covering medical health status, physical measurements, laboratory test results, diet, demographic characteristics, and socioeconomic status.[17] NHANES data, released biennially since 1999, is used for epidemiological investigation, medical science research, and to guide public health policies.[18] Mendelian randomization (MR), a statistical analysis method proposed by Katan in 1986, uses genotypes as tools to explore the causal relationships between phenotypes and diseases.[19] Based on Mendel's law of random allele distribution to offspring and employing single nucleotide polymorphisms (SNPs) as instrumental variables (IVs), this method seeks to uncover the true causal relationships between exposures and outcomes, minimizing the impact of reverse causality and confounding factors.[20]

In this study, we will employ a combined NHANES and MR approach to investigate the association between gallstones and depressive symptoms. We will first analyze the large-scale survey data from NHANES 2017-March 2020 to examine the association between gallstones and depressive symptoms, followed by using the MR study to analyze their causal relationship, thereby clarifying the connection between the two.

Materials and Methods

Study Populations and Variables from NHANES

This study analyzes data from the NHANES spanning from 2017 to March 2020, involving 15,560 participants who provided informed consent prior to participation. The survey protocol and content received approval from the Research Ethics Review Board of the National Center for Health Statistics, and secondary analysis of publicly available data did not require specific informed consent.

In this study, the presence of gallstones was self-reported by participants. A positive gallstone status was indicated if the participant answered "Yes" to the question, "Has a doctor ever said you have gallstones?" Conversely, a "No" response indicated the absence of gallstones. The Patient Health Questionnaire-9 (PHQ-9), an effective depressive symptoms screening tool comprising nine items (Supplementary Table 1), was employed to assess participants' depressive status.[21] Each item was scored on a four-point scale ranging from 0 ("Not at all") to 3 ("Nearly every day"), with a total score of 27. A PHQ-9 score of 10 or higher indicates the presence of clinically significant

depressive symptoms, as it is considered to have high sensitivity and specificity.[22,23] Participants' PHQ-9 scores are available from the "Mental Health – Depression Screener" section of the NHANES 2017 – March 2020.

To minimize confounding factors and enhance precision, the study incorporated relevant covariates from the NHANES database, including gender, age, race/ethnicity, education level, marital status, poverty income ratio (PIR), smoking status, alcohol consumption, Body Mass Index (BMI), physical activity status, and the presence of hypertension and diabetes. The study categorized age into three groups: 18–39, 40–59, and 60 years old or older. Race/ethnicity was classified into five categories: Mexican American, other Hispanic, non-Hispanic White, non-Hispanic Black, and other race. Educational levels were divided into more than high school education, high school education, and less than high school education. Marital status was categorized into married/living with partner, widowed/divorced/separated, and never married. Economic status, reflected by the PIR (ratio of family income to poverty threshold), was classified into low income (PIR $\leq$ 1), middle income (1 < PIR < 4), and high income (PIR $\geq$ 4).[17] Smoking status was indicated by serum cotinine levels, categorized into low (<0.015 ng/mL), medium (0.015–3 ng/mL), and high (>3 ng/mL).[24] Alcohol consumption was classified based on survey responses into none, moderate (1–2 drinks/day for men or 1 drink/day for women), heavy (3–4 drinks/day for men or 2–3 drinks/day for women), and binge (≥5 drinks/day for men or ≥4 drinks/day for women).[25] BMI was categorized into normal or below (BMI < 25 kg/m^2), overweight (25 kg/m^2 $\leq$ BMI < 30 kg/m^2), and obese (BMI $\geq$ 30 kg/m^2). Physical activity guidelines for Americans suggest adults engage in either 75 minutes of vigorous physical activity or 150 minutes of moderate intensity physical activity per week.[26] Participants meeting this standard were considered active, while others were classified as less active. Hypertension was defined as having an average systolic blood pressure $\geq$ 130mmHg or diastolic blood pressure $\geq$ 80mmHg.[27] Diabetes was identified with a glycated hemoglobin (HbA1C) level $\geq$ 6.5%.[28]

Study Design for Cross-Sectional Investigation Using NHANES Data

The study design involved a cross-sectional survey using NHANES data from 2017 – March 2020, with 15,560 participants' health survey data included (Figure 1). After excluding participants under 18 years old (n=5867), pregnant (n=87), missing gallstone records (n=482), missing PHQ-9 scores (n=1327), and missing information on education and marital status (n=10), 7787 participants were included in the analysis. To minimize confounding factors, the study used propensity score matching (PSM) to pair participants with and without gallstones, matching factors including gender, age, race, education level, marital status, PIR, serum cotinine levels, alcohol use, BMI, physical activity, hypertension, and diabetes status, with a match tolerance of 0.02. Multivariate logistic regression analysis was conducted to evaluate the association between gallstones and depressive symptoms, adjusting for covariates such as gender, age, race, education level, marital status, PIR, serum cotinine levels, alcohol use, BMI, physical activity, hypertension, and diabetes. Three models were used: an unadjusted model, a minimally adjusted model (adjusting for gender, age, race), and a fully adjusted model (including all covariates).To minimize the interference of confounding factors and refine the outcomes of the study, and to explore the association between gallstone disease and depressive symptoms in various demographics, a multivariate logistic regression analysis was conducted, stratifying the model by gender, age, educational level, marital status, serum cotinine levels, hypertension, and diabetes.

Statistical Analysis of NHANES Cross-Sectional Study

Statistical analyses were performed in accordance with NHANES analytical and reporting standards. Considering the clustered design and differential sampling probabilities, NHANES-provided sample weights were utilized. Categorical variables were described using weighted percentages (95% confidence interval, 95% CI), and compared using the Chi-square test. Continuous variables were represented by mean ± standard deviation, and inter-group comparisons were conducted by independent sample *T*-test or Mann–Whitney test. Multi-factor adjusted logistic regression analysis was performed on the data of the two groups of patients matched by the PSM method to assess the association between gallstones and depressive symptoms.

All statistical analyses were conducted using R programming language (R version 4.1.2), SPSS 26.0, and Stata 17.0. Two-sided levels of significance were calculated, and *P* values less than 0.05 were considered statistically significant.

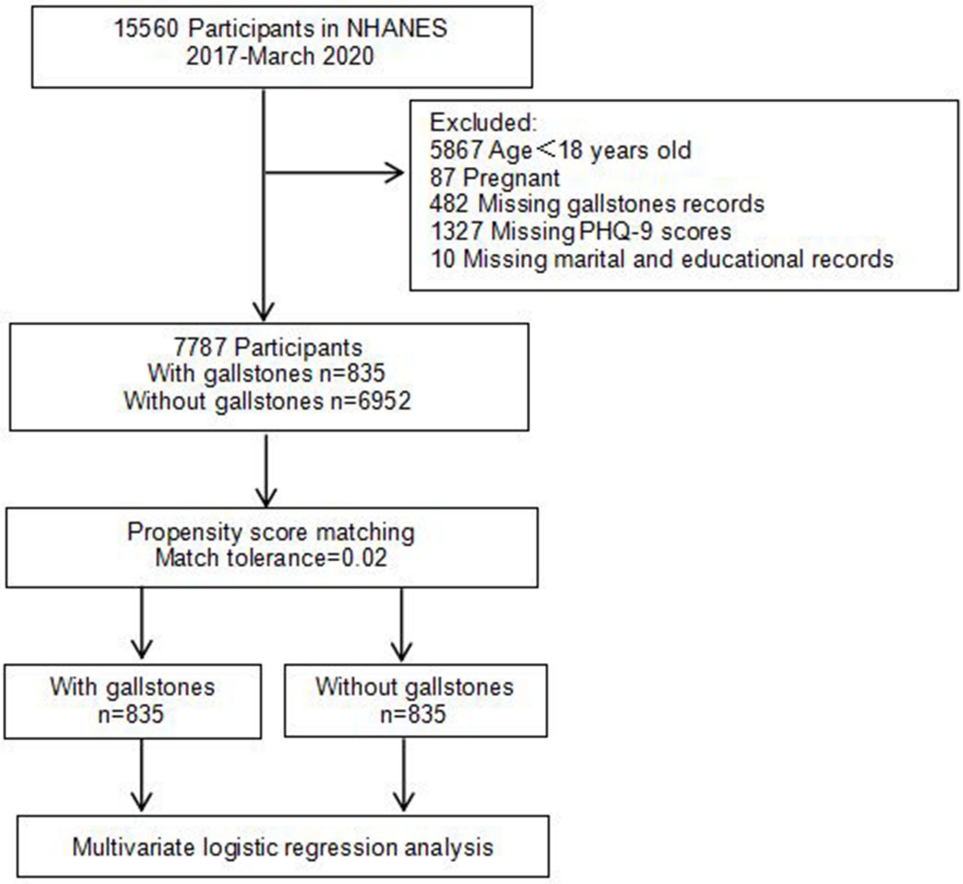

Figure 1 Flowchart for selecting participants in the NHANES study.
Abbreviation: NHANES, National Health and Nutrition Examination Survey.

Data Acquisition for Exposure and Outcome in MR Study

Based on MR principles, the extracted IVs were required to adhere to three criteria:[20] (1) strong correlation with exposure and independence ($P < 5*10^{-8}$ to $1*10^{-5}$); (2) no correlation with confounding factors; (3) no direct correlation with or influence on the outcome. According to the above three principles, we carried out the extraction of IVs. SNPs associated with gallstones were extracted from the European Bioinformatics Institute (EBI) database. The gallstones database included 487,553 European samples and 24,173,391 SNPs, from which strongly associated SNPs ($P < 5*10^{-8}$) were selected and subjected to clumping with a linkage disequilibrium (LD) $r^2 < 0.001$. To further eliminate confounding factors, SNPs directly related to depressive symptoms were identified using PhenoScanner and excluded. The SNP's F-statistic was calculated as $F = \beta^2 / se^2$ [β, effect size (exposure); se, standard error (exposure)], with an F-statistic > 10 indicating strong association with exposure and suitability for MR analysis and sensitivity testing. SNPs with F-statistic < 10 were excluded.[29] Similarly, depressive symptoms-related SNPs were extracted from the Integrative Epidemiology Unit (IEU) database, a multidisciplinary research institute affiliated with the University of Bristol, integrating epidemiology, genetics, and related fields. The IEU database includes genome-wide association study (GWAS) data from various research fields, with the depression-related dataset comprising 47,517 samples and 8,281,749 SNPs. Given previous reports suggesting diabetes and obesity as potential risk factors for depressive symptoms,[1] this study included SNPs related to these conditions for multivariate MR analysis. SNPs related to diabetes and obesity were extracted from the FinnGen Biobank database, which contains genome information and healthcare data of 500,000 participants over 18 years of age living in Finland, established by the University of Helsinki with the primary aim of enhancing the understanding of disease etiology and promoting the development of diagnostic, preventive, and therapeutic methods. The diabetes-related GWAS dataset consisted of 186,765 samples and 16,380,339 SNPs, while the obesity-related dataset

comprised 218,735 samples and 16,380,465 SNPs. Details of these datasets are summarized in the Supplementary Table 2. All GWAS datasets from the EBI, the IEU, and FinnGen Biobank have been publicly published and received appropriate ethical approval, thus no additional ethical approval was required for use in this study.

MR Statistical Analysis

This study employed a two-sample MR approach, including bidirectional univariate and multivariate analyses, to investigate the causal association between gallstones and depressive symptoms (Figure 2A and B).

Bidirectional Univariate MR Analysis

For the univariate MR analysis, in the absence of heterogeneity and pleiotropy among the IVs related to exposure and outcome, the multiplicative random effects-inverse variance weighted (MRE-IVW) method was the primary analytical technique for assessing the causal relationship between exposure and outcome, with P value < 0.05 indicating a significant causal association. To ensure the reliability of the MR analysis results, further sensitivity analyses were conducted, including tests for heterogeneity and pleiotropy. Heterogeneity was assessed using Cochran's Q statistic, and a Q value < 0.05 indicated the presence of heterogeneity.[30] In the presence of heterogeneity, the MR-PRESSO method is utilized to identify and exclude IVs causing heterogeneity, and MR analysis is subsequently reconducted after eliminating heterogeneity.[31] The intercept of the MR-Egger regression line serves as an indicator for assessing horizontal pleiotropy; a greater deviation from zero indicates a higher likelihood of horizontal pleiotropy.[31] A P value greater than 0.05 is considered indicative of the absence of horizontal pleiotropy, suggesting reliability in the MR analysis results.[31] In the leave-one-out test, each IV is sequentially excluded, and the MRE-IVW method is applied to determine if the removal of the SNP significantly alters the MR results. Consistency in the distribution of the "ALL" line with the MR estimate results indicates stability and reliability in the MR findings.[32] Data visualization techniques such as scatter plots, forest plots, and funnel plots are employed to present the MR study results more intuitively and graphically.

Initially, gallstones are analyzed as the exposure and depressive symptoms as the outcome using the MRE-IVW method in a forward univariable MR analysis, accompanied by tests for heterogeneity, pleiotropy, and data visualization. Although MR analysis significantly minimizes the possibility of reverse causality, a reverse univariable MR analysis is

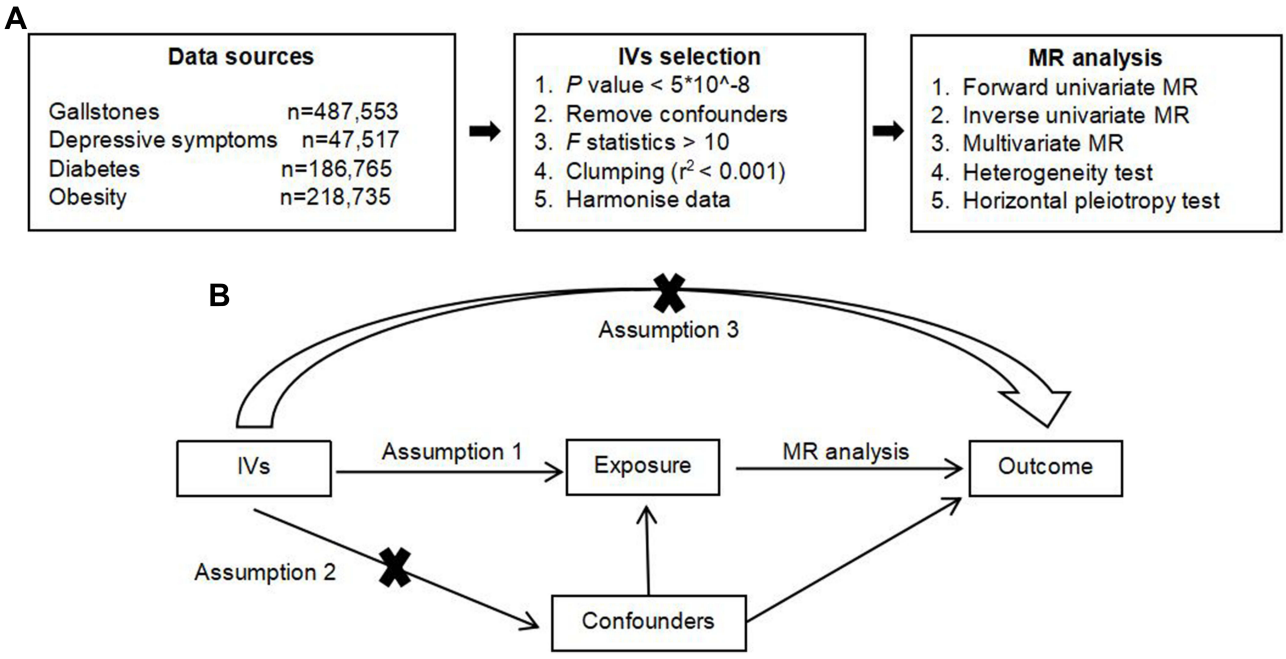

Figure 2 Flowchart of study and assumptions of Mendelian randomization. (**A**) Flowchart of study. (**B**) Assumptions of Mendelian randomization.
Abbreviations: IVs, instrumental variables; MR, Mendelian randomization.

conducted to further ascertain the potential reverse causal relationship between gallstones and depressive symptoms, with depressive symptoms as the exposure and gallstones as the outcome.

Multivariate MR Analysis

In the multivariate MR analysis, the aim is to mitigate the influence of undetected confounding factors. Given that diabetes and obesity are reported as significant risk factors for depressive symptoms,[1] these conditions are adjusted for using multivariate MR analysis methods, verifying the causal association between gallstones and depressive symptoms in the absence of interference from diabetes and obesity.

All statistical analyses in the MR study are performed using the R programming language (R version 4.1.2). The software packages employed include TwoSampleMR, MR-PRESSO, and openxlsx.

Results

Observational results Between Gallstones and Depressive Symptoms in NHANES

Overall Characteristics of the Participants in NHANES

In the NHANES 2017 – March 2020, a total of 7787 participants aged 18 and older were included, with 835 individuals diagnosed with gallstones and 6952 without (Figure 1). Table 1 presents the overall characteristics of these participants, revealing significant differences between those with and without gallstones. Compared to those without gallstones, individuals with gallstones are predominantly female, aged 60 and above, non-Hispanic White, with a status of widowed/divorced/separated, belonging to a middle-income group, and are obese. Additionally, those with gallstones have significantly lower serum cotinine levels, a lower proportion of binge drinkers, lower physical activity levels, and a higher prevalence of diabetes and hypertension. Furthermore, the study notes a significantly higher incidence of depressive symptoms among participants with gallstones compared to those without [13.1% (95% CI, 10.1–16.9%) vs 7.9% (95% CI, 7.0–8.8%), $P<0.001$].

Table 1 General Characteristics of Included Participants (n=7787) by the Presence (n=835) or Absence (n=6952) of Gallstones in the NHANES 2017–March 2020

	Gallstones (No) n=6952	Gallstones (Yes) n=835	P
Characters			
Gender			<0.001
Male	51.8% (50.0%, 53.6%)	26.4% (22.0%, 31.2%)	
Female	48.2% (46.4%, 50.0%)	73.6% (68.8%, 78.0%)	
Age (years old)			<0.001
18–39	37.5% (35.8%, 39.3%)	15.9% (12.8%, 19.7%)	
40–59	35.1% (33.4%, 36.9%)	34.2% (29.3%, 39.4%)	
More than 60	27.4% (25.9%, 28.9%)	49.9% (44.7%, 55.1%)	
Race/ethnicity			<0.001
Mexican American	8.3% (7.6%, 9.0%)	7.4% (5.9%, 9.4%)	
Other Hispanic	7.4% (6.8%, 8.1%)	7.1% (5.5%, 9.0%)	
Non-Hispanic White	63.1% (61.6%, 64.6%)	70.5% (66.5%, 74.1%)	
Non-Hispanic Black	11.6% (10.9%, 12.3%)	7.1% (5.8%, 8.6%)	
Other Race	9.6% (8.9%, 10.4%)	8.0% (6.0%, 10.5%)	

(*Continued*)

Table 1 (Continued).

	Gallstones (No) n=6952	Gallstones (Yes) n=835	P
Education levels			0.498
Less than high school	10.2% (9.4%, 11.0%)	10.5% (8.3%, 13.3%)	
High school or equivalent	26.7% (25.1%, 28.3%)	31.1% (26.3%, 36.4%)	
More than high school	63.1% (61.4%, 64.8%)	58.3% (53.1%, 63.4%)	
Marital status			<0.001
Married/living with partner	62.1% (60.4%, 63.8%)	63.6% (58.7%, 68.2%)	
Widowed/divorced/separated	17.9% (16.7%, 19.2%)	24.4% (20.5%, 28.7%)	
Never married	20.0% (18.6%, 21.4%)	12.0% (9.1%, 15.7%)	
PIR			0.048
PIR≤1	11.2% (10.3%, 12.1%)	10.2% (7.5%, 13.7%)	
1<PIR<4	41.3% (39.6%, 43.0%)	50.2% (45.0%, 55.4%)	
PIR≥4	36.6% (34.7%, 38.5%)	30.9% (26.1%, 36.2%)	
Not recorded	11.0% (10.0%, 12.1%)	8.7% (6.3%, 11.8%)	
Serum cotinine levels (ng/mL)			<0.001
<0.015	35.6% (33.9%, 37.4%)	43.5% (38.3%, 48.7%)	
0.015–3	35.6% (33.9%, 37.3%)	30.6% (26.3%, 35.3%)	
>3	23.8% (22.4%, 25.4%)	21.5% (17.2%, 26.4%)	
Not recorded	5.0% (4.2%, 5.8%)	4.5% (2.9%, 6.7%)	
Alcohol drinking status			<0.001
Non-drinkers	6.5% (5.7%, 7.3%)	8.1% (5.8%, 11.0%)	
Moderate-drinkers	39.0% (37.3%, 40.8%)	33.7% (29.1%, 38.8%)	
Heavy-drinkers	27.8% (26.2%, 29.4%)	23.6% (19.4%, 28.4%)	
Binge-drinkers	12.0% (10.8%, 13.2%)	6.5% (4.6%, 9.2%)	
Not recorded	14.8% (13.7%, 15.9%)	28.1% (23.5%, 33.1%)	
Body Mass Index			<0.001
<25	27.7% (26.1%, 29.4%)	13.2% (9.6%, 17.8%)	
25–29.9	31.5% (29.9%, 33.2%)	29.3% (24.5%, 34.5%)	
≥30	40.1% (38.4%, 41.9%)	55.8% (50.5%, 61.0%)	
Not recorded	0.6% (0.5%, 0.9%)	1.7% (1.0%, 3.0%)	
Physical activity level			0.009
Active	45.2% (43.4%, 47.0%)	37.3% (32.3%, 42.5%)	
Less active	8.4% (7.5%, 9.5%)	9.4% (6.8%, 13.0%)	
Not recorded	46.4% (44.6%, 48.2%)	53.3% (48.1%, 58.5%)	

(*Continued*)

Table 1 (Continued).

	Gallstones (No) n=6952	Gallstones (Yes) n=835	P
Hypertension			0.003
Yes	35.8% (34.2%, 37.5%)	38.7% (33.9%, 43.8%)	
No	58.6% (56.8%, 60.3%)	53.0% (47.9%, 58.1%)	
Not recorded	5.6% (4.9%, 6.4%)	8.2% (6.2%, 10.9%)	
Diabetes			<0.001
Yes	8.9% (8.1%, 9.9%)	16.3% (13.2%, 20.0%)	
No	87.4% (86.2%, 88.5%)	79.4% (75.3%, 83.0%)	
Not recorded	3.7% (3.1%, 4.4%)	4.2% (2.6%, 6.9%)	
Depressive symptoms			<0.001
Yes	7.9% (7.0%, 8.8%)	13.1% (10.1%, 16.9%)	
NO	92.1% (91.2%, 93.0%)	86.9% (83.1%, 89.9%)	

Abbreviations: NHANES, National Health and Nutrition Examination Survey; PIR, poverty-income ratio.

Characteristics of Participants After PSM

Given the substantial differences in baseline characteristics between participants with gallstones and those without, this study incorporated covariates such as gender, age, race, education level, marital status, PIR, serum cotinine levels, alcohol use, BMI, physical activity, hypertension, and diabetes prevalence to perform PSM. Post-PSM, we obtained 835 matched pairs, comprising 835 participants with gallstones and an equal number without. Subsequent to PSM, comparability was achieved between the two groups in terms of education, marital status, PIR, serum cotinine levels, alcohol usage, BMI, physical activity, and prevalence of hypertension and diabetes. However, the gallstones group still showed a higher proportion of female, individuals aged 60 and above, and non-Hispanic Whites. Additionally, the prevalence of depressive symptoms was significantly higher among those with gallstones compared to their counterparts [13.1% (95% CI, 10.1–16.9%) vs 7.8% (95% CI, 5.8–10.4%), P= 0.001] (Table 2).

Table 2 General Characteristics of Included Participants (n=1670) by the Presence (n=835) or Absence (n=835) of Gallstones After Propensity Score Matching in the NHANES 2017–March 2020

	Gallstones (No) n=835	Gallstones (Yes) n=835	P
Characters			
Gender			0.020
Male	31.4% (26.8%, 36.4%)	26.4% (22.0%, 31.2%)	
Female	68.6% (63.6%, 73.2%)	73.6% (68.8%, 78.0%)	
Age (years old)			0.041
18–39	21.9% (18.0%, 26.4%)	15.9% (12.8%, 19.7%)	
40–59	35.0% (30.0%, 40.2%)	34.2% (29.3%, 39.4%)	
More than 60	43.2% (38.2%, 48.3%)	49.9% (44.7%, 55.1%)	

(*Continued*)

Table 2 (Continued).

	Gallstones (No) n=835	Gallstones (Yes) n=835	P
Race/ethnicity			<0.001
Mexican American	8.9% (7.1%, 11.1%)	7.4% (5.9%, 9.4%)	
Other Hispanic	6.7% (5.1%, 8.8%)	7.1% (5.5%, 9.0%)	
Non-Hispanic White	64.3% (59.9%, 68.4%)	70.5% (66.5%, 74.1%)	
Non-Hispanic Black	12.3% (10.4%, 14.5%)	7.1% (5.8%, 8.6%)	
Other Race	7.7% (5.9%, 10.2%)	8.0% (6.0%, 10.5%)	
Education levels			0.763
Less than high school	9.8% (7.9%, 12.3%)	10.5% (8.3%, 13.3%)	
High school or equivalent	29.1% (24.2%, 34.5%)	31.1% (26.3%, 36.4%)	
More than high school	61.1% (55.8%, 66.1%)	58.3% (53.1%, 63.4%)	
Marital status			0.412
Married/living with partner	60.9% (55.8%, 65.8%)	63.6% (58.7%, 68.3%)	
Widowed/divorced/separated	26.0% (21.7%, 30.8%)	24.4% (20.5%, 28.7%)	
Never married	13.1% (10.3%, 16.5%)	12.0% (9.1%, 15.7%)	
PIR			0.318
PIR≤1	10.4% (8.3%, 13.0%)	10.2% (7.5%, 13.7%)	
1<PIR<4	43.1% (38.1%, 48.2%)	50.2% (45.0%, 55.4%)	
PIR≥4	34.2% (29.2%, 39.7%)	30.9% (26.1%, 36.2%)	
Not recorded	12.3% (9.3%, 15.9%)	8.7% (6.3%, 11.8%)	
Serum cotinine levels (ng/mL)			0.223
<0.015	41.8% (36.7%, 47.0%)	43.5% (38.3%, 48.7%)	
0.015–3	36.7% (31.9%, 41.7%)	30.6% (26.3%, 35.3%)	
>3	18.2% (14.6%, 22.6%)	21.5% (17.2%, 26.4%)	
Not recorded	3.3% (1.8%, 5.8%)	4.5% (2.9%, 6.7%)	
Alcohol drinking status			0.409
Non-drinkers	6.7% (4.7%, 9.5%)	8.1% (5.8%, 11.0%)	
Moderate-drinkers	39.7% (34.6%, 45.1%)	33.7% (29.1%, 38.8%)	
Heavy-drinkers	26.1% (21.8%, 31.0%)	23.6% (19.4%, 28.4%)	
Binge-drinkers	8.8% (6.5%, 11.7%)	6.5% (4.6%, 9.2%)	
Not recorded	18.7% (15.4%, 22.5%)	28.1% (23.5%, 33.1%)	
Body Mass Index			0.956
<25	15.0% (11.6%, 19.4%)	13.2% (9.6%, 17.8%)	
25–29.9	24.3% (20.2%, 28.9%)	29.3% (24.5%, 34.5%)	

(*Continued*)

Table 2 (Continued).

	Gallstones (No) n=835	Gallstones (Yes) n=835	P
≥30	59.0% (53.8%, 64.0%)	55.8% (50.5%, 61.0%)	
Not recorded	1.7% (1.0%, 2.9%)	1.7% (1.0%, 3.0%)	
Physical activity level			0.723
Active	35.6% (30.7%, 40.8%)	37.3% (32.3%, 42.5%)	
Less active	8.0% (5.7%, 11.1%)	9.4% (6.8%, 13.0%)	
Not recorded	56.4% (51.2%, 61.5%)	53.3% (48.1%, 58.5%)	
Hypertension			0.789
Yes	35.4% (30.7%, 40.4%)	38.7% (33.9%, 43.8%)	
No	57.9% (52.8%, 62.8%)	53.0% (47.9%, 58.1%)	
Not recorded	6.8% (5.2%, 8.7%)	8.2% (6.2%, 10.9%)	
Diabetes			0.187
Yes	15.7% (12.4%, 19.7%)	16.3% (13.2%, 20.0%)	
No	82.7% (78.7%, 86.1%)	79.4% (75.3%, 83.0%)	
Not recorded	1.6% (1.0%, 2.8%)	4.2% (2.6%, 6.9%)	
Depressive symptoms			0.001
Yes	7.8% (5.8%, 10.4%)	13.1% (10.1%, 16.9%)	
NO	92.2% (89.6%, 94.2%)	86.9% (83.1%, 89.9%)	

Abbreviations: NHANES, National Health and Nutrition Examination Survey; PIR, poverty-income ratio.

Association Between Gallstones and Depressive Symptoms

Exploring the association between gallstones and depressive symptoms, multivariate logistic regression after PSM revealed that, in the unadjusted analysis (Model 1), participants with gallstones had an odds ratio (OR) of 1.781 (95% CI, 1.154–2.748, P=0.009) for depressive symptoms. This OR increased to 1.807 (95% CI, 1.168–2.796, P=0.008) in the analysis adjusted for gender, age, and race (Model 2), and remained statistically significant [OR=1.821 (95% CI, 1.181–2.808), P=0.007] in the fully adjusted analysis (Model 3). Subgroup analysis indicated a notably higher prevalence of depressive symptoms among gallstones patients who were female, aged 40–59, had less than a high school education, were married/living with partner and widowed/divorced/separated, had serum cotinine levels ≥0.015 ng/mL, and had no hypertension or diabetes. The association between gallstones and depressive symptoms was particularly significant in these subgroups according to the fully adjusted logistic regression analyses (Table 3).

Causal Association Between Gallstones and Depressive Symptoms in MR Study

Forward Univariate MR Analysis

In the MR analysis investigating the causal association between gallstones and depressive symptoms, the univariate MR analysis initially identified 49 SNPs strongly associated with gallstones but not with depressive symptoms or confounding factors, each with an F-statistic not less than 10 (Supplementary Table 3). After harmonizing exposure and outcome data, 37 SNPs were used as IVs (Supplementary Table 4). The MRE-IVW method suggested a causal association [OR=1.04 (95% CI, 1.01–1.06), P=0.002], implicating gallstones as a risk factor for depressive symptoms (Table 4). Subsequently, we conducted a sensitivity analysis encompassing heterogeneity testing and horizontal pleiotropy testing. The results indicate that there is no heterogeneity in the forward univariate MR analysis concerning gallstones on

Table 3 Association Between Gallstones and Depression Based on Multivariate Logistic Regression Analysis in NHANES 2017-March 2020

	Model 1		Model 2		Model 3	
	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
Gallstones						
No	Reference		Reference		Reference	
Yes	1.781 (1.154, 2.748)	0.009	1.807 (1.168, 2.796)	0.008	1.821 (1.181, 2.808)	0.007
Stratified by gender						
Male	1.741 (0.818, 3.706)	0.151	1.799 (0.833, 3.883)	0.135	1.633 (0.719, 3.709)	0.241
Female	1.768 (1.047, 2.983)	0.033	1.813 (1.076, 3.055)	0.025	1.758 (1.056, 2.926)	0.030
Stratified by age						
18–39 years old	2.345 (1.031, 5.331)	0.042	2.578 (1.134, 5.864)	0.024	2.024 (0.876, 4.675)	0.099
40–59 years old	3.712 (1.735, 7.941)	0.001	3.604 (1.697, 7.655)	0.001	3.887 (1.851, 8.159)	<0.001
60-years old	0.894 (0.486, 1.644)	0.718	0.934 (0.513, 1.700)	0.824	1.035 (0.582, 1.840)	0.907
Stratified by education levels						
Less than high school education	2.239 (1.066, 4.701)	0.033	2.588 (1.184, 5.655)	0.017	2.981 (1.269, 7.002)	0.012
High school education	1.324 (0.583, 3.008)	0.503	1.366 (0.589, 3.169)	0.468	/	
More than high school education	1.992 (1.084, 3.662)	0.026	2.003 (1.095, 3.662)	0.024	/	
Stratified by marital status						
Married/living with partner	2.241 (1.083, 4.639)	0.030	2.266 (1.106, 4.642)	0.025	2.040 (1.139, 3.652)	0.016
Widowed/divorced/separated	1.911 (1.002, 3.646)	0.049	2.089 (1.033, 4.226)	0.040	2.627 (1.305, 5.289)	0.007
Never married	0.987 (0.398, 2.445)	0.977	0.803 (0.276, 2.334)	0.687	0.749 (0.315, 1.781)	0.513
Stratified by serum cotinine levels						
Low level	0.718 (0.33, 1.563)	0.404	0.617 (0.286, 1.331)	0.218	0.594 (0.273, 1.290)	0.188
Moderate level	2.935 (1.558, 5.529)	0.001	2.965 (1.567, 5.611)	0.001	2.883 (1.529, 5.435)	0.001
High level	2.656 (1.162, 6.073)	0.021	2.657 (1.117, 6.323)	0.027	3.142 (1.403, 7.036)	0.005
Stratified by hypertension						
Yes	1.314 (0.653, 2.644)	0.444	1.336 (0.656, 2.718)	0.425	1.360 (0.707, 2.616)	0.357
No	2.282 (1.218, 4.273)	0.010	2.377 (1.269, 4.453)	0.007	2.263 (1.204, 4.254)	0.011
Stratified by diabetes						
Yes	1.880 (0.705, 5.012)	0.207	1.925 (0.680, 5.449)	0.217	2.003 (0.936, 4.285)	0.073
No	1.865 (1.135, 3.065)	0.014	1.840 (1.123, 3.016)	0.016	1.907 (1.173, 3.102)	0.009

Notes: Model 1: non-adjusted model. Model 2 adjusted for: gender; age; race. Model 3 adjusted for: gender, age, race, education level, marital status, PIR, serum cotinine levels, alcohol use, BMI, physical activity, hypertension, and diabetes status. "/" means that values can not be calculated.
Abbreviations: NHANES, National Health and Nutrition Examination Survey; OR, odds ratio; 95% CI, 95% confidence interval; PIR, poverty-income ratio; BMI, body mass index.

depressive symptoms (Supplementary Table 5), and the horizontal pleiotropy is not observed (Supplementary Table 6). This signifies that our MR analysis outcomes are robust and credible. Additionally, leave-one-out analysis (Figure 3), scatter plots (Figure 4), forest plots (Supplementary Figure 1), and funnel plots (Supplementary Figure 2) further substantiated the causal association between gallstones and depressive symptoms.

Inverse Univariate MR Analysis

In inverse univariate MR analysis, we investigated depressive symptoms as the exposure and gallstones as the outcome, identifying 3 SNPs as IVs (Supplementary Table 7). The MRE-IVW method revealed no reverse causality between depressive symptoms and gallstone disease [OR=1.28 (95% CI, 0.90–1.83), *P*=0.17] (Table 4), and there were no indications of heterogeneity (Supplementary Table 5) or horizontal pleiotropy (Supplementary Table 6).

Multivariate MR Analysis

In the multivariate MR analysis, factors such as diabetes and obesity were included for adjustment. The results demonstrated a significant causal association between gallstones and depressive symptoms [OR=1.03 (95% CI,

Table 4 Two-Sample Univariable MR Analyses of the Association Between Gallstones and Depressive Symptoms

Exposure	Outcome	MR Analysis Method	Number of SNP	OR	95% CI	P
Gallstones	Depressive symptoms	MR Egger	37	1.03	(1.00, 1.07)	0.067
		WME		1.03	(1.00,1.07)	0.046
		MRE-IVW		1.04	(1.01,1.06)	0.002
		SM		1.02	(0.96,1.09)	0.498
		WM		1.03	(1.00,1.06)	0.060
Depressive symptoms	Gallstones	MR Egger	3	0.79	(0.48,1.30)	0.524
		WME		1.15	(0.86,1.54)	0.355
		MRE-IVW		1.28	(0.90,1.83)	0.171
		SM		1.08	(0.73,1.60)	0.728
		WM		1.10	(0.79,1.51)	0.635

Abbreviations: MR, Mendelian randomization; SNP, single nucleotide polymorphism; OR, odds ratio; 95% CI, 95% confidence interval; WME, weighted median; MRE-IVW, multiplicative random effects-inverse variance weighted; SM, simple mode; WM, weighted mode.

1.01–1.05), P=0.009] (Table 5). The consistency between multivariate and univariate analyses reinforces the robustness and reliability of our findings, further substantiating gallstones as a risk factor for depressive symptoms.

Discussion

This study found a causal association between gallstones and depressive symptoms through cross-sectional study from NHANES 2017-March 2020 data and MR study. To our knowledge, this is the first study to elucidate this causal relationship using extensive observational data for a cross-sectional survey and large-scale genetic data for MR analysis. Our analysis of NHANES 2017- March 2020 data revealed an association between gallstones and depressive symptoms. The specific causal relationship was clarified in both bidirectional univariate MR and multivariate MR analyses, indicating that genetically determined gallstone disease is a risk factor for depressive symptoms. Previous researches[15,16] have reported a potential link between gallstones and depressive symptoms. However, these studies have been limited by small sample sizes. This study, utilizing extensive cross-sectional survey data and MR analysis, overcomes the limitations of prior research and further substantiates the aforementioned findings. Previous cross-sectional study[14] has indicated a significantly higher prevalence of gallstones among individuals with depressive disorder, but our MR analysis reveals that genetically predicted gallstones are a risk factor for depressive symptoms and that there is no reverse causation. By analyzing genetic data, this study more accurately identifies the causal relationship between gallstones and depressive symptoms, mitigating the interference of reverse causation.

The exact mechanism by which gallstones influence the onset and progression of depressive symptoms remains unclear, with several potential mechanisms suggested. Some studies propose that gallstones may trigger depressive symptoms by affecting the microbiota-gut-brain (MGB) axis.[33] There is a bidirectional communication between the gut and the brain through neural, endocrine, and immune pathways. Microbiota and their metabolic products in the gut can regulate gut-brain signal transmission, thereby influencing the nervous system.[34] Studies have found significant alterations in the gallbladder and intestinal microbiota in patients with gallstones compared to those without, leading to dysbiosis in the gastrointestinal tract.[35,36] This dysregulated gut microbiota can directly or indirectly produce neurotransmitters like serotonin and gamma-aminobutyric acid, regulating gut physiology locally and modulating the distant organs' function via the gut-brain axis and circulatory system.[37] An increase in the Bacteroides genus, identified as a specific species associated with depressive disorder, has been noted in the intestines of gallstone patients, increasing susceptibility to depressive disorder.[38–40] However, due to the complexity of gut microbiota, how gallstones affect them and thus lead to depressive symptoms is a complex question that requires further in-depth research and exploration. In

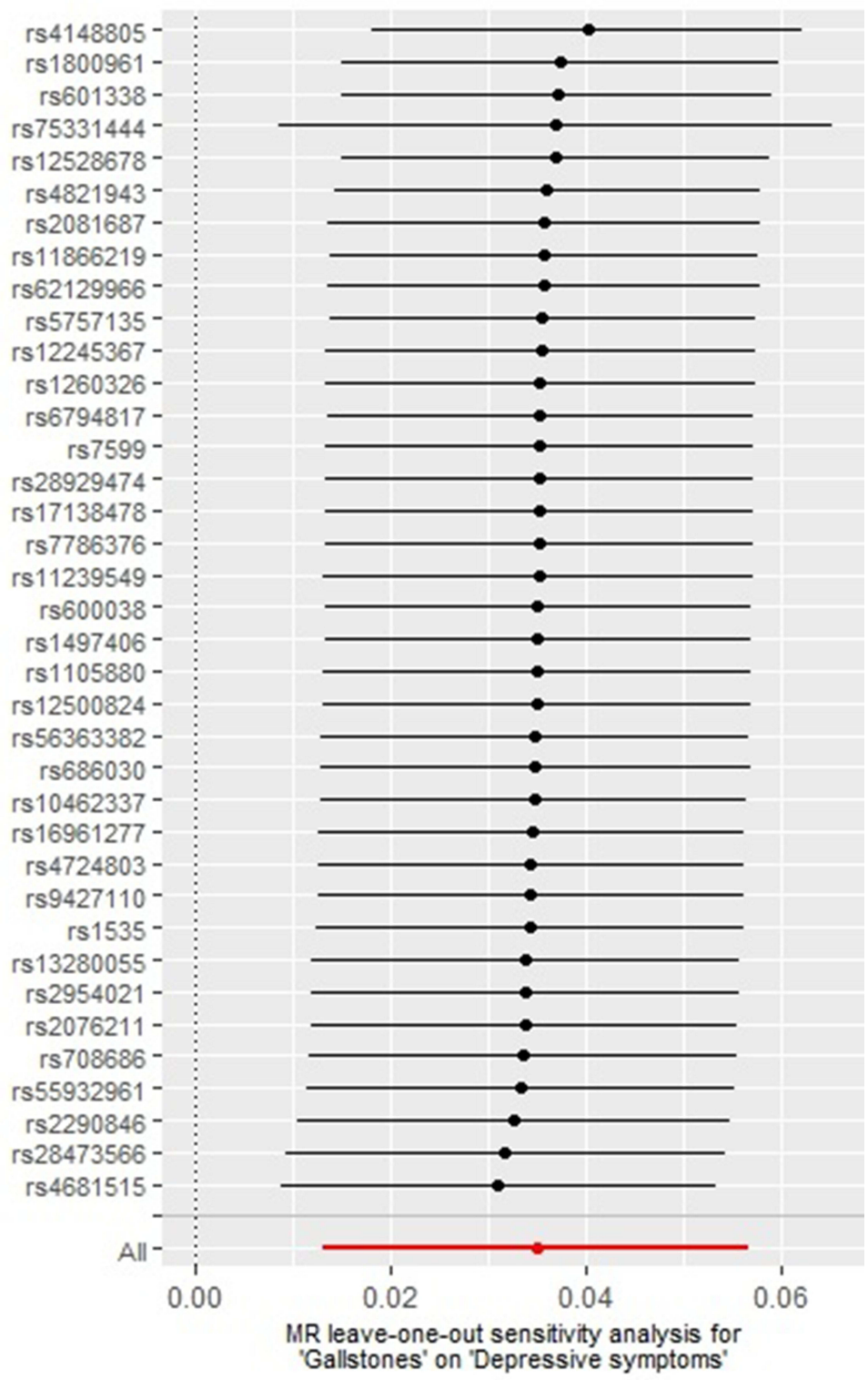

Figure 3 Leave-one-out test plot of causal effect estimates by MRE-IVW for gallstones on depressive symptoms, with all 37 valid IVs.
Abbreviations: MRE-IVW, multiplicative random effects-inverse variance weighted; IVs, instrumental variables; MR, Mendelian randomization.

addition, gallstones could cause depressive symptoms by changing gut microbiota, so whether depressive symptoms can affect gut microbiota through BGM axis or even form a vicious cycle to further aggravate depressive symptoms is a question worthy of further discussion. Another aspect is that gallstones can cause chronic, low-grade inflammation in the gallbladder wall.[41] Chronic inflammation, by mediating the body's immune system and compensatory anti-

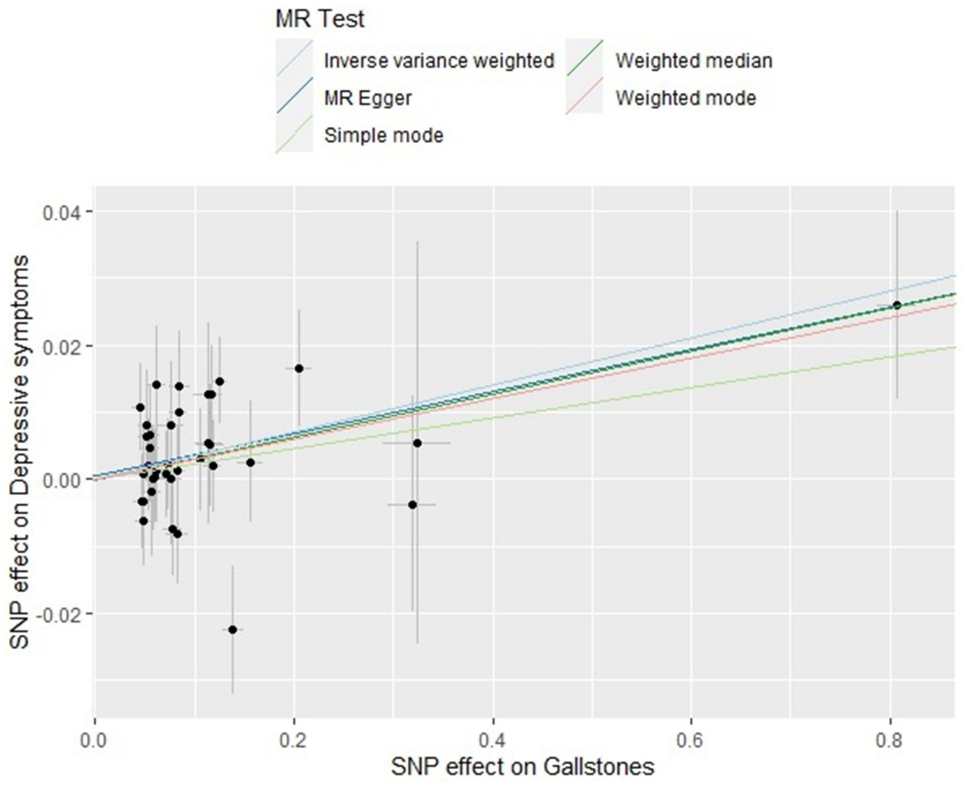

Figure 4 Scatter plot of causal effect estimates for gallstones on depressive symptoms, with all 37 valid IVs.
Abbreviations: IVs, instrumental variables; MR, Mendelian randomization; SNP, single nucleotide polymorphism.

inflammatory reflex system, can lead to the onset of depressive symptoms.[42,43] The body's inflammatory response, altering signaling in the prefrontal cortex, hippocampus, and nucleus accumbens through brain-derived neurotrophic factor (BDNF)-tropomycin receptor kinase B (TrkB), can result in a depressive phenotype.[44] In addition, peripheral inflammatory factors caused by bacterial infection in gallstones and lipopolysaccharides from Gram-negative bacteria can cross the blood-brain barrier, causing neuroinflammation and, through the activation of specific cells, induce neuropathological changes including synaptic defects, demyelination, and neurotransmitter release, thereby influencing depressive symptoms.[45,46] Furthermore, dysbiosis in gut microbiota causing disturbances in the intestinal microenvironment can lead to gut inflammation and barrier dysfunction.[47] Metabolites and microbiota moving through the compromised gut barrier can cause systemic inflammation, potentially linked to the pathogenesis of depressive symptoms.[47–49] The specific underlying mechanisms of how gallstones lead to depressive symptoms remain to be elucidated, awaiting more clinical and basic medical research for deeper exploration. In the context of increasingly prevalent mental health issues such as depressive symptoms,[50] these studies will hold significant importance.

Table 5 Multivariate MR Analysis of Association Between Gallstones and Depressive Symptoms

Exposure	Outcome	Number of SNP	OR	95% CI	P
Gallstones	Depressive symptoms	38	1.03	(1.01,1.05)	0.009
Diabetes		3	0.96	(0.92,1.00)	0.054
Obesity		4	0.96	(0.91,1.02)	0.220

Abbreviations: MR, Mendelian randomization; SNP, single nucleotide polymorphism; OR, odds ratio; 95% CI, 95% confidence interval.

This study employed cross-sectional analysis using publicly available NHANES data from the National Center for Health Statistics. The questionnaire's design was scientifically sound, and the test and examination data were reliable. Participants showed good compliance, and the original data had high authenticity and credibility. Additionally, the large sample size and strong representativeness overcame the limitations of previous small-scale cross-sectional studies. Before conducting logistic regression analysis, we performed PSM to ensure comparability between the gallstones group and the non-gallstones group in terms of basic participant characteristics. The multivariate logistic regression analysis incorporated demographic characteristics, chronic disease conditions, smoking and drinking habits, and socio-economic status to adjust the analysis model, effectively reducing confounding factor interference. We also conducted stratified analysis, enhancing the precision of our results.

As cross-sectional studies from NHANES can only establish associations between gallstones and depressive symptoms, not direct causality, we performed bidirectional univariate and multivariate MR study. In conducting the MR analysis, we extracted SNPs related to gallstones, depressive symptoms, diabetes and obesity from the EBI, IEU, and FinnGen Biobank databases. This approach was chosen to obtain IVs from databases with large sample sizes while addressing the need to avoid sample overlap. It is worth noting that although the SNPs came from three different databases, these three databases were only affiliated with different academic research institutions, and their surveyed populations were all European, with similar ethnic and genetic backgrounds, which supports the feasibility of our MR analysis. The inherent nature of MR analysis significantly minimized confounding interference, and our bidirectional MR approach avoided reverse causation issues, overcoming the drawbacks of observational studies. Multivariate MR analysis included obesity and diabetes factors, mitigating their potential impact on the results. Additionally, sensitivity analyses were conducted to exclude heterogeneity and pleiotropy, enhancing the robustness and reliability of our findings. Through MR analysis, we further clarified the relationship, identifying gallstones as a risk factor for depressive symptoms, increasing the likelihood of its onset.

Limitations

Despite these findings, our study has limitations. The NHANES data used were for adults over 18, excluding analysis for minors and children, and thus, our results do not apply to individuals under 18. Moreover, as the NHANES data are derived from an American population and the genetic database for MR analysis from a European population, the applicability of our conclusions to Asian, African, and Latin American populations requires further in-depth exploration. Additionally, the NHANES survey's design limited our ability to ascertain gallstone presence, relying on patient questionnaires rather than abdominal ultrasound or radiological diagnosis, potentially introducing recall bias. It is noteworthy that the NHANES survey questionnaire does not include questions regarding the number of gallstones, clinical symptoms, pain intensity, gallbladder inflammation, or the extent of inflammation. This omission prevents a more in-depth analysis of the relationship between these factors and depressive symptoms and their severity. In the future, the research team intends to carry out subsequent studies at affiliated medical institution to delve deeper into how the severity of gallstone pain and inflammation correlates with depressive symptoms. Also, the inclusion of some covariates from the NHANES database had partial missing values ("not recorded"), inevitably affecting the analysis.

Conclusion

This study, utilizing epidemiological data from the NHANES 2017- March 2020, found an association between depressive symptoms and gallstones. MR analysis further identified genetically determined gallstones as a risk factor for depressive symptoms, with no evidence of reverse causation. In summary, this study demonstrates that gallstones increase the risk of depressive symptoms in adults. The prevalence of depressive symptoms is on a steady rise, inflicting severe physical and psychological trauma on patients and contributing to an escalating economic strain on society. Gallstones are notably common in clinical settings. We hope our research findings will guide public health policy makers and medical professionals, advocating for prompt depressive symptoms screenings and diligent follow-up for patients diagnosed with gallbladder calculi. This approach is aimed at early detection and timely psychological interventions and treatments for depressive symptoms, thereby mitigating the afflictions and socio-economic impact of the condition on patients and society.

Data Sharing Statement

No additional data are available. NHANES data and GWAS datasets are publicly available.

Ethics Statement

All NHANES data and GWAS datasets obtained in this study have been publicly published and received corresponding ethical approvals. Due to the use of publicly published public database, the study was reviewed by the Ethics Committee of the First Affiliated Hospital of Chongqing Medical University and approved the application for exemption from the research ethics review of this study.

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Disclosure

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