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Brain-body interactions underlying comorbid depression and other mood disorders

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Brain-body interactions underlying comorbid depression and other mood disorders

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Table of contents

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Editorial: Brain-body interactions underlying comorbid depression and other mood disorders

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Editorial on the Research Topic

Brain-body interactions underlying comorbid depression and other mood disorders

Depression is among the most prevalent psychiatric disorders globally with a disabling impact on individual psychological well-being and overall health (1). Beyond its psychological symptoms, depression is increasingly recognized as a systemic illness with complex interactions between the brain and peripheral organ systems (2). Chronic stress plays a pivotal role in these interactions, exerting profound effects on the central nervous system (CNS) and disrupting brain's regulatory influence over physiological processes. This dysregulation contributes to the onset and progression of psychiatric and systemic illnesses, including cardiovascular disease, diabetes, chronic pain, and neurodegenerative disorders (3).

The mechanisms underlying the brain–body interactions remain incompletely understood, but growing evidence points to neuroendocrine, immune, and inflammatory pathways as key drivers (4). Stress can compromise the integrity of the blood–brain barrier, enabling peripheral inflammatory signals to affect brain function and behavior (5, 6). This interplay contributes to the development of mood disorders but also complicates the management and treatment of both depression and systemic illnesses. Neuroendocrine dysregulation, immune activation, and inflammatory cascades collectively impair brain's control over peripheral systems, allowing inflammatory mediators to penetrate the CNS and alter neural functions. Understanding these mechanisms is critical for developing integrated treatment strategies that address both mental and physical health outcomes.

The studies featured in this Research Topic add complementary evidence that depression is not an isolated disorder, but a multidimensional illness shaped by genetic, metabolic, and immunological factors. They highlight 1) potentially novel biomarkers such as lipid ratios and inflammatory proteins, 2) important comorbidities spanning chronic diseases and neurodegenerative conditions, and 3) innovative therapeutic interventions including digital health technologies. Collectively, these findings advance our understanding of depression as a systemic illness requiring holistic approaches that

bridge neuroscience, immunology, endocrinology, and emerging digital therapeutics. This perspective accentuates the need for interdisciplinary research and clinical models that move beyond symptom management towards strategies designed to improve outcomes and quality of life for individuals affected by depression and its comorbidities.

Biomarkers and physiological correlates of depression

Compared to neurodegenerative illnesses, cancer or cardiovascular disorders, finding biomarkers for depression has been challenging despite the need of measurable indicators for improving either diagnosis, prognosis, or treatment efficacy. Several papers in this Research Topic raise the possibility that biomarkers of depression could be found when considering other systems than the brain and underscore the role of systemic inflammation, lipid metabolism, and neurophysiological dysfunction in depressive disorders. Li et al. identify the Platelet/High-Density Lipoprotein Cholesterol Ratio (PHR) as a potential biomarker for depression among individuals with chronic opioid use. Their analysis of the National Health and Nutrition Examination Survey (NHANES; 2007–2018) data reveals that elevated PHR is independently associated with depression, even after adjusting for demographic and clinical covariates. They also suggest that beyond a certain threshold, PHR sharply increases depression risk. Subgroup analyses indicate stronger associations in younger individuals and those with obesity, pointing to systemic inflammation and lipid dysregulation as key contributors. These findings emphasize the need to incorporate metabolic and inflammatory markers into mental health assessments especially in depressed individuals with chronic opioid use.

Furthermore, Jiang et al. investigated the Framingham Steatosis Index (FSI), a diagnostic indicator of accumulation of fat in liver, and its potential association with depression. Using data from nearly 20,000 participants, they demonstrate that higher FSI values correlate with increased depression risk. This study highlights a role of metabolic dysfunction as well as hormonal imbalances, and inflammation in depression pathophysiology. The authors suggest that screening for fatty liver indicators and related metabolic disturbances could improve preventive strategies for depression.

Sleep disturbances and cognitive impairments are hallmarks of depression. Fang et al. showed a potential link between sleep efficiency, event-related potentials (ERPs), and levels of serum C-reactive protein (CRP) in patients with depression. Their results indicate that poor sleep efficiency is associated with prolonged ERP latencies and high levels of CRP and suggest that chronic inflammation can mediate cognitive dysfunction in depression. This study fuels the literature suggesting that addressing sleep quality and inflammatory status is important when considering therapeutic interventions for depression.

Genetic and immunological factors also contribute to the depression pathology and its comorbidities. Liu et al. report shared biomarkers—CD163 and KLRB1—between depression and diabetic kidney disease, suggesting involvement of common inflammatory and immune pathways as risk factors. Their comprehensive analysis, including linkage disequilibrium score regression and gene expression profiling, identifies 83 crosstalk genes and eight hub genes involved in immune regulation. These findings further reinforce the systemic nature of depression and open avenues for targeted therapies addressing shared mechanisms across comorbid conditions. Finally, Zhang et al. provide evidence of neuroinflammation and brain function abnormalities in HIV-associated depressive disorders. Using resting-state fMRI and peripheral immune profiling, they demonstrate altered connectivity patterns and elevated inflammatory markers in HIV-positive individuals with depression, underscoring the detrimental effects of immune dysregulation on the brain function.

Collectively, these studies position potential biomarkers—ranging from lipid ratios and liver indices to inflammatory proteins and genetic signatures—as critical tools for advancing precision psychiatry. They also emphasize the interplay between peripheral and central processes in stratifying depressive phenotypes.

Depression in specific populations and comorbid conditions

Depression manifests differently across populations and comorbidities, highlighting the heterogeneity of the disorder and the need for tailored interventions. Several studies in this Research Topic showcase these differences and nuances. As mentioned above, Li et al. highlight the role of lipid metabolism and systemic inflammation as a depression risk in depressed individuals with chronic opioid use. These findings call for integrated treatment approaches that encompass substance use disorder, depression, and metabolic health. In depressed patients with Parkinson's disease, Xu et al. reveal a bidirectional relationship between depression and activities of daily living (ADL) that are mediated by cognitive function. Their longitudinal analysis shows that depression predicts functional decline, while impaired ADL exacerbates depressive symptoms. In PD-associated depression, decline in cognitive function is a key mediator, suggesting that interventions aimed at enhancing cognition could improve quality of life for PD patients.

Moreover, Zhang et al. investigated HIV-positive individuals as another vulnerable group. The authors report high rates of depression among men who have sex with men living with HIV, linking these symptoms to aberrant immune responses and brain connectivity alterations. These findings emphasize the need for mental health services that are integrated into HIV care, addressing both neuropsychiatric and immunological dimensions. Interestingly, Chen et al. uncover a genetic overlap between psychiatric disorders—including depression, bipolar disorder, and schizophrenia—and hemorrhoidal disease. Their genome-wide cross-trait analysis identifies pleiotropic genes and causal relationships, suggesting shared biological mechanisms. While the clinical implications of this association remain to be fully

elucidated, these findings expand our understanding of depression as a systemic disorder with unexpected genetic links.

These studies collectively reinforce the concept that depression is deeply intertwined with physical health, genetic predispositions, and social determinants. Personalized treatment plans that consider these factors are essential for improving outcomes across diverse patient populations.

Innovative therapeutic interventions

Advancements in therapeutic technologies are creating new opportunities for managing depression and its primary comorbidities, such as anxiety. Premkumar et al. contributed a study to this Research Topic focusing on evaluating the effect of self-guided Virtual Reality Exposure Therapy (VRET) on social anxiety. The authors conducted a randomized controlled trial demonstrating that VRET significantly reduces public speaking anxiety and physiological arousal, especially when augmented with biofeedback such as heart rate and frontal alpha asymmetry monitoring. These benefits were sustained at one-month follow-up, indicating that immediate biofeedback enhances VRET by empowering individuals to regulate anxiety responses in real time, potentially allowing them to develop adaptive coping strategies. The emergence of innovative therapies such as VRET with biofeedback offers hope for more effective and personalized treatment strategies, in particular when systemic parameters are included.

Conclusion

The studies featured in this Research Topic collectively accentuate and support the multifaceted nature of depression. From biomarkers and genetic predispositions to systemic inflammation and neurophysiological dysfunction, the evidence points to a complex web of brain–body interactions that shape the onset, progression and the severity of depressive disorders. The high prevalence of comorbidities—spanning chronic pain, diabetes, HIV, Parkinson's disease, and less commonly considered conditions such as hemorrhoidal disease—further highlights the urgent need for integrated, interdisciplinary approaches to psychiatric research and treatments. Digital therapeutics, including VR-based interventions, represent a shift toward delivering personalized, technology-driven mental health care, offering scalable and accessible solutions for anxiety and depression. With continued evolution of technology, incorporating these tools into mainstream psychiatric care could revolutionize treatment paradigms, making mental health support more inclusive and effective. As we continue to unravel the biological and systemic underpinnings of depression, future research must prioritize translational approaches that bridge neuroscience, immunology, endocrinology, and technology for disorder monitoring and therapeutics. This editorial highlights the need for sustained collaboration and innovation to advance the field of psychiatric research. By embracing a holistic view of depression that encompasses both brain and body, we can move toward more comprehensive care models that improve outcomes and quality of life for individuals affected by depression and its various comorbidities.

Author contributions

MB: Conceptualization, Writing – original draft, Writing – review & editing. KM: Writing – review & editing, Writing – original draft, Conceptualization. DC: Conceptualization, Writing – review & editing, Writing – original draft. VD: Writing – original draft, Conceptualization, Supervision, Writing – review & editing.

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Shared genetic architecture of psychiatric disorders and hemorrhoidal disease: a large-scale genome-wide cross-trait analysis

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Background: The genetic association between psychiatric disorders and hemorrhoidal disease (HEM) is still not well known. The work aims to investigate their comorbidity at a genetic level.

Methods: Utilizing recent large-scale genome-wide association studies (GWAS), we investigated the genetic overlap at the single nucleotide polymorphism (SNP), gene, and molecular level between depression and HEM, bipolar disorder (BD) and HEM, neuroticism and HEM, as well as schizophrenia (SCZ) and HEM. The cross-trait genes were validated through the utilization of transcriptome and proteome methodologies. The causal link was assessed using bidirectional two-sample Mendelian randomization analysis (MR) analysis. MRlap corrects for the potential bias in estimation caused by sample overlap.

Results: We discovered significant positive genetic associations between these four types of psychiatric disorders and HEM. Cross-phenotypic association analyses identified shared SNPs along with 17 specific loci between psychiatric disorders and HEM. MAGMA identified a total of 2304 pleiotropic genes, several of which showed significant expression in the results of transcriptome and proteome analyses. We observed that these genes are mostly associated with the regulation of transcription factors and particular DNA binding activities. Lastly, MR analysis provided evidence supporting a correlation between these conditions.

Conclusion: This study revealed a genetic correlation between four psychiatric disorders and HEM, identified pleiotropic loci, found multiple candidate genes, and confirmed causal relationships. This has enhanced our comprehension of the common genetic mechanisms of psychiatric disorders and HEM.

KEYWORDS

hemorrhoidal disease, psychiatric disorders, genetic overlap, pleiotropic loci, Mendelian randomization

1 Introduction

Hemorrhoidal disease (HEM) is a prevalent anorectal condition characterized by symptomatic enlargement and distal displacement of the normal anal cushions (1). The psychiatric concerns of patients with HEM have been consistently present in clinical practice, yet are frequently disregarded. The symptoms of pain, bleeding, itching, swelling, and other discomforts associated with HEM can have a substantial influence on a patient's daily life and reduce their overall quality of life (2). Patients suffering from this private ailment may accumulate distressing stress over an extended period (3), which can potentially trigger psychiatric problems. Observational studies indicate that psychological and psychiatric factors may play a crucial role in hemorrhoid development (4). Akkoca et al.'s study found that the levels of psychological symptoms and aggression among patients with HEM were significantly higher than those in the control group (5). A national health and nutrition examination survey I dentified depression as a risk factor for HEM (6). The coexistence of psychiatric disorders and HEM has emerged as a significant global challenge (7). Nevertheless, the connection between psychiatric disorders and HEM cannot be conclusively proven due to inconsistencies in research methodology, the diversity of populations studied, and the inherent limits of observational settings, including the possibility of reverse causality effects and environmental factors that may influence the results. In order to further examine the cause-and-effect relationship between the two variables, it is necessary to utilize instrumental factors such as genetic variations.

Large-scale genome-wide association studies (GWAS) have made substantial progress in identifying genetic variations linked to psychiatric disorders and HEM. Through a comprehensive GWAS analysis, 102 independent risk loci for HEM were identified, revealing significant associations with neuroaffective disorders (8). This could be mediated by similar mechanisms involving intestinal motility to mediate genetic risk effects. However, our understanding of the common etiology and genetic susceptibility underlying both psychiatric disorders and HEM remains incomplete. To address this gap, a Mendelian randomization (MR) study was conducted to assess the causal relationship between psychiatric disorders and HEM (9). While MR addresses confounding factors and reverse causality, it does not account for pleiotropy, where genetic variants are associated with multiple traits simultaneously (10, 11). Therefore, more advanced approaches are needed to comprehensively understand the association between psychiatric disorders and HEM as well as elucidate the underlying biological mechanisms in order to facilitate effective prevention and management of this comorbid condition.

This study utilized GWAS summary data at both the SNP and gene levels to conduct a comprehensive pleiotropic analysis of four major psychiatric disorders (depression, bipolar disorder, neuroticism, and schizophrenia) and HEM, aiming to systematically investigate the genetic correlation, shared genetic loci and genes. Bidirectional two-sample MR analysis was employed to explore the causal components of the genetic association. A multifaceted approach was adopted for genome-wide cross-trait analysis.

2 Methods

2.1 GWAS datasets

The overall study design is depicted in Figure 1. Participants were selected from European descent public data sets for the study. Summary statistics on HEM were obtained through a meta-analysis of GWAS datasets, which included a total of 944,133 subjects (8). We acquired GWAS summary data for depression from a meta-analysis that encompassed 807,553 subjects and combined the three largest available depression genetic studies (UK Biobank, 23andMe, and Psychiatric Genomics Consortium) (12). Due to the availability of data from 23andMe, we obtained summary data for approximately 500199 individuals (170756 cases and 329443 controls). Additionally, we acquired GWAS summary data for bipolar disorder (BD) and schizophrenia (SCZ) from the Psychiatric Genomics Consortium (PGG) (https://pgc.unc.edu/for-researchers/download-results/). The data set of neuroticism was included as an additional component. While neuroticism is not officially classified as a psychiatric disorder, it is recognized as a personality trait closely associated with psychiatric conditions such as depression and anxiety. Its genetic architecture has demonstrated significant overlap with common psychiatric disorders (13). The GWAS summary data for Neuroticism were obtained from the Center for Neurogenomics and Cognitive (CNCR) (14). Specifically, Supplementary Table S1 provides detailed information about each GWAS study. We excluded SNPs with duplicate or missing rsID numbers from the dataset and mapped the chromosomal positions of all SNPs to the hg19 human reference genome. For quality control purposes, we retained only SNPs in the 1000 Genomes European population (15).

2.2 General genetic correlation analysis

We employed linkage disequilibrium (LD) (LDSC; https://github.com/bulik/ldsc) score regression (16) and high-definition likelihood (HDL) (17) methods to examine the overall genetic association between four psychiatric disorders and HEM. The LDSC method recognizes that the estimated effect size of a particular SNP reflects not only its own impact but also the combined effects of all other SNPs in LD with it. Genetic correlation between different traits can be estimated using GWAS summary statistics by considering this relationship (18). Univariate LDSC was initially utilized to estimate the heritability (h2) of individual traits, while bivariate LDSC with unconstrained intercepts calculated the genetic correlation (rg) and genetic covariance for the four psychiatric disorders and HEM,

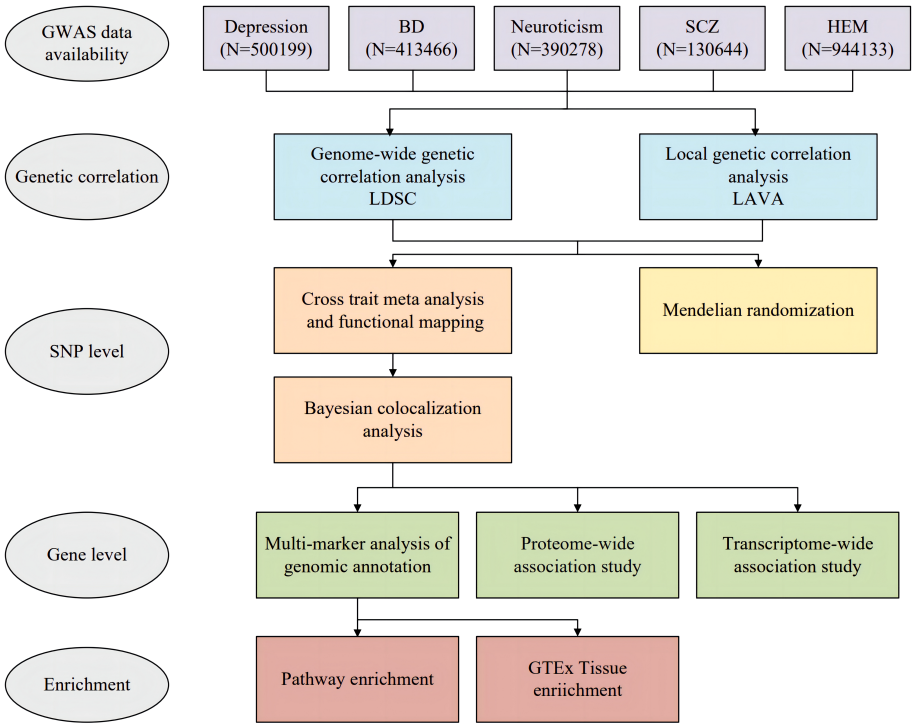

FIGURE 1
Overall study design of genome-wide cross-trait analysis. BD, bipolar disorder; SCZ, schizophrenia; HEM, hemorrhoidal disease.

respectively. The range of genetic correlation estimates is from -1 to +1. LD Scores were estimated from European ancestry samples in the 1000 Genomes Project (18). As an extension of the LDSC approach, The HDL method naturally expands upon the regression formula of LDSC and provides a more precise estimation of genetic correlation (17).

2.3 Local genetic correlation analysis

To tackle the issue that conventional global approaches may fail to detect signals localized in specific regions or in opposing directions at different loci, the local analysis of covariant association (LAVA) method was supplemented with (19). Using the 1000 Genomes Project's European panel as a reference for linkage disequilibrium (LD), we stratified the GWAS dataset into 2495 loci and used multivariate genetic association analysis to identify common genetic association regions and conditional local genetic relationships between the four psychiatric disorders and HEM.

2.4 Cross-trait meta-analysis

To investigate the genetic pleiotropy of a specific locus on two distinct traits, we employed a comprehensive cross-phenotype association analysis (CPASSOC) at the genome-wide level to identify shared loci between psychiatric disorders and HEM. As one of the statistical methods utilized in CPASSOC, unlike Shom, which is a linear combination of univariate test statistics, SHet statistics can be effectively approximated by a shift gamma distribution that accommodates heterogeneous effects resulting from different studies on diverse traits (20).

2.5 Genomic loci characterization and functional annotation

The SNP2GENE method of Functional mapping and annotation (FUMA) platform was employed for functional mapping and annotation to further ascertain independent genomic loci (21). SNPs meeting the criteria of $P_{CPASSOC}$<5×10−8 and LD r2<0.6 were defined as independent significant SNPs, while the lead SNP was determined based on its independence significance and LD r2<0.1. Genomic loci closer than a distance of 500 kb were merged into a single locus, with reference panel data from the European population of the 1000 Genomes Project Phase 3 being applied. For each single trait, we established a threshold of $P_{single\text{-}trait}$<5e-08 to obtain its respective genomic loci, which were then compared against those obtained from previous meta-analyses to identify novel pleiotropic. ANNOVAR provided functional information for SNPs located within genes. The CADD score in the annotated information was used as the harm score, which below 12.37 was considered indicative of reduced potential adverse protein effects.

2.6 Bayesian colocalization analysis

Bayesian colocalization analyses (COLOC) (22) were conducted using the combined SNPs from two GWAS in each study group to assess the likelihood of shared genetic causal variants between two traits by exploring potential pleiotropic loci. The COLOC analysis was based on five exclusive posterior probabilities: H0 (no association with either trait), H1 (genetic association only with trait 1), H2 (genetic association only with trait 2), H3 (association with both traits but independent SNPs), and H4 (association with both traits and only one shared SNP). Co-localized loci in cross-trait analysis were identified as those having PP4 > 0.70.

2.7 Multi-marker analysis of GenoMic Annotation

Gene and gene set analyses have been identified as potentially more effective alternatives to SNP analysis. In gene analysis, genetic marker data are aggregated at the level of the entire gene to assess the collective association of all markers in the gene with the phenotype. Gene set analysis can also provide additional insights into the functional and biological mechanisms underlying the genetic components of traits. Multi-marker Analysis of GenoMic Annotation (MAGMA) is a rapid and flexible method for conducting gene and gene-set analyses within a two-tiered parametric framework (23). MAGMA's gene analysis utilizes a multiple regression approach that effectively combines LD between markers and identifies multi-marker effects, demonstrating superior computational efficiency compared to alternative tools. We obtained overlapping loci by aligning coding genes' positions in NCBI build 37.3 with all pleiotropy loci identified by CPASSOC. Subsequently, MAGMA was utilized to perform association analysis on candidate overlapping pleiotropy genes. The gene set generated by MAGMA was intersected with both FUMA's physically annotated gene set and MAGMA's own generated gene set, resulting in the identification of pleiotropic genes at the individual level after BH correction.

2.8 Transcriptome-wide association studies

The utilization of diverse methodologies for identifying overlapping genes can mitigate the potential for errors and enhance the elucidation of causal mechanisms underlying interrelated traits. We used GTEx (Genotype-Tissue Expression, version 8) transcriptome data from 16 different regions of brain tissue, sigmoid colon, and whole blood as a reference panel to perform transcriptome-wide association studies (TWAS) using FUSION software (24). The results of single-trait TWAS were then combined to identify gene-tissue pairs that are shared among the four psychiatric disorders and HEM.

2.9 Proteome-wide association studies

The enhanced comprehension of genetic regulation in the proteome facilitates the identification of causal mechanisms underlying complex traits. In comparison to trans-associations, cis-associations exhibit greater reproducibility across diverse proteomic platforms (25). Proteome-wide association studies (PWAS) were performed to identify cross-trait protein expression using plasma protein cis-pQTL data from individuals of European ancestry.

2.10 Biological pathway and tissue enrichment analysis

We evaluated the enrichment of overlapping genes between CPASSOC and MAGMA in gene Ontology (GO) (26) biological processes and Kyoto Encyclopedia of Genes and Genomes (KEGG) (27) pathways using MAGMA Gene-set analysis (23) to better understand the biological significance of genes across traits. Gene sets corresponding to colocalized loci were focused on for further exploration. In addition, the FUMA analysis platform was utilized to perform tissue enrichment analysis on 53 tissue types provided by GTEx v.8, enabling the identification of tissues associated with shared genes (21).

2.11 Mendelian randomization

We conducted a comprehensive Bidirectional two-sample MR analysis of the relationship between HEM and depression, BD, neuroticism, and SCZ. We applied a genome-wide association significance threshold of $P < 5.0 \times 10\text{-}8$, excluded single nucleotide polymorphisms (SNPs) with palindromic SNPs and linkage disequilibrium structures ($r2 < 0.001$ within 10000 kb), and removed weak instrumental variables with an F-statistic less than 10. To ensure consistent SNP effects, we harmonized the exposure and outcome data to account for allele differences. Given the heterogeneity among SNPs, we primarily employed random effects inverse variance weighting (IVW) in our MR analysis (28), supplemented by MR-Egger regression to detect potential bias caused by directional pleiotropy (29). Due to the possibility of sample overlap in the summary data from European populations, as well as the possibility of sample overlap in the pooled UK Biobank (UKB) data for depression and HEM, we applied MRlap to correct for the estimated bias in the IVW results. MRlap uses cross-feature LDSC to approximate overlap, allowing it to evaluate and correct for the bias introduced by sample overlap in MR analyses (30). We assessed heterogeneity using the MR-Egger intercept test and Cochran's Q test. Additionally, we utilized MR-Egger regression as well as MR-pleiotropy residual and outlier (MR-PRESSO) methods to identify and address any potential horizontal pleiotropy (31).

3 Results

3.1 Genetic correlations

Univariate LDSC showed a significant genetic effect for each trait. After adjusting for multiple tests, significant genetic associations were

identified between HEM and all four psychiatric disorders. Depression exhibited the most pronounced genetic effect (rg = 0.28, P = 2.04E-34), followed by notable positive associations observed for BD (rg = 0.142, P = 1.42E-10), neuroticism (rg = 0.197, P = 1.55E-20), and SCZ (rg = 0.101, P = 6.58E-07). The HDL model we utilized further validated the presence of a significant positive correlation (Table 1). The results of local genetic correlation analysis of LAVA revealed significant local genetic correlations between psychiatric disorders and HEM. Following FDR multiple corrections, we identified 49 specific associations between depression and HEM, with the most prominent position at chr3: 113657666-115649909(P = 4.38E-07, rho = 0.59). Additionally, we observed twenty-four distinct relationships between BD and HEM, forty-eight connections between neuroticism and HEM, as well as twenty-four links between SCZ and HEM (Supplementary Table S2–S5).

3.2 Cross-trait meta-analysis and SNP annotation

The CPASSOC analysis identified SNPs significantly associated with at least one psychiatric disorder or HEM in each cohort through four genome-wide meta-analyses. Using FUMA, we further annotated the results and discovered independent pleiotropic loci, including 134 loci shared between depression and HEM, 140 loci shared between BD and HEM, 170 loci shared between neuroticism and HEM, as well as 266 loci shared between SCZ and HEM (Figure 2; Supplementary Table S6–S9). Multiple loci expressed significance across all pairwise traits, indicating broad pleiotropy at these loci, such as Index SNP rs9847710 (mapped gene: SFMBT1:RP11-894J14.5), Index SNP rs55646585 (mapped gene: PLEC), and Index SNP rs12474027 (mapped gene: MYT1L). Interestingly, the index SNP rs4910165 mapped in MRVI1 and rs6498573 mapped in MYH11, which showed significance in all four groups of analyses, were related to extracellular matrix organization and muscle function and have been elucidated to be associated with HEM (6). The MYH11 encodes a smooth muscle myosin that is involved in muscle contraction, relaxation and gastrointestinal motility disorders and shows increased expression in HEM transcriptome analysis. Non-coding variants in MYH11 have also been found to be associated with dementia in women with differential expression in microarray study of frontal cortex (32). The Index SNP rs2186797, located at chr11:69971277-70028543 and mapped in ANO1, exhibited significant expression in the meta-analysis of SCZ and HEM. It demonstrated a CADD score of 27.6, which was the highest among all pairwise traits. After ANO1 activation, there is an efflux of chloride ions, resulting in cell depolarization and elevated membrane potential. This leads to the activation of calcium channels and enhanced cell contraction, which are essential for normal gastrointestinal peristalsis and have also been implicated in the pathogenesis of HEM (8, 33). Variants in non-coding regions accounted for the highest proportion of identified SNPS, and a summary of SNP annotations for each pair of traits is shown in Supplementary Figure S1.

Known pleiotropic loci overlapping in each trait pair were obtained by alignment with FUMA annotation results from single-trait GWAS (P<5e-08). In addition, SNPS that did not overlap with the significant loci of the two single-trait GWAS were compared with the P values of the two single-trait GWAS, and 5× 10-08 <P<1× 10-03 loci were considered as novel pleiotropic loci of greatest interest. Finally, we identified 21 of the most important novel pleiotropic loci (Supplementary Table S10), including 9 pleiotropic loci between depression and HEM, 6 pleiotropic loci between BD and HEM, 3 pleiotropic loci between neuroticism and HEM, and 3 novel pleiotropic loci between SCZ and HEM. The most significant novel pleiotropic locus (Index SNP: rs12705959, $P_{CPASSOC}$ = 4.95E-11) between depression and HEM was located near the intron of FOXP2. The expression of FOXP2 in brain tissue is significantly elevated (34), indicating its association with genetic speech and language disorders, and its involvement in regulating motor function (35). For BD and HEM, a novel pleiotropic topSNP rs144767533 was identified, which was located near the non-coding RNA intron of CUL9. It was also the mapped gene for the most significant novel locus between neuroticism and HEM.

TABLE 1 Genome-wide genetic correlation between psychiatric disorders and HEM by LDSC and HDL.

Method	Trait1	$h2_{trait1}$ (SE)	Trait2	$h2_{trait2}$ (SE)	rg (95%CI)	*P*-value	Intercept (SE)
LDSC	Depression	0.06 (0.0024)	HEM	0.029 (0.0012)	0.28 (0.2347,0.3245)	2.04E-34	0.016 (0.0064)
	BD	0.071 (0.0027)			0.142 (0.0988,0.1854)	1.42E-10	0.004 (0.0062)
	Neuroticism	0.102 (0.0035)			0.197 (0.1552,0.2384)	1.55E-20	0.014 (0.0068)
	SCZ	0.359 (0.0115)			0.101 (0.0611,0.1403)	6.58E-07	0.008 (0.0066)
HDL	Depression	0.05(0.0018)	HEM	0.025 (0.0009)	0.27 (0.2221,0.3173)	1.14E-28	0.009 (0.0007)
	BD	0.059 (0.0020)			0.142 (0.1053,0.1779)	2.03E-14	0.005 (0.0007)
	Neuroticism	0.074 (0.0023)			0.194 (0.1566,0.2320)	4.84E-24	0.008 (0.0008)
	SCZ	0.301 (0.0087)			0.122 (0.0926,0.1510)	3.24E-16	0.011 (0.0013)

h2, heritability; rg, genetic correlation; SE, standard error; HEM, hemorrhoidal disease; BD, bipolar disorder; SCZ, schizophrenia.

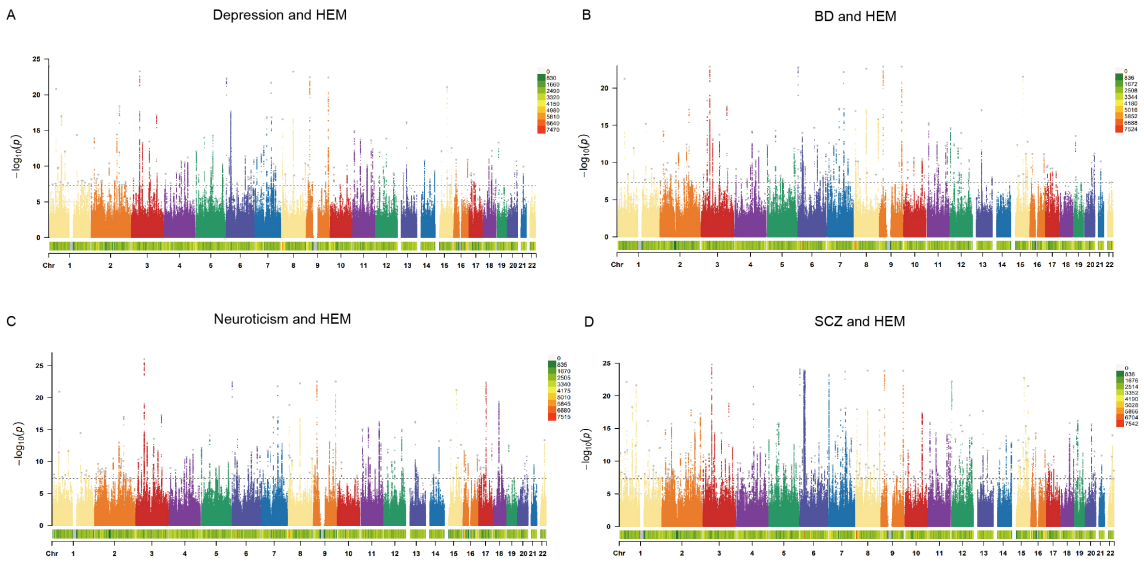

FIGURE 2
The Manhattan plots of CPASSOC. The x-axis represents the chromosomal location of the SNPs, and the y-axis represents the significance (-log10P). The bottom of the Manhattan plots represents the chromosome density. The gray dots represent the independent pleiotropic loci annotated by FUMA. **(A)** The Manhattan plot of shared SNPs based on results of CPASSOC between depression and HEM. **(B)** The Manhattan plot of shared SNPs based on results of CPASSOC between BD and HEM. **(C)** The Manhattan plot of shared SNPs based on results of CPASSOC between neuroticism and HEM. **(D)** The Manhattan plot of shared SNPs based on results of CPASSOC between SCZ and HEM. HEM: hemorrhoidal disease, BD: bipolar disorder, SCZ: schizophrenia.

3.3 Colocalization analysis

A colocalization analysis was conducted to determine whether the multi-effective SNPs driving the association between the two traits are the same. The COLOC analysis identified shared genetic causal variants between each pair of traits in the four sets of CPASSOC outcomes for psychiatric disorders and HEM (Table 2). A total of 17 loci were identified in the four analyses. Among the 134 significant pleiotropy loci associated with depression and HEM, three loci exhibited a PP4 value exceeding 0.7, indicating a potential shared genetic basis underlying these two traits. The significant locus with the highest posterior probability of association between depression and HEM (PPH4 = 88.46%, mapped gene: CELF4) was located in the intergenic region on chromosome 18. It is worth noting that locus 17 (PPH4 = 82.04%, mapped gene: MYT1L) was also identified as one of the most important novel pleiotropic loci in the previous CPASSOC analysis of depression and HEM. The most significant locus 137 (PPH4 = 96.03%) between BD and HEM was mapped to the intergenic region of OSBPL2 on chromosome 20, which plays a key role in lipid transport (36). Three loci overlapped with previous novel pleiotropy loci between BD and HEM. In addition, locus 122 (PPH4 = 71.46%) between SCZ and HEM also coincided with a novel pleiotropic locus in this group.

3.4 Gene-level analysis

A total of 2304 pleiotropic genes with significant associations were identified by MAGMA across the four pairs of traits after correction (FDR qvalue < 0.05). These genes overlapped with the shared loci identified by CPASSOC, including 416 genes between depression and HEM, 495 genes between BD and HEM, 487 genes between neuroticism and HEM, and 906 genes between SCZ and HEM (Supplementary Table S11-S14). Secondly, we identified 17 candidate genes associated with both depression and HEM by matching the aforementioned genes to previously identified colocalization regions. Additionally, we discovered 25 candidate genes shared between BD and HEM, 6 candidate genes between neuroticism and HEM, as well as 18 candidate genes between SCZ and HEM (Supplementary Table S15). MTCH2 (P adjust = 1.08E-11) was the most significant gene between depression and HEM, and it was also a mapped gene for one of the shared loci identified in the previous colocalization analysis between depression and HEM. In addition, the significant gene MYT1L (P adjust = 4.55E-4) was also consistent with the FUMA annotation results based on CPASSOC analysis. For BD and HEM, several significant genes, such as PLEC (P adjust = 5.25E-13) on chromosome 8 overlapped with mapped genes of colocalized genetic loci. ESR1 (P adjust = 1.11E-12) was the most significant gene between neuroticism and HEM. After correction, significant SNP-heritability enrichment across multiple organizations was identified, mainly enriching in various brain tissues. The cerebellum exhibited significant enrichment in all four pairs of traits (Supplementary Figure S2). We further used FUSION for TWAS based on the significant genes identified by MAGMA to investigate the effects of gene expression levels and tissue specificity. We also conducted PWAS to identify plasma protein-trait associations.

We obtained the set of tissue-gene pairs shared by each trait after tissue-specific FDR correction. There were 440 shared gene-

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TABLE 2 Results of colocalization analysis of pleiotropic loci between psychiatric disorders and HEM identified from CPASSOC.

GenomicLocus	TopSNP	Chr: position	A1	A2	BETA		*P*-value		$P_{CPASSOC}$	PP4	NearestGene
					Trait	HEM	Trait	HEM			
Depression and HEM											
126	rs1557339	18:35129076	A	C	0.029	0.024	4.37E-09	2.98E-07	1.14E-11	0.884580042	CELF4
95	rs10838738	11:47663049	A	G	-0.021	0.025	2.99E-06	1.69E-09	1.76E-14	0.82780693	MTCH2
17	rs55933406	2:2297348	C	G	0.021	0.02	1.07E-05	2.35E-06	6.31E-09	0.820422933	MYT1L
BD and HEM											
137	rs13044225	20:60865815	A	G	0.055	-0.02	8.50E-09	8.34E-07	6.08E-12	0.960301673	OSBPL2
132	rs62109878	19:13105333	C	G	-0.046	-0.018	2.39E-06	9.47E-06	9.43E-09	0.90269178	NFIX
36	rs2007403	4:106131210	C	T	0.039	0.028	4.53E-05	1.10E-11	6.57E-15	0.84862958	TET2
55	rs144767533	6:43186138	C	T	-0.062	0.032	4.67E-05	3.52E-07	3.70E-10	0.840720864	CUL9
49	rs12153515	5:164631794	C	T	0.055	0.037	6.99E-05	7.33E-10	1.26E-12	0.817408791	CTB-181F24.1
82	rs55646585	8:144999621	C	T	0.058	0.027	6.95E-09	5.10E-11	1.58E-16	0.781187052	PLEC
115	rs12908161	15:85207825	A	G	-0.065	-0.022	4.68E-10	6.40E-07	6.61E-12	0.711169399	SEC11A
10	rs11684360	2:26942156	C	T	0.042	0.025	3.09E-04	2.68E-07	3.27E-09	0.70348687	KCNK3
Neuroticism and HEM											
46	rs827186	3:158047235	C	T	-0.023	-0.033	1.31E-07	1.39E-05	2.48E-08	0.854796741	RSRC1
77	rs7749650	6:152044872	A	T	0.012	-0.026	4.27E-06	6.80E-09	4.76E-13	0.836922977	ESR1
SCZ and HEM											
116	rs144767533	6:43186138	C	T	-0.065	0.032	3.62E-06	3.52E-07	8.91E-09	0.919688426	CUL9
23	rs6715366	2:2327295	A	G	0.054	0.018	2.49E-08	3.17E-05	3.10E-08	0.84936598	MYT1L
228	rs11638554	15:85148231	G	T	-0.065	-0.02	7.58E-12	3.28E-06	5.10E-12	0.786392303	ZSCAN2
122	rs12207616	6:111608797	A	T	0.055	0.029	1.45E-05	2.78E-07	2.93E-08	0.714630982	RP5-1112D6.4

A1, effect allele; A2, alternative allele; HEM, hemorrhoidal disease; BD, bipolar disorder; SCZ, schizophrenia; PP4, the posterior probabilities of these two features are correlated and share a single causal variant; NearestGene, the nearest gene of the SNP based on ANNOVAR annotations.

tissue pairs observed between depression and HEM (with 109 shared genes), 436 shared gene-tissue pairs observed between BD and HEM (with 108 shared genes), 460 shared gene-tissue pairs observed between neuroticism and HEM (with 122 shared genes), and 763 shared gene-tissue pairs observed between SCZ and HEM (with 219 shared genes) (Supplementary Tables S16–19). A total of 11 loci fell within the colocalization analysis (Figure 3). Of the 14 genes shared between depression and HEM, MTCH2 was shared simultaneously by 2 traits in 13 of the tissues. Two traits share PLEC in 15 of the tissues among the 20 genes between BD and HEM. There are several shared genes that overlap with previously MAGMA co-localized regions, such as DNPH1, ZSCAN2, ALPK3, CUL9, and PTK7 between SCZ and HEM. Based on the significant genes identified by MAGMA, PWAS identified a total of 86 plasma proteins as significantly associated with four traits after multiple testing corrections (FDR qvalue< 0.05) (Supplementary Table S20). There are four plasma proteins shared between depression and HEM, six plasma proteins shared between BD and HEM, two plasma proteins shared between neuroticism and HEM, and seven plasma proteins shared between SCZ and HEM. All pairwise traits share two protein-coding genes (ITIH1, ITIH3). Comparing the shared genes in TWAS of whole blood with the shared proteins in PWAS, we found that the protein-coding gene ITIH4 reached significant levels of mRNA and protein in three pairs of traits of depression, BD and SCZ.

3.5 Functional enrichment analysis

GO analysis revealed that the target genes associated with psychiatric disorders significantly enriched in biological processes such as positive regulation of the nucleobase-containing compound metabolic process, positive regulation of transcription by RNA polymerase ii, neurogenesis, stem cell differentiation, and muscle organ development. The molecular functions focused on transcription factor binding, specific DNA binding, nuclear receptor binding, and calcium channel activity (Figure 4; Supplementary Table S21). The KEGG analysis of SCZ indicates

that pleiotropic genes are enriched in the MAPK signaling pathway. However, no significant KEGG analysis after FDR correction was found for the other pairwise traits.

3.6 Mendelian randomization analysis

Finally, we assessed the causal relationship between psychiatric disorders and HEM using a bidirectional two-sample MR analysis. There were 40, 52, 90, and 150 instrumental variables for depression, BD, neuroticism, and SCZ, respectively, that reached genome-wide significance. Each SNP had an F-value greater than 10 (Supplementary Table S22). Random effects IVW analysis indicated significant positive associations between depression (OR = 1.15, 95% CI = 1.08-1.22, P = 1.72E-05), neuroticism (OR = 1.16, 95% CI = 1.06-1.27, P = 9.21E-04) and SCZ (OR = 1.03, 95% CI = 1.01-1.04, P = 4.34E-04) and increased HEM risk (Supplementary Table S23, Supplementary Figure S3, S4). The results of MRlap indicate that MR outcomes are affected by sample overlap. However, by correcting for sample overlap using MRlap, the causal effect of psychiatric disorders on HEM determined by MRlap is consistent with the primary MR analysis's causal effect, ensuring the robustness of the IVW method (Supplementary Table S25). Despite heterogeneity, the MR-Egger intercept test indicates the absence of horizontal pleiotropy. The leave-one-out results confirmed the reliability of the overall causality (Supplementary Figure S5). However, the inverse MR analysis did not reveal a definitive causal relationship of genetic susceptibility between HEM and psychiatric disorders (Supplementary Table S24).

4 Discussion

To our knowledge, this study was the first comprehensive genome-wide cross-trait analysis of the shared genetic basis between common psychiatric disorders and HEM using large GWAS. Using genetic variation as an instrumental variable, we found some genetic associations between psychiatric disorders and

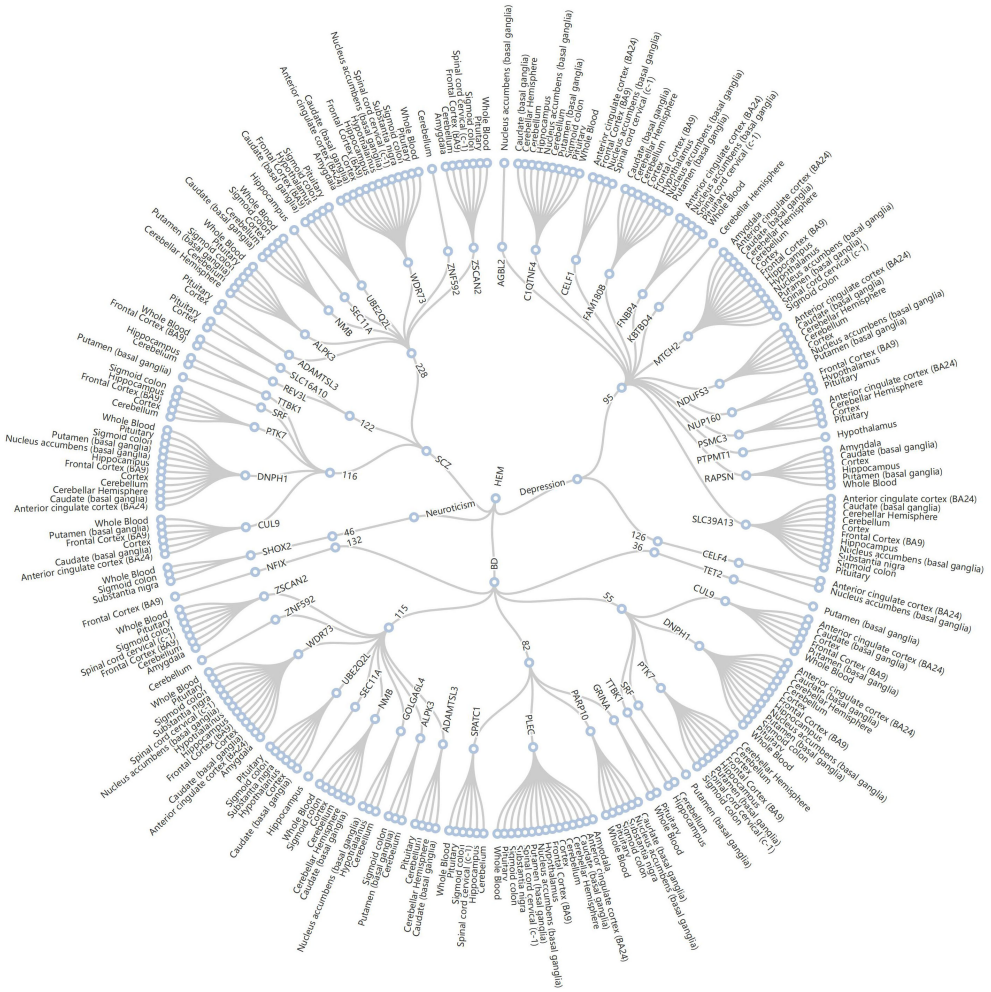

FIGURE 3
Radial dendrogram of TWAS based on colocalized regions. 11 loci for HEM (center point) and four psychiatric disorders (inner circle) fell within the colocalization region (circle 2). The 50 shared genes involved (circle 3) were shared in 16 tissues (circle 4). HEM, hemorrhoidal disease; BD, bipolar disorder; SCZ, schizophrenia.

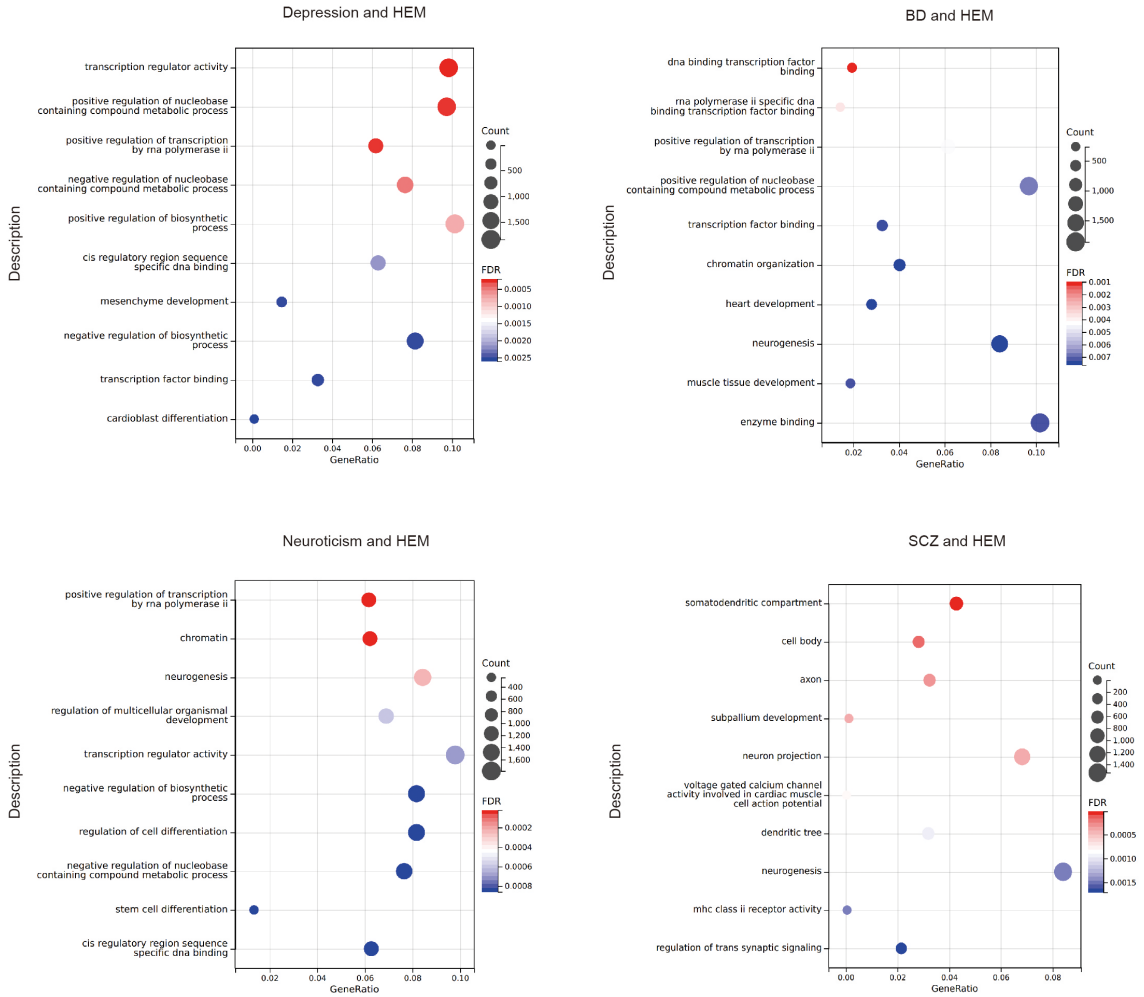

FIGURE 4
The bubble plot of the GO analysis. HEM, hemorrhoidal disease; BD, bipolar disorder; SCZ, schizophrenia.

HEM. First, we assessed the genetic correlations between depression and HEM, BD and HEM, neuroticism and HEM, and SCZ and HEM at both global and local levels. A significant positive correlation was found in all four pairs of traits. Secondly, our study identified multiple shared loci and colocalization evidence through pairwise analysis. Furthermore, we localized to 2304 genes and identified enriched biological processes. Subsequently, the cross-trait genes were validated at the transcriptome and proteome levels. Finally, the MR Analysis provided evidence for the causal relationship between depression, neuroticism, and SCZ and HEM.

We identified 17 pleiotropic loci between psychiatric disorders and HEM through cross-trait and colocalization analysis, obtaining multiple candidate genes. Multiple loci carried genes related to nervous system (MTCH2, MYT1L, CELF4, RSRC1, ZNF592, SHOX2) (37–41), cell proliferation and differentiation (CUL9) (42), and lipid transport (OSBPL2) (36). MYT1L, CELF4 and MTCH2 were given priority as novel pleiotropic genes between depression and HEM. MYT1L is associated with neurons in the brain and encodes MYT1L protein, a member of a zinc finger superfamily of neuronal transcription (37). MYT1L plays an important role in neural development, and is involved in intercellular synaptic transmission, axon development, and neurite growth (43). The CUGBP Elav-like family (CELF) can regulate RNA stability and protein translation, affecting neural development and closely related to neurological diseases (39). CELF4 is significantly enriched in neurons and neuroblasts, mainly in peptidergic neurons. The mitochondrial carrier homolog 2 (MTCH2) was the most significant candidate gene between depression and HEM. As a mitochondrial outer membrane protein, MTCH2 can regulate mitochondrial metabolism and related cell death (44). Research has shown that mitochondrial dysfunction is significantly associated with severe depression (MDD) (45). Kuffner et al. found that fibroblasts from patients with depression had significantly impaired mitochondrial function, as indicated by decreased respiration and decreased adenosine triphosphate (ATP) -related oxygen consumption (46). Single-cell transcriptomics studies indicated that fibroblasts played a critical role in the development, stability, and disease processes of the intestine (47). Our study also found that pleiotropic SNPS between depression and HEM were significantly enriched in fibroblasts (Supplementary Figure S2). Additionally, the effects of

MTCH2 on neurological diseases and adipocyte differentiation have been increasingly recognized in recent years. The study by Cristen et al. pointed to the importance of MTCH2 as a highly expressed gene in the central nervous system in obesity susceptibility (48). Obesity-related oxidative stress tends to promote mitochondrial dysfunction and DNA damage (49). In summary, MTCH2 may be a common risk gene for psychiatric disorders and HEM, highlighting the role of mitochondrial dysfunction in the nervous system.

CUL9 is a member of the Cullin protein family, which plays a crucial role in regulating DNA damage response, cell proliferation, and apoptosis (42). Additionally, CUL9 is involved in the ubiquitination process of various substrates associated with cellular functions (50, 51). The polymorphism of RSRC1 and the brain functional changes in SCZ have been reported in previous studies (52). The mutations of RSRC1 triggered the decay of RSRC1 transcript mRNA in the fibroblasts of patients. ESR1 encodes estrogen receptor α (ER). Estrogen regulates mood-related neurotransmission through receptor-mediated and involved in the interaction of the hypothalamic-pituitary-adrenal axis (53). Plec-encoded lectin located in the chr8:144973183-145086428 (index SNP rs55646585) is a large cell junction protein widely distributed in many tissues, which is highly expressed in skin, muscle, and brain (54). Lectins promote cell-to-cell adhesion. In the brain, PLEC is co-localized with glial fibrillary acidic protein and tau protein to help the structural integrity of astrocytes and neurons (54). In a recent study, PLEC alterations were found to impair the adhesion of EBS-MD fibroblasts (55). The occurrence of muscle malnutrition can cause the intestinal mucosa and muscles to become weak, leading to the downward migration of the mucosa (56). Likewise, further studies are needed to elucidate the complex underlying biological mechanisms.

We obtained evidence of other genes through the results of TWAS and PWAS. PTK7, a pseudo tyrosine kinase lacking catalytic activity, is involved in the occurrence and development of a variety of cancers and is associated with cell survival, growth, and migration (57). Animal experiments showed that PTK7 cleavage in enteroendocrine cells activated the non-canonical Wnt signaling pathway in intestinal stem cells and promoted the migration of stem cells to the wound (58). Two roles of PTK7/Otk in patterning and neurogenesis were observed in a recent study, with OTk-1 expression observed in epithelial and neuronal cells during embryogenesis (59). This may suggest a role for PTK7 in neuronal cell migration. ITIH family genes are common genetic risk factors for a variety of psychiatric disorders (60, 61). The regulatory variant rs2535629 in the ITIH3 intron contributes to SCZ risk (62). ITIH4 was identified as a gene expressed by vascular smooth muscle cells in atherosclerotic plaques and was also associated with inflammatory responses (63).

Additionally, shared genes are enriched in various brain tissues. The shared genes of all pairwise traits showed significant enrichment in the cerebellum, which expressed that the occurrence of comorbidity may mainly depend on abnormal neurological dysfunction. In recent years, the role of the cerebellum in non-motor functions such as cognition and emotion has gradually been understood (64). Studies have shown that changes in the morphology or connectivity of the cerebellum can be observed in dominant or threatened SCZ and BD (65). Another study found that patients with SCZ, BD, or severe depression had altered expression levels of multiple GABAA receptor proteins in the cerebellum lateral region, which helps explain the similarity in the underlying basis of these diseases (66). The enrichment of functions and pathways indicated that pleiotropic genes affect the transcription and expression of downstream genes by regulating the binding process of transcription factors to DNA. It is well known that even within the same organism, the same transcription factors can recognize and control different genes through different assembly modes (67), and are widely involved in embryonic development and cell differentiation (68). Pleiotropy of transcription factors explained the identified targets of comorbidity may play important roles in two different diseases. This is validated by biological process enrichment, where mutations in shared genes may already affect our health during development.

Finally, the MR analysis provided evidence of a causal effect of the three psychiatric disorders on HEM. Consistent with previous findings, genetic susceptibility to HEM was significantly associated with the risk of depression. Our MR results extend the existing MR analysis. For depression, we used a larger sample size for GWAS and explained the potential sample overlap in pairwise traits using MRlap. Future studies with larger sample sizes or more observational studies are needed to complement the work on genetic information.

Our study has several limitations. First, we only focused on large European datasets to reduce population stratification. More data sets on psychiatric disorders and HEM in other populations are needed to generalize our conclusions. Second, there was a certain degree of sample overlap in the selected GWAS dataset. However, the bias introduced by overlapping samples was small, and the study was not affected by this. Finally, we need more experimental studies to validate the potential mechanisms in our research.

5 Conclusion

This study revealed a genetic correlation between four psychiatric disorders and HEM, identified 17 multi-effective genetic loci and multiple candidate genes, and confirmed causal relationships. This has facilitated our understanding of the common genetic mechanism, providing new targets for the treatment of patients with dual chronic burdens of psychiatric disorders and HEM.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

Ethical approval was not required for the study involving humans in accordance with the local legislation and institutional

requirements. Written informed consent to participate in this study was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and the institutional requirements.

Author contributions

ZSC: Data curation, Methodology, Software, Writing – original draft. BH: Software, Writing – review & editing. JS: Data curation, Visualization, Writing – review & editing. YJ: Data curation, Writing – review & editing. ZC: Data curation, Writing – review & editing. CY: Formal analysis, Writing – review & editing. HH: Investigation, Project administration, Supervision, Writing – review & editing. WW: Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2024.1456182/full#supplementary-material

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Augmenting self-guided virtual-reality exposure therapy for social anxiety with biofeedback: a randomised controlled trial

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Introduction: We previously found that self-guided Virtual Reality Exposure Therapy (VRET) improved Public Speaking Anxiety (PSA) and reduced heartrate. Elevated heartrate characterises social anxiety and the self-guided VRET seemed to reduce heartrate. Thus, receiving continuous biofeedback about physiological arousal during the VRET could help socially anxious individuals to manage their anxiety. The present study aimed to determine whether biofeedback enhances the responsiveness of VRET.

Methods: Seventy-two individuals with high self-reported social anxiety were randomly allocated to VRET-plus-biofeedback (n=38; 25 completers) or VRET alone (n=35; 25 completers). Three hour-long VRET sessions were delivered over three consecutive weeks. During each session, participants delivered a 20-minute public speech in front of a virtual audience. Participants in the VRET-plus-biofeedback group received biofeedback on heartrate and frontal alpha asymmetry (FAA) within the virtual environment and were asked to lower their arousal accordingly. Participants in both groups completed psychometric assessments of social anxiety after each session and at one-month follow-up.

Results: PSA improved by the end of treatment and overall social anxiety improved one month after the VRET across both groups. The VRET-plus-biofeedback group showed a steadier reduction in FAA in the first VRET session and a greater reduction in self-reported arousal across the three sessions than the VRET-alone group.

Conclusion: Biofeedback can steady physiological arousal and lower perceived arousal during exposure. The benefits of self-guided VRET for social anxiety are sustained one month after therapy.

KEYWORDS

social anxiety, longitudinal, perceived control, physiological arousal, presence

1 Introduction

Social Anxiety Disorder (SAD) is a marked fear of social situations especially when it involves scrutiny by others (1). People with SAD fear being observed (e.g., eating or drinking), interacting with others and performing before an audience (e.g., public speaking), and they may avoid these situations altogether (1). SAD is the third most reported psychiatric disorder after depression and alcoholism (2), with a lifetime prevalence of 4% worldwide (3). One in three young people now meet the criteria for SAD globally, while one in six deny having social anxiety (4). In the U.K., 0.6% (n=63 of 10,108 residents) were diagnosed with SAD and SAD was most often comorbid with depression (5). While this proportion is low, it is underdiagnosed (4, 6) and undertreated (7, 8). Thus, SAD poses a significant public health risk.

1.1 Self-guided virtual-reality therapy for social anxiety

Recent advances in virtual-reality (VR) technology have resulted in VR-based psychological therapy where realistic scenarios elicited similar emotional responses to that of *in vivo* situations (9, 10). VRET is especially effective for anxiety because clients can encounter anxiety-provoking cues in a controlled manner (11–13). Consequently, VR exposure therapy (VRET) has been found to be as effective as *in vivo* exposure therapy for SAD (14). Patients even prefer VRET over *in vivo* exposure therapy (15, 16).

A further development in VRET is the switch from therapist-led to self-guided intervention (17). Self-guided VRET circumvents the involvement of a therapist, as a non-specialist practitioner can oversee the users' adherence to the digital intervention (11, 18–23). Thus, the benefits of self-guided VRET are that it has a minimal need for a trained therapist (24, 25) and it has reduced economic costs. Self-guided VRET produces notable decreases in self-reported anxiety among those with panic disorder (22) and specific phobias (19, 26). Furthermore, self-guided VRET is already adopted by the National Health Services in England to reduce agoraphobia (25). When considering SAD, self-guided VRET reduced social anxiety more than waitlist after four sessions, with improvement being sustained for six months after exposure (23). In another study (21), the improvement in Public Speaking Anxiety (PSA) following a session of self-guided VRET was comparable to that of a session of therapist-led VRET and this improvement was sustained for 6-12 months after exposure. However, a single session of VRET (21) may not be reliable. A more involved three-week self-guided VRET for PSA was delivered to adolescents at home (20). Here, adolescents could engage in 15 short (two-minute) public-speaking tasks amounting to 60 minutes of VRET each week. The VRET improved PSA more than being on a waitlist. However, this improvement relied on self-report, rather than objective measures of PSA. Thus, further research is needed to objectively test the benefits of several sessions of self-guided VRET for social anxiety.

1.2 Avoidance behaviour and perceived control

Avoidance behaviour is a hallmark of anxiety disorder, where the intolerance to uncertainty generates excessive anxiety and perpetuates avoidance behaviour (27). Anxiety is associated with a reduced sense of control (28), but importantly, the avoidance behaviour itself can facilitate a sense of perceived control over the uncertainty of events, which will then further reinforce avoidance behaviour to maintain control (29). Thus, the more anxious individuals are the less control they perceive and the more they are motivated to avoid the situation. However, 'perceived' control could also be facilitated by the amount of control one has over the exposure to a specific threat when it must be approached, such as control over the perceived distance from threat (11). As such, increasing a sense of control over the gradual exposure to threat in self-guided VRET may help reduce uncertainty and avoidance when one must approach an unknown/risky environment and facilitate engagement and exploration of the virtual environment. Indeed, greater perceived control over exposure to fearful stimuli relates to greater willingness to approach threat (30). According to the Health Belief Model (HBM), individuals engage better with treatment when they believe they have fewer barriers to action (31, 32) and possess control over the therapy (31, 32). Thus, socially anxious people are more willing to engage in performing and even give better performances when they have greater perceived control (33).

Having a sense of presence in the virtual environment could facilitate such perceived control and it is another mechanism of improving the efficacy of VRET. Evidence suggests that having a sense of presence in the virtual environment determines the level of improvement in anxiety in both therapist-led VRET and self-guided VRET (18, 34). These studies of self-guided VRET for social anxiety (11, 20, 21, 23) did not examine role of perceived control or sense of presence as of improvement in social anxiety.

1.3 Using biofeedback to measure treat anxiety

Elevated physiological arousal, such as increased heartrate during an oral presentation (35, 36), is a hallmark of social anxiety. A month of therapist-led VRET for PSA reduced elevated heartrate (37). Likewise, Premkumar and colleagues (2021) found a reduction in heartrate that co-occurred with a reduction in self-reported social anxiety and PSA over two sessions of self-guided VRET. Heartrate can also be measured as variability (HRV), the variation in beat-to-beat heartrate intervals (38). Here, elevated HRV is linked to an adaptive and healthy cardiovascular system (39). Reduced HRV indicates a maladaptive autonomic nervous system (40–44) and is associated with greater psychological distress and fear and avoidance of social interaction (45).

Frontal alpha asymmetry (FAA), especially rightward (right > left hemisphere activity) asymmetry, represents another biomarker of avoidance behaviour. Conversely, leftward FAA indicates an

inclination to approach threat (46) and, in the context of social anxiety, it could denote a willingness for social interaction. Accordingly, highly socially withdrawn individuals have greater rightward FAA when preparing for a speech than less socially withdrawn individuals (47). However, groups high and low in social anxiety did not differ in FAA before and after delivering a speech (48) which could imply that heightened FAA may only relate to social withdrawal and not social anxiety.

Biofeedback about such physiological arousal is important for treating anxiety disorders (49–52) because participants can see the real-time feedback of their physiological reactions and alter their arousal (53). Biofeedback improves the sensation and interpretation of internal physiological signals (54, 55), and aids the practice of emotion regulation (56). Several meta-analyses have noted that biofeedback based on HRV is associated with lower self-reported stress and anxiety (54, 56–58). Biofeedback could even enhance perceived control since learning to synchronise respiration rate with observed heartrate and to relax increases perceived control over situations (55, 59) and enhances creativity (60). In turn, greater perceived control through effective emotional regulation lowers heartrate (61). Thus, therapies use biofeedback to manage anxiety (58) in both clinical (50, 62–65) and non-clinical populations (51, 52, 66). A meta-analysis of randomised controlled trials of biofeedback for anxiety disorders revealed greater improvement in anxiety in the biofeedback-based intervention (broadly defined) than waitlist, but weaker improvement than an active control (67), thus implying the modest benefits of biofeedback as a standalone intervention. If VRET reduces physiological arousal and distress, giving continuous biofeedback to participants about their physiological arousal could help socially anxious individuals to manage their distress. When VR therapy for anxiety includes biofeedback, five out of seven studies reported significant, albeit modest, reductions in self-reported anxiety (Hedge's g=0.28) and heartrate (g=-0.45) (53). However, these studies mostly delivered a single session of treatment (53).

1.4 Aims and hypotheses

The current study aimed to evaluate the benefit of biofeedback on heartrate, FAA and responsiveness to self-guided VRET in social anxiety (11, 68). It was hypothesised that compared to self-guided VRET alone, improvements will be greater for self-guided VRET +biofeedback in terms of:

1. PSA, social anxiety and confidence as a speaker,
2. Continuous self-assessment of anxiety and arousal during VRET sessions, and
3. Physiological arousal (heartrate and leftward FAA).

In addition, it was hypothesised that across both groups

4. Improvement in social anxiety would be sustained for one month,
5. Perceived control would explain the long-term improvement in PSA and social anxiety, and
6. A greater sense of presence in the virtual environment would predict greater responsiveness to treatment.

2 Methods

2.1 Participants

Six-hundred and sixty-five participants from the general population completed the initial screening survey. Participants were recruited by placing posters around the university, in local general medical practices, libraries and community centres and on social networking platforms, such as Twitter and Facebook. Three hundred and ninety-seven participants (60%) scored 32 and above on the Social Phobia Inventory (SPIN), indicating moderate-to-high social anxiety (69). Participants were invited to the randomized controlled trial (RCT), the next stage of the study, if they scored >19 on the SPIN, a score which has 79% accuracy with detecting social anxiety (69). Participants were recruited for the RCT until a target of n=75 was reached, namely VRET +Biofeedback, n=38 and VRET alone n=37. Other barriers to participation might have led to participants self-selecting for the RCT, such as motivation to travel to attend an in-person session, meet strangers and confront anxiety in the intervention. Seventy-three participants were recruited and successfully completed the first session. SPIN scores ranged from 20 to 67 (mean=46 ± 10) in the final sample (n=73), suggesting high levels of social anxiety. Participants mostly represented those from the East Midlands region of England who were aged 18 years and above, and had normal or corrected vision with contact lenses as they needed to see the virtual environment clearly. Participants were randomized to VRET+biofeedback and VRET-alone groups and age, sex and ethnicity were similarly distributed between the groups. Likewise, the groups were matched in the level of social anxiety and the number diagnosed with social anxiety disorder or other psychiatric disorder (Table 1).

2.2 Materials

2.2.1 Social Phobia inventory

The SPIN (69) was used to screen for social anxiety. The 17 items assess self-reported fear, avoidance and physical sensations associated with social anxiety. Items were rated on a five-point Likert scale from 0 = "Not at all" to 4 = "Extremely". Scores range from 0 to 68, and individuals who meet the DSM-IV diagnostic criteria for social anxiety typically have a mean score>40 (70). The SPIN has adequate to good internal consistency (Cronbach's alpha >0.80 in 69; 0.94 in the current study), test-retest reliability (r=0.78 and 0.89) and convergent validity (69).

2.2.2 Personal Report of Confidence as a Speaker

The short form of the PRCS scale (71) was used to measure PSA. It consists of 12 true-or-false items on fear of public-speaking. Its psychometric properties include convergent validity with measures of social anxiety and divergent validity with a measure of sociability (71, 72). The PRCS has good internal reliability (Cronbach's alpha=0.85, 72) (Table 2). The summary score was the average rating of all the items.

TABLE 1 Comparison between VRET+biofeedback and VRET alone groups on demographic characteristics and change in anxiety over the course of the three sessions of the VRET and one-month follow-up.

Outcome measure	VRET+Biofeedback (n=38)	VRET alone (n=35)	Cohen's d	Chi-square or F-value (*p*)
*Age	25.47 (9.72)	30.44 (12.34)	0.45	3.54 (0.065)
Sex (% female)	86.8	62.9		5.97 (0.051)
Ethnicity (% White)	76.3	57.1		3.03 (0.081)
Social anxiety disorder diagnosis (% with current or past diagnosis)	21.1	28.6		0.55 (0.457)
Other psychiatric diagnosis (% with current or past diagnosis)	48.0	29.2		1.83 (0.176)
SPIN at baseline	46.4 (10.89)	45.71 (9.72)	0.68	0.08 (0.772)

*Welch test was performed due to significant heterogeneity of variance.

2.2.3 Public-Speaking Anxiety

The PSA scale (73) is a 17-item measure of cognitive, behavioural and physiological dimensions of PSA. Items were rated on a five-point Likert scale from 0 = "Not at all" to 4 = "Extremely". The sum of individual items was calculated after reverse scoring some items. Scores range from 17 to 85. The scale's significant correlations with other self-reported measures of speech anxiety and measures of anxiety, but weak correlation with a measure of depression evidence the scale's good concurrent validity, convergent validity and discriminant validity, respectively (73). The internal consistency was good in the original development study (Cronbach's alpha=0.94; 73) and the current study (Table 2).

2.2.4 Liebowitz Social Anxiety Scale

The LSAS (74) measures fear and avoidance in different social and performance situations. Twelve statements concern social interactions, such as going to a party and meeting strangers. A further 12 items concern performance situations, such as eating in public spaces and working under observation. For each statement, participants were asked how much they feared that situation and how much they avoided the situations. The Likert scale ratings of fear were 0 = "None", 1 = "Mild", 2 = "Moderate" and 3 = "Severe". The Likert scale ratings of avoidance were 0 = "Never (0%)", 1 = "Occasionally (1—33%)", 2 = "Often (33—67%)" and 3 = "Usually (67—100%)". Thus, there are four subscales, Fear of Performance situations, Avoidance of Performance situations, Fear of Social interaction situations and Avoidance of Social interaction situations. The summary score of each subscale was the sum of all the items in that subscale. The scale has good convergent validity with other measures of social anxiety (75). The four subscales had acceptable to good internal consistency in the current study (Table 2).

2.2.5 Brief Fear of Negative Evaluation

The BFNE scale (76) has 12 items on the fear of being evaluated by others. FNE is where the person is concerned that others will think badly of them and criticise them (77). FNE is a hallmark of PSA and social anxiety (77, 78). The items were rated on a five-point Likert scale from 0 = "Not at all characteristic of me" to 0 = "Extremely characteristic of me". The summary score was the sum of all the items after reverse scoring some items. The scale demonstrated good internal reliability in previous studies (76, 79, 80) and the current study (Table 2), and good test-retest reliability (76, 81). The BFNE has good construct validity since patients with social anxiety score higher than non-anxious patients on the BFNE (80).

2.2.6 Visual analogue scale ratings of behavioural avoidance, arousal and anxiety

Participants rated VASs from 0 to 100 within the virtual environment at each session (a) before entering the virtual lecture room, (b) during each pause and (c) after leaving virtual lecture room. The VASs measured avoidance of giving a speech (this alone was assessed before and after each VRET session), arousal and anxiety. Arousal was defined as feeling vigorous, lively, energetic and alert. Anxiety was defined as dryness of mouth, difficulty breathing, trembling, feeling panicked, increased heartrate and scared.

2.2.7 Presence questionnaire

Nineteen items measure sense of presence in the virtual environment (82). Items were rated on a seven-point Likert scale with the descriptors of the anchor points varying from item to item. The scale has five subscales with poor to good internal reliability (Table 2). *Realism* refers to how natural and compelling the environment was. *Possibility to Act* enquires about controlling and surveying the environment. *Quality of Interface* gauges delays to one's actions appearing in the environment and being distracted by the environment when completing the task. *Possibility to Examine* refers to examining the environment closely. *Self-evaluation of Performance* involves adjusting to and being proficient with interacting with the environment.

2.2.8 Behavioural Inhibition System – appraisal subscale

The Behavioural Inhibition System (BIS)-appraisal subscale of an inhouse measure of reinforcement sensitivity (83) was used as a proxy measure of perceived control. BIS relates to perceived control

TABLE 2 Comparison between completers and non-completers of the three VRET sessions on the outcome measures at baseline.

Outcome measure	Cronbach's alpha (n=73)	Completers Mean (SD)	Non-completers Mean (SD)	Cohen's d	*F* (df)	*p* value	Group-by-Completion status interaction *F* (df)[†]	*p* value
Baseline								
		n=50	n=23					
PRCS	0.85	0.73 (0.19)	0.68 (0.26)	0.24	0.87 (1,71)	0.353	0.92 (3,69)	0.435
Avoid giving a presentation	NA	78.20 (16.97)	74.39 (25.78)	0.19	0.56 (1,71)	0.455	1.26 (3,69)	0.295
PSA	0.94	65.12 (9.93)	62.56 (9.98)	0.26	1.04 (1,71)	0.311	0.48 (3,69)	0.697
BFNE	0.88	35.44 (7.53)	33.48 (10.49)	0.23	0.83 (1,71)	0.366	0.54 (3,69)	0.659
LSAS – Fear of Performance	0.85	20.02 (7.99)	19.39 (10.29)	0.07	0.08 (1,71)	0.777	0.69 (3,69)	0.652
LSAS – Fear of social situations	0.91	18.72 (8.27)	17.74 (9.70)	0.11	0.20 (1,71)	0.657	0.84 (3,69)	0.475
[‡]LSAS – Avoidance of Performance	0.82	17.77 (7.45)	17.00 (8.29)	0.10	0.15 (1,71)	0.698	0.77 (3,66)	0.517
[‡]LSAS – Avoidance of social situations	0.88	17.45 (8.14)	16.30 (8.29)	0.14	0.30 (1,71)	0.585	0.86 (3,66)	0.464
RST – BIS appraisal	0.82	3.29 (0.69)	3.26 (0.75)		0.01 (1,71)	0.885	1.27 (3,66)	0.292
Post-session 1								
		n=48	n=15					
Presence – realism	0.82	34.18 (6.73)	31.20 (7.67)	0.43	2.11 (1,61)	0.152	0.63 (1,58)	0.432
Presence – possibility to act	0.63	20.42 (3.56)	19.47 (4.60)	0.25	0.70 (1,61)	0.405	2.20 (1,58)	0.143
Presence – quality of interface	0.66	12.17 (5.15)	9.80 (3.86)	0.48	2.68 (1,61)	0.107	2.46 (1,58)	0.122
Presence – possibility to examine	0.71	14.29 (3.41)	13.27 (4.06)	0.29	0.94 (1,61)	0.335	0.71 (1,58)	0.402
Presence – self-evaluation of performance	0.76	11.12 (2.01)	9.47 (2.12)	0.81	**7.46 (1,61)**	**0.008**	0.50 (1,58)	0.484

[†]Completers and non-completers in the VRET+biofeedback group = 25 and 13; completers and non-completers in the VRET-alone group = 25 and 10; [‡]Completers and non-completers in the VRET+biofeedback group = 22 and13; completers and non-completers in the VRET-alone group = 25 and 10; BFNE, Brief Fear of Negative Evaluation scale; LSAS, Liebowitz Social Anxiety Scale; NA, Not applicable because the scale is a single item; PRCS, Personal Report of Confidence as a Speaker scale; PSA, Public-Speaking Anxiety scale; RST_BIS, Reinforcement Sensitivity Theory – Behavioural Inhibition Scale; RST_BAS, Reinforcement Sensitivity Theory – Behavioural Approach Scale. Values in bold are statistically significant.

over negative life events (28). Furthermore, locus of control partly explains the relationship between BIS and trait anxiety (84). BIS-appraisal is seen as an essential component of anxiety that involves monitoring risk and carefully appraising information about uncertainty, weighing up the pros and cons of a situation before engaging in approach or avoidance behaviour (27). This in-house BIS-appraisal subscale forms part of a measure of trait anxiety and consists of four items that are rated on a four-point Likert scale from 1 = "Very false for me" to 4 = "Very true for me".

2.3 Physiological arousal measures

2.3.1 Heartrate

Heartrate was measured continuously during the public speech in each VRET session. Data were collected from a Microsoft Band 2 biometric wristband which has 11 sensors for tracking heartrate and blood pressure (Figure 1A). Heartrate was sampled at 10 Hz and the average beats per minute were calculated for each four-minute speech block during the VRET session.

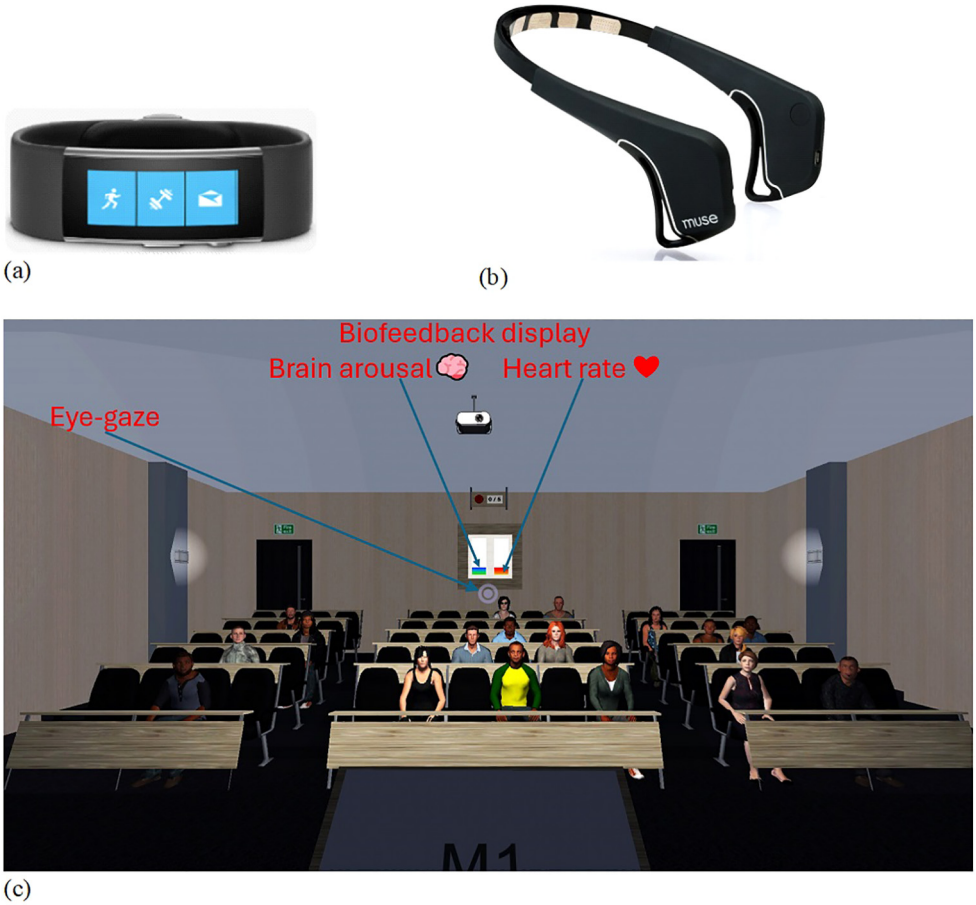

FIGURE 1
Physiological arousal measurement using **(A)** Microsoft Band 2 biometric wrist band to measure heart rate and **(B)** Muse brain sensing headband to measure frontal alpha asymmetry, and **(C)** virtual-reality lecture hall with biofeedback display.

2.3.2 Electroencephalography

Frontal electrical signals were recorded continuously from a Muse wireless EEG headband (Figure 1B) (85). Frontal alpha power was sampled at 220 Hz from the AF7 and TP9 channels on the left and AF8 and TP10 channels on the right with the FPz site as the reference. Average frontal alpha power was calculated from the 60 samples per minute during each four-minute speech block. FAA was calculated as (the average of AF7 and TP9 on the left) minus (the average of AF8 and TP10 on the right).

2.3.3 Self-guided VRET software and hardware

A Samsung Gear VR headset housed a Samsung Galaxy S7 smartphone through which the VRET application displayed the virtual environment. The VRET application was developed using the Unity real-time 3D development platform (86). The Unity-based VRET smartphone application was deployed to the Android operating system on the Samsung Galaxy S7 smartphone. Heartrate data were collected through the smartphone application, which was connected to Microsoft Band 2. A bespoke plugin developed in Java bridged the Java-based official Microsoft Band software to the VRET smartphone application.

2.4 Virtual environment design and self-guided manipulation

Participants gave a 20-minute speech about "going on a holiday". The session was broken into five four-minute speech blocks. Participants spoke spontaneously using prompts (e.g., dream destination and sight-seeing) that appeared on a podium in the virtual environment. After each speech block, participants had a brief (one minute) pause to perform the following tasks, namely respond to VASs on anxiety and arousal, navigate to a 'settings menu' and change the exposure level of five exposure elements. Each modifiable element had three grades (G) of exposure ranging from low to high: (i) audience size consisting of 6 (G1), 12 (G2) or 20 people (G3); (ii) audience reaction comprising approving (G1), neutral (G2) or disapproving reactions (G3); (iii) speaker's distance from the audience being far (G1), near (G2) or nearest (G3); (iv) number of speech prompts per slide, each slide having many (G1), moderate (G2) or few prompts (G3); and (v) salience of self having no poster (G1), a silhouette with the label "Speaker" (G2), or a photo of the participant and their full name (G3). Participants were encouraged to increase their exposure to threat

at their own pace (see 11, for further details). Those in the VRET +biofeedback group received continuous biofeedback about their arousal level and were asked to monitor and lower their arousal accordingly. The biofeedback consisted of two vertical bars displayed on the rear of the virtual lecture theatre that oscillated, with the red bar denoting heartrate and the blue bar denoting FAA (Figure 1C).

2.5 Procedure

Ethical approval was obtained from the university's Research Ethics Committee, ethics application number No. 2017/115. Participants gave informed consent and received a £15 shopping voucher for each VRET session (69). Participants were randomly allocated to the two interventions single-blind using a randomisation list. Before session one, participants completed an online survey containing the BFNE, LSAS, PRCS and PSA scale for the baseline assessment. Participants in the two groups did not differ across those measures at baseline, $F(1,71)<2.14$, $p>0.148$. The PRCS was readministered after each VRET session and at four-week follow-up. The Presence Questionnaire was administered after the first VRET session. The BFNE, LSAS and PSA scale were readministered at the end of therapy (after the three sessions) and at one-month follow-up. The sessions were held in the same laboratory throughout the study. Thus, the ambient room temperature was monitored to ensure that the change in temperature did not alter physiological arousal.

2.6 Statistical analysis

2.6.1 Missing data analysis and manipulation checks

Chi-square tests compared the number of completers in each group at each session. Analyses of variance (ANOVAs) compared treatment completers and non-completers on each self-reported scale at baseline. The analyses were repeated with Group as an additional independent variable (IV). These analyses determined whether multiple imputation could replace missing data from non-completers at subsequent sessions. Multiple imputation was then performed using the iterative Markov Chain Monte Carlo method.

2.6.2 Hypothesis-testing

A series of 2x2 mixed-design ANOVAs was performed with group (VRET+biofeedback and VRET-alone) and time (baseline, post-treatment) as the IVs and measures of social anxiety as the dependent variables (hypothesis 1). Two 2x3x4 mixed-design ANOVAs were performed with Group (VRET+biofeedback vs. VRET-alone), Time (sessions 1, 2 and 3) and Block (1, 2, 3 and 4) as IVs and the self-reported VASs of arousal and anxiety as the dependent variables (DVs) (hypothesis 2). Two further 2x2 ANOVAs were performed with Group (VRET+biofeedback vs. VRET-alone) and Time (first minute of the first session and last minute of the third session) as IVs, and heartrate and FAA as the DVs (hypothesis 3). Another ANOVA was performed with Group (VRET+biofeedback vs. VRET-alone), Minute (1, 2, 3 and 4) and Block (1, 2, 3 and 4) as IVs, and heartrate and FAA at just the first VRET session as the DVs (hypothesis 3).

2x3 ANOVAs using quadratic contrasts as the model of comparison were performed with Group (VRET+biofeedback vs. VRET-alone) and Time (baseline, post-treatment and one-month follow-up) as the IVs, and the scores on BFNE, LSAS, PRCS and PSA scale as the DVs to test long-term improvement in social anxiety (hypothesis 4). Then, analyses of covariance (ANCOVAs) were performed on the same measures with the appraisal subscale of the rRST as a covariate (hypothesis 5). Multiple linear regressions were performed with the subscales of the Presence Questionnaire at session one as the predictor variables and change-relative-to-baseline on PSA and social anxiety as the criterion variables (hypothesis 6). Change relative to baseline was calculated as follows,

$$\text{Change relative to baseline} = \frac{\text{Score at baseline} - \text{Score at end of treatment}}{\text{Score at baseline}} \times 100$$

.

3 Results

3.1 Comparison between completers and non-completers on outcome measures

The number of completers in the VRET+biofeedback group was 34 (89%), 29 (76%), 25 (66%) and 19 (45%) at sessions 1, 2 and 3 and one-month follow-up, respectively. The number of completers in the VRET-alone group was 29 (81%), 26 (72%), 25 (69%) and 23 (64%) at sessions 1, 2 and 3 and one-month follow-up respectively. There was no difference between groups in the rate of dropout at any session, Chi-square<1.25, P>0.228. Completers and non-completers did not differ on any social anxiety measure at baseline (Table 2). Therefore, missing data of non-completers were replaced with predicted scores obtained from multiple imputation on all social anxiety measures. Completers also rated the 'self-evaluation of performance' subscale of the Presence Questionnaire at the end of session 1 higher than the non-completers.

3.2 Change in PSA and social anxiety from baseline to end-of-treatment

There was a main effect of Time on PSA, $F(1, 71)=42.23$, $p<0.001$, PRCS, $F(1, 71)=53.39$, $p<0.001$ and avoidance of giving a speech, $F(1, 71)=116.24$, $p<0.001$, suggesting improvement in PSA by the end of treatment (Figures 2A-C). The reliable change index (RCI) (20, 87) was used to determine if the change was clinically meaningful,

$$\text{RCI} = \frac{x_2 - x_1}{\sqrt{2(SE)^2}}$$

where x_2 is the score at end of treatment (or follow-up) and x_1 is the score at baseline. SE was the standard error of the difference between the two sets of scores. An RCI>1.96 is considered clinically meaningful. The change was clinically meaningful for each measure, RCI_{PSA} = -4.4, RCI_{PRCS} = -5.4, $RCI_{avoidance}$ = -7.6, where a negative sign means a reduction in the scores.

However, the main effect of Time was not significant for BFNE or LSAS, $F(1,71)<2.66$, $p>0.108$ (Figures 2D-H). There was a trend for greater improvement in PSA in the VRET-alone group than the VRET+biofeedback group (Table 3; Figure 2C). Correspondingly, RCI_{PSA}=-2.1 in the VRET+biofeedback group and RCI_{PSA}=-4.5 in the VRET alone group. There was a trend for greater improvement on LSAS-avoidance of performance in the VRET+biofeedback group than the VRET-alone group (Figure 2G). However, these changes did not correspond to clinical significance with clinical significance, $RCI_{LSPS\ -\ avoidance\ of\ performance}$=-1.4 in the VRET +biofeedback group and $RCI_{LSPS\ -\ avoidance\ of\ performance}$=-0.9 in the VRET alone group.

Some participants in the VRET+biofeedback group (n=12) and the VRET-alone group (n=13) completed the VASs of anxiety and arousal during every pause of all three VRET sessions. There was a marginal gender bias in the likelihood of completing the VASs, with women in the VRET+biofeedback group (n=11) being more likely to complete these VASs than in the VRET-alone group (n=7), $\chi^2(1)=3.55$, $p=0.059$. There was a Group-by-Session-by-Block interaction for VAS-arousal, $F(5.6, 130)=2.24$, $p=0.046$. According to the plot of VAS arousal (Figure 3), self-reported arousal was lower at sessions 2 and 3 of the VRET+biofeedback intervention compared to session

TABLE 3 Comparison between VRET+biofeedback and VRET alone groups on demographic characteristics and change in anxiety over the course of the three sessions of the VRET and one-month follow-up.

Outcome measure	VRET+Biofeedback (n=38) Relative change from baseline to follow-up, mean (SD)	VRET alone (n=35) Relative change from baseline to follow-up, mean (SD)	Cohen's d for group difference in relative change from baseline to follow-up	Change from baseline to end-of-treatment F statistic of Group-by-time interaction (H1)	Change from baseline to follow-up (H2, time points)	*Group-by-time F (H3)	*Change from baseline to follow-up Main effect of time F (*p*)	*Time (P1 to FU) with BIS appraisal as a covariate F (*p*) (H4)
†PRCS	25.14 (39.23)	23.8 (34.96)	0.04	0.612 (0.433)	P1, S1, S2, S3, FU	0.04 (0.834)	**10.09 (0.002)**	0.12 (0.731)
†Avoidance of giving a presentation	34.20 (37.66)	29.49 (30.09)	0.14	1.039 (0.312)	P1, S1, S2, S3, FU	**4.03 (0.049)**	**37.75 (<0.001)**	0.57 (0.451)
†PSA	15.36 (15.75)	17.83 (15.54)	0.16	3.91 (0.052)	P1, S3, FU	**5.83 (0.018)**	**10.58 (0.002)**	0.11 (0.746)
†BFNE	8.53 (22.04)	9.06 (24.05)	0.25	0.18 (0.675)	P1, S3, FU	0.28 (0.599)	**22.85 (<0.001)**	0.26 (0.608)
†LSAS – Fear of Performance	30.10 (35.79)	51.74 (69.79)	0.40	3.077 (0.084)	P1, S3, FU	**6.22 (0.015)**	**48.88 (<0.001)**	1.84 (0.179)
†LSAS – Fear of social situations	22.08 (75.72)	38.68 (40.98)	0.27	2.42 (0.124)	P1, S3, FU	**5.29 (0.024)**	**52.00 (<0.001)**	2.13 (0.149)
†LSAS – Avoidance of Performance	42.89 (33.64)	33.36 (40.49)	0.26	3.82 (0.055)	P1, S3, FU	1.62 (0.207)	**36.41 (<0.001)**	2.34 (0.129)
†LSAS – Avoidance of social situations	33.48 (38.87)	52.34 (71.30)	0.33	0.002 (0.960)	P1, S3, FU	0.001 (0.979)	**19.49 (<0.001)**	2.47 (0.120)

*F-statistic is based on quadratic contrasts between time points due to the lag in the level of change during the one-month follow-up after the three sessions, unless otherwise specified; †VRET +biofeedback group, n = 38, VRET alone group, n = 35; BFNE, Brief Fear of Negative Evaluation scale; FU, follow-up; LSPS, Liebowitz Social Anxiety Scale; H1, H2 and H3, Hypotheses 1, 2 and 3; P1, baseline, PRCS, Personal Report of Confidence as a Speaker scale; PSA, Public-Speaking Anxiety scale; S1, S2, S3, Sessions 1, 2 and 3, respectively; SPIN, Social Phobia Inventory. Values in bold are statistically significant.

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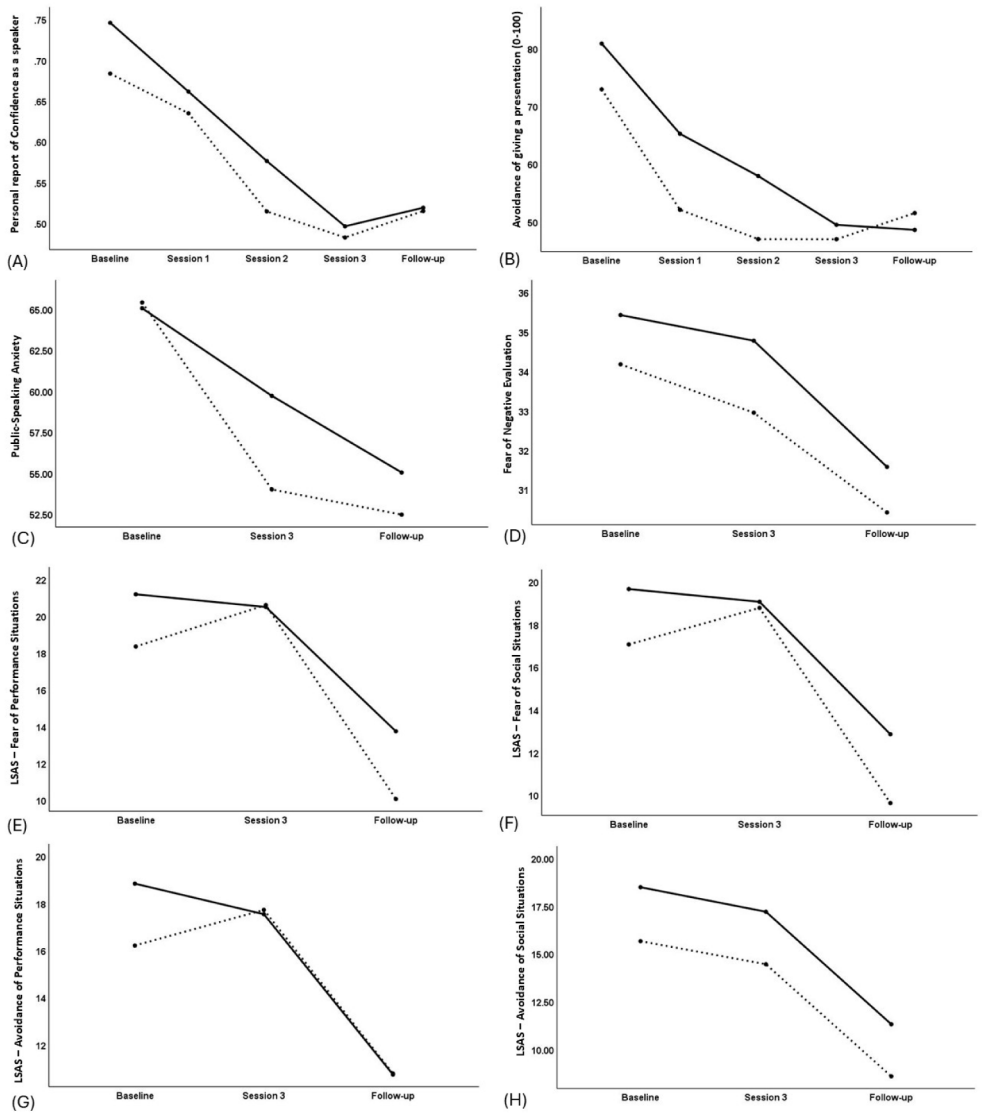

FIGURE 2
Plot of time (x-axis) by outcome measure (y-axis) in the VRET+biofeedback group (solid line) and VRET-alone group (broken line) for the following outcome measures, **(A)** Personal Report of Confidence as a Speaker, **(B)** Avoidance of giving a presentation, **(C)** Public-speaking Anxiety, **(D)** Brief Fear of Negative Evaluation, **(E)** Liebowitz Social Phobia Scale – Fear of Performance situations, **(F)** Liebowitz Social Anxiety Scale – Fear of Social Situations, **(G)** Liebowitz Social Anxiety Scale – Avoidance of Performance situations, **(H)** Liebowitz Social Anxiety Scale – Avoidance of Social situations. For all scales, greater reduction relative to baseline means greater improvement in anxiety.

1, and the decline was steadier from one pause to the next in each session. In contrast, self-reported arousal changed haphazardly between sessions and pauses in each session in the VRET-alone group. There was no Group-by-Session-by-Block interaction for VAS-anxiety, $F(4, 91.8)=0.66$, $p=0.620$.

3.3 Change in PSA and social anxiety at one-month follow-up

The main effect of Time was significant for all measures of PSA and social anxiety, $F>10$, $p<0.001$ (Table 3). Again, these changes were clinically meaningful, RCI_{PRCS} = -5.2, $RCI_{avoidance}$ = -6.3, RCI_{PSA} = -6.3, RCI_{BFNE} = -3.4, $RCI_{LSPS\ -\ Fear\ of\ performance}$ = -7.3, $RCI_{LSPS\ -\ Fear\ of\ social\ situations}$ = -6.7; $RCI_{LSPS\ -\ Avoidance\ of\ performance}$ = 6.5, $RCI_{LSPS\ -\ Avoidance\ of\ social\ situations}$ = -6.4. Furthermore, the Group-by-Time interaction was significant for avoidance of giving a presentation, PSA, LSAS-Fear of Performance and LSAS-Fear of Social Situations (Table 3). The effect size (Cohen's d) of the difference in the improvement between the VRET-alone group and the VRET+biofeedback group was medium for LSAS-Fear of Performance, small for BFNE, LSAS – Fear of social situations, LSAS – Avoidance of Performance and LSAS – Avoidance of social situations and negligible for PRCS, Avoidance of giving a presentation and PSA. Thus, the VRET-alone group showed greater improvement on avoidance of giving a presentation and PSA than the VRET+biofeedback group at the end of treatment, but the improvement levelled between the groups at follow-up

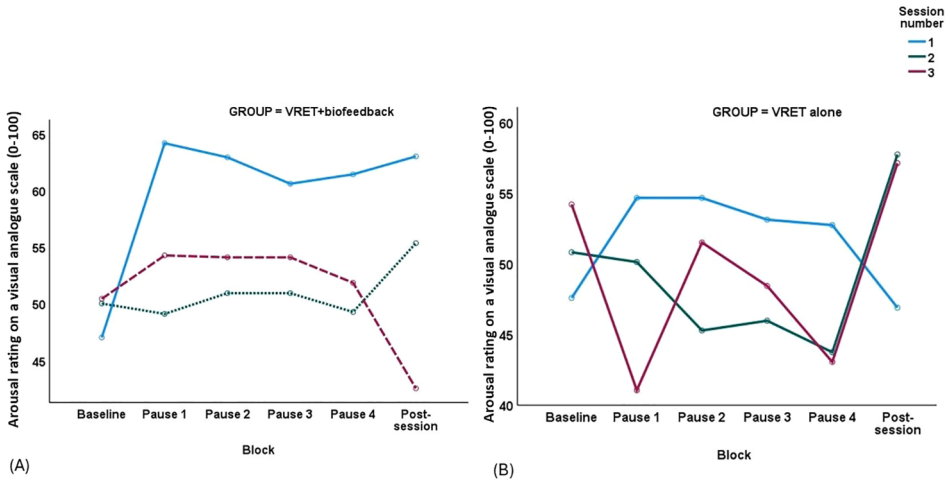

FIGURE 3
Plot of block (x-axis) by self-reported arousal (y-axis) in **(A)** the VRET+biofeedback group and **(B)** VRET alone group across the three sessions, session 1 – continuous line, session 2 – dotted line and session 3 – broken line.

(Figures 2B, C). The improvement from end-of-treatment to follow-up on LSAS-Fear of Performance and LSAS-Fear of Social Situations was greater in the VRET-alone group than the VRET +biofeedback group (Figures 2E, F, respectively).

3.4 Improvement social anxiety after covarying for BIS-appraisal at baseline

The main effect of time was no longer significant after covarying for BIS-appraisal for any measure of PSA or social anxiety (Table 3).

3.5 Change in physiological arousal during the VRET

There was a main effect of time on heartrate among participants with heartrate data at every minute of the four speech blocks and across all three sessions (VRET+biofeedback, n=8, and VRET-alone, n=10), F (1, 16)=6.24, p=0.024. When examining the change in heartrate at just the first VRET session (VRET+biofeedback, n=22, and VRET-alone, n=16), the Group-by-Block-by-Minute interaction with non-linear (fourth order) contrasts was not significant, F(3, 48.3)=0.32, p=0.81. Still, a decline in heartrate from one block to the next after the first block appeared steadier the VRET+biofeedback group (Figures 4A and B).

There was no effect of time on FAA among participants with FAA data at every minute of each VRET session (10 VRET+biofeedback, n=10, and VRET-alone participants, n=12), F(1, 20) = 0.33, p=0.573. However, the Group-by-Block interaction with non-linear (cubic) contrasts was significant when studying FAA in 20 VRET +biofeedback participants and 16 VRET-alone participants at the first VRET session alone, F(1)=5.86, p=0.021. The VRET+biofeedback group showed a steady decline in FAA, while the FAA in the VRET-alone group changed haphazardly from one block to the next (Figure 5).

3.6 Prediction of change in PSA and social anxiety by sense of presence

A greater sense of presence at session 1 predicted improvement in PSA at end of session 3, F (5,55) = 5.58, p<0.001 (Table 4). Specifically, greater ability to examine significantly predicted improvement in PSA, standardised beta = 0.53, p<0.001.

4 Discussion

This is the first RCT to assess the role of biofeedback in the responsiveness of individuals with social anxiety to self-guided VRET. The hypothesis of greater improvement in PSA and social anxiety in the VRET+biofeedback group than the VRET-alone group was not supported (hypothesis 1). However, the hypothesis of greater continuous improvement in self-reported arousal during each VRET session in the VRET+biofeedback group was supported (hypothesis 2). Furthermore, the VRET+biofeedback group showed a steadier reduction in FAA from one block to the next than the VRET-alone group at the first VRET session alone (hypothesis 3). In addition, the hypothesized improvement in PSA and social anxiety one month after therapy in both groups was supported (hypothesis 4). Another hypothesis that perceived control, as measured by BIS-appraisal in this study, would explain the improvement in PSA and social anxiety following self-guided VRET (hypothesis 5) was upheld. Lastly, a greater sense of presence when examining the virtual environment predicted greater improvement in PSA at end of treatment (hypothesis 6).

4.1 Improvement at end of treatment and at follow-up

Our findings strengthen the case for self-guided VRET as a potential treatment of choice. Our first study tested university students with high PSA (11). There, a post-treatment improvement was observed in PSA, but not other measures of social anxiety. In the current study too, PSA, but not other measures of social anxiety improved at the end of treatment regardless of the presence or absence of biofeedback. However, the benefits of the self-guided VRET extended to measures of social anxiety at follow-up in the present study, with all measures of PSA and anxiety

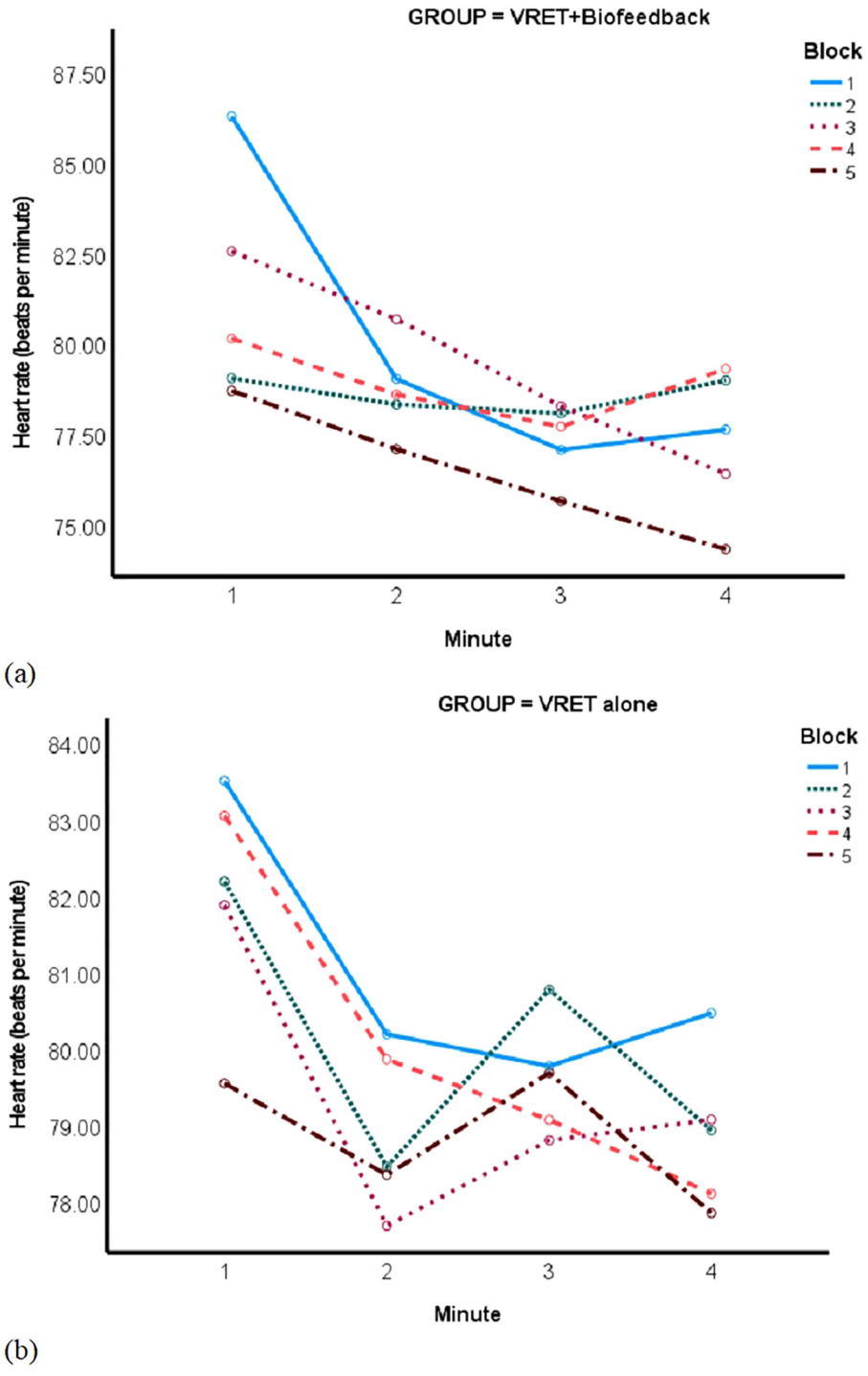

FIGURE 4
Plot of time in minutes (x-axis) by heartrate (y-axis) by block (separate lines) in **(A)** VRET+biofeedback and **(B)** VRET alone groups.

having a clinically meaningful improvement. The current study tested participants with high social anxiety from the general community and so, this study tested whether the improvement in PSA and social anxiety would generalise to socially anxious individuals. The improvement in PSA at end of treatment was clinically meaningful. This improvement in PSA corresponded with a reduction in heartrate in both groups during each session, suggesting that the improvement in PSA was both perceived and real.

This improvement could be attributed to the sense of presence in the virtual environment in terms of being able to examine elements of the self-guided VRET, such as the five modifiable elements, because it predicted improvement in PSA. A sense of presence within a virtual environment consists of existing in a physical space, in social interaction and experiencing a sense of togetherness with others (88). The association between presence and treatment outcome in the present study may exist because a sense of presence elicits the anxiety that VRET alleviates (89). Sense of presence accounts for improvement in symptoms of acrophobia following self-guided VRET (18). However, sense of presence did not predict treatment outcome following therapist-led VRET for arachnophobia in one study (26), yet it did for agoraphobia in another study (34). Thus, the predictive value of sense of presence may vary by type of phobia, especially when virtual avatars are involved. VRET for SAD is as effective as *in vivo* exposure therapy for SAD (14) and this suggests that patients with SAD can meaningfully perceive threat from a virtual audience and experience a meaningful improvement in FNE. The greater improvement on the BFNE from baseline to follow-up (small effect size) in the VRET-alone group compared with the VRET +biofeedback group suggests that the VRET-alone group may have engaged with the VRET better which may have lasting improvement in how socially anxious individuals perceive the threat of negative evaluation from others. Thus, the threat from a virtual audience could affect how participants interpret others' evaluation of them even though participants know that the audience is not real.

4.1.1 The role of biofeedback

The VRET+biofeedback group displayed greater improvement in self-reported arousal at sessions 2 and 3 than at session 1, and participants rated their arousal more consistently at each pause than the VRET-alone group. Furthermore, a steadier decline in FAA from the first to the last block of the first VRET session featured in the VRET +biofeedback group. Participants in the VRET+biofeedback group were told to lower the biofeedback bars if they went up. This process of controlling the visual display of physiological arousal may have steadied the participants' FAA and heartrate and improved their perceived control. The steadier decline in FAA suggests that participants could apply greater cognitive control and gradually lower their arousal. Greater awareness of physiological sensations through biofeedback (54, 55) could improve cognitive control and address the heightened physiological arousal that is a key symptom of anxiety. The findings of the study support the evidence of the benefits of biofeedback as an intervention for anxiety (67) and in combination with VR therapy (53).

Lower heartrate relates to greater perceived control when emotion regulation is high (61). Participants in the VRET +biofeedback group may have learned to use the biofeedback to lower their arousal at sessions 2 and 3, a technique that the VRET-alone group did not learn, and this would have resulted in the lower self-reported arousal at each pause of the self-guided VRET. People with high FNE have a greater P2 amplitude, an event-related potential, during angry faces relative to neutral faces which denotes heightened early attention to negative facial expressions (90). Biofeedback could reduce arousal from such negative attentional bias. Furthermore, biofeedback produces improvement in anxiety, with effect sizes varying from moderate to large due to

TABLE 4 Multiple regression analysis between subscales of the Presence Questionnaire and change in measures of social anxiety and public-speaking anxiety from baseline to end of therapy (n=62).

	F	R (R^2)	Standardised Beta				
			Realism	Possibility to act	Quality of interface	Possibility to examine	Self-evaluation of performance
PRCS	1.08	0.30 (0.09)	-0.13	0.14	0.03	0.15	0.16
Avoidance of giving a presentation	2.22	0.41 (0.17)	-0.10	-0.02	-0.01	0.40	0.15
PSA	**5.85**	0.57 (0.35)	-0.22	0.24	-0.13	**0.53**	0.10
BFNE	0.57	0.22 (0.05)	0.16	-0.01	-0.12	0.07	-0.03
LSAS – Fear of Performance	0.44	0.20 (0.04)	0.14	0.14	-0.04	-0.20	-0.07
LSAS – Fear of social situations	0.97	0.29 (0.08)	0.02	0.28	-0.05	-0.08	-0.24
LSAS – Avoidance of Performance	0.42	0.20 (0.04)	0.10	0.05	0.09	-0.12	0.12
LSAS – Avoidance of social situations	0.58	0.23 (0.05)	0.06	0.18	0.09	-0.07	0.03

Values in bold denote correlations that were significant at $p<0.001$.

variation between studies in number of sessions, age and sample size (91). Thus, greater awareness of arousal during social interactions could help socially anxious individuals to regulate their arousal and report lower arousal. Accordingly, the VRET +biofeedback group showed a marginally higher improvement on LSAS–avoidance of performance at the end of treatment. The findings of the study support the evidence of the benefits of biofeedback as an intervention for anxiety (67) and as an intervention alongside VR therapy (53).

4.1.2 Effect of self-guided VRET on long-term improvement

Regardless of the greater reduction of physiological arousal in the VRET+biofeedback group, the VRET-alone group improved more than the VRET+biofeedback group at one month follow-up on LSAS-Fear of Performance and to a lesser extent LSAS-Fear of Social Situations. Thus, learning to regulate arousal from biofeedback over three self-guided VRET sessions may be insufficient to sustain a long-term improvement in social anxiety. Sustaining attention to the stimulus display of the biofeedback is a challenge of biofeedback training (53). Thus, scaffolding self-guided VRET with biofeedback may have temporary rather than sustained benefits, making participants more reliant on this ongoing feedback to maintain improvement in social anxiety. Simply showing biofeedback is ineffective (Weerdmeester, J. W. et al., 2020). Instead, training in breathing in relaxation during the biofeedback may be more rewarding and retain learning (53, 55). Awareness of physiological arousal through biofeedback may diminish confidence in developing active coping strategies to reduce physiological arousal beyond the VRET session which the VRET-alone group may have developed better. Indeed, heightened introceptive awareness increases social anxiety (55). Biofeedback and neurofeedback as a form of therapy must involve operant learning, such as training in interpreting the feedback and being rewarded for achieving learning goals (91, 92). Gamifying the response to the biofeedback, such as receiving a star rating to successful down-regulation of arousal could reinforce learning (20). Participants in the present may have found down-regulating one's arousal during the biofeedback without being commended for their success frustrating. The challenge of down-regulating one's arousal during the biofeedback may have diminished the perceived benefits of the self-guided VRET on PSA and social anxiety. Nonetheless, the sustained improvements following self-guided VRET, regardless of biofeedback, espouse the long-term benefits of the self-guided VRET for social anxiety.

The lived experiences of self-guided VRET may give further insight into the observed effects of the self-guided VRET. Participants provided written feedback about the benefits of the self-guided VRET at one-month follow-up. Participants expressed that the therapy made them more relaxed and less anxious, and it increased their confidence with delivering presentations, even helping some to get a distinction on an assessed presentation,

> "I definitely felt more relaxed towards the end of the experiment. It helped me to talk slower and focus on my breathing to relax myself. It also made me aware of certain verbal ticks [sic] that I use when giving a speech."

Participants also benefitted from the repeated practice even if they felt that the virtual environment could have been more realistic,

> "It was very interesting, the environment was very cool, but could be improve upon if slightly more realistic. I'm glad I took part, I think the repetition and having a talk prepared was a really good idea. I think it definitely improved my nervousness."

4.2 Perceived control and risk appraisal as a mechanism for the efficacy of the SGV for social anxiety

Avoidance behaviour as a hallmark of anxiety disorder is facilitated through perceived control over exposure to uncertainty (29). Higher intolerance of uncertainty over future events is linked to greater perceived control over avoidant behaviour (29). Here (29), perceived control was measured as a relief over averting an aversive unconditioned stimulus (a loud noise) after encountering the conditioned stimuli (innocuous images) (29). Such intolerance of uncertainty could perpetuate avoidant behaviour. BIS-appraisal – the tendency to monitor and appraise risk under conditions of uncertainty – underpins elevated trait anxiety and avoidance motivation (27). BIS-appraisal (83) was used a proxy measure of perceived control in this study and it fully explained the improvement in PSA and social anxiety following self-guided VRET. The BIS-appraisal measure denotes the ability to weigh the pros and cons of a situation before engaging with it. This ability to appraise situations could aid appraisal of threat during self-guided VRET, inspire confidence in the benefits of the therapy and reduce avoidant behaviour. Indeed, greater perceived control encourages socially anxious individuals to give better performances (33). Thus, a prior ability to carefully understand situations and stay in control could give anxious individuals more control over their self-guided graded exposure, more confidence in self-guided VRET and improve social anxiety.

4.3 Limitations and future research

This study had a high attrition rate by the end, with the percentage of dropout increasing from 10% at session 2 to 55% at follow-up in the VRET+biofeedback group and from 19% at session 2 to 36% at follow-up in the VRET-alone group (Table 3). Non-completers were less likely to feel present in the virtual environment. The immersion and realism of the self-guided VRET experience could be improved to address the dropout rate and participant written feedback. Adverse effects were not routinely monitored, but some participants withdrew due to adverse events.

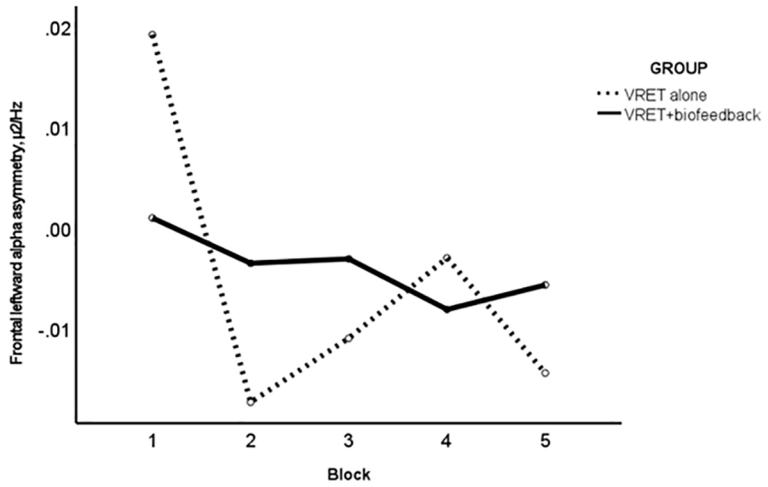

FIGURE 5
Plot of block (x-axis) by frontal alpha asymmetry (μ^2/Hz; y-axis) in session 1; VRET+biofeedback (solid line) and VRET alone (broken line).

Adverse events included distress arising from exposure to the virtual audience, finding the public-speaking challenging, having physical discomfort after the first session and becoming anxious. Participants were not guided about how to lower heartrate during biofeedback which could have undermined confidence in coping strategies and hindered the sustained improvement in social anxiety. We have developed a machine learning algorithm for integrating heartrate, FAA and other cognitive-performance-based measures into multi-sensory integrated feedback (93). Such machine-learning algorithms are based on prolonged, rather than momentary, physiological responses and could prove more reliable to participants. Lastly, asking participants to give a presentation in front of a real audience would help to understand how participants apply learning about arousal from biofeedback in real-world situations. The limited realism of the virtual audience was noted by participants in their written feedback. Encountering animated emotions in the virtual environment may have limited relevance when faced with the social judgements of a real audience. Thus, the fidelity of the intervention must be tested in front of a real audience.

5 Conclusion

Three weekly sessions of self-guided VRET produce a clinically meaningful improvement in PSA and social anxiety up to one month after therapy. The accompanied reduction in heart rate reinforces the objective benefits of this self-guided VRET. People with a sub-clinical level of social anxiety could do the self-guided VRET as homework (20) before social situations, such as interacting with family and peers, use of public spaces, job interviews and other performance situations. Furthermore, VRET+biofeedback reduced heartrate and FAA and maintained a steady level of physiological and self-reported arousal. VRET+biofeedback also reduced social avoidance in performance situations marginally more than VRET-alone. The heightened physiological awareness from biofeedback may explain the responsiveness of self-guided VRET since FAA is linked to social withdrawal (47). Being able to examine the virtual environment and focus on the assigned activities was important in improving the experience of the self-guided VRET, since this ability predicted the improvement in PSA. These benefits of the self-guided VRET could help socially anxious individuals who are on a waitlist for treatment from a therapist. These benefits could also help meet the targets of clinical services to offer treatment within six weeks, reduce the burden on clinical services, reduce costs of a trained therapist and reduce therapist burnout that causes errors of judgement (94).

Furthermore, greater perceived control in terms of weighing the pros and cons of a situation before engaging with it explained the improvement in PSA and social anxiety. Teaching participants practical strategies to manage perceived control over impending threat and uncertainty during VRET sessions could sustain the long-term benefits of self-guided VRET (29). Deterioration rates of virtual-reality therapies are comparable with other active therapies (95); this finding alongside the practical benefits of our self-guided VRET increases the credibility of self-guided VRET as a viable accessible therapy to encourage engagement with *in vivo* therapies.

Data availability statement

The raw data presented in the study are publicly available. This data can be found here: https://doi.org/10.5281/zenodo.13995305. Further inquiries can be directed to the corresponding author/s.

Ethics statement

The studies involving humans were approved by the ethics committee of the Nottingham Trent University School of Social Sciences, ethics application number No. 2017/115. The studies were conducted in accordance with the local legislation and institutional

requirements. The participants provided their written informed consent to participate in this study.

Author contributions

PP: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. NH: Conceptualization, Funding acquisition, Investigation, Methodology, Resources, Validation, Visualization, Writing – review & editing. JM: Investigation, Methodology, Resources, Visualization, Writing – review & editing. PF: Validation, Visualization, Writing – original draft. SB: Data curation, Methodology, Resources, Software, Visualization, Writing – review & editing. AS: Conceptualization, Funding acquisition, Investigation, Methodology, Software, Validation, Visualization, Writing – review & editing. DB: Conceptualization, Funding acquisition, Investigation, Methodology, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Corrigendum: Augmenting self-guided virtual-reality exposure therapy for social anxiety with biofeedback: a randomised controlled trial

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A Corrigendum on

Augmenting self-guided virtual-reality exposure therapy for social anxiety with biofeedback: a randomised controlled trial

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In the published article, there were errors in the **Abstract**, *Methods* and *Results*. The number of therapy sessions and position of the subheadings were incorrect. The **Abstract**, *Methods* and *Results* previously stated:

"**Methods**: Seventy-two individuals with high self-reported social anxiety were randomly allocated to VRET-plus-biofeedback (n=38; 25 completers) or VRET alone (n=35; 25 completers). Three hour-long VRET sessions were delivered over two consecutive weeks. During each session, participants delivered a 20-minute public speech in front of a virtual audience.

Results: Participants in the VRET-plus-biofeedback group received biofeedback on heartrate and frontal alpha asymmetry (FAA) within the virtual environment and were asked to lower their arousal accordingly. Participants in both groups completed psychometric assessments of social anxiety after each session and at one-month follow-up. PSA improved by the end of treatment and overall social anxiety improved one month after the VRET across both groups. The VRET-plus-biofeedback group showed a steadier reduction in FAA in the first VRET session and a greater reduction in self-reported arousal across the two sessions than the VRET-alone group."

The corrected version appears below:

"**Methods:** Seventy-two individuals with high self-reported social anxiety were randomly allocated to VRET-plus-biofeedback (n=38; 25 completers) or VRET alone (n=35; 25

completers). Three hour-long VRET sessions were delivered over three consecutive weeks. During each session, participants delivered a 20-minute public speech in front of a virtual audience. Participants in the VRET-plus-biofeedback group received biofeedback on heartrate and frontal alpha asymmetry (FAA) within the virtual environment and were asked to lower their arousal accordingly. Participants in both groups completed psychometric assessments of social anxiety after each session and at one-month follow-up.

Results: PSA improved by the end of treatment and overall social anxiety improved one month after the VRET across both groups. The VRET-plus-biofeedback group showed a steadier reduction in FAA in the first VRET session and a greater reduction in self-reported arousal across the three sessions than the VRET-alone group."

The authors apologize for these errors and state that this does not change the scientific conclusions of the article in any way. The original article has been updated.

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Brain function abnormalities and inflammation in HIV-positive men who have sex with men with depressive disorders

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Background: Depressive disorders are highly prevalent among people with HIV (PWH) and are related to aberrant inflammation and immune responses. However, there is currently a lack of investigation into the neurological, inflammatory, endocrine, and immune aspects of HIV-associated depressive disorders (HADD).

Methods: The study involved 33 HIV-positive men who have sex with men with depressive disorders (HADD group) and 47 without neuropsychiatric disorders (HIV control group). Participants underwent resting-state functional magnetic resonance imaging (rs-fMRI) scans and assessments of peripheral blood. Peripheral blood cytokines, plasma concentrations of hormone and neurotrophic factors, and immune cell levels were determined using liquid chip, enzyme-linked immunosorbent assay, and flow cytometry, respectively. The correlation of imaging alterations with clinical variables and peripheral blood indicators was assessed.

Results: Compared to the HIV control group, the HADD group exhibited a higher fractional amplitude of low-frequency fluctuations in the left superior parietal gyrus, lower regional homogeneity in the left precentral gyrus, and reduced voxel-wise functional connectivity for the seed region in the right precentral gyrus with clusters in the right cuneus, etc. Furthermore, the HADD group had higher levels of interferon-gamma, a higher frequency of non-classical monocytes, and higher expression levels of perforin and CD38 on specific cells. These imaging results were significantly correlated with peripheral blood indicators and clinical variables.

Conclusion: This rs-fMRI study provides considerable evidence for abnormal intrinsic brain activity in people with HADD. Furthermore, our data also indicate the detrimental effects of depression-related inflammation on PWH. Therefore, it is imperative to increase attention to HADD and implement effective preventive interventions accordingly.

KEYWORDS

human immunodeficiency virus, depressive disorders, resting-state functional magnetic resonance imaging, peripheral immunity, inflammation

1 Introduction

HIV-associated depressive disorders (HADD) are highly prevalent in the era of potent antiretroviral therapy, even when patients are virally suppressed (1). The presence of depressive disorders negatively affects medication adherence, disease progression, and mortality in people with HIV (PWH), placing a serious burden on patients, their families, and society (2). Several factors associated with HIV/acquired immunodeficiency syndrome (AIDS) status contribute to the high prevalence of depressive disorders, including infectious-immunological, psychosocial, and external factors (3–5). Substantial evidence suggests that HIV reservoirs in the central nervous system (CNS) may cause brain injury through chronic inflammation (6). Chronic inflammation and immune activation significantly contribute to non-AIDS-related neuropsychiatric adverse events (including HADD) in PWH (7, 8). Nonetheless, the pathogenesis of HADD is highly complex and the relationship between the neurological, inflammation, and immune systems of HADD is unclear. Investigations of brain function may help clarify the impact of HADD on the brain.

Magnetic resonance imaging (MRI) is the most frequently employed method in depressive disorder studies, and it has provided invaluable insights into the neuropathology of HIV (9). Previous studies have illustrated that PWH present brain activation abnormalities and gray matter atrophy compared to healthy controls (10, 11). Nevertheless, current studies on HADD by MRI are lacking. Firstly, more attention has been paid to the neuropsychiatric conditions in PWH. However, depressive disorders are extremely underdiagnosed in HIV/AIDS largely due to the lack of professional evaluation by highly-trained psychiatrists. Some clinicians prefer to use scales and questionnaires rather than specialized diagnostic tools for neuropsychiatric disorders. Secondly, in the field of HIV research, studies mainly focus on patients with HIV infection as well as those without infection. In particular, there have been numerous studies on HIV-associated neurocognitive disorders using MRI in this area. However, there is limited research on HIV-associated neuropsychiatric conditions such as depressive disorders in imaging. Thus, empirical data on brain imaging alterations in people with HADD is scarce.

To investigate changes in brain function among people with HADD, we examined the resting-state functional MRI (rs-fMRI) in a cohort of men who have sex with men (MSM) with HADD, comparing them to a well-matched group of HIV-positive MSM without neuropsychiatric disorders. Additionally, given the association between depression, inflammation, neurotrophins, endocrine, and immunity (12–15), we aimed to explore the effects of these factors on brain imaging as well as depressive disorders among PWH. Finally, correlation analyses were conducted to explore the relationships between imaging alterations, clinical data, inflammation-related markers, neurotrophic factors, endocrine indicators, and immune variables. The results of this investigation can provide potential theoretical foundations and data support for future studies on neuronal imaging and inflammation related to HADD.

2 Materials and methods

2.1 Participants

This cross-sectional study obtained approval from the Institutional Ethics Committee of Beijing Youan Hospital, Capital Medical University (2023/057). Prior to signing a written informed consent form, all participants were informed of the entire process and potential risks. The inclusion criteria for this study were as follows: (1) virologically suppressed HIV-infected individuals; (2) Chinese MSM; (3) aged at least 18 years; (4) right-handed; (5) not taking antidepressants; and (6) capable of signing an informed consent form. The exclusion criteria were: (1) individuals with current or previous opportunistic CNS infections; (2) individuals with a history of neurological disorders such as epilepsy, multiple sclerosis, Parkinson's disease, or dementia; (3) individuals with MRI contraindications or claustrophobia; (4) previously experienced head injury with loss of consciousness for more than 30 minutes; and (5) substance abuse. Eventually, 106 participants were enrolled in our research project between May 2022 and November 2022.

We selected participants with HADD (defined as the HADD group) and those without neuropsychiatric disorders (defined as the HIV control group) for subsequent analysis. Sixteen participants

with other types of neuropsychiatric disorders were excluded from the study. All participants underwent clinical, MRI, and peripheral blood assessments on the same day.

2.2 Clinical assessments

2.2.1 Diagnosis of neuropsychiatric disorders

Psychiatric diagnoses were determined by a psychiatrist following the diagnostic criteria in the *Diagnostic and Statistical Manual of Mental Disorders*, 5th edition (16).

2.2.2 Neurocognitive, mood, and sleep assessments

Neurocognitive function assessment was conducted using the Montreal Cognitive Assessment (MoCA) (17). Anxiety and depression levels of all participants were evaluated using the Self-Anxiety Scale (SAS) and the Self-Depression Scale (SDS), respectively (18, 19). Psychological health status was evaluated using the Symptom Checklist 90 (SCL-90) (20, 21). Sleep quality was assessed by the Pittsburgh Sleep Quality Index (PSQI) (22).

2.2.3 Other assessments

Childhood maltreatment history was evaluated utilizing the Childhood Trauma Questionnaire (CTQ) (23). The assessment of alcohol craving included the administration of the Alcohol Urge Questionnaire (AUQ) and the Visual Analogue Scale (VAS) (24, 25).

2.3 MRI data acquisition

All imaging data were obtained using a 1.5 T MRI scanner (Philips, Amsterdam, The Netherlands) at the Second Hospital of Beijing. Foam cushions were utilized to restrain head movement. All participants were instructed to lie down, relax, close their eyes, and not think about anything specific to avoid falling asleep.

Whole-brain resting-state functional MRI data were acquired using a gradient echo planar imaging sequence. The acquisition parameters were as follows: repetition time/echo time (TR/TE) = 4019.8/30 ms, slices = 40, matrix = 64 × 62, flip angle = 90°, slice thickness = 3.5 mm, no gap, volumes = 102, and scanning time = 6 min 52 s. The structural images were used for the registration process of functional images, with the following acquisition parameters: shortest TR/TE = 8.3/3.9 ms, matrix = 256 × 227, field of view = 256 mm × 256 mm, flip angle = 12°, slice thickness = 1 mm, and slice number = 384.

2.4 Image preprocessing

Image preprocessing and statistical analyses were conducted using Matlab R2023a (The MathWorks, Natick, MA, USA). Initially, the raw data were inspected for anatomical abnormalities and scanner artifacts. All image data obtained in digital imaging and communications in medicine format were converted to neuroimaging informatics technology initiative format for further processing and analysis.

The rs-fMRI data were preprocessed using Statistical Parametric Mapping 12 (SPM12, https://www.fil.ion.ucl.ac.uk/spm/software/spm12/) and the Rs-fMRI Data Analysis Toolkit (REST, http://www.restfmri.net) (26). The processing pipeline included the following steps: first, removal of initial volumes (n = 5), followed by slice timing correction and motion correction. Then, the functional images were co-registered with individual T1-weighted structural images and spatially normalized by the Montreal Neurological Institute brain template (27). The normalized functional images were resampled to an isotropic voxel size (3.0 × 3.0 × 3.0 mm), smoothed with a full-width half maxima (FWHM) Gaussian kernel (6 mm), and underwent linear drift removal. The Friston 24-parameter head motion model was applied to eliminate the impacts of head motion (28, 29). To further remove nuisance signals, regression was performed on the average white matter and cerebrospinal fluid signals. Finally, a temporal band-pass filter (0.01-0.08 Hz) was applied to eliminate drifts and physiological noise.

Nine participants were removed from further analysis due to excessive head motion (displacements > 2.0 mm or rotations > 2.0°).

2.5 Computation of brain rs-fMRI metrics

2.5.1 Computation of amplitude of low-frequency fluctuations (ALFF)/fractional ALFF maps

The ALFF value of each voxel was obtained by computing the mean square root of the power spectrum within the frequency range (0.01-0.08 Hz). The ALFF maps of all participants were computed to measure spontaneous brain activity (30). The fALFF measures the ratio of the power spectrum within a specific frequency range to the power spectrum across the entire frequency range. In order to standardize the variation between participants, the ALFF/fALFF maps of each individual were normalized to the mean ALFF/fALFF map and used for between-group comparisons.

2.5.2 Computation of regional homogeneity (ReHo) maps

The ReHo value for each voxel was computed using Kendall's coefficient of concordance for that voxel and its 26 neighboring voxels (31). Subsequently, Gaussian smoothing with a FWHM of 6 mm was applied to ReHo maps to diminish residual differences and noise in gyral anatomy. To mitigate the overall impact of differences between subjects, we computed the mean ReHo for each subject for group comparison.

2.5.3 Computation of seed-based whole-brain functional connectivity (FC) maps

Previous neuroimaging studies have indicated that depressive disorders are related to focal functional and structural abnormalities in various brain regions, including the precentral gyrus, dorsolateral

prefrontal cortex, medial prefrontal cortex, insula, hippocampus, anterior cingulate cortex, posterior cingulate cortex, precuneus, and caudate nucleus (32, 33). For FC analysis, the average blood-oxygen level-dependent time series from twenty-two seed regions were calculated. These seed regions included the bilateral precentral gyrus, medial part of the superior frontal gyrus, dorsolateral part of the superior frontal gyrus, medial orbital part of the superior frontal gyrus, anterior cingulate and paracingulate gyri, median cingulate and paracingulate gyri, posterior cingulate gyrus, insula, hippocampus, amygdala, precuneus, and caudate nucleus. In addition, brain regions showing group differences in ALFF, fALFF, and ReHo analyses were also selected as seed regions for FC analysis. The detailed information of these seed regions is shown in Supplementary Table 1. These seed region masks were derived from the automated anatomical labeling atlas (34). Before conducting intergroup comparisons using these FC maps, Fisher *r*-to-*z* conversion was applied to elevate the normality of the FC maps.

2.6 Cytokine and chemokine assay: Luminex® xMAP® technology

Inflammation has been reported to be associated with depression as well as some brain functional alterations (35–37). We examined the plasma levels of 37 cytokines and chemokines in the participants. The cytokine and chemokine assays on plasma samples were conducted using the MILLIPLEX® MAP Human Cytokine/Chemokine/Growth Factor Panel A MAGNETIC BEAD PANEL 96-Well Plate Assay (EMD Millipore, Billerica, MA, USA), which is based on the cutting-edge Luminex® xMAP® technology. All steps were conducted according to the manufacturer's instructions. Detailed methods can be found in the Supplementary Material.

2.7 Assessment of hormones and neurotrophic factors

The correlations of endocrine hormones and neurotrophic factors were determined using enzyme-linked immunosorbent assay kits. We primarily analyzed seven hormones and neurotrophic factors. For detailed information on these analyses, please refer to Supplementary Table 2. All measurements were conducted according to the manufacturers' protocols.

2.8 Mass cytometry and data analysis

This study used 23 custom antibodies to identify various immune cells. These antibodies were purchased preconjugated from Fluidigm (South San Francisco, USA). Detailed information about the antibodies and reporter isotopes is available in Supplementary Table 3. The cell labeling process followed established protocols (38). Please refer to the methods section of the Supplementary Material for detailed methods and operational procedures. Using a doublet-filtering approach, the raw data of each pre-processed sample was de-barcoded utilizing distinct mass-tagged barcodes. The.fcs files produced by various batches were standardized using the bead normalization technique. Subsequently, meticulous gating was performed using FlowJo software (version 10.9.0) to remove debris and dead cells. Lymphocytes and monocytes were then artificially gated for further analysis using the R language. The PhenoGraph clustering technique was used to separate the cells into many clusters based on the expression levels of surface markers. To reduce dimensionality and visualize the high-dimensional data, a visual dimensionality reduction approach called *t*-distributed stochastic neighbor embedding was used. The distribution of each cluster, the expression of markers, and differences between groups or sample types were analyzed using R software (version 4.2.3).

2.9 Statistical analysis

The statistical analysis was conducted using SPSS software (version 25.0; IBM Corp., Armonk, New York, USA). The significance threshold (α) was set at 0.05. The normality of the data was assessed using the Kolmogorov-Smirnov and Shapiro-Wilk tests. For normally distributed data, continuous variables were expressed as mean ± standard deviation; for non-normally distributed data, continuous variables were expressed as median and interquartile range. Based on the results of the normality test, a two-sample *t*-test or Mann-Whitney *U*-test was employed to compare continuous variables between groups. Categorical data were presented as proportions. Chi-square tests and Fisher's exact tests were employed to compare categorical variables between groups. In correlation analysis, Pearson correlation analysis was performed for normally distributed data, and Spearman correlation analysis was performed for non-normally distributed data. An additional analysis was conducted to explore whether the observed group differences were attributable to the primary effects of depressive disorders, ART regimens, or the interaction between them.

In voxel-based comparisons, two sample *t*-tests were used to examine differences between the two groups in terms of ALFF, fALFF, ReHo, and FC. Age and years of education were used as covariates in the analysis process. The significance level was determined using a voxel threshold of $P < 0.001$ and a cluster threshold of $P < 0.05$ (corrected for multiple comparisons using false discovery rate (FDR), Gaussian random field, and AlphaSim correction). For further exploratory analyses, a threshold of $P < 0.001$ was applied (uncorrected for multiple comparisons). Imaging results were displayed using MRIcroGL software (https://www.nitrc.org/projects/mricrogl/). The imaging results were correlated with clinical data and peripheral blood metrics. Statistical results were graphed using GraphPad Prism software (version 9.5.1; GraphPad Software, San Diego, CA, USA).

3 Results

3.1 Demographic and clinical characteristics of participants

A total of 80 participants successfully completed the study, with 33 participants (41.25%) in the HADD group and 47 participants

(58.75%) in the HIV control group. Most demographic variables were well-matched between the two groups. The demographics and clinical characteristics are comprehensively outlined in Table 1.

In comparison to the HIV control group, the HADD group exhibited significantly higher SAS scores ($P < 0.001$), SDS scores ($P < 0.001$), PSQI scores ($P = 0.004$), and SCL-90 scores ($P < 0.001$) (see Table 1 and Supplementary Table 4 for details). There were no differences between the two groups in CTQ, AUQ, VAS for alcohol craving, MoCA, or medication status (Table 1).

3.2 Comparison of brain rs-fMRI metrics

3.2.1 ALFF/fALFF

The HADD group exhibited higher fALFF in the left superior parietal gyrus compared to the HIV control group (FDR correction, voxel-level $P < 0.001$, cluster-level $P < 0.05$; see Figure 1 and Supplementary Table 5 for details). In this final result, the largest cluster size consists of less than 10 continuous voxels, and there was no difference in the ALFF comparison between the two groups.

3.2.2 ReHo

The HADD group exhibited lower ReHo in the left precentral gyrus compared to the HIV control group (voxel-level uncorrected $P < 0.001$; see Figure 1 and Supplementary Table 6 for details).

3.2.3 FC

Compared to the HIV control group, the HADD group displayed decreased voxel-wise FC for the seed region in the right precentral gyrus with clusters in the right cuneus, the right medial orbital part of the superior frontal gyrus with clusters in the left inferior parietal, but supramarginal and angular gyri, and the right insula with clusters in the right calcarine fissure and surrounding cortex. (AlphaSim correction, voxel-level $P < 0.001$, cluster-level $P < 0.05$; Table 2).

3.3 Peripheral blood indicators

Higher plasma levels of interferon-gamma (IFN-γ) were found in the HADD group compared to the HIV control group (Figure 2).

TABLE 1 Demographic and clinical characteristics of all participants.

Demographic and clinical data	HADD group (N = 33)	HIV control group (N = 47)	Statistic	*P* value
Age (years)	33.00 (26.50 - 40.00)	33.00 (29.00 - 38.00)	$Z = -0.166$	0.868[a]
Height (m)	1.74 ± 0.05	1.75 ± 0.06	$t = -0.913$	0.364[b]
Weight (kg)	68.24 ± 10.27	69.32 ± 9.47	$t = -0.483$	0.630[b]
BMI (kg/m²)	22.15 (20.37 - 24.05)	22.10 (20.68 - 23.89)	$Z = -0.093$	0.926[a]
Education (years)	16.00 (14.50 - 16.50)	16.00 (15.00 - 16.00)	$Z = -0.010$	0.992[a]
Period of diagnosed HIV infection				
CD4 at diagnosis (cells/μL)	353.32 ± 151.96	329.55 ± 208.75	$t = 0.589$	0.557[b]
CD8 at diagnosis (cells/μL)	962.93 (844.50 - 1305.00)	936.00 (741.00 - 1183.00)	$Z = -1.173$	0.241[a]
CD4/CD8 ratio at diagnosis	0.33 (0.26 - 0.47)	0.38 (0.15 - 0.45)	$Z = -0.259$	0.796[a]
VL at diagnosis (log10 copies/mL)	4.03 (3.80 - 4.90)	4.07 (3.56 - 4.72)	$Z = -0.963$	0.336[a]
Period of initial ART start				
CD4 at initiation of ART (cells/μL)	381.82 ± 177.36	326.8 ± 208.07	$t = 1.236$	0.220[b]
CD8 at initiation of ART (cells/μL)	1114.95 (745.00 - 1329.09)	936 (749.00 - 1209.82)	$Z = -1.300$	0.194[a]
CD4/CD8 ratio at initiation of ART	0.33 (0.25 - 0.51)	0.37 (0.15 - 0.45)	$Z = -0.562$	0.574[a]
VL at initiation of ART (log10 copies/mL)	3.93 (3.77 - 4.71)	4.08 (3.56 - 4.82)	$Z = -0.112$	0.910[a]
ART regimen at initiation (INSTI/Non-INSTI - based regimen)	6/27	10/37	$\chi^2 = 0.116$	0.733[c]
Period of clinical and MRI assessment				
Current CD4 (cells/μL)	589.00 (450.00 - 808.32)	559.00 (385.00 - 750.00)	$Z = -0.821$	0.412[a]
Current CD8 (cells/μL)	1003.47 (573.00 - 1250.00)	854.00 (645.00 - 1028.00)	$Z = -0.582$	0.561[a]
Current CD4/CD8 ratio	0.68 (0.41 - 0.90)	0.72 (0.43 - 0.90)	$Z = -0.171$	0.864[a]
Current virus not detectable (yes/no)	33/0	47/0	NA	NA

(Continued)

TABLE 1 Continued

Demographic and clinical data	HADD group (N = 33)	HIV control group (N = 47)	Statistic	*P* value
Current ART regimen (INSTI/Non-INSTI - based regimen)	20/13	34/13	χ^2 = 1.217	0.270[c]
Duration between diagnosis and initiation of ART (months)	0.60 (0.35 - 5.15)	0.50 (0.40 - 2.30)	*Z* = -0.383	0.702[a]
Duration of ART (months)	61.90 (20.20 - 97.25)	63.80 (43.10 - 88.00)	*Z* = -0.186	0.853[a]
Duration of HIV diagnosis (months)	78.50 (26.40 - 103.80)	70.80 (44.50 - 92.70)	*Z* = -0.034	0.973[a]
SAS	41.00 (33.00 - 44.00)	31.00 (24.00 - 34.00)	*Z* = -4.566	<0.001[a]
SDS	42.00 (34.50 - 49.50)	30.00 (26.00 - 36.00)	*Z* = -4.192	<0.001[a]
PSQI	7.00 (4.00 - 10.00)	4.00 (3.00 - 7.00)	*Z* = -2.913	0.004[a]
CTQ	61.00 (52.00 - 64.00)	57.00 (52.00 - 61.00)	*Z* = -1.645	0.100[a]
SCL-90	166.00 (132.50 - 215.50)	110.00 (99.00 - 133.00)	*Z* = -4.507	<0.001[a]
AUQ	9.00 (8.00 - 18.00)	11.00 (8.00 - 14.00)	*Z* = -0.610	0.542[a]
VAS	2.00 (1.00 - 4.00)	2.00 (1.00 - 4.00)	*Z* = -0.639	0.523[a]
MoCA	27.00 (25.50 - 28.00)	27.00 (25.00 - 28.00)	*Z* = -0.396	0.692[a]

The continuous data were expressed as mean ± standard deviation or median interquartile range and the categorical data were expressed as numbers. Two-sample *t*-tests were used for continuous data with a normal distribution, while Mann-Whitney *U*-tests were used for continuous data that did not obey a normal distribution. Chi-square and Fisher's exact tests were used to compare categorical variables. aMann-Whitney *U*-test; btwo-sample *t*-test; cchi-square test. HADD, HIV-associated depressive disorders; HIV control, HIV-infected individuals without neuropsychiatric disorders; NA, not available; BMI, body mass index; CD4, CD4+ T cell count; CD8, CD8+ T cell count; VL, viral load; ART, antiretroviral therapy; INSTI, integrase strand transfer inhibitor; MRI, magnetic resonance imaging; SAS, self-rating anxiety scale; SDS, self-rating depression scale; SCL-90, symptom checklist 90; PSQI, Pittsburgh sleep quality index; CTQ, childhood trauma questionnaire; AUQ, alcohol urge questionnaire; VAS, visual analogue scale for alcohol craving; MoCA, Montreal cognitive assessment.

3.4 Flow cytometry analysis results

Compared to the HIV control group, we observed an elevated frequency of non-classical monocytes (NCM) in the HADD group. The results showed that the HADD group expressed greater levels of perforin (in cluster 3, which represents the $CD8^+$ effector memory T cells (T_{EM}); cluster 5, which represents the NCM; cluster 12, which represents the double-negative T cells (DNT)) and CD38 (in cluster 1, which represents the classical monocytes (CM); cluster 3; cluster 4, which represents the $CD8^+$ naïve T cells (T_N); cluster 14, which represents the NCM; cluster 19, which represents the NCM) compared to the HIV control group (Figure 3).

3.5 Analysis of the main effects and interactions between groups and ART regimens on findings

Depressive disorder showed significant main effects on the intergroup differences in most imaging, cytokine, and

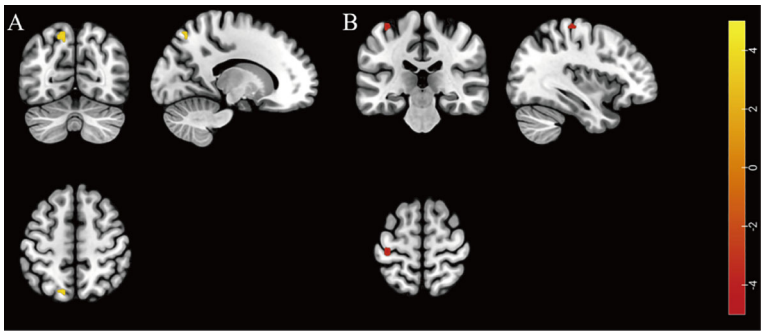

FIGURE 1
Individuals with HIV-associated depressive disorders exhibit abnormal functional activity in their brains. **(A)** The brain region with higher fALFF was located at the left superior parietal gyrus in the HADD group compared to the HIV control group (FDR correction, voxel-level $P < 0.001$, cluster-level $P < 0.05$). **(B)** The brain region with lower ReHo was located at the left precentral gyrus in the HADD group compared to the HIV control group (voxel-level uncorrected $P < 0.001$). fALFF, fractional amplitude of low-frequency fluctuation; ReHo, regional homogeneity; HADD, HIV-associated depressive disorders; HIV control, HIV-infected individuals without neuropsychiatric disorders; FDR, false discovery rate. The color bars indicate T-statistics (red/yellow).

10.3389/fpsyt.2024.1438085

TABLE 2 Functional connectivity differences between the HADD and HIV control groups.

Seed region number	Contrast/Seed regions	Connected regions	Peak MNI coordinates			T value	Cluster Size
			X	Y	Z		
	HADD < HIV control						
2	R precentral gyrus	R cuneus	15	-87	39	5.1858	33[a]
		L middle occipital gyrus	-33	-87	15	4.1676	25
		L middle frontal gyrus	-27	51	15	4.7906	23
		L inferior parietal, but supramarginal and angular gyri	-33	-39	45	4.2157	10
8	R medial orbital part of the superior frontal gyrus	L inferior parietal, but supramarginal and angular gyri	-36	-54	51	4.4441	21[a]
10	R insula	R calcarine fissure and surrounding cortex	21	-63	6	4.6126	27[b]
		R lingual gyrus	24	-54	0	3.9103	12
		R postcentral gyrus	54	-21	57	3.6817	10
14	R posterior cingulate gyrus	L inferior temporal gyrus	-48	-51	-21	4.2577	12

Coordinates (X, Y, Z) refer to the peak MNI coordinates of brain regions with peak intensity. [a]Corrected for multiple comparisons (GRF correction, voxel-level $P < 0.001$, cluster-level $P < 0.05$, two-tailed); [b]Corrected for multiple comparisons (AlphaSim correction, voxel-level $P < 0.001$, cluster-level $P < 0.05$). GRF, gaussian random field; HADD, HIV-associated depressive disorders; HIV control, HIV-infected individuals without neuropsychiatric disorders; FC, functional connectivity; MNI, Montreal Neurological Institute; L, left; R, right.

immunological indicators between the two groups. No main effects of ART regimens or interactions between groups and ART regimens were identified in the results (Supplementary Table 7).

3.6 Correlations of the imaging results with the clinical data and peripheral blood indicators

In the analysis of all participants, there was a positive correlation between the brain-derived neurotrophic factor (BDNF) levels and ReHo values in the left precentral gyrus. The FC values (voxel-wise FC for the seed region in the right precentral gyrus with clusters in the left middle occipital gyrus) were negatively correlated with SAS scores, etc. (Figure 4).

4 Discussion

In this study, we conducted comparisons between the HADD and HIV control groups in terms of clinical, neuroimaging, neurotrophic factors, endocrinological indicators, and immunological markers. Compared to the HIV control group, we found that the HADD group exhibited higher fALFF in the left superior parietal gyrus, lower ReHo in the left precentral gyrus, and decreased voxel-wise FC for the seed region in the right precentral gyrus with clusters in the right cuneus, etc. Additionally, we observed higher levels of inflammation-related immune markers in the HADD group. These imaging changes were correlated with clinical variables and peripheral blood indicators. These results may provide evidence that depressive disorders are associated with brain function abnormalities and inflammation in HIV-infected MSM.

In brain functional imaging comparisons, we observed higher fALFF in the left superior parietal gyrus and lower ReHo in the left precentral gyrus in the HADD group compared to the HIV control group. The parietal gyrus is involved in the processing of sensory, emotional, and cognitive functions in the human brain (39). Relevant literature in neurology and psychiatry has reported that people with schizophrenia exhibit higher fALFF in the left superior parietal lobule compared to healthy control groups (40). We also discovered that the HADD group exhibited higher fALFF in the left superior frontal gyrus, indicating abnormal activity in this region in people with HADD. The precentral gyrus, located in the dorsal portion of the frontal lobe, is responsible for processing sensory input and motor output as well as planning, controlling, and executing voluntary movements (41, 42). Interestingly, previous studies have reported similar changes between people with major depressive disorder and healthy controls (43, 44), suggesting that abnormal ReHo in the precentral gyrus may serve as a prospective neuroimaging biomarker for depressive disorder (44). Therefore, our results suggest that people with HADD may exhibit abnormal brain activity in multiple brain regions compared to HIV controls.

According to the definition of seed regions of interest, we observed decreased voxel-wise FC in the HADD group for the seed region in the right insula with clusters in the right calcarine fissure and surrounding cortex, right postcentral gyrus, and right lingual gyrus. Previous research has also indicated similar results in the general population (45). These findings provide further proof that depression is closely associated with functional coordination deficits of the insula with the calcarine, postcentral gyrus, and lingual gyrus. It is suggested that this may serve as a relatively stable biomarker for depressive disorders. Thus, these findings indicate that individuals with HADD exhibit related abnormalities in brain network connections.

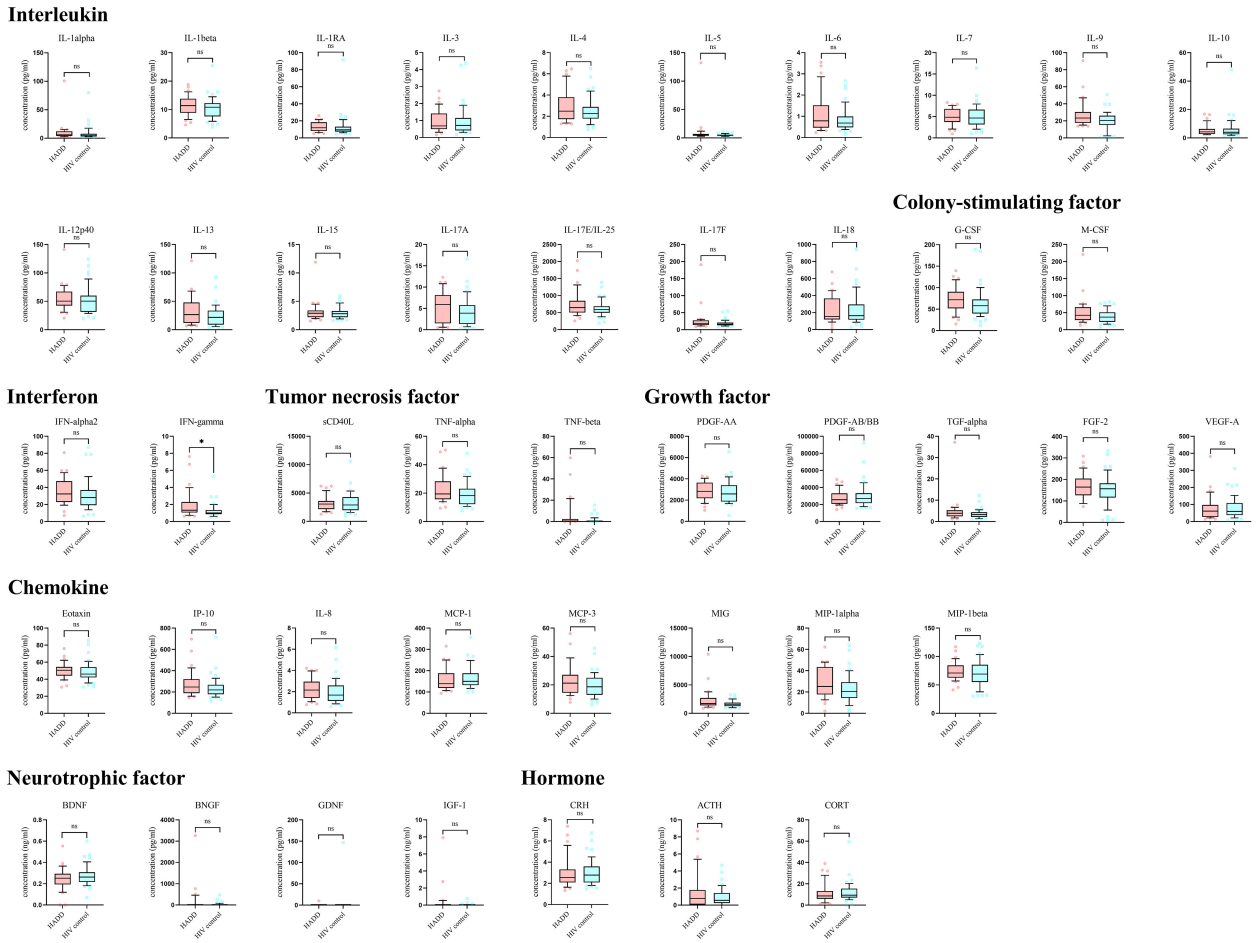

FIGURE 2
Differences in inflammatory, endocrine, and neurotrophic indicators between the HADD and HIV control groups. HADD, HIV-associated depressive disorders; HIV control, HIV-infected individuals without neuropsychiatric disorders; IL, interleukin; IL-1RA, interleukin-1 receptor antagonist; G/M-CSF, granulocyte/macrophage colony-stimulating factor; IFN, interferon; sCD40L, soluble CD40 ligand; TNF, tumor necrosis factor; PDGF, platelet-derived growth factor; TGF, transforming growth factor; FGF, fibroblast growth factor; VEGF, vascular endothelial growth factor; IP, interferon-inducible protein; MCP, monocyte chemotactic protein; MIG, monokine induced by gamma interferon; MIP, macrophage inflammatory protein; BDNF, brain-derived neurotrophic factor; BNGF, beta-nerve growth factor; GDNF, glial-derived neurotrophic factor; IGF-1, insulin-like growth factor-1; CRH, corticotropin-releasing hormone; ACTH, adrenocorticotropic hormone; CORT, cortisol; ns, non-significant. *P<0.05.

Current research on the mechanism of depression in PWH is relatively common, and it is now believed that HIV infection leads to dysfunction of the hypothalamic-pituitary-adrenal axis, causing sustained immune activation, which in turn leads to the occurrence of depression (37, 46). We found that levels of IFN-γ and the frequency of NCM were higher in the HADD group compared to the HIV control group. IFN-γ is a pro-inflammatory cytokine that plays a pivotal role in regulating immune and inflammatory events (47). This aligns with literature describing how HIV infection increases the release of tumor necrosis factor-α, IFN-γ, interleukin (IL)-1, and IL-6, resulting in reduced 5-hydroxytryptamine transmission and ultimately leading to depression in PWH (37). There are three subpopulations of monocytes in whole blood: classical monocytes (phagocytosis), intermediate monocytes (pro-inflammatory, phagocytosis), and NCM (patrolling, antiviral, pro-inflammatory) (48, 49). In some inflammatory diseases, NCM have been shown to exhibit pro-inflammatory features (50–52). The frequency of NCM is higher in the HADD group, and the levels of perforin and CD38 expression in NCM are also elevated, indicating increased inflammation levels in HADD patients. DNT cells are a unique antigen-specific regulatory T cell (53, 54), and the increased expression of perforin in the HADD group indicates enhanced cytotoxic function in these cells. Since DNT cells also play a role in promoting neuroinflammation (55), their active function in the HADD group may have implications for the promotion of inflammation. Overall, the results of these peripheral blood assessments suggest a higher level of inflammation in individuals with HADD.

In the correlation investigation, we discovered a positive correlation between the ReHo values of the left precentral gyrus and BDNF levels. BDNF, as the most abundant member of the neurotrophic factor family of growth factors in the CNS, plays key roles in neuronal survival and growth as well as synaptic plasticity (56). Previous studies have shown an etiological link between depression and BDNF (57–59). One of the most significant biological findings in

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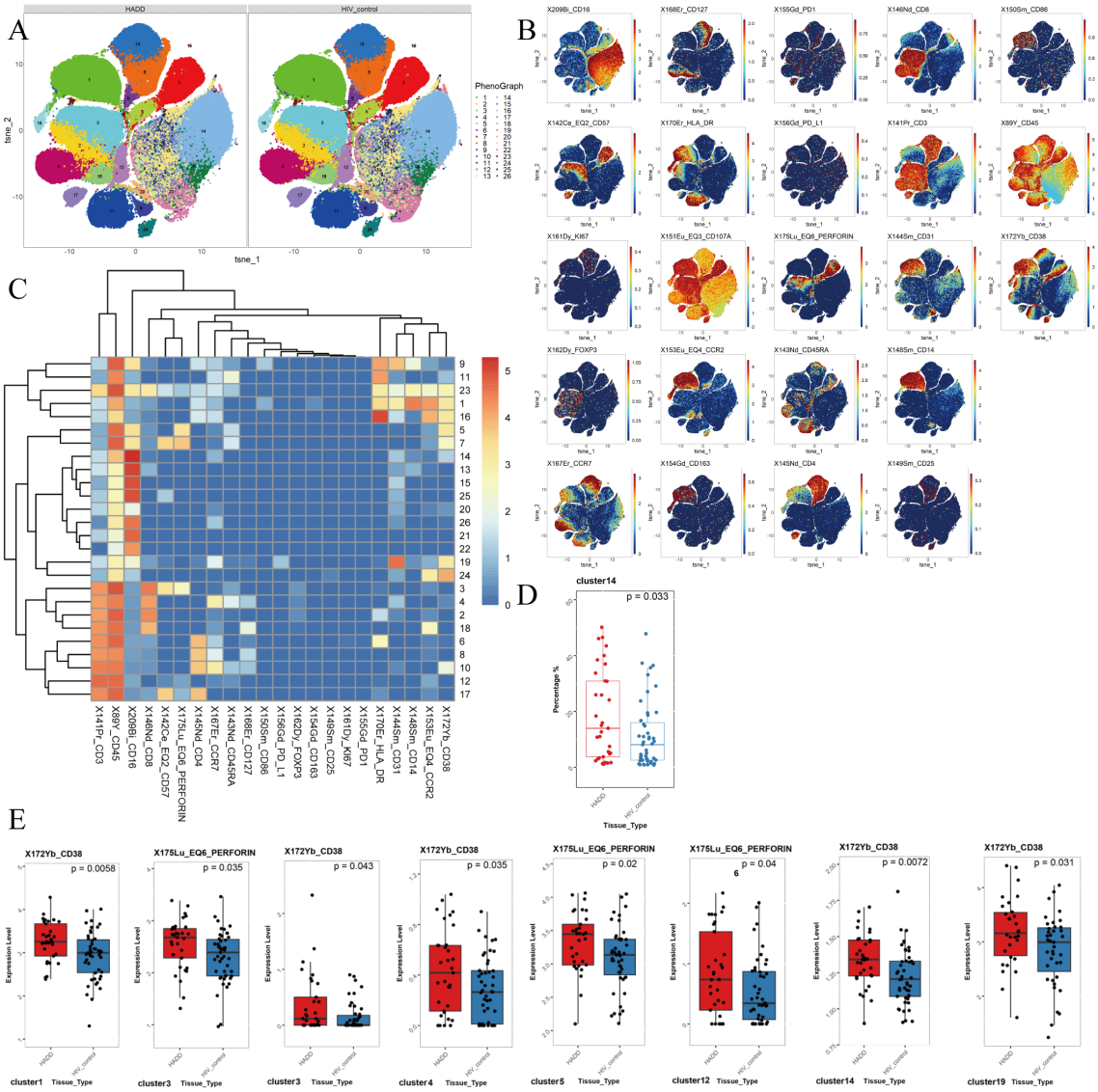

FIGURE 3
The results of flow cytometric analysis. **(A)** *t*-SNE plots of monocytes and CD3$^+$ T cells in HADD and HIV control groups. **(B)** Expression distribution of selected markers across clusters. Dark red color: high expression; dark blue color: no expression. **(C)** Heatmap of marker expression for 26 monocytes and CD3$^+$ T cell clusters. Among the 26 clusters of monocytes and CD3$^+$ T cells, cluster 1 represented classical monocytes (CD14$^+$ CD16$^-$); cluster 2, 3, 18 represented CD8$^+$ effector memory T cells (CD3$^+$ CD4$^-$ CD8$^+$ CD45RA$^-$ CCR7$^-$); cluster 4, 23 represented CD8$^+$ naïve T cells (CD3$^+$ CD4$^-$ CD8$^+$ CD45RA$^+$ CCR7$^+$); cluster 5, 7, 11, 13, 14, 15, 19, 20, 21, 22, 24, 25, 26 represented nonclassical monocytes (CD14$^-$ CD16$^+$); cluster 17 represented CD4$^+$ effector memory T cells (CD3$^+$ CD4$^+$ CD8$^-$ CD45RA$^-$ CCR7$^-$); cluster 9 represented intermediate monocytes (CD14$^+$ CD16$^+$); cluster 10 represented CD4$^+$ naïve T cells (CD3$^+$ CD4$^+$ CD8$^-$ CD45RA$^+$ CCR7$^+$); cluster 12 represented double negative T cells (CD3$^+$ CD4$^-$ CD8$^-$); cluster 6, 8, 16 represented CD4$^+$ central memory T cells (CD3$^+$ CD4$^+$ CD8$^-$ CD45RA$^-$ CCR7$^+$). **(D)** Different frequencies of cluster 14 (nonclassical monocytes) were observed between the HADD and HIV control groups. **(E)** Cluster showing significant differences in perforin and CD38 expression levels between the HADD and HIV control groups. Statistical significance was defined as a *P* value < 0.05. HADD, HIV-associated depressive disorders; HIV control, HIV-infected individuals without neuropsychiatric disorders; *t*-SNE, *t*-distributed stochastic neighbor embedding.

depression disorders is the decline in peripheral (plasma or serum) BDNF levels (59). ReHo measures the consistency of time signals between a voxel and its surrounding voxels (31). Our study found that lower ReHo in the left precentral gyrus among individuals with HADD, which was related to lower levels of BDNF. The correlation between BDNF levels and the ReHo values of the left precentral gyrus seems to indicate a consistent correlation between imaging changes and neurotrophic factors.

This study has certain strengths. First, the combined use of multiple technologies and interdisciplinary methods allows for further investigation into the associations between clinical, imaging, endocrine, and immune results. Second, the comprehensive psychiatric disorders were diagnosed by expert psychiatrists using standard diagnostic criteria to ensure the accuracy of this study, avoiding potential biases that have arisen from using psychiatric screening measures such as the general health questionnaire in other clinical studies.

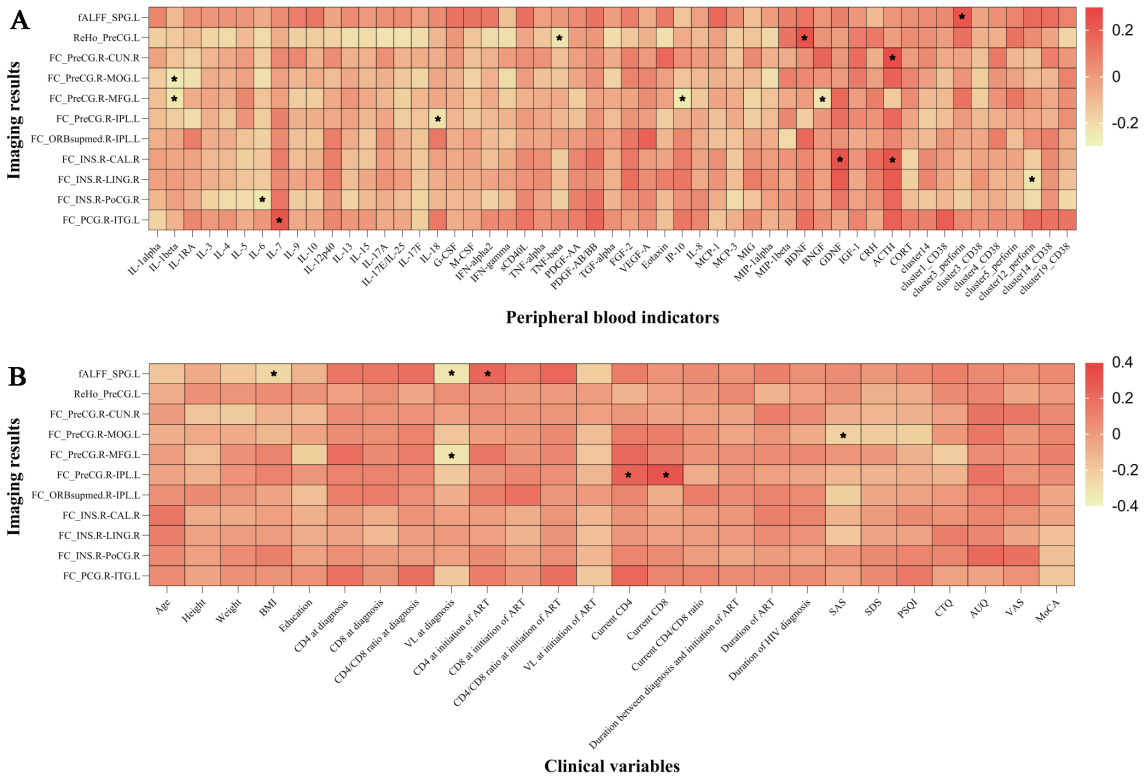

FIGURE 4
The imaging results were correlated with peripheral blood indicators and clinical variables. **(A)** Correlation between imaging results and peripheral blood indicators. **(B)** Correlation between imaging results and clinical variables. *$P < 0.05$. IL, interleukin; IL-1RA, interleukin-1 receptor antagonist; G/M-CSF, granulocyte/macrophage colony-stimulating factor; IFN, interferon; sCD40L, soluble CD40 ligand; TNF, tumor necrosis factor; PDGF, platelet-derived growth factor; TGF, transforming growth factor; FGF, fibroblast growth factor; VEGF, vascular endothelial growth factor; IP, interferon-inducible protein; MCP, monocyte chemotactic protein; MIG, monokine induced by gamma interferon; MIP, macrophage inflammatory protein; BDNF, brain-derived neurotrophic factor; BNGF, beta-nerve growth factor; GDNF, glial-derived neurotrophic factor; IGF-1, insulin-like growth factor-1; CRH, corticotropin-releasing hormone; ACTH, adrenocorticotropic hormone; CORT, cortisol; ALFF, fractional amplitude of low-frequency fluctuation; ReHo, regional homogeneity; FC, functional connectivity; SPG.L, left superior parietal gyrus; PreCG.L, left precentral gyrus; PreCG.R, right precentral gyrus; CUN.R, right cuneus; MOG.L, left middle occipital gyrus; MFG.L, left middle frontal gyrus; IPL.L, left inferior parietal, but supramarginal and angular gyri; ORBsupmed.R, right medial orbital part of the superior frontal gyrus; INS.R, right insula; CAL.R, right calcarine fissure and surrounding cortex; LING.R, right lingual gyrus; PoCG.R, right postcentral gyrus; PCG.R, right posterior cingulate gyrus; ITG.L, left inferior temporal gyrus; BMI, body mass index; CD4, $CD4^+$ T cell count; CD8, $CD8^+$ T cell count; VL, viral load; ART, antiretroviral therapy; INSTI, integrase strand transfer inhibitor; MRI, magnetic resonance imaging; SAS, self-rating anxiety scale; SDS, self-rating depression scale; PSQI, Pittsburgh sleep quality index; CTQ, childhood trauma questionnaire; AUQ, alcohol urge questionnaire; VAS, visual analogue scale for alcohol craving; MoCA, Montreal cognitive assessment. Cluster 14 represented nonclassical monocytes, cluster 1 represented nonclassical monocytes, cluster 3 represented $CD8^+$ effector memory T cells, cluster 4 represented $CD8^+$ naïve T cells, cluster 5 represented nonclassical monocytes, cluster 12 represented double negative T cells, and cluster 19 represented nonclassical monocytes.

However, there are also limitations and shortcomings in this research. First, the cross-sectional design limited our capacity to study temporal relationships and draw causal inferences. Second, the sample of this study, primarily composed of HIV-positive MSM, limits the generalizability of the findings to other demographics. To improve applicability, future research should include more diverse populations, encompassing varied genders, sexual orientations, and socioeconomic backgrounds. Expanding the target population to other high-risk groups and the general population is also recommended to validate the robustness and broader relevance of the findings. Third, the relatively small sample size in this study may reduce statistical power. Future research should recruit larger and more diverse sample populations through multi-center cohort studies to ensure the findings are representative of broader HIV-positive populations. Fourth, the 1.5 T MRI scanner utilized in this study has inherent limitations in imaging resolution and signal-to-noise ratio. Compared to higher-field scanners, it may be less effective in capturing fine structural details and subtle signal variations. Future studies employing higher-field scanners could enhance resolution and signal-to-noise ratio, yielding more precise imaging data to validate our findings and provide deeper mechanistic insights for clinical applications.

In summary, this study has revealed that individuals with HADD display abnormalities in brain function and higher levels of chronic inflammation compared to PWH without neuropsychiatric disorders. Moreover, there is a clear correlation between imaging results, clinical data, and inflammation markers. These findings suggest that integrating imaging and immunological data in future research may deepen our understanding of the neurofunctional changes associated with HADD.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by the Institutional Ethics Committee of Beijing Youan Hospital, Capital Medical University (2023/057). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Author contributions

YZ: Data curation, Formal analysis, Funding acquisition, Investigation, Writing – original draft. YH: Data curation, Formal analysis, Writing – original draft. YF: Data curation, Formal analysis, Writing – original draft. MC: Data curation, Formal analysis, Writing – original draft. GS: Data curation, Formal analysis, Writing – original draft. RW: Data curation, Formal analysis, Writing – original draft. JZ: Data curation, Formal analysis, Writing – original draft. YLZ: Conceptualization, Funding acquisition, Supervision, Writing – review & editing. ZL: Conceptualization, Formal analysis, Funding acquisition, Supervision, Writing – review & editing. YM: Conceptualization, Formal analysis, Funding acquisition, Supervision, Writing – review & editing. TZ: Conceptualization, Formal analysis, Funding acquisition, Supervision, Validation, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2024.1438085/full#supplementary-material

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The curvilinear relationship between Framingham Steatosis Index and depression: insights from a nationwide study

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Background: The Framingham Steatosis Index (FSI) serves as a diagnostic metric for fatty liver. While research has established a link between depression and fatty liver, the association with the Framingham Steatosis Index (FSI) remains undocumented. The aim of this study is to explore the potential correlation between FSI and depression, addressing this research void.

Methods: Our data originates from the National Health and Nutrition Examination Survey (NHANES) database. We employed the PHQ-9 questionnaire for the evaluation of depressive symptoms. We investigated the association between FSI and depression using a weighted multiple logistic regression model and stratified analysis. Non-linear associations were explored using fitted smooth curves. A recursive method was employed to identify inflection points. Subgroup analyses were conducted to examine differences in the association between FSI and depression within subgroups.

Results: Our research encompassed a total of 19,697 participants. Multivariate logistic regression analysis, adjusted for potential confounding factors, demonstrated a significant positive association between FSI and depression, with OR of 1.14 (95% CI: 1.10, 1.18). Stratified analysis indicated that a significant positive correlation exists between FSI and depression among all groups except those with BMI below 30. The non-linear relationship was further confirmed by the restricted cubic splines analysis, which revealed an inflection point at an FSI value of 29.72. Below this threshold, there was no significant correlation, while above it, a positive correlation was observed. Subgroup analysis revealed statistically significant interactions between FSI and depression within the educational attainment groups.

Conclusion: Our study's discovery is the curvilinear relationship between FSI and depression. Factors such as inflammation, hormonal levels, and metabolic disruptions could be the underlying mechanisms driving this relationship. This finding offers valuable insights that could inform the development of comprehensive intervention strategies for managing depression in clinical settings.

KEYWORDS

Framingham Steatosis Index, depression, curvilinear, NHANES, cross-sectional study

1 Introduction

Depression, recognized as a pervasive mood disorder, poses a significant threat to global public health, affecting an estimated 280 million individuals around the world (1) and showing an upward trend in prevalence (2). A cross-sectional study conducted by Neyazi A et al. indicated an alarmingly high prevalence of depression in Afghanistan, reaching 72.05% (3). This condition not only erodes social, psychological, and physical well-being but also amplifies the risk of suicide (4, 5). Individuals with Major Depressive Disorder (MDD) are alarmingly prone to suicidal tendencies, with a mortality rate nearly 20 times higher than that of the broader population (6). In addition, depression is associated with a spectrum of health challenges, such as cardiovascular diseases, breast cancer, sleep disorders, and non-alcoholic fatty liver disease (7–10).

The Framingham Steatosis Index (FSI), developed by Long MT et al. in 2016, is a key diagnostic tool for hepatic steatosis (11). It was established through analysis of a cross-sectional study with 1,181 participants, incorporating factors like age, gender, BMI, triglycerides, hypertension, diabetes, and the ALT: AST ratio. The accuracy of the FSI has been confirmed through validation and it is currently being applied in clinical settings. A cohort study by Nima Motamed and colleagues has demonstrated that FSI is a potent tool for diagnosing non-alcoholic fatty liver disease (NAFLD) and has the additional capability to predict new incidences of NAFLD (12). Furthermore, a community-based evaluation of liver steatosis by Jung TY and other researchers revealed that the FSI outperformed both the Hepatic Steatosis Index (HSI) and the Fatty Liver Index (FLI) in terms of diagnostic accuracy (13). Previous research has already documented associations between liver-related markers and depression. The link between NAFLD and depression could be attributed to the disruption of inflammation, oxidative stress pathways, and mitochondrial dysfunction (14). For example, Manusov EG and colleagues have shown that the AST/ALT ratio is significantly correlated with depression (15). Furthermore, in a cohort study by Cho YK and colleagues, FSI demonstrated a significant predictive ability for cardiovascular risk (16). Considering that cardiovascular diseases, including hypertension, are significantly linked to depression (17), we have grounds to hypothesize a potential relationship between FSI and depression. Nonetheless, the relationship between FSI and depression remains largely unexplored. The National Health and Nutrition Examination Survey (NHANES) constitutes a large, meticulously collected, and extensively detailed database. Utilizing NHANES dataset, this study endeavors to investigate the association between FSI and depression. Our research endeavors to elucidate the intricate interplay among multiple factors and mental health, offering insights to inform the creation of more holistic prevention and intervention strategies.

Abbreviations: FSI, Framingham Steatosis Index; ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; BMI, Body Mass Index; GGT, Gamma-Glutamyl Transferase; TG, Triglycerides; CVD, Cardiovascular Disease; CV, Cardiovascular; OGTT, Oral Glucose Tolerance Test; NHANES, National Health and Nutrition Examination Survey; DSM-IV, Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition; RCS, Restricted Cubic Spline; CDC, Centers for Disease Control and Prevention; NCHS, National Center for Health Statistics; MECs, Mobile Examination Centers; OR, Odds Ratios; CI, Confidence Intervals.

2 Materials and methods

2.1 Study participants

Our research drew upon data from the National Health and Nutrition Examination Survey (NHANES) in the United States, spanning nine cycles from 2003 to 2020. The NHANES database offers a rich array of information, including demographic details, lifestyle practices, self-reported health metrics, and blood biochemistry assessments. Data collection is conducted through household interviews, mobile examination centers (MECs), and laboratory tests. This resource is openly available to the research community without the need for specific permissions. The study protocol was granted approval by the National Center for Health Statistics Research Ethics Review Board, and all participants provided their informed written consent. To safeguard the privacy of the individuals involved, all personal identifiers were anonymized. In the data preparation phase of our study, we

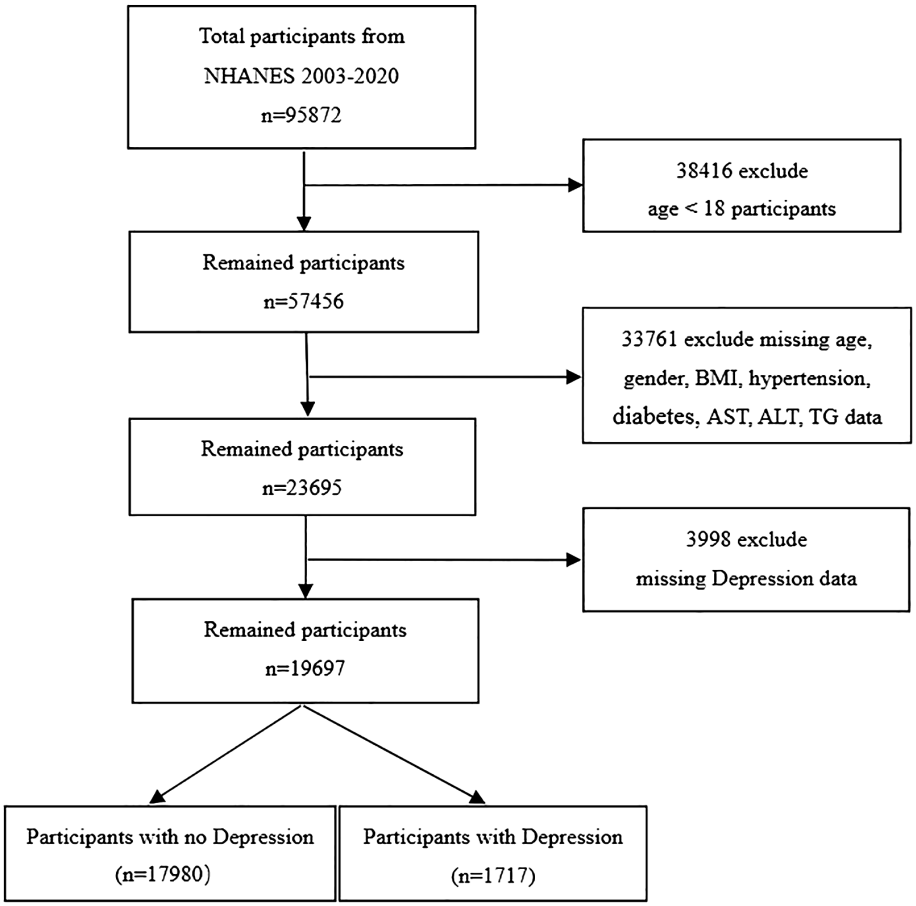

FIGURE 1
Flow chart of sample selection from the 2003-2020. BMI, body mass index; AST, Aspartate Aminotransferase; ALT, Alanine Aminotransferase; TG, Triglycerides.

excluded participants who were under 18 years of age, accounting for 38,416 individuals. Furthermore, we omitted 33,761 individuals due to incomplete data on gender, age, BMI, diabetes status, hypertension, TG, AST, and ALT levels. An additional 3,998 individuals were excluded for lacking depression-related data. Consequently, our study encompassed a total of 19,697 participants, as depicted in Figure 1.

2.2 Study variables

2.2.1 Definition of depression

The 9-item Patient Health Questionnaire (PHQ-9), recognized as a prevalent self-assessment tool, is fashioned to gauge the intensity of depressive symptoms experienced within a two-week period. This instrument adheres to the criteria for major depressive episodes as detailed in the DSM-IV, the fourth edition of the Diagnostic and Statistical Manual of Mental Disorders (18). This scale is a reliable tool for diagnosing depression, exhibiting high specificity and sensitivity. The questionnaire comprises nine items that evaluate symptoms such as sadness, loss of interest, sleep disturbances, fatigue, feelings of worthlessness, appetite issues, difficulty concentrating, psychomotor agitation or retardation, and suicidal thoughts. Each item is scored from 0 to 3, with 0 indicating "not at all", 1 reflecting "a few days", 2 corresponding to "more than half the days", and 3 signifying "nearly every day". The total score ranges from 0 to 27, with a score of 10 or above indicating the presence of depression. A total score of 9 or below is considered to indicate no depressive symptoms, while a score of 10 or above is used for the diagnosis of depression, demonstrating 88% sensitivity and 88% specificity. The PHQ-9 exhibits strong internal reliability, as evidenced by a Cronbach's alpha coefficient of 0.89.

2.2.2 Definition of FSI

We employed FSI, as formulated by Long MT and colleagues in 2016 (11), in our analysis. It is calculated using the formula:

FSI = -7.981 + 0.011 × age (years) - 0.146 × sex (female = 1, male = 0) + 0.173 × BMI (kg/m2) + 0.007× triglycerides (mg/dL) + 0.593× hypertension (yes = 1, no = 0) + 0.789× diabetes (yes = 1, no = 0) + 1.1× ALT: AST ratio>= 1.33(yes = 1, no = 0).

Assessment of TG, AST and ALT Levels: Peripheral blood samples were collected in the morning from participants who had fasted for at least eight hours. Serum alanine TG, ALT levels were assessed using an enzymatic method, while AST levels were measured in serum or plasma by a kinetic rate assay.

2.2.3 Assessment of other variables

CDC collects comprehensive participant data, including demographics, lifestyle, self-reported health status, physical measurements, and biochemical indicators, through computer-assisted personal interviews. Demographic factors encompass age, sex, ethnicity, educational attainment, marital status, and the income-to-poverty ratio. Lifestyle factors, including smoking and drinking habits as well as recreational activities, were also taken into account. Self-reported health data included diabetes, hypertension, stroke, and cardiovascular disease, while anthropometric data included BMI. Biochemical data encompassed gamma-glutamyl transferase (GGT), triglycerides (TG), ALT, and AST. Smoking status was categorized into three distinct groups: "Never" was defined as having smoked fewer than 100 cigarettes in one's lifetime; "Former" referred to individuals with a history of smoking who had since quit; and "Now" was designated for those who continued to smoke (19). Engagement in recreational activities was binary, recorded as "Yes" or "No." The diagnosis of diabetes, including pre-diabetes, is based on meeting at least one of the following criteria: 1. Fasting blood glucose levels above 7.0 mmol/L; 2. Hemoglobin A1c (HbA1c) levels of 6.5% or higher; 3. Random blood glucose levels of at least 11.1 mmol/L; 4. Blood glucose levels of 11.1 mmol/L or higher after a 2-hour oral glucose tolerance test (OGTT); 5. A formal diagnosis of diabetes by a healthcare provider; 6. Impaired fasting glucose ranging from 6.11 to 7.0 mmol/L or impaired glucose tolerance, with OGTT levels between 7.7 and 11.1 mmol/L. Hypertension was determined by one or more of the following conditions: 1. Systolic blood pressure readings of 140 mmHg or higher; 2. Diastolic blood pressure of 90 mmHg or higher; 3. Current use of antihypertensive medications; 4. Self-reported hypertension. Alcohol consumption levels were categorized as follows: "Heavy" drinking was characterized by women consuming three or more drinks per day or four or more drinks on a single occasion; men consuming four or more drinks per day or five or more drinks on a single occasion, with at least five heavy drinking days per month. "Moderate" drinking was defined as women consuming two drinks per day and men consuming three drinks per day, with at least two heavy drinking days per month. "Mild" drinking referred to women having one drink per day and men having two drinks per day. "Never" drinking was designated for those who had fewer than 12 drinks in their lifetime, while "Former" drinkers were individuals with a history of drinking who no longer consumed alcohol. CVD was determined through a medical history questionnaire, which recorded whether participants had been diagnosed with coronary artery disease, congestive heart failure, or had a history of a heart attack (20).

2.3 Statistical analysis

The research data were properly weighted to accurately represent a more extensive demographic profile. We managed missing data by imputation, utilizing predictive mean matching for continuous variables and logistic regression for binary variables. Participants were categorized into groups with and without depression based on their baseline characteristics. Continuous variables are displayed as the mean ± standard error, while categorical variables are shown as percentages of the overall sample. To explore the association between FSI and depression, we utilized weighted logistic regression analysis. The outcomes are expressed as odds ratios (ORs) along with their respective 95% confidence intervals (95% CI). To substantiate the stability of the association between FSI and depression, we conducted a linear trend analysis. Subsequently, generalized additive models were applied to assess any potential non-linear relationships. After establishing non-linearity, the inflection point was determined using a recursive algorithm, which was then utilized to build a two-piecewise linear regression model. Further subgroup analyses and interaction tests were conducted to identify any additional risk factors that could potentially influence the relationship between FSI and depression.

TABLE 1 Baseline characteristics of participants.

	No Depression (n=17980)	With Depression (n=1717)	P value
Age (year)	46.87 ± 17.48	46.18 ± 16.41	0.0249
Sex (%)			<0.0001
Female	49.66	63.42	
Male	50.34	36.58	
Race/ethnicity (%)			<0.0001
Mexican American	8.52	8.11	
Non-Hispanic White	67.48	63.29	
Non-Hispanic Black	10.99	13.19	
Other Hispanic	5.51	7.80	
Other Race	7.51	7.61	
Marry status (%)			<0.0001
Never married	18.00	21.74	
Married/Living with partner	64.53	47.82	
Divorced/ Widowed/Separated	17.47	30.44	
Education status (%)			<0.0001
Less than high school	4.44	7.94	
High school	34.00	44.61	
More than high school	61.57	47.45	
Recreational activity (%)			<0.0001
No	43.65	65.11	
Yes	56.35	34.89	
Drinking status (%)			<0.0001
Never	11.16	9.31	
Mild	38.01	27.67	
Moderate	18.02	19.09	

(Continued)

TABLE 1 Continued

	No Depression (n=17980)	With Depression (n=1717)	P value
Heavy	21.64	27.05	
Former	11.17	16.88	
Smoking status (%)			<0.0001
Never	56.81	39.45	
Now	18.18	38.02	
Former	25.00	22.53	
Diabetes (%)			<0.0001
No	78.58	71.91	
Yes	21.42	28.09	
Hypertension (%)			<0.0001
No	64.27	52.81	
Yes	35.73	47.19	
Stroke (%)			<0.0001
No	97.32	92.54	
Yes	2.68	7.46	
CVD (%)			<0.0001
No	91.76	83.04	
Yes	8.24	16.96	
Income to poverty ratio	3.10 ± 1.63	2.15 ± 1.57	<0.0001
BMI (kg/m2)	28.95 ± 6.85	30.70 ± 8.27	<0.0001
FSI	-1.32 ± 1.74	-0.83 ± 1.98	<0.0001
TG (mmol/L)	1.38 ± 1.16	1.56 ± 1.33	<0.0001
AST (U/L)	24.71 ± 13.53	25.29 ± 22.54	0.0284
ALT (U/L)	24.83 ± 17.54	25.44 ± 29.47	0.0779
GGT (U/L)	27.56 ± 37.52	34.78 ± 54.93	<0.0001

Means ± SE are reported for continuous variables, p value was calculated by the weighted linear regression model. Categorical variables are presented as % with p-values calculated using a weighted chi-square test. BMI, body mass index; CVD, Cardiovascular Disease; AST, Aspartate Aminotransferase; GGT, Gamma-Glutamyl Transferase; ALT, Alanine Aminotransferase; TG, Triglycerides; FSI, Framingham steatosis index.

Statistical analyses were performed using R (version 3.5.3) and EmpowerStats software (http://www.empowerstats.com), with a P-value < 0.05 for statistical significance.

3 Results

3.1 Baseline characteristics

Table 1 delineates the foundational traits of participants, distinguishing those afflicted with depression from their non-depressed counterparts. Notably, the prevalence of women (63.42%) is markedly higher within the depressed cohort compared to the non-depressed group (49.66%). Individuals with depression are less likely to be married or living with a partner compared to those without depression, and are also less likely to have attained higher education, or to participate in recreational activities. They also have a higher incidence of health issues such as diabetes, hypertension, stroke, and cardiovascular disease. Moreover, they have a lower income-to-poverty ratio and are younger on average, yet they exhibit higher levels of BMI, FSI, TG, and GGT.

3.2 Association between FSI and depression

Table 2 details the correlation between FSI and depression. The unadjusted Model 1 revealed a significant positive association, with an OR of 1.17 (95% CI: 1.14, 1.20). This correlation persisted in Model 2, even after accounting for race and education status, with an OR of 1.15 (95% CI: 1.12, 1.18). Model 3, which included all covariates, still showed a positive significant association, with an OR of 1.14 (95% CI: 1.10, 1.18).In the subsequent trend test, the ORs (95% CI) for the association between FSI and depression were Q2 (OR: 0.90, 95% CI: 0.74, 1.09), Q3 (OR: 1.07, 95% CI: 0.89, 1.30), and Q4 (OR: 1.68, 95% CI: 1.41, 2.01), using Q1 as a reference, indicating a potential non-linear relationship between FSI and depression.

Sex-stratified analysis reveals that both females and males exhibit significant associations between FSI and depression, with females showing a more pronounced relationship (Female: OR 1.15, 95% CI: 1.11, 1.21; Male: OR 1.13, 95% CI: 1.07, 1.19). BMI-stratified analysis indicates that individuals with BMI >=30 demonstrate a significant association, while those with BMI <30 show no significant link. Age-stratified analysis confirms a consistent association across different age groups, with a stronger association observed in participants younger than 60 years old (<60: OR 1.12, 95% CI: 1.08, 1.16; >=60: OR 1.19, 95% CI: 1.10, 1.29).

In order to further explore the relationship between FSI and depression, we utilized a two-piecewise linear regression model enhanced by RCS analysis. The findings, after accounting for all relevant covariates, exposed a non-linear dynamic between FSI and the incidence of depression, as graphically represented in Figure 2. A pivotal point was discerned at an FSI value of -2.4. At values below this threshold, a negative correlation was observed, with OR of 0.89 (95% CI: 0.75, 1.07), which did not achieve statistical significance. Conversely, at values surpassing this threshold, a positive correlation emerged, with an OR of 1.17 (95% CI: 1.13, 1.22), signifying an enhanced probability of depression with an increase in FSI levels, as detailed in Table 3.

We performed subgroup analyses to investigate the potential Interaction between FSI and the risk of depression among different demographic groups, based on factors such as education level, marital status, smoking and drinking habits. The results indicate that there is a significant interaction between FSI and depression within subgroups defined by education level (Table 4). As education levels increase, the association between FSI and depression becomes more pronounced.

TABLE 2 Association of FSI and depression.

Exposure	Model 1 OR (95% CI)	P value	Model 2 OR (95% CI)	P value	Model 3 OR (95% CI)	P value
FSI	1.17 (1.14, 1.20)	<0.0001	1.15 (1.12, 1.18)	<0.0001	1.14 (1.10, 1.18)	<0.0001
FSI quartile						
Q1	Reference		Reference		Reference	
Q2	0.98 (0.83, 1.14)	0.7519	0.93 (0.79, 1.09)	0.3456	0.90 (0.74, 1.09)	0.2906
Q3	1.27 (1.09, 1.47)	0.0019	1.16 (1.00, 1.35)	0.0500	1.07 (0.89, 1.30)	0.4698
Q4	1.92 (1.67, 2.20)	<0.0001	1.76 (1.53, 2.03)	<0.0001	1.68 (1.41, 2.01)	<0.0001
P for trend	<0.0001		<0.0001		<0.0001	
SEX						
Female	1.21 (1.17, 1.25)	<0.0001	1.19 (1.15, 1.23)	<0.0001	1.15 (1.11, 1.21)	<0.0001
Male	1.13 (1.08, 1.18)	<0.0001	1.12 (1.08, 1.17)	<0.0001	1.13 (1.07, 1.19)	<0.0001
Age						
< 60	1.16 (1.13, 1.19)	<0.0001	1.15 (1.12, 1.18)	<0.0001	1.12 (1.08, 1.16)	<0.0001
>= 60	1.21 (1.14, 1.29)	<0.0001	1.20 (1.13, 1.28)	<0.0001	1.19 (1.10, 1.29)	<0.0001
BMI						
<25	1.03 (0.92, 1.15)	0.6227	0.99 (0.88, 1.11)	0.8227	0.86 (0.73, 1.01)	0.0667
25-30	1.08 (1.00, 1.17)	0.0446	1.07 (0.99, 1.17)	0.0991	1.04 (0.95, 1.15)	0.3926
>=30	1.16 (1.11, 1.21)	<0.0001	1.16 (1.11, 1.21)	<0.0001	1.14 (1.08, 1.20)	<0.0001

Model 1: no adjustment.
Model 2: adjusted for race and education status.
Model 3: adjusted for race, education status, smoking status, marriage status, drinking status, physical activity, family income to poverty ratio, CVD, and stroke.
In the subgroup analyses, which are stratified by sex, age, or BMI the model does not incorporate adjustments for the stratification variables themselves. BMI, body mass index; FSI, Framingham steatosis index; OR, odds ratios; CI, confidence intervals.

4 Discussion

As far as we are aware, this research pioneers the exploration of the association between FSI and the inclination toward depression. The multivariable logistic regression analysis, meticulously adjusted for a spectrum of potential confounding variables, has uncovered a notably positive correlation between FSI and depressive symptoms, with an OR of 1.14 (95% CI: 1.10, 1.18). Our sophisticated curve-fitting analysis has brought to light a nonlinear dynamic between FSI and the presence of depressive disorders, establishing an FSI threshold at -2.4. Below this threshold, no significant link is observed between FSI and the propensity for depression. Above this threshold, however, a 17% heightened risk of depression is associated with each incremental unit increase in FSI. These insights underscore the intricate interrelationship between FSI and the susceptibility to depressive tendencies. The intricate interconnection between physical and mental health is both pervasive and profound. For example, research indicates that an increase of 100μmol/L in uric acid levels is associated with a 21.7% reduction in the risk of depressive symptoms (21). Additionally, studies on the Dietary Inflammatory Index reveal that each unit increase in DII corresponds to a 12% rise in the likelihood of depression (22). These insights not only highlight the

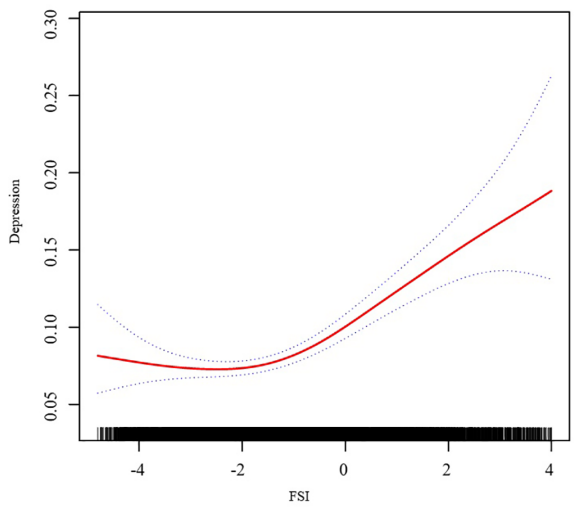

FIGURE 2
Association of FSI and Depression. We adjusted for race, education status, smoking status, marriage status, drinking status, physical activity, family income to poverty ratio, CVD, and stroke.

TABLE 3 Threshold effect analysis of FSI on depression using a two-piecewise linear regression model.

Outcome:	OR (95% CI), P
Fitting by standard linear model	1.14 (1.10, 1.18) <0.0001
Fitting by two-piecewise linear model	
Inflection point	-2.4
< -2.4	0.89 (0.75, 1.07) 0.2127
> -2.4	1.17 (1.13, 1.22) <0.0001
Log-likelihood ratio	0.007

We adjusted for race, education status, smoking status, marriage status, drinking status, physical activity, family income to poverty ratio, CVD, and stroke.

significance of the relationship between FSI and depression but also imply that a comprehensive, collaborative approach may be necessary for effective depression management.

Examining the FSI calculation formula reveals that the Framingham Steatosis Index is a composite indicator associated with sex, age, BMI, diabetes, hypertension, ALT/AST, and TG levels. While no research has yet investigated the link between FSI and depression, the relationships between depression and the individual components of the FSI formula have been previously explored in existing studies. Research indicates that women are roughly twice as likely as men to experience depression, a disparity that may stem from differences in sex hormones (23). Among the elderly, a study has found that individuals aged 70 and above who suffer from high blood pressure are at a higher risk of developing depression compared to those aged between 60 and 69 (24). Moreover, it's been established that an individual's BMI during adulthood, not during childhood, is causally linked to an increased risk of major depressive disorder (25). This underscores the significant role that age plays in the development of depression. A substantial link has been established between BMI and psychological well-being. Studies have demonstrated that obesity is associated with a 55% increased risk of depression, and individuals with depression are 58% more likely to become obese (26). Additionally, there is a correlation between depression and triglyceride levels; research by Segoviano-Mendoza M et al. has demonstrated that lower cholesterol levels are associated with mood disorders and suicidal behaviors, including Major Depressive Disorder (27). Furthermore, a robust association exists between depression and diabetes, hinting at a potential two-way causality (28). Hypertension, a condition that can severely impact the well-being of older adults, has also been linked to depression (29). There is also evidence to suggest that depression can influence the expression of genetic factors in liver enzymes, particularly the ratio of AST to ALT (30).

TABLE 4 Subgroup analysis.

Subgroup	OR, (95%CI)	P value	P interaction
Drinking status			0.7885
Never	1.10 (1.02, 1.20)	0.0180	
Mild	1.17 (1.10, 1.25)	<0.0001	
Moderate	1.13 (1.04, 1.21)	0.0019	
Heavy	1.13 (1.06, 1.20)	0.0002	
Former	1.16 (1.07, 1.27)	0.0006	
Smoking status			0.3422
Never	1.16 (1.11, 1.22)	<0.0001	
Now	1.10 (1.04, 1.17)	0.0007	
Former	1.15 (1.07, 1.23)	0.0002	
Education status			0.0194
Less than high school	1.06 (0.97, 1.16)	0.1822	
High school	1.10 (1.05, 1.16)	0.0002	
More than high school	1.19 (1.14, 1.25)	<0.0001	
Marriage status			0.9287
Never married	1.13 (1.06, 1.21)	0.0003	
Married/Living with partner	1.14 (1.08, 1.19)	<0.0001	
Divorced/ Widowed/Separated	1.15 (1.08, 1.22)	<0.0001	

We adjusted for race, education status, smoking status, marriage status, drinking status, physical activity, family income to poverty ratio, CVD, and stroke but not adjusted for the subgroup analysis variables themselves.

The precise mechanisms underlying the interaction between FSI and depression remain unclear. However, evidence suggests that inflammation, hormonal imbalances, and metabolic disruptions may be pivotal in this relationship. Obesity could trigger immune-inflammatory pathways (31), causing adipose tissue to release inflammatory cytokines like tumor necrosis factor-α and interleukin-6, which can impair brain function and precipitate depressive symptoms (32). Moreover, depression might intensify inflammatory responses by affecting the serotonin system and the hypothalamic-pituitary-adrenal axis (33). Research indicates that inflammatory markers such as AST/ALT are significantly involved in the pathophysiology of depression in individuals with diabetes (34). Fluctuations in sex hormone levels could also influence the development of depression by altering immune responses and inflammatory markers like C-reactive protein (35). Depression may further stimulate the hypothalamic axis, leading to increased cortisol secretion and subsequent insulin resistance (36). This insulin resistance could hasten liver fat accumulation and result in the overproduction of very-low-density lipoprotein-triglycerides, causing abnormal triglyceride levels (37). Conditions linked to metabolic disorders, such as hypertension and diabetes, may also contribute to the onset of depression (24). Studies suggest that obesity might elevate the risk of mental health issues through stress responses mediated by the hypothalamic-pituitary-adrenal axis (38). The progression of late-life depression is multifaceted and associated with cognitive decline (24). Educational level also impacts the prevalence of depression, largely due to its influence on memory capacity and, consequently, the expression of

depressive symptoms (39). Overall, the interplay between FSI and depression appears to be a complex dynamic involving the dysregulation of multiple physiological systems.

Our data is sourced from the NHANES database, recognized for its stringent and expert-driven data collection, as well as its large sample size, thereby providing robust credibility and reliability to our research findings. Employing stratified and subgroup analyses, we thoroughly investigated the link between FSI and depression, examining its variation across different population subsets. Nonetheless, our study has inherent limitations. As a cross-sectional observational study, it does not establish a causal relationship between FSI and depression. Moreover, unadjusted residual confounders or potential unknown factors may still affect our results. There is currently a gap in research elucidating the relationship and underlying mechanisms between FSI and depression.

Further longitudinal and experimental studies are required to delve into the biological mechanisms in detail. We hope that through our collective efforts, we can improve the understanding of the etiology and pathophysiological mechanisms of depression related to FSI, potentially refining comprehensive prevention and treatment strategies for depression.

5 Conclusion

Drawing from the NHANES database spanning 2003 to 2020, our research delineated a non-linear association between FSI and depression, pinpointing a critical inflection point at an FSI value of 29.72, as identified by restricted cubic spline analysis. Below this threshold, no significant correlation was observed, but an affirmative link materialized above it. Stratified analysis consistently showed a positive correlation between FSI and depression across all groups, with the exception of those with BMI below 30. Subgroup analysis further disclosed significant interactions between FSI and depression within educational attainment cohorts, underscoring the influence of demographic factors on this nexus. These findings accentuate the critical role of demographic and clinical parameters, along with tailored management strategies, in the evaluation of depression risk. The observed relationship between FSI and depression implies that interventions such as lifestyle adjustments and specific medical treatments for correlated chronic conditions may be crucial in preventing or alleviating the advancement of depressive symptoms. It is imperative that further clinical and foundational research be conducted to elucidate the mechanisms at play in this association and to devise more effective strategies for the prevention and treatment of depression.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: www.cdc.gov/nchs/nhanes/Default.aspx.

Ethics statement

The study was approved by the Ethics Review Committee of the National Center for Health Statistics. These studies were conducted in accordance with local legislation and institutional requirements. Written informed consent was obtained from participants or their legal guardians/next of kin as required by national legislation and institutional mandates.

Author contributions

CJ: Data curation, Formal analysis, Methodology, Software, Validation, Visualization, Writing – original draft. BW: Data curation, Formal analysis, Methodology, Software, Validation, Visualization, Writing – original draft. NW: Data curation, Formal analysis, Methodology, Software, Validation, Visualization, Writing – original draft. JW: Data curation, Methodology, Software, Validation, Visualization, Writing – original draft. YQ: Data curation, Methodology, Software, Validation, Visualization, Writing – original draft. GZ: Conceptualization, Methodology, Writing – review & editing. XZ: Conceptualization, Methodology, Project administration, Supervision, Writing – review & editing.

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Conflict of interest

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Bidirectional relationship between depression and activities of daily living and longitudinal mediation of cognitive function in patients with Parkinson's disease

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Objective: To investigate the bidirectional relationship between depression and activities of daily living (ADL) in Parkinson's disease (PD) patients and explore the mediating role of cognitive function over time.

Methods: Data from 892 PD patients from the Parkinson's Progression Markers Initiative (PPMI) database were included in this study, and depression, cognitive function, and ADL were measured using the Geriatric Depression Scale (GDS-15), Montreal Cognitive Assessment Scale (MoCA), and Unified Parkinson's Disease Rating Scale, Part II (UPDRS II) respectively. The cross-lagged panel model (CLPM) was employed to analyze the reciprocal relationship between depression and ADL. Then, we explored the mediating role of cognitive function in the bidirectional relationship between depression and ADL in patients with PD, and the mediation effect test was carried out using a bias-corrected nonparametric percentile bootstrap approach.

Results: Depression in patients with PD predicted their subsequent ADL (β = 0.079, p < 0.01), and ADL also predicted their subsequent depression (β = 0.069, p < 0.05), In addition, Bootstrap analysis showed that cognitive function played a significant mediating role in prediction of depression to ADL in patients with PD (β = 0.006, p = 0.074, 95%CI = 0.001 ~ 0.014), and cognitive function also played a significant mediating role in prediction of depression to ADL (β = 0.006, p = 0.067, 95%CI = 0.001 ~ 0.013).

Conclusion: There is a bidirectional relationship between depression and ADL in patients with PD. Furthermore, we found that cognitive function mediates the relationship that exists between depression and ADL in patients with PD. Interventions aimed at enhancing cognitive function could potentially lessen the vicious cycle of depression and ADL in PD, thus improving patient quality of life (QOL).

KEYWORDS

Parkinson's disease, depression, cognitive function, activities of daily living, cross-lagged panel model, longitudinal mediation analysis

1 Introduction

Parkinson's disease (PD) is the second most prevalent neurological disorder after Alzheimer's. As global aging advances, its incidence is increasing, particularly affecting older individuals, which places a greater burden on societal and economic structures (Dorsey et al., 2018; Santos García et al., 2019). While PD patients commonly exhibit motor symptoms, non-motor symptoms such as depression, cognitive impairment, and autonomic dysfunction often manifest earlier. These symptoms can significantly impact the quality of life (QOL) more profoundly than motor symptoms (Hussein et al., 2021).

One of the most prevalent non-motor symptoms among people with PD is depression, which usually manifests in the beginning phases of the illness, and affects approximately 40–50% of patients (Camargo et al., 2017; Cong et al., 2022). Activities of Daily Living (ADL) are crucial indicators of PD severity and directly reflect patients the QOL (Santos García et al., 2019). Several research have examined a link between depression and ADL in PD patients. Several cross-sectional studies have discovered that depression is a strong predictor of ADL, and depression is inversely linked with ADL in patients with PD (Lawrence et al., 2014; He et al., 2021a). Additionally, longitudinal research has shown that lower ADL scores are a risk factor for depression in PD patients (Antar et al., 2021). However, the studies mentioned above all explored the unidirectional relationship between depression and ADL in PD patients. Although a recent cohort study showed a bivariate relationship between depression and both ADL and instrumental activities of daily living (IADL) in older adults, this study included ADL in addition to IADL, and different scales were used to assess ADL (Zhu et al., 2024).

Another prevalent non-motor symptom of PD is cognitive impairment, about 25% of people with PD are diagnosed with mild cognitive impairment at initial diagnosis, and up to 80% experience Parkinson's disease dementia (PDD) within 15–20 years of diagnosis (Hely et al., 2008; Litvan et al., 2011). It has been shown that depression in PD patients is a risk factor for cognitive impairment, and that cognitive impairment in PD patients is associated with ADL difficulties (Rosenthal et al., 2010; Hemphill et al., 2023), In a cross-sectional study, Jones et al. assessed 214 US patients and found that those with Parkinson's Disease Mild Cognitive Impairment (PD-MCI) exhibited more severe depression than those without cognitive deficits (Jones et al., 2016). And impaired ADL in PD patients increase the risk of cognitive impairment (Jones et al., 2016; D'Iorio et al., 2018).

The above studies are two-by-two relationship between depression, ADL, and cognitive function in PD patients. There are some studies on the relationship between cognitive function, ADL, and depression. Sun et al. found that cognitive function can influence depressive status through ADL in older adults (Sun et al., 2024). Ai et al. found a significant interaction between ADL limitation and cognitive dysfunction in older adults, both of which are risk factors for depression (Ai et al., 2024). However, these studies were conducted on older adults, not PD patients. One Korean study of 32 MCI patients found that cognitive function mediated the effect of depression on ADL (Jung et al., 2023). However, this study was a cross-sectional study and could not explore the causal relationship between variables. Survey-based research often employ Cross-lagged panel model (CLPM) to establish causality because it can eliminate autoregressive effects (Schuurman et al., 2016). The examination of the interaction between variables has been applied widely (Cole and Maxwell, 2003).

Our study analyzed the relationship between cognitive function, ADL, and depression in PD patients to fill the gap in longitudinal research on the relationship between depression, cognitive function, and ADL in PD patients. This study explored the bidirectional relationship between depression and ADL. Then, we also investigated the longitudinal mediating role of cognitive function in the bidirectional relationship between depression and ADL in PD patients. Patients with PD presenting with depression can have an impact on their QOL by affecting their motor symptoms (Nagarajan et al., 2024). PD patients with depression affect their ADL, which serious affects QOL (Cong et al., 2022). Depression and ADL in PD patients can mediate the impact of their cognitive function on the QOL (He et al., 2021a). So as to provide theoretical basis for enhancing the QOL of PD patients.

2 Methods

2.1 Data and sample

Data used in the preparation of this article were obtained (on January 29, 2024) from the Parkinson's Progression Markers Initiative (PPMI) database,[1] RRID:SCR_006431. For up-to-date information on the study, visit http://www.ppmi-info.org. PPMI is an extensive global, multicenter, longitudinal observational study. In order to better understand the etiology and progression of the disease, a longitudinal clinical, imaging, and biomarker assessment was carried out at 21 clinical sites in the US, Europe, and Australia using standardized data collecting. We chose data from the PPMI database from 2010 to 2024 at baseline and at two-year follow-up (with follow-up at 1-year intervals), which includes PD diagnoses. Participants with only baseline data or one-year follow-up, missing data from both the baseline and two-year follow-up, or lacking essential demographic information were excluded. Figure 1 shows the specific sample inclusion criteria, and our ultimate sample size was 892 people. None of our participants received any therapy at baseline. However, they underwent confirmatory tests, including clinical and cognitive assessments, imaging examinations, and biological specimen collection, all approved by the local Central Institutional Review Board. Each participant provided written informed consent prior to enrollment.

2.2 Measurements

Depression was assessed using the Geriatric Depression Scale (GDS). We selected the GDS-15, a shorter version with demonstrated reliability and validity in clinical assessments (Acosta Quiroz et al., 2020). The total score ranged from 0 to 15, with ≥5 indicating mild depression and ≥10 indicating moderate to severe depression.

1 https://www.ppmi-info.org/access-data-specimens/download-data

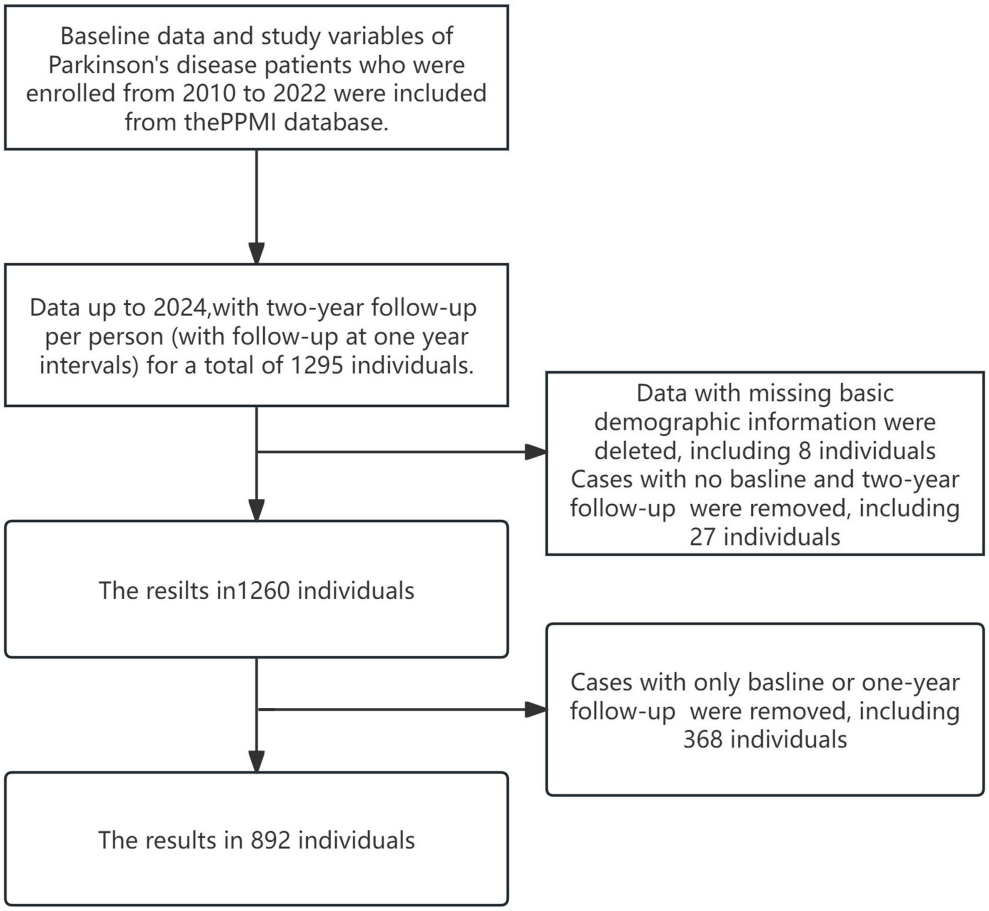

FIGURE 1
Sample selection.

ADL were assessed using the UPDRS II, the second part of the Unified Parkinson Disease Rating Scale, consisting of 13 items and a score range of 0–52. Higher scores indicate poorer functionality (Goetz et al., 2007). The UPDRS II has been shown to have good reliability and validity in patients with PD (Ramsay et al., 2020), and this is a useful marker for tracking the progression of the illness.

The Montreal Cognitive Assessment (MoCA) was used to assess cognitive function, which consists of 11 items with scores ranging from 0 to 30 and ≥26 normal. It has high sensitivity and specificity in the detection of cognitive function (Nasreddine et al., 2005).

2.3 Covariates

Including the age (<56 years, 56–65 years, and >65 years), gender (female, male), education (<13 years, 13–23 years, and >23 years), race white, black, Asian, and other, family history (1st degree family, no 1st degree family, and no family), and duration of disease (<5 years, 5–10 years, and >10 years) in patients with PD. In addition, patients with PD recorded in their medical history the use of levodopa in dopaminergic drug therapy during the follow-up period, indicated as Levodopa Equivalent Daily Dose (LEDD). LEDD1 indicates first year follow-up Levodopa Equivalent Daily Dose and LEDD2 indicates second year follow-up Levodopa Equivalent Daily Dose.

2.4 Statistical analysis

Data analysis was conducted using SPSS27.0 Missing data were imputed using multiple interpolation techniques. Quantitative data were expressed as mean ± standard deviation, and qualitative data were reported as frequencies (percentage). Independent samples t-test and one-way ANOVA were used to compare the scores of depression, cognitive function, and ADL across patients with different characteristics. Pearson correlation analysis was utilized to estimate relationships between variables. Mplus8.3 was used to fit the CLPM for depression, cognitive function, and ADL. $\alpha = 0.05$ was used as the test level. Holographic great likelihood estimation was used for parameter estimation, and the mediation effect was tested using the bias-corrected nonparametric percentile Bootstrap 1,000 iterations were taken (Holm et al., 2021; Yang and Zhao, 2024). And the coefficient product was significant and the mediation effect was significant if the confidence interval did not contain zero. The fit of the model was evaluated using several indices: chi-square/degrees of freedom (χ^2/df), Comparative Fit Index (CFI), Tucker-Lewis Index (TLI), Root Mean Square Error of Approximation (RMSEA), and Standardized Root Mean Square Residual (SRMR). A good model fit was indicated by (χ^2/df) < 5, CFI and TLI > 0.90, and RMSEA and SRMR < 0.08 (Bentler, 1990; Hu and Bentler, 1999; Schermelleh-Engel et al., 2003).

10.3389/fnagi.2025.1513373

3 Results

3.1 Basic information on the subject of the study

This study included 892 PD patients in total, 551 (61.8%) of them were male and 341 (38.2%) were female, most of them were white (93.6%), and most of them had 13–23 years of education (80.3%). Age > 65 years was 45.3%, and disease duration of <5 years was 92.6%, The percentage of LEDD1 > 500 mg in the first year of follow-up was 77.6%, and the percentage of LEDD2 > 500 mg in the second year of follow-up was 63.1%, as shown in Table 1.

Between-group comparisons of baseline ADL scores among demographic subgroups revealed statistically significant differences within age and disease duration groups ($p < 0.05$). Between-group comparisons of patients' depression scores at baseline based on demographic subgroups showed statistically significant differences in baseline patients' depression scores within education level, race, and disease duration groups ($p < 0.05$). Comparisons of baseline cognitive function scores among demographic subgroups demonstrated statistically significant differences within educational level, racial, family history, age, and disease duration groups ($p < 0.05$), as shown in Table 2.

Between-group comparisons of patients' ADL scores, depression scores, and cognitive function scores at the first year of follow-up were made according to LEDD subgroups ($p < 0.001$), as shown in Table 3.

Comparison of the patients' ADL scores, depression scores and cognitive function scores at the second year follow-up was made between groups according to LEDD subgroups, and the results showed that the differences of ADL scores and depression scores at the second year follow-up were statistically significant within LEDD subgroups ($p < 0.001$), and the differences of the patients' cognitive function score at the second year follow-up were statistically significant within LEDD subgroups ($p < 0.05$), as shown in Table 4.

TABLE 1 Demographic information on the study population ($N = 892$).

Variables	*N* (%)
Gender	
Female	341 (38.2%)
Male	551 (61.8%)
Education (years)	
<13	160 (17.9%)
13–23	716 (80.3%)
>23	16 (1.8%)
Race	
White	835 (93.6%)
Black	14 (1.6%)
Asian	14 (1.6%)
Other	29 (3.3%)
Family history	
1st degree family	205 (23.0%)
No-1st degree family	111 (12.4%)
No family	576 (64.6%)
Age (years)	
<56	229 (25.7%)
56–65	259 (29.0%)
>65	404 (45.3%)
Duration of disease (years)	
<5	826 (92.6%)
5–10	64 (7.2%)
>10	2 (0.2%)
LEDD1 (mg)	
<500	692 (77.6%)
500–1,000	156 (17.5%)
>1,000	44 (4.9%)
LEDD2 (mg)	
<500	563 (63.1%)
500–1,000	249 (27.9%)
>1,000	80 (9.0%)

3.2 Correlates of depression, ADL and cognitive function

Table 5 presents the mean and standard deviation of the scores for depression, cognitive function, and ADL at baseline and at two-year follow-up assessments. It also showed the interrelationships among these variables via correlation coefficients. The Pearson correlation analysis indicated that depression was positively correlated with ADL ($p < 0.01$), while both depression and ADL were negatively associated with cognitive function ($p < 0.01$).

3.3 Longitudinal mediation analysis of cognitive function in the relationship between depression and ADL in patients with PD

To evaluate the longitudinal mediating role of cognitive function in the relationship between depression and ADL, we constructed the CLPM incorporating three time points. This model adjusted for factors including age, gender, education, race, family history, and disease duration and LEDD in PD patients, positioning depression as the independent variable, cognitive function as the mediator, and ADL as the dependent variable. After stepwise removal of non-significant paths, the final results are shown in Figure 2. Model fit indices were favorable, with $\chi^2/df = 3.10$, CFI = 0.979, TLI = 0.946, RMSEA = 0.049, and SRMR = 0.045. T1 depression significantly predicted T2 ADL ($\beta = 0.079$, $p < 0.01$), T1 ADL significantly predicted T2 depression ($\beta = 0.069$, $p < 0.05$), which can indicate that, depression and ADL are causative of each other, and they can predict each other. T1 depression significantly predicted T2 cognitive function ($\beta = -0.082$, $p < 0.01$), T2 cognitive function significantly predicted T3 ADL ($\beta = -0.077$, $p < 0.01$). The results of Bootstrap analysis showed a significant mediating effect of T2 cognitive function ($\beta = 0.006$, $p = 0.074$, 95%CI = 0.001 ~ 0.014) in prediction of T1 depression on T3 ADL. T1 ADL significantly predicted T2 cognitive function ($\beta = -0.077$, $p < 0.01$), T2 cognitive function significantly predicted T3 depression ($\beta = -0.080$, $p < 0.05$). The results

10.3389/fnagi.2025.1513373

TABLE 2 Study participants' scores on Baseline ADL, depression, and cognitive function.

Variables	Mean ± SD			*t/F*			*p*		
	UPDRSII (Max score: 30)	GDS (Max score: 14)	MOCA (Max score: 30)	UPDRSII	GDS	MOCA	UPDRSII	GDS	MOCA
Gender									
Female	6.26 ± 4.56	2.71 ± 2.88	26.79 ± 2.89	−0.384	1.598	1.607	0.701	0.11	0.108
Male	6.38 ± 4.67	2.41 ± 2.61	26.48 ± 2.75						
Education (years)									
<13	6.16 ± 4.51	2.94 ± 2.86	25.80 ± 3.52	0.517	3.275	8.115	0.596	0.038	<0.001
13–23	6.34 ± 4.63	2.46 ± 2.70	26.76 ± 2.61						
>23	7.38 ± 5.68	1.50 ± 1.67	27.19 ± 1.94						
Race									
White	6.33 ± 4.66	2.45 ± 2.67	26.69 ± 2.75	0.049	5.273	5.923	0.986	0.001	<0.001
Black	6.71 ± 4.76	4.71 ± 4.21	25.71 ± 3.15						
Asian	6.29 ± 3.93	2.79 ± 2.42	26.14 ± 3.11						
Other	6.14 ± 4.07	3.72 ± 2.80	24.59 ± 3.45						
Family history									
1st degree family	6.76 ± 4.98	2.75 ± 2.74	25.74 ± 3.43	1.396	0.927	12.978	0.248	0.396	<0.001
No-1st degree family	5.91 ± 4.07	2.41 ± 2.56	27.05 ± 2.32						
No family	6.26 ± 4.59	2.47 ± 2.72	26.81 ± 2.58						
Age (years)									
<56	6.10 ± 4.78	2.80 ± 2.95	27.31 ± 2.80	4.482	2.717	25.131	0.012	0.067	<0.001
56–65	5.77 ± 4.24	2.24 ± 2.75	27.06 ± 2.22						
>65	6.82 ± 4.72	2.56 ± 2.54	25.89 ± 2.99						
Duration of disease (years)									
<5	6.14 ± 4.40	2.43 ± 2.64	26.70 ± 2.65	12.137	9.896	8.545	<0.001	<0.001	<0.001
5–10	9.21 ± 6.62	4.05 ± 3.41	25.16 ± 4,23						
>10	4.00 ± 2.83	1.00 ± 1.41	26.59 ± 2.81						

GDS, Geriatric Depression Scale score; MOCA, Montreal Cognitive Assessment Scale score; UPDRS II, Unified Parkinson's Disease Rating Scale Part II score.

TABLE 3 Study participants' scores on ADL, depression, and cognitive function in the first year follow-up.

Variables	Mean + SD			*F*			*p*		
	UPPRSII	GDS	MOCA	UPDRSII	GDS	MOCA	UPDRSII	GDS	MOCA
LEDD1									
<500 mg	7.16 ± 4.82	2.38 ± 2.74	26.51 ± 2.88	15.210	17.386	9.233	<0.001	<0.001	<0.001
500–1,000 mg	9.28 ± 7.03	3.73 ± 3.58	25.32 ± 4.43						
>1,000 mg	10.20 ± 6.37	3.95 ± 3.72	25.64 ± 4.44						

GDS, Geriatric Depression Scale score; MOCA, Montreal Cognitive Assessment Scale score; UPDRS II, Unified Parkinson's Disease Rating Scale Part II score; LEDD1, first year follow-up Levodopa Equivalent Daily Dose.

of Bootstrap analyses showed a significant mediating effect of T2 cognitive function ($\beta = 0.006$, $p = 0.067$, 95%CI = 0.001 ~ 0.013) in prediction of T1 ADL on T3 depression.

4 Discussion

This study utilized a longitudinal research design and focused on the relationship between depression, cognitive function, and ADL in patients with PD, with special attention to the mediating role of cognitive function. We confirmed a bidirectional relationship between depression and ADL in PD patients. Mediation analysis showed that cognitive function mediated the bidirectional relationship between depression and ADL in PD patients.

The present study provided evidence for a bidirectional relationship between depression and ADL in PD patients, which complements studies showing that PD patients' ADL predicted

TABLE 4 Study participants' scores on ADL, depression, and cognitive function in the second year follow-up.

Variables	Mean + SD			*F*			*p*		
	UPDRSII	GDS	MOCA	UPDRSII	GDS	MOCA	UPDRSII	GDS	MOCA
LEDD2									
<500 mg	7.45 ± 5.43	2.49 ± 2.70	26.53 ± 3.25	13.972	8.823	4.449	<0.001	<0.001	0.012
500–1,000 mg	8.73 ± 6.83	3.00 ± 3.05	26.37 ± 3.52						
>1,000 mg	11.03 ± 7.38	3.83 ± 3.59	25.30 ± 4.40						

GDS, Geriatric Depression Scale score; MOCA, Montreal Cognitive Assessment Scale score; UPDRS II, Unified Parkinson's Disease Rating Scale Part II score; LEDD2, second year follow-up Levodopa Equivalent Daily Dose.

TABLE 5 Correlation analysis among variables.

Variables	1	2	3	4	5	6	7	8	9
GDS1	1								
GDS2	0.642**	1							
GDS3	0.609**	0.704**	1						
MOCA1	−0.124**	−0.141**	−0.185**	1					
MOCA2	−0.188**	−0.205**	−0.251**	0.627**	1				
MOCA3	−0.180**	−0.234**	−0.283**	0.609**	0.790**	1			
UPDRSII1	0.328**	0.297**	0.298**	−0.142**	−0.194**	−0.222**	1		
UPDRSII2	0.299**	0.365**	0.349**	−0.183**	−0.254**	−0.310**	0.682**	1	
UPDRSII3	0.258**	0.318**	0.367**	−0.198**	−0.283**	−0.338**	0.637**	0.772**	1
Mean	2.53	2.70	2.75	26.59	26.26	26.37	6.33	7.68	8.13
SD	2.72	3.01	2.91	2.80	3.32	3.46	4.62	5.44	6.12

GDS, the Geriatric Depression Scale score; MOCA, Montreal Cognitive Assessment Scale score; UPDRS II, Unified Parkinson's Disease Rating Scale Part II score.
**$p < 0.01$.

subsequent depression (Xu et al., 2015; Antar et al., 2021) and that PD patients' depression predicted subsequent ADL (Randver, 2018; Jin et al., 2020). Elderly people with limited ADL are in great need of help and care from others, their QOL is lower than before, they have fewer opportunities for social support, and they have difficulty in regulating their negative emotions in a timely manner. In turn depression can lead to fatigue, decreased energy levels and disrupted sleep patterns, further hindering the ability to perform basic daily tasks and affecting ADL (Pearson et al., 2023). It is that although the results of a recent longitudinal study showed that ADL in PD patients can influence the progression of depression, the reverse pathway could not confirm that depression in PD patients influences ADL (Wang et al., 2024), which is contradictory to the results of our findings, and the reason for this analysis may be due to the relatively large sample size of our study as well as the fact that we adjusted for a number of factors such as age and LEDD. In comparison with the study by Sperens et al. (2020), our study only found that baseline depression predicted ADL, which may have been related to the fact that the patients received treatment during the follow-up period and the shorter follow-up period of the current study.

Our study found that depression went on to affect ADL by affecting cognitive function. Depression predicted subsequent cognitive function in patients may be related to Locus coeruleus (LC). LC is considered to be the main noradrenergic nucleus in the CNS, and the accumulation of synaptic nuclear proteins in the LC leads to the loss and degeneration of LC neurons, which may result in reduced innervation of LC target nuclei and reduced levels of NE in several regions of the brain in patients with PD, leading to the onset of depression, which further reduces responsiveness to sensory inputs and impairs cognitive flexibility and vigilant attention (Zarow et al., 2003; Paredes-Rodriguez et al., 2020). Cognitive function in PD patients predicted subsequent ADL. The likely reason for this is that cognitive decline not only impairs older adults in areas such as memory and processing speed, but also reduces their sense of self-efficacy, which ultimately affects ADL (Sun et al., 2024). Therefore, depressive symptoms in older adults reduce mental flexibility and their ability to consolidate and retrieve memories, thus affecting overall cognitive function (Yatawara et al., 2016), which in turn can lead to reduced memory, difficulty in decision making, and impaired executive function can affect the patient's independence and ability to perform daily tasks, thus affecting ADL. Additionally, depression in PD patients can affect cognitive function by affecting their non-motor symptoms of apathy, and when their cognitive function declines, it can also affect ADL by affecting apathy (Szymkowicz et al., 2022; Cui et al., 2024). Therefore, apathy also plays a role in the relationship between depression, ADL, and cognitive function, and could be included in future studies to examine apathy.

Furthermore, our study found that ADL went on to affect depression by influencing cognitive function in patients with PD. ADL predicted subsequent cognitive function, the reason for

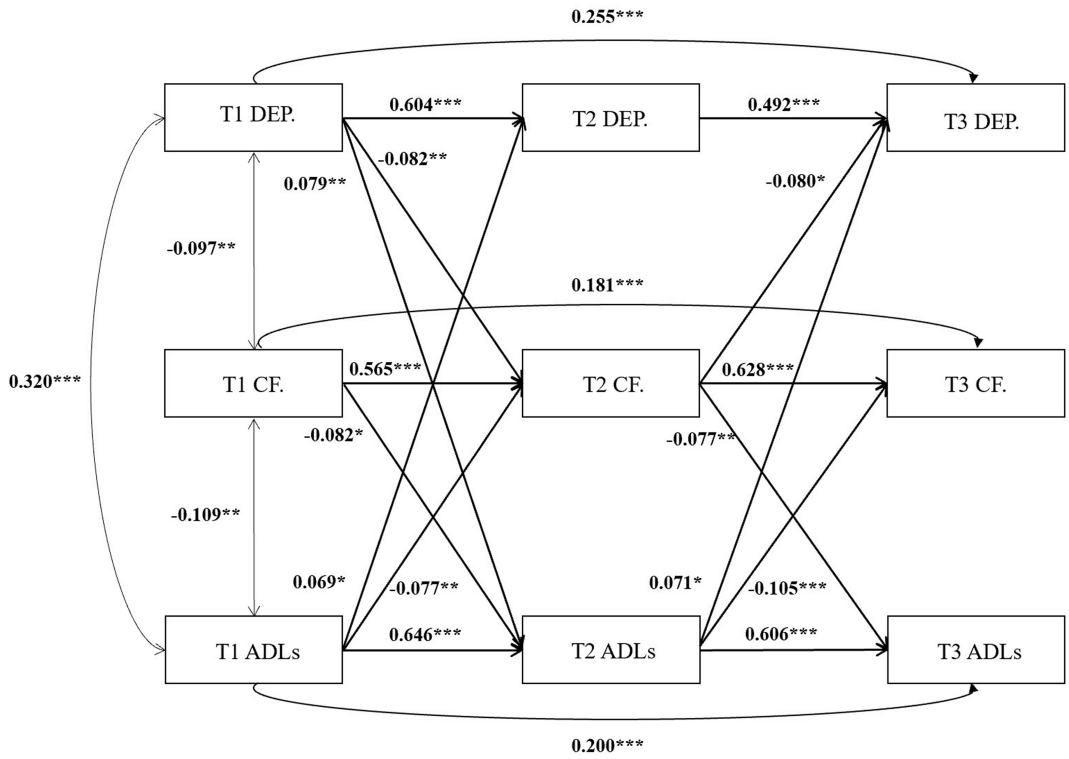

FIGURE 2
Cross-lagged panel model of depression, cognitive function, and ADL. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ADL, Activities of daily living; CF, Cognitive Function; DEP: Depression; T1, Baseline; T2, First year follow-up; T3, Second year follow-up.

this may be that people with PD have limited ADL, and this physical helplessness increases the risk of cognitive deficits. Cognitive function in PD patients predicted subsequent depression. Interpretation of PD in terms of its neuropathologic features, especially the deterioration of the striatum, loss of dopamine and disruption of the cortico-striatal pathway, may first affect cognitive function and then increase anxiety and depression (Weintraub et al., 2005), these brain regions are affected in PD and play an important role in cognitive function as well as in emotion regulation and motivation (Lago et al., 2017). Cognitive function can mediate the interpretation of the effects of ADL on depression is complex. Elderly people's body functions are affected by ageing, which inevitably leads to a decline in ADL levels, further leading to further cognitive impairment, and they have difficulty in dealing with a variety of situations in daily life, such as memorizing, learning, and problem solving, which can increase frustration and helplessness, thus aggravating depression (Hammar et al., 2022). Additionally, difficulties with ADL in PD patients lead to higher levels of stigma, a decrease in their social engagement interactions, which are an important way of obtaining information and social support, which in turn leads to cognitive dysfunction with lower executive function, exacerbating the development of depressive (Eccles et al., 2023; Zhang and Yang, 2024).

Since there is a bidirectional relationship between depression and ADL in PD patients, both the individual's ADL and depression may be addressed in PD patients. Clinicians can follow up with PD patients on a regular basis, distribute depression scales for testing, and conduct psychological counseling, relaxation training, and sleep interventions for patients with depressive symptoms, thus further reducing the burden of disabling illnesses brought about by depression. When a decline in ADL has occurred, PD patients should be helped to cope positively to avoid exacerbating depressive symptoms. Given that cognitive function mediates the bidirectional relationship between depression and ADL in PD patients, special attention should be paid to improving cognitive function in PD patients. In addition to administering conventional pharmacological treatments, mainly cholinesterase inhibitors such as carbapenems and doxorubicin that have been shown to be useful in clinical practice (Rolinski et al., 2012; Seppi et al., 2019), clinicians can also inform patients that they can engage in appropriate physical exercise and cognitive training, which may have a beneficial effect on cognitive function in patients with PD (Weintraub et al., 2022).

PD patients in this study were predominantly white, older, and more educated, which is related to the criteria PPMI chose for inclusion of PD patients, and there is now recent research that suggests that ethnic minority groups such as Latinos or Hispanics have more severe non-motor symptoms such as depression and cognitive deficits compared to whites, which may have a greater impact on ADL (Duarte Folle et al., 2023), which may be due to their low economic income, are less likely to visit an outpatient neurologist, and do not receive timely treatment, leading to the increasing severity of these non-motor symptoms (Saadi et al., 2017). Therefore, future studies could examine the relationship

between depression, ADL and cognitive function in PD patients of different races.

The findings of this study offer theoretical and practical insights into mitigating the negative cycle of depression and ADL in PD patients. In theory, the model explains the correlation mechanism between depression, ADL, and cognitive function, which lays the foundation for the next step of the study. In practice, the results have guiding significance for improving the vicious cycle of depression and ADL in PD patients, and intervention on cognitive function might be an appropriate choice to alleviate the vicious cycle of depression and ADL in PD patients.

Despite its strengths, the study has several limitations. Primarily, the study population was predominantly white, with most participants hailing from North America and Europe, which may limit the generalizability of the findings, given the global diversity represented in the PPMI database (Duarte Folle et al., 2023). In the future, we can study the relationship between depression, cognitive function, and ADL in PD patients of other races. Although our study adjusted for factors such as age, LEDD, etc. However, the use of antidepressants in patients with PD was not taken into account, as well as the possible influence of other non-motor symptoms such as apathy, which could be further included for analysis in the future. Finally, our study was selected as a longitudinal study with data from baseline and two-year follow-up, and although three time points were required to set up to satisfy the CLPM, more in-depth studies could be considered in the future by increasing the number of follow-up and the duration of the study (Cole and Maxwell, 2003; Little, 2024).

5 Conclusion

Individuals with PD exhibit a reciprocal relationship between depression and ADL, with cognitive function serving as a mediator. Given the interdependence of these variables, interventions aimed at improving cognitive function could potentially lessen the vicious cycle of depression and ADL in PD, thus improving patient the QOL. Additionally, it is essential to explore other potential pathways between ADL and depression to significantly improve the QOL in PD patients.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by the local Central Institutional Review Board. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Author contributions

YX: Conceptualization, Investigation, Methodology, Validation, Writing – original draft, DC: Formal analysis, Visualization, Writing – original draft. MD: Data curation, Writing – review & editing. YZ: Writing – review & editing. HY: Project administration, Writing – review & editing. YH: Project administration, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Platelet/high-density lipoprotein cholesterol ratio as a biomarker of depression in individuals with chronic opioid use

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Background: The comorbidity of depression and opioid use is increasingly recognized as a significant public health concern. Chronic opioid use can alter biological systems, including lipid metabolism and inflammatory responses, potentially contributing to depressive symptoms. The platelet/high-density lipoprotein cholesterol ratio (PHR) has emerged as a biomarker associated with both cardiovascular and mental health outcomes. This study investigates the relationship between PHR and depression in individuals with chronic opioid use.

Methods: A cross-sectional analysis was conducted using data from the National Health and Nutrition Examination Survey (NHANES) (2007–2018). A total of 843 participants with prescription opioid use were included. Depression was assessed using the Patient Health Questionnaire-9 (PHQ-9), with a score ≥10 indicating clinically significant depression. PHR was calculated from platelet counts and HDL cholesterol levels and categorized into quartiles. Weighted logistic regression and restricted cubic spline regression were employed to evaluate associations and potential nonlinear relationships, adjusting for demographic, socioeconomic, lifestyle, and clinical covariates.

Results: Higher PHR quartiles were significantly associated with increased odds of depression, even after full adjustment for confounders (OR for Q3: 3.40; 95% CI: 1.95–5.94; OR for Q4: 4.12; 95% CI: 2.21–7.12). A nonlinear relationship was observed, with depression risk increasing sharply beyond a specific PHR threshold. Subgroup analyses revealed stronger associations in younger participants and those with obesity, with significant interaction effects for age and BMI.

Conclusion: Elevated PHR is independently associated with depression in individuals with chronic opioid use, suggesting its potential as a biomarker for identifying at-risk populations. The findings underscore the need to address systemic inflammation and lipid dysregulation as part of integrated mental health care for opioid users.

KEYWORDS

platelet/HDL cholesterol ratio, depression, chronic opioid use, biomarkers, inflammation, lipid metabolism, NHANES

1 Introduction

The association between opioid use and mental health disorders, particularly depression, has been a subject of extensive research in recent years (1). Opioids, especially prescription painkillers, are commonly used for the management of chronic pain but have been linked to numerous adverse outcomes, including the development of substance use disorders and mood disturbances (2). One of the key factors influencing the relationship between opioid use and depression is the alteration of various biological systems, including inflammation and lipid metabolism (3). Among these, the platelet/high-density lipoprotein cholesterol ratio (PHR) has emerged as a potential biomarker for cardiovascular and mental health disorders (4). This ratio is thought to reflect a state of systemic inflammation and dyslipidemia, both of which have been implicated in the pathogenesis of depression (5).

Opioid use, particularly chronic prescription opioid consumption, is known to have significant effects on various biological systems, including lipid metabolism and platelet activity. Recent studies have highlighted the bidirectional nature of this relationship, with opioid use exacerbating depressive symptoms and vice versa. Additionally, the potential role of biological markers in improving early identification and stratification of high-risk groups in depression, including treatment-resistant depression (TRD), has gained attention. Maina et al. (2023) emphasize the importance of biological markers in the management of TRD, which is highly relevant when considering the role of biomarkers like the platelet/high-density lipoprotein cholesterol ratio (PHR) in opioid users (6). Opioids can increase platelet aggregation, which is linked to inflammatory responses that may play a role in mood disturbances (7). Chronic opioid use, by altering lipid profiles and inducing inflammation, may lead to dysregulated platelet function and changes in the platelet/high-density lipoprotein cholesterol ratio (PHR), a marker associated with systemic inflammation and vascular health. In turn, these biological alterations could contribute to the development of depressive symptoms, as inflammation and lipid dysregulation have been implicated in the pathophysiology of depression (8). Opioid receptors, including the mu, delta, and kappa receptors, are expressed on the membranes of platelets. These receptors play a critical role in platelet aggregation and activation, which could contribute to the inflammatory processes associated with depression in individuals using opioids. In addition to inflammation, other neurotransmission systems, particularly the serotonergic system, are critical in the pathophysiology of depression. The serotonin transporter, present on both platelets and neurons, regulates serotonin uptake. Alterations in serotonin function, including reduced serotonin transporter activity, may impair platelet function and further contribute to depression by enhancing the inflammatory response and disrupting the balance of neurotransmitters. This relationship is particularly concerning in the context of opioid use, where these changes may be amplified. The PHR itself has been proposed as a marker of vascular dysfunction and inflammatory processes, which are common in individuals with both opioid use disorders and depression. Numerous studies have highlighted the bidirectional relationship between depression and inflammation, with both conditions exacerbating each other's severity (9, 10). Similarly, emerging research has linked altered lipid profiles, including reduced HDL cholesterol levels and increased platelet aggregation, to an increased risk of depressive symptoms (11).

In the context of opioid use, it is plausible that chronic opioid consumption could lead to a dysregulation of lipid metabolism, contributing to changes in the PHR that may predispose individuals to depressive symptoms. This hypothesis is supported by evidence showing that individuals with opioid use disorders often exhibit altered lipid profiles and increased inflammatory markers (12). Furthermore, research suggests that depression itself can influence platelet activity and lipid metabolism, creating a complex interplay between these factors in individuals with opioid use history (13).

Recent epidemiological studies have underscored the importance of understanding how these biological markers interact with the neurobiological mechanisms of depression in opioid users. For example, a study by Macedo et al. found that individuals with a history of opioid misuse exhibited significantly higher platelet counts and lower HDL cholesterol levels, which were associated with more severe depressive symptoms (14). Additionally, the PHR has been shown to be a reliable marker of cardiovascular risk (15), which is often heightened in individuals with substance use disorders, particularly those using opioids.

Despite the growing interest in understanding the interplay between opioids, depression, and cardiovascular health, studies specifically examining the role of PHR in this context remain scarce. Research to date has primarily focused on PHR as a marker of cardiovascular risk, with limited investigations exploring its potential relevance to mental health, particularly in opioid users. While some evidence suggests that PHR might serve as an early indicator of depressive symptoms (16), its predictive value in the context of opioid-related disorders remains largely unexplored. Furthermore, the biological mechanisms linking PHR, opioid use, and depression are not well understood, highlighting the need for more targeted research to elucidate these complex interactions.

In this study, we aim to investigate the relationship between PHR and depression in a cohort of adults with a history of prescription opioid use between 2007 and 2018. We hypothesize that alterations in the PHR are associated with an increased risk of depression, due to underlying inflammation and lipid dysregulation induced by opioid use. Understanding this relationship could provide valuable insights into the complex biological interactions between opioids, depression, and cardiovascular risk, potentially leading to improved therapeutic strategies for individuals with opioid use disorders.

2 Methods

2.1 Study design and population

This cross-sectional study utilized data from the National Health and Nutrition Examination Survey (NHANES) conducted

between 2007 and 2018, a program designed to assess the health and nutritional status of the U.S. population through a complex, multistage probability sampling design. In the exclusion criteria, we specifically excluded participants who were undergoing pharmacological treatments that could influence depressive states or biochemical indicators, such as antidepressants, statins, and anti-inflammatory drugs. These medications can affect lipid profiles, platelet activity, and inflammatory markers, which are relevant to the study's focus on platelet/high-density lipoprotein cholesterol ratio (PHR). To ensure the results accurately reflect the relationship between opioid use and depression, only individuals not currently on these treatments were included in the study. The initial dataset included 48,291 participants. To identify a relevant study population, we sequentially excluded individuals based on specific criteria. First, participants with missing data on depression, assessed using the Patient Health Questionnaire-9 (PHQ-9), were excluded (n = 16,822), leaving 31,469 participants. Second, individuals with incomplete laboratory data for calculating the platelet/high-density lipoprotein cholesterol ratio (PHR) were excluded (n = 1,656), resulting in 29,813 participants. Third, we identified 1,025 individuals who provided complete information on prescription opioid use. Chronic opioid use was defined as the regular use of prescription opioids for at least three months. Participants who reported using opioid medications such as oxycodone, hydrocodone, morphine, or fentanyl (whether oral, transdermal, or through other routes) were classified as chronic opioid users. The most common opioids in the study were oxycodone and hydrocodone, with oral consumption being the predominant route. Participants using opioid patches (e.g., fentanyl dermal patches) for therapeutic purposes or those using opioids recreationally (e.g., fentanyl or heroin) were also included in the chronic opioid use group. The type of opioid and the route of administration (oral, dermal, intravenous, or others) were recorded to allow for detailed analysis of the impact of opioid consumption on depression and biochemical markers. Lastly, participants with missing data for key covariates, including demographic, socioeconomic, lifestyle, and clinical variables, were excluded, yielding a final analytical sample of 843 participants. A flowchart illustrating the selection process is provided in Figure 1.

2.2 Depression assessment

Depressive symptoms were assessed using the Patient Health Questionnaire-9 (PHQ-9), a validated tool widely used in epidemiological studies. The PHQ-9 evaluates the frequency of nine depressive symptoms over the past two weeks, with response options ranging from 0 ("not at all") to 3 ("nearly every day"). The

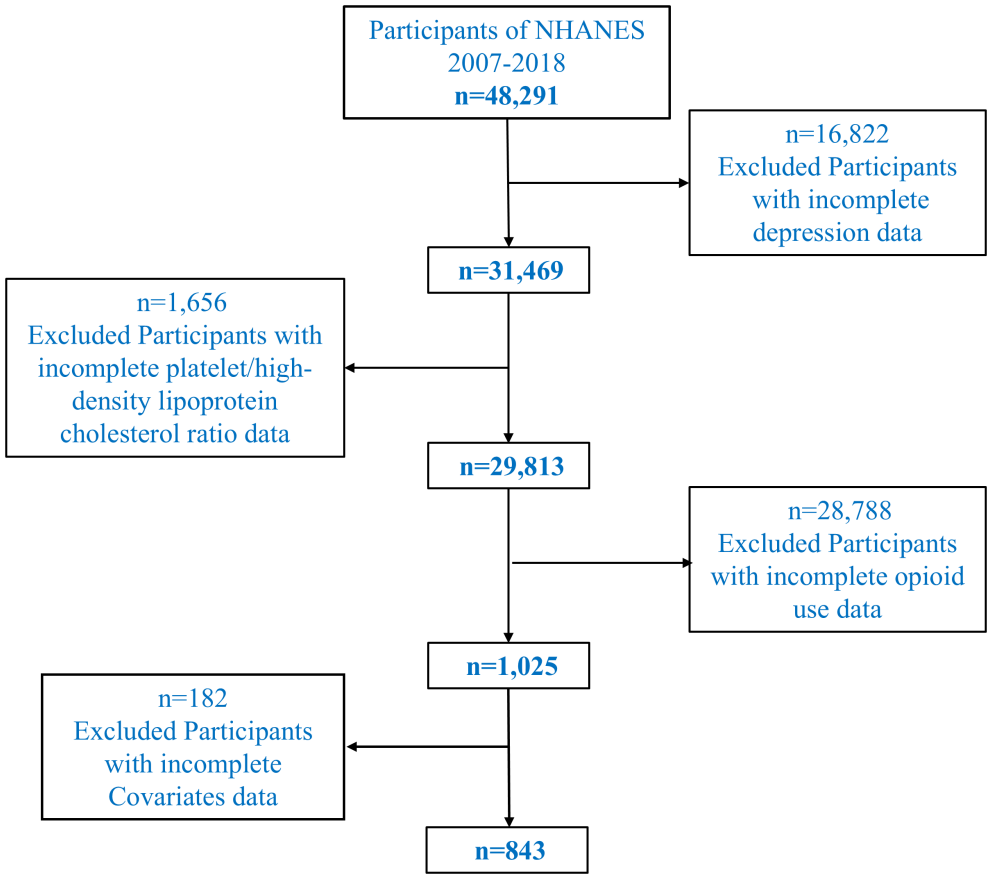

FIGURE 1
Population screening diagram.

total score ranges from 0 to 27, with higher scores indicating greater depressive symptomatology. For this study, a PHQ-9 score of 10 or higher was used to define clinically significant depression, consistent with its established sensitivity and specificity for diagnosing major depressive disorder (17). This binary classification allowed us to differentiate individuals with significant depressive symptoms from those without.

2.3 Platelet/high-density lipoprotein cholesterol ratio

The platelet/high-density lipoprotein cholesterol ratio (PHR) was calculated as the ratio of platelet count to HDL-C level, both of which were measured following NHANES standardized protocols. Platelet count, expressed as $\times 10^9$/L, was determined using a complete blood count (CBC) with a five-part differential analyzer, a reliable tool for hematological analysis. HDL-C concentration, expressed in mg/dL, was measured enzymatically after dextran sulfate-magnesium precipitation of other lipoproteins (18). The platelet/high-density lipoprotein cholesterol ratio (PHR) was calculated as the ratio of platelet count ($\times 10^9$/L) to high-density lipoprotein cholesterol (HDL-C) level (mg/dL), both measured following NHANES standardized protocols. Specifically, the PHR is calculated using the following formula:

$$\text{PHR} = \text{Platelet count}(\times 10^9/\text{L})/\text{HDL} - \text{C}(\text{mg/dL})$$

All laboratory procedures adhered to NHANES quality control standards to ensure accuracy and reliability of the measurements. PHR was then derived as a continuous variable and subsequently categorized into quartiles based on the weighted distribution within the study population.

2.4 Covariates

Several covariates were included to account for potential confounders in the association between PHR and depression. Demographic variables comprised age (measured in years), gender (male or female), race/ethnicity (Mexican American, other Hispanic, non-Hispanic Black, non-Hispanic White, and other races), and marital status (married, widowed, divorced, separated, never married, or living with a partner). Socioeconomic factors included educational attainment (less than 9th grade, 9–11th grade, high school graduate, some college or associate degree, and college graduate or above) and poverty-income ratio (PIR, categorized as ≤1, 1–3, and >3). Lifestyle factors such as smoking status (current smoker or non-smoker) and alcohol consumption (yes or no, based on self-reported alcohol use in the past 12 months) were also considered. Clinical characteristics included hypertension, hyperlipidemia, diabetes (classified as yes, no, or borderline), body mass index (BMI; categorized as underweight, normal weight, overweight, and obese), and self-reported average sleep duration in hours. These covariates were selected based on their theoretical or empirical associations with both PHR and depression.

2.5 Statistical analysis

All statistical analyses were conducted using SPSS version 27.0 (IBM Corp., Armonk, NY) and R version 4.4.2, with the application of NHANES survey weights to account for the complex sampling design and ensure nationally representative estimates. Continuous variables were expressed as means with standard deviations (SDs), while categorical variables were summarized as frequencies and percentages. Differences between individuals with and without depression were assessed using the independent-samples t-test for continuous variables and the chi-square test for categorical variables. To evaluate the association between PHR and depression, weighted logistic regression models were constructed with three levels of adjustment. Model 1 adjusted for age and gender. Model 2 included additional adjustments for race/ethnicity, marital status, education level, and PIR. Model 3 further adjusted for smoking, alcohol consumption, hypertension, hyperlipidemia, diabetes, BMI, and sleep duration. Results were expressed as odds ratios (ORs) with 95% confidence intervals (CIs), with the lowest PHR quartile (Q1) serving as the reference group.

Restricted cubic spline regression was employed to explore potential nonlinear relationships between PHR and depression. Knots were placed at the 10th, 50th, and 90th percentiles of PHR to provide a flexible yet parsimonious model for capturing dose-response relationships. Subgroup analyses were performed to investigate whether the association between PHR and depression varied by key factors such as gender, age, and BMI. Interaction terms were tested in the logistic regression models to formally evaluate effect modification, and results from these analyses were visualized using forest plots.

2.6 Ethical considerations

The NHANES study was approved by the National Center for Health Statistics (NCHS) Research Ethics Review Board. All participants provided written informed consent prior to participation. The present study, being a secondary analysis of publicly available and de-identified data, was exempt from additional ethical review by our institutional ethics committee.

3 Results

3.1 Baseline characteristics of the study participants

The baseline characteristics of the 843 participants included in the final analysis are presented in Table 1. The table is divided into two sections: sociodemographic variables and clinical variables.

TABLE 1 Baseline characteristics of the study participants.

Characteristics	Overall	Depression		*P*-value
		No	Yes	
n	843	599	244	
Age, years	56.72 ±14.93	58.54 ±13.73	52.25 ±14.49	0.002
Gender, n (%)				0.149
Female	404(47.9%)	287(34.0%)	117(13.9%)	
Male	439(52.1%)	312(37.0%)	127(15.1%)	
Race, n (%)				0.057
Mexican American	86(10.2%)	55(6.5%)	31(3.7%)	
Other Hispanic	71(8.4%)	48(5.7%)	23(2.7%)	
Non-Hispanic Black	461(54.7%)	320(38.0%)	141(16.7%)	
Non-Hispanic White	169(20.0%)	137(16.3%)	32(3.8%)	
Other races	56(6.6%)	39(4.6%)	17(2.0%)	
Education, n (%)				0.542
Less than 9th grade	83(9.8%)	57(6.8%)	26(3.1%)	
9-11th grade	150(17.8%)	103(12.2%)	47(5.6%)	
High school graduate	211(25.0%)	142(16.8%)	69(8.2%)	
Some college or AA degree	280(33.2%)	199(23.6%)	81(9.6%)	
College graduate or above	119(14.1%)	98(11.6%)	21(2.5%)	
Marital Status, n(%)				0.815
Married	415(49.2%)	297(35.2%)	118(14.0%)	
Widowed	76(9.0%)	63(7.5%)	13(1.5%)	
Divorced	151(17.9%)	108(12.8%)	43(5.1%)	
Separated	48(5.7%)	31(3.7%)	17(2.0%)	
Never married	105(12.5%)	68(8.1%)	37(4.4%)	
Living with partner	48(5.7%)	32(3.8%)	16(1.9%)	
PIR, n (%)				0.209
<=1	247(29.3%)	160(19.0%)	87(10.3%)	
1-3	376(44.6%)	264(31.3%)	112(13.3%)	
>3	220(26.1%)	175(20.8%)	45(5.3%)	
Smoke, n (%)				0.007
Yes	557(66.1%)	374(44.4%)	183(21.7%)	
No	286(33.9%)	225(26.7%)	61(7.2%)	
Alcohol Use, n (%)				0.079
Yes	644(76.4%)	463(54.9%)	181(21.5%)	
No	199(23.6%)	136(16.1%)	63(7.5%)	
Hypertension, n (%)				0.015
Yes	509(60.4%)	359(42.6%)	150(17.8%)	
No	334(39.6%)	240(28.5%)	94(11.2%)	
Hyperlipidemia, n (%)				0.046
Yes	438(52.0%)	297(35.2%)	141(16.7%)	
No	405(48.0%)	302(35.8%)	103(12.2%)	
Diabetes, n (%)				<0.001
Yes	209(24.8%)	144(17.1%)	65(7.7%)	
Borderline	18(2.1%)	16(1.9%)	2(0.2%)	
No	615(73.0%)	438(52.0%)	177(21.0%)	
BMI, n (%)				<0.001
Underweight	15(1.8%)	10(1.2%)	5(0.6%)	
Normal weight	181(21.5%)	151(17.9%)	30(3.6%)	
Overweight	246(29.2%)	179(21.2%)	67(7.9%)	
Obesity	401(47.6%)	259(30.7%)	142(16.8%)	

Sociodemographic variables include age, gender, race/ethnicity, and marital status, while clinical variables are further categorized into non-pathological personal history (e.g., smoking status, alcohol consumption) and pathological history (e.g., hypertension, hyperlipidemia, diabetes). Comorbidities are classified under pathological history to differentiate them from non-pathological factors. The mean age of the participants was 56.72 ± 14.93 years, with individuals experiencing depression being significantly younger than those without depression (52.25 ± 14.49 *vs.* 58.54 ± 13.73 years, p = 0.002). A quartile graph by gender and for the total population was used to visualize the distribution of participants' ages. This distribution was further examined to assess the potential impact of menopause and climacteric symptoms in women, which are strongly associated with depression. Among the study population, 47.9% were female, and while gender distribution did not differ significantly between groups with and without depression (p = 0.149), a higher proportion of depressed individuals were female (13.9%) compared to males (15.1%). Race and ethnicity also showed marginal differences (p = 0.057), with a larger percentage of non-Hispanic Black individuals (54.7%) comprising the total sample, and depressed individuals being more represented in this group (16.7%).

Educational level and marital status did not show significant differences between groups (p = 0.542 and p = 0.815, respectively). This simplification allowed for clearer assessment of whether companionship influences depression. However, lifestyle and clinical

characteristics highlighted notable disparities. Smokers were significantly more likely to be depressed compared to non-smokers (21.7% *vs.* 7.2%, p = 0.007). Similarly, hypertension (p = 0.015), hyperlipidemia (p = 0.046), and diabetes (p < 0.001) were more prevalent in depressed individuals. Body mass index (BMI) distributions also differed significantly (p < 0.001), with obesity being more common among depressed individuals (16.8%) compared to those without depression.

3.2 Association between PHR and depression in individuals with chronic opioid use

Weighted logistic regression analyses assessing the association between PHR quartiles and depression are summarized in Table 2. In the unadjusted model, higher PHR quartiles were positively associated with depression. After adjusting for age and gender (Model 1), participants in the third quartile (Q3) had significantly higher odds of depression compared to the lowest quartile (OR: 3.83, 95% CI: 2.23–5.55, p < 0.001). This association remained robust in Model 2, which additionally adjusted for race, marital status, education, and PIR (OR: 3.62, 95% CI: 2.09–6.24, p < 0.001), and in Model 3, which further controlled for lifestyle and clinical variables (OR: 3.40, 95% CI: 1.95–5.94, p < 0.001). Participants in the highest quartile (Q4) also exhibited significantly higher odds of depression compared to Q1 across all models, with a consistent trend (p for trend < 0.001). These findings suggest a strong and independent association between elevated PHR and depression among individuals with opioid use.

3.3 Nonlinear relationship between PHR and depression

The restricted cubic spline analysis revealed a nonlinear relationship between PHR and the odds of depression, as shown in Figure 2. The association became particularly pronounced at higher levels of PHR, indicating a dose-response relationship. The risk of depression remained relatively stable at lower PHR levels but increased sharply after a specific threshold, suggesting that elevated PHR might serve as a critical biomarker for identifying individuals at greater risk of depression.

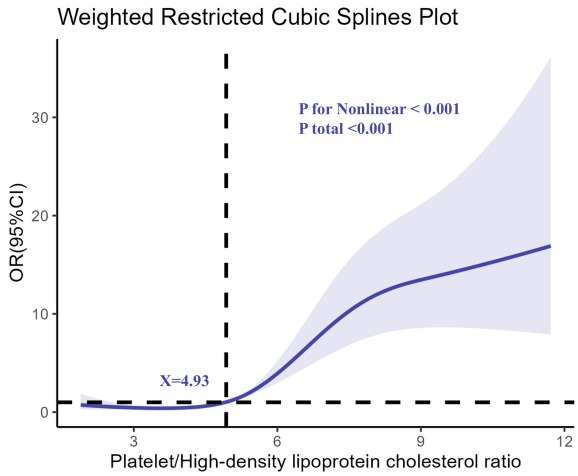

FIGURE 2
The nonlinear relationship between PHR and the odds of depression.

3.4 Subgroup and interaction analyses

Subgroup analyses stratified by age, gender, BMI, and other key covariates are summarized in Figure 3. The association between PHR and depression was consistent across most subgroups but exhibited variations in magnitude. For instance, the effect of higher PHR on depression was more pronounced in younger individuals (age < 60 years) and in those with obesity (BMI ≥ 30). The interaction analyses revealed significant effect modification by age (p for interaction = 0.01) and BMI (p for interaction = 0.03), indicating that these factors may amplify the impact of elevated PHR on depression risk. Gender-stratified analysis showed a slightly stronger association among females compared to males, although this interaction did not reach statistical significance (p for interaction = 0.08). These results

TABLE 2 Weighted logistic regression analyses of association between the platelet/high-density lipoprotein cholesterol ratio and depression in Opioid-Using Populations.

PHR	Model 1		Model 2		Model 3	
	OR 95%CI	P value	OR 95%CI	P value	OR 95%CI	P value
Q1	Ref		Ref		Ref	
Q2	0.55(0.27,1.16)	0.118	0.52(0.25,1.09)	0.083	0.50(0.24,1.06)	0.071
Q3	3.83(2.23,5.55)	<0.001	3.6192.09,6.24)	<0.001	3.40(1.95,5.94)	<0.001
Q4	2.09(1.22,3.58)	<0.001	1.97(1.13,3.42)	<0.001	1.81(1.03,3.19)	<0.001
p for trend		<0.001		<0.001		<0.001

Model 1: Adjusted for gender, age.
Model 2: Additionally, adjusted for race, marital status, education and poverty-income ratio.
Model 3: Additionally, adjusted for smoke, alcohol Use, hypertension, hyperlipidemia, diabetes, BMI, sleep time.

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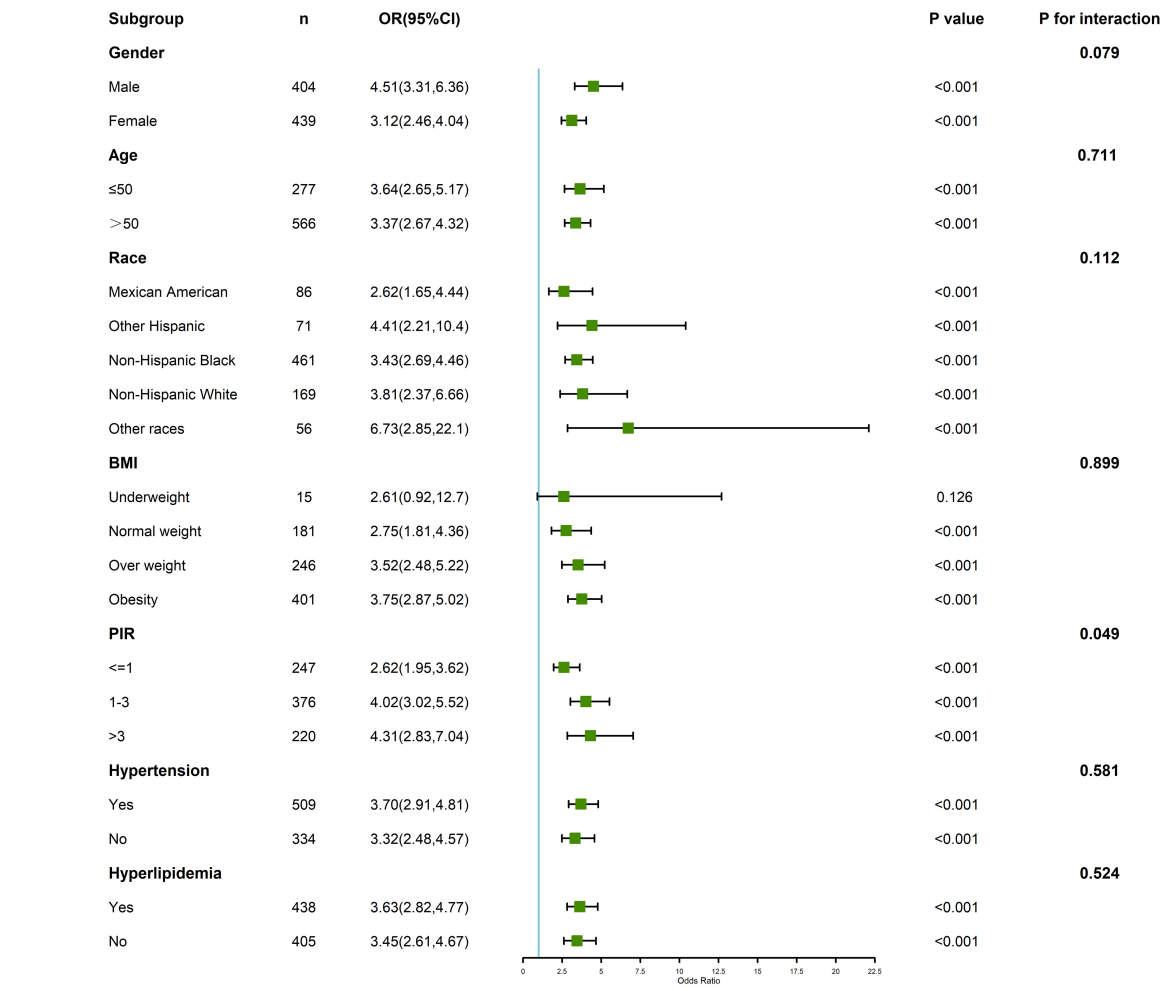

FIGURE 3
Forest plot of subgroup analysis and interaction analysis.

highlight the potential importance of individual-level characteristics in modifying the relationship between PHR and depression.

4 Discussion

Depression, a common comorbidity among opioid users, is often mediated by biological alterations in inflammatory and metabolic pathways (19, 20). Platelet/high-density lipoprotein cholesterol ratio (PHR), a novel biomarker that integrates inflammatory and lipid profiles, offers a unique lens to understand these mechanisms. In this study, PHR was examined as a potential biomarker for depression among opioid users, contributing to a deeper understanding of how systemic inflammation and lipid metabolism may influence mental health outcomes.

A plausible mechanism underlying the association between PHR and depression lies in the role of chronic inflammation. Elevated platelet counts and reduced HDL cholesterol levels, which define higher PHR values, are both hallmarks of systemic inflammation. One possible mechanism underlying the increase in platelet counts is the activation of opioid receptors on platelet membranes. Opioids, particularly those commonly prescribed for pain management, may bind to these receptors and stimulate platelet aggregation. This process could lead to increased platelet counts and contribute to the inflammatory state observed in opioid users (21, 22). Chronic inflammation is well established as a key factor in the development of depression, and it can disrupt neurotransmitter pathways, alter hypothalamic-pituitary-adrenal (HPA) axis function, and impair neurogenesis. However, the mechanisms by which opioid use induces neuroinflammation are not fully understood. It is important to distinguish between peripheral and central nervous system mechanisms. While peripheral inflammation has been widely studied, opioids may also activate neuroinflammatory pathways in the brain. Previous research has shown that opioids can increase the production of pro-inflammatory cytokines, such as prostaglandins, in the central nervous system, which may contribute to mood disturbances and depression (23, 24). HDL cholesterol, beyond its lipid-transport role, has anti-inflammatory properties and modulates endothelial function, oxidative stress, and immune responses (25, 26). Thus, reduced HDL levels in individuals with high PHR could

exacerbate inflammatory damage, creating a feedback loop that perpetuates both cardiovascular and neuropsychiatric risks.

Opioid use further complicates this interplay. Chronic opioid consumption induces immune dysregulation, increasing pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), while simultaneously suppressing anti-inflammatory mechanisms (27–29). Opioid-induced reductions in HDL levels have also been documented, likely driven by alterations in hepatic lipid metabolism and peripheral immune activity (30, 31). These dual effects may heighten the vulnerability of opioid users to both cardiovascular and mental health disorders. The observed relationship between PHR and depression may therefore reflect a broader, multifaceted disruption of systemic homeostasis caused by opioid use.

From a comparative perspective, prior research has identified PHR as a marker of cardiovascular disease risk (32), with growing evidence linking it to psychiatric outcomes. While studies have highlighted the role of inflammatory markers such as CRP and IL-6 in depression, PHR offers distinct advantages by integrating two measurable and routinely assessed parameters: platelet count and HDL cholesterol (33–35). Unlike standalone markers, PHR captures a composite picture of systemic inflammation and lipid dysregulation, making it particularly relevant in populations with overlapping risks, such as opioid users. This study extends the application of PHR into a novel context, emphasizing its potential utility in mental health research and clinical practice.

Despite these strengths, the relationship between inflammation, lipid metabolism, and depression is complex and not fully understood. For instance, The nonlinear association observed in this study suggests that PHR influences depression risk only above a certain threshold, which may reflect the point at which the inflammatory response becomes significant enough to affect mood regulation. This threshold may be influenced by factors such as resilience and allostatic overload, which are common in stress-related depressive disorders. It is crucial to recognize that opioid treatment serves as an additional factor contributing to depression development, and individuals with high PHR may represent those at risk for allostatic overload, where the body's ability to adapt to chronic stressors is compromised. The PHR index could therefore be useful in identifying individuals who may be more vulnerable to depression due to both systemic inflammation and the stress imposed by opioid use (36, 37). These pathways may interact with inflammation and lipid metabolism to influence mental health outcomes (38). Future research should integrate a broader range of biomarkers to disentangle these overlapping mechanisms.

An important aspect to consider in understanding the heterogeneity of depression risk among opioid users is the role of temperament traits. Recent studies have highlighted the significance of affective temperaments in modulating individual susceptibility to depressive symptoms. For example, Favaretto et al. (2024) (39) provide a comprehensive synthesis of clinical experiences and empirical evidence, emphasizing how distinct affective temperaments can either mitigate or exacerbate the psychological impact of chronic stressors, including opioid use. This perspective aligns with our findings, as individuals with certain temperament profiles may experience a heightened inflammatory response or altered lipid metabolism, thereby increasing their vulnerability to depression. Understanding these temperament-linked differences could inform personalized prevention and intervention strategies in clinical practice.

In addition to biological mechanisms, it is critical to consider the clinical and public health implications of these findings. Depression among opioid users is often underdiagnosed, due in part to overlapping symptoms with opioid withdrawal and the stigma surrounding both conditions (40, 41). Identifying simple, cost-effective biomarkers such as PHR could facilitate earlier recognition and intervention, particularly in resource-limited settings. Moreover, the association between PHR and depression underscores the importance of addressing systemic inflammation and lipid dysregulation as part of comprehensive mental health care strategies. Anti-inflammatory therapies, HDL-raising interventions, and lifestyle modifications targeting these pathways may hold promise for mitigating depression risk in this population (42, 43).

Nevertheless, this study is not without limitations. The cross-sectional design precludes causal inference, leaving open the question of whether elevated PHR is a cause or consequence of depression. Additionally, the broad age range of the studied population, along with factors such as menopause and climacteric states in women, may act as confounding variables influencing the results. Life stressors and comorbid conditions such as diabetes, which were not fully accounted for in this analysis, may also contribute to depression and alter PHR levels. Future studies should aim to address these factors more comprehensively. Residual confounding by unmeasured variables, such as dietary patterns, physical activity, and genetic predispositions, cannot be ruled out (44). Additionally, while PHR integrates key markers of inflammation and lipid metabolism, it does not capture the full spectrum of biological processes involved in depression. Longitudinal studies are needed to validate these findings and to explore the potential of PHR as a predictive marker for depression onset or treatment response (45).

5 Conclusion

In conclusion, this study provides valuable insights into the relationship between systemic inflammation, lipid dysregulation, and depression in opioid users, suggesting that PHR could serve as a potential biomarker for identifying individuals at risk for depression. However, further research, including longitudinal studies and clinical trials, is needed to validate the use of PHR as a predictive marker for depression and to better understand the underlying mechanisms. By integrating insights from cardiovascular and psychiatric research, it offers a novel framework for understanding and addressing the unique challenges faced by individuals with opioid use. Further investigation is warranted to elucidate the mechanisms underlying these associations and to translate these findings into targeted interventions that improve both mental and physical health outcomes in this vulnerable population.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

QL: Data curation, Formal analysis, Investigation, Methodology, Software, Writing – original draft, Writing – review & editing. CL: Writing – review & editing, Investigation. SC: Investigation, Writing – review & editing. HC: Conceptualization, Formal analysis, Methodology, Software, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

Publisher's note

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Sleep efficiency and event–related potentials in patients with depression: the mediating role of serum C-reactive protein

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Background: Patients with major depressive disorder (MDD) may experience cognitive dysfunction and sleep disorders. Limited research exists on the neurophysiological mechanisms that connect sleep efficiency and cognitive function in individuals with MDD. The study aims to investigate the link between sleep efficiency, mental abilities, and levels of serum C-reactive protein (CRP) in individuals diagnosed with MDD.

Methods: A total of 101 individuals diagnosed with MDD were selected and categorized into two groups: the normal sleep efficiency group (NSE) with SE ≥90% and the group with lower sleep efficiency (LSE) with SE <Eth) All patients underwent polysomnography (PSG), event-related potentials (ERPs) tests, and CRP detection. The study used multiple linear regression and bootstrapped mediation analysis to explore the correlation between SE, ERPs latency, and CRP.

Results: The N2, P3a, and P3b latencies were longer in the LSE group compared to the NSE group (p = 0.036, p = 0.013, p < 0.001). N2 (Pr = -122.182), P3a (Pr = -109.597), P3b (Pr = -151.960), and CRP (Pr = -3.768) are significantly associated with SE. A strong correlation was found between CRP (Pr = 9.414) and P3b latency. After controlling for gender and other pertinent variables, the subsequent investigation revealed a direct correlation between CRP and P3b latency, specifically within the cohort of depression patients exhibiting low SE. CRP mediated the association between SE and P3b latency.

Conclusion: Low SE with MDD was associated with chronic inflammation and impaired cognitive function, suggesting that inflammation may act as a potential mediating factor in the relationship between SE and impaired cognitive function.

KEYWORDS

depression, event-related potentials, sleep efficiency, chronic systemic low-grade inflammation, cognitive function

Introduction

Major depressive disorder (MDD), influenced by social, psychological, and biological factors, is prevalent worldwide (1). Severe MDD can lead to decreased quality of life, impaired social functioning, cognitive impairment, neurophysiological alterations, and increased financial burden (2). Sleep disturbances affect over 80% of individuals with MDD across community and clinical populations (3). Sleep is often particularly associated with cognitive health, such as slow-wave sleep deficits that can impair cognitive function through the mechanisms that may be relevant to SE, and sleep is increasingly recognized as a modifiable risk factor for cognitive decline (4–6). Sleep disorders often accompany MDD in clinical settings, and sleeping is essential for maintaining cognitive health (4, 5). While the neurophysiological basis of cognitive dysfunction in MDD remains incompletely understood (7–10), emerging evidence implicates prefrontal cortex glutamate dysregulation in executive function and working memory deficits, along with amygdala hyperactivity, contributing to negative cognitive biases (11–13). The efficacy of existing drug and non-drug therapies remained unsatisfactory in clinical practice (14–16). Given the limited treatment options for cognitive impairment, investigating modifiable factors and underlying mechanisms becomes particularly crucial.

Event-related potentials (ERPs), particularly P3b (17, 18), are valuable for elucidating cognitive processes and detecting abnormal brain activity associated with potential disorders. Moreover, ERPs have been extensively utilized in the assessment of various neurological conditions such as dementia (19, 20) and Parkinson's syndrome (21, 22). However, their application in research on MDD has been relatively limited (23–25). Currently, there is a lack of objective indicators (26, 27) to evaluate the relationship between cognition and sleep, primarily relying on subjective assessment questionnaires (28, 29).

Accumulating evidence shows that sleep quality has a significant impact on the regulation of inflammatory factors, which, in turn, influences overall health (30–32). C-reactive protein (CRP) is a reliable measurable acute-phase protein commonly used as an inflammation marker, indicating both peripheral and central inflammation (33, 34). A previous meta-analysis reports that individuals with major depressive disorder (MDD) who experience poor sleep patterns tend to demonstrate elevated levels of CRP (35). Recent studies indicate that MDD accompanied by sleep problems usually leads to elevated levels of inflammatory markers (31, 36, 37) as well as cognitive decline. Disrupted circadian rhythms not only heighten inflammatory responses but also contribute to the onset of neuropsychiatric disorders that adversely affect mood and cognition (30, 38, 39). In the context of cognitive decline, it is well established that inflammatory factors play a significant role (30, 31, 40). The correlation between CRP levels and sleep/cognitive indicators implies that routine CRP screening in MDD patients could potentially serve as a valuable tool to identify early cognitive impairment. While our study highlights CRP as a plausible mediator, the exact neurobiological pathways require further investigation through longitudinal imaging studies combined with cytokine profiling. We hypothesize that improving sleep quality may potentially facilitate a reduction in the body's inflammatory response, while improvement in CRP levels could ameliorate cognitive impairment resulting from poor sleep quality. This approach holds promise for the treatment of cognitive impairment in MDD; however, there is currently limited research on this topic within MMD. It is unclear if high CRP levels affect the link between poor sleep and cognitive impairment in MDD. Future trials could stratify patients by baseline CRP levels to evaluate whether anti-inflammatory adjunct therapies yield differential benefits in sleep and cognition.

The study seeks to investigate the possible associations of sleep and serum CRP levels with cognitive function in depressed patients in a cross-sectional study. Furthermore, we aimed to explore the potential mediating role of higher CRP levels in the relationship between poorer sleep quality and cognitive decline.

Materials and methods

Inclusion criteria

From February 2021 to April 2023, 101 hospitalized MDD patients were recruited from the Sleep Disorders Department of Hefei Fourth People's Hospital. During the registration process, initial screening was carried out by two or more attending physicians, using both the ICD-10 diagnostic criteria for depression and specific study criteria. The inclusion criteria included meeting the ICD-10 diagnostic criteria for depressive symptoms, age between 18 and 60 years, education level equivalent to junior high school or higher, right-handedness, and Han nationality. The exclusion criteria included the following: mental disorders such as schizophrenia, substance-induced mood disorders, anxiety disorders, bipolar disorder, and substance abuse or dependence; history of important physical diseases related to nervous system dysfunction, metabolism issues, or endocrine system abnormalities; conditions or medications known to influence systemic inflammation (like statins or NSAIDs); pregnancy status, including lactating women and those planning pregnancy; severe physical ailments or autoimmune-related problems; history of head trauma resulting in seizures lasting over 5 min, leading to consciousness disorders; auditory or sensory abnormalities; and diagnosis of atherosclerosis and/or hypertension since hypertension is linked to an elevated risk in CRP levels synergistically when considering body mass index (BMI).

Data on demographics and clinical characteristics included gender, age, education level, BMI, illness duration, smoking and alcohol habits, the utilization of benzodiazepines medications and the utilization of conventional antidepressant medications such as serotonin norepinephrine reuptake inhibitors (SNRIs), selective serotonin reuptake inhibitors (SSRIs), or noradrenaline and specific serotonergic antidepressants (NaSSA). As all participants were hospitalized, diet, physical activity, and stress levels were

institutionally standardized and thus not recorded in demographic data. These measures will be included in subsequent studies. Trained researchers obtained informed consent from all patients after receiving approval from the Ethics Committee of Hefei Fourth People's Hospital.

Event-related potential

Professional technicians specializing in electroencephalography within our hospital employed the American NicoletEDX electromyography evoked potential system to assess the patients. This took place in the electromyography room of our hospital from 9:00 to 11:30 a.m. upon admission. During the data collection process, the patients are usually seated upright in a comfortable chair, maintaining a relaxed state and focusing as much as possible. They are positioned approximately 1 m away from the computer screen, with the keyboard or button pad typically placed on their knees or in a tray. Electroencephalogram (EEG) signals are non-invasively recorded using electrodes, primarily made of silver/silver chloride or tin, which are attached to the scalp surface. Our electrode selection was primarily based on equipment limitations adhering to the 10/20 system and Cz's superior signal-to-noise ratio compared to Pz in our sample. Additionally, while we analyzed all midline electrodes (Fz, Cz, and Pz), Cz demonstrated the most stable data for cognitive tasks, consistent with known P300 scalp distribution patterns. Thus, the electrodes were secured in an elastic nylon cap according to the International 10/20 System (American Neuromagnetic Society, 1994) (41), with Cz as the recording electrode, M2 (right ear) as the reference, and FPz (forehead center) as the ground lead. The signals were first amplified by scalp-mounted preamplifiers and then transmitted to a main amplifier (gain: ×10,000–50,000) for precise measurement. The parameters included the following: electrode impedance, <5 kΩ; bandpass filter, 0.5–100 Hz; and analysis time, 1,000 ms. The auditory stimuli comprised non-target (80% probability; 70 dB; 1,000 Hz) and target (20%; 90 dB; 2,000 Hz) tones presented randomly across two sessions; the responses were averaged for analysis. The electrodes are connected to a set of preamplifiers, which are located close to the participant's scalp to provide sufficient initial amplification to transmit the weak signal to the main amplifier in the laboratory.

After enrollment, we collected the ERP parameters. MMN reflects the brain's automatic processing function in response to diverse stimulus signals (42). N100 primarily indicates the integrity of the auditory conduction pathway and residual function of the primary auditory cortex, with minimal impact on higher cognitive assessment (43). The P200 component delineates the distinction between task-related stimuli and those unrelated to the task (44). The endogenous N200 is associated with selective response and also signifies the process of stimulus classification (45). P300 predominantly represents the initial processing of information stimuli in the brain, sensitively reflecting higher cognitive abilities and requirements for task processing speed (19, 46). We extracted the latency period of MMN, N1, P2, N2, P3a, and P3b (the horizontal linear distance from the onset of stimulation to the peak point of the maximum amplitude wave component) from ERP variables for analysis purposes.

Polysomnography examination

Considering the first-night effect in sleep monitoring, we opted to analyze PSG data from patients on the second day. Throughout the night, all subjects were continuously monitored using the Embla N7000 device to assess neurophysiological and cardiopulmonary parameters. Sleep efficiency (SE) was calculated from the PSG data as a measure of sleep quality, with higher values indicating better sleep quality. SE is determined by the ratio of total sleep time [TST; TST is total sleep in minutes for all stages of sleep (stages 1, 2, and 3 non-REM and REM)] to time in bed [TIB; TIB begins with lights out and ends with lights on and is calculated in hours and minutes (also occasionally referred to as total bedtime time or TSP)], with higher values indicating improved sleep quality. Previous reviews have considered reduced SE in the PSG of MDD patients as a characteristic manifestation with high credibility (47). While difficulty falling asleep is commonly seen in various mental disorders, when it comes to evaluating the sleep patterns of MDD patients in clinical practice, sleep efficiency (SE) is a more appropriate measure. SE offers a more accurate depiction of the characteristics of sleep disturbances in MDD patients (47, 48). In normal individuals, SE values typically decrease with age, but they commonly remain above 90% for both youthful and middle-aged adults (49, 50).

C-reactive protein

Peripheral cubital venous blood of 5 mL was collected from the patients upon admission, and the serum CRP level was determined by immunoturbidimetry. The blood samples were centrifuged at a low speed of 3,000 r/min for 10 min to harvest the upper layer of serum. The specimens were either promptly collected and analyzed within 1 h or stored at -80°C for further analysis. The level of CRP in the serum was analyzed using an automatic biochemical analyzer, following the manufacturer's instructions.

Scale assessment

The depression and anxiety symptoms of depressed patients were assessed using the 24-item Hamilton Depression Scale (HAMD) (51) and Hamilton Anxiety Scale (HAMA) (52). According to the HAMD scoring standard, a total score of 8~19 is mild depression, 20~34 is moderate depression, and ≥ 35 is severe depression. According to the HAMA scoring standard, a total score of more than 29 may indicate severe anxiety, more than 21 must have obvious anxiety, more than 14 may indicate anxiety, more than 7 may be anxiety, and less than 7 is not anxiety. All scale assessments are completed by trained researchers and entered into statistical software by professional statisticians.

Sensitivity analysis

The SPSS 26.0 software package was utilized for data analysis, with count data being presented as frequency and percentage. The comparison of gender across different groups was conducted utilizing the χ^2 test. In cases where continuous variables did not follow a normal distribution, we described them using median (quartile range) [M(QR)] and conducted group comparisons using Mann–Whitney *U*-test. Measurement data were assessed with an independent sample *T*-test and presented as (x ± s). A repeated-measures ANOVA was employed to compare the differences in event-related potentials between the two groups with age, gender, BMI, education level, smoking habits, alcohol consumption, antidepressant usage, and use of benzodiazepine medication as covariates. Logistic regression analysis and mediation analyses were used to test the association between two variables and the mediating associations among three variables. Furthermore, we performed linear regression analysis on the latency of CRP and ERP in two groups of MDD. Moreover, to examine other variable-mediated relationships within the logistic regression analysis, we utilized the PROCESS plug-in tool. Finally, ordinary least squares path analysis evaluated both direct and indirect mediating effects of LnCRP on sleep efficiency and ERP-P300 latency. The statistical significance level was set at $P < 0.05$. As CRP data showed skewness, CRP is measured in mg/L, and CRP was transformed to natural logarithms in the analysis (34).

Results

Demographic, clinical, and polysomnography characteristics

A total of 101 individuals diagnosed with MDD were enrolled and admitted to the Sleep Disorders Department at Hefei Fourth People's Hospital between February 2021 and April 2023. They were categorized into two groups based on their sleep efficiency: one of which is a group with normal sleep efficiency (SE ≥90%; $N = 45$). The average age of the participants in the normal sleep efficiency group was 39.11 ± 13.78 years, consisting of 11 male and 34 female individuals. The low sleep efficiency group had an average age of 41.27 ± 12.93 years, including 23 male and 33 female individuals. Statistically significant differences were not observed in terms of age distribution, educational background, gender distribution, residential status, body mass index (BMI), alcohol consumption history, smoking history, scores on the Hamilton Depression Rating Scale (HAMD), scores on the Hamilton Anxiety Rating Scale (HAMA), use of antidepressant medication between, or use of benzodiazepines medication in these two groups ($P > 0.05$). However, a significant disparity was noted in C-reactive protein (CRP) levels (see Table 1).

Comparison of indicators related to event-related potential

The latency differences of event-related potentials between the normal sleep efficiency group and the low sleep efficiency group were compared using an independent-samples *t*-test. No significant outliers were observed in the study data, and both groups exhibited distributions that approximated a normal distribution while meeting the assumptions of homogeneity of variance. The results indicated that the latencies of N2, P3a, and P3b in the low sleep efficiency group (239.16 ± 26.18, 292.27 ± 26.14, and 356.91 ± 27.32) were significantly higher than those in the normal sleep efficiency group (228.42 ± 27.78, 280.15 ± 24.63, and 336.46 ± 24). These differences reached statistical significance with effect sizes represented by *t*-values of -1.99 and -2.38 ($P < 0.05$) for N2 and P3a, respectively, and a *t*-value of -3.92 ($P < 0.01$) for P3b. After controlling for age and BMI through covariance analysis (ANCOVA), significant differences remained between groups regarding latencies of N2, P3a, and P3b [$F = 4.50$ ($P = 0.036$); $F = 6.35$ ($P = 0.013$); $F = 15.22$ ($P < 0.001$)]. No significant differences in latencies of MMN, N1, or P2 were observed between groups ($P > 0.05$) (see Table 2).

Direct association of SE with ERP latency

A significant correlation exists between sleep efficiency and ERP latency in patients diagnosed with MDD. After controlling for variables including age, gender, BMI, education level, smoking habits, alcohol consumption, and antidepressant usage, we discovered significant inverse associations between sleep efficiency and N2 latency (Pr = -122.182, $P = 0.013$), P3a latency (Pr = -109.597, $P = 0.020$), and P3b latency (Pr = -151.960, $P = 0.003$) within our study population (Figures 1A–C). These findings suggest that individuals with lower sleep efficiency display a noticeable cognitive impairment.

Analysis of the correlation between SE, LnCRP, and LnCRP and ERP latency

A significant correlation exists among CRP and SE as well as ERP latency in patients with MDD. After adjusting for age, gender, BMI, education level, smoking habits, alcohol consumption, and antidepressant usage, we discovered significant inverse associations between SE and CRP (Pr = -3.768, $P = 0.020$) within our study population (Figure 2A). The results of the examination revealed as well a positive correlation between the level of CRP and the latency curves of N2 (Pr = 3.276, $P = 0.300$), P3a (Pr = -0.595, $P = 0.845$), and P3b (Pr = 9.414, $P = 0.004$) (Figures 2B–D). Specifically, there was a significant positive correlation with P3b, indicating that patients with elevated CRP levels may be at risk for a more severe cognitive impairment (Figure 2D).

TABLE 1 Comparison of traits in depressed individuals with low vs. normal sleep effectiveness (n = 101).

Characteristics	SE ≥ 90% (n 45)	SE<90% (n 56)	$Z/t/\chi^2$	P
Age (year)[a]	39.11 ± 13.78	41.27 ± 12.93	-0.81	0.420
Gender, n (%)[b]			2.39	0.079
Male	11 (24.4)	23 (41.1)		
Female	34 (75.6)	33 (58.9)		
BMI (kg/m^2)[a]	22.46 ± 3.28	23.74 ± 3.53	-1.87	0.064
Education level[b]			0.26	0.609
High school—below	24 (53.3)	27 (48.2)		
High school—above	21 (46.7)	29.7el—)		
Drink ≥1 time/week[b]	1 (2.2)	3 (5.4)	0.65	0.422
Smoking ≥1 branch/day[b]	3 (6.7)	5 (8.9)	0.18	0.676
Use of antidepressant medication[b]			0.398	0.941
SSRIs	19 (42.2)	24 (42.9)		
SNRIs	8 (17.8)	9 (16.0)		
NaSSA	4 (8.9)	7 (12.5)		
No use	14 (31.1)	16 (28.6)		
Use of benzodiazepines medication[b]	18 (40)	23 (41.1)	0.01	0.913
$HAMD_{24}$[a]	31.80 ± 8.77	30.20 ± 9.24	0.89	0.377
HAMA[a]	21.23 ± 6.10	19.44 ± 6.26	1.41	0.161
CRP (mg/L)[c]	0.40 (0.55)	0.60 (0.88)	-2.29	0.022

SE, sleep efficiency; BMI, body mass index; SSRIs, selective serotonin reuptake inhibitors; SNRIs, serotonin norepinephrine reuptake inhibitors; NaSSA, noradrenergic and specific serotonergic antidepressant; HAMA, Hamilton Rating Scale for Anxiety; HAMD, Hamilton Rating Scale for Depression; CRP, C-reactive protein.
[a]n(%) description, and χ^2 test is employed for inter-group comparisons.
[b]$\overline{x}$ ± s description, and t-test is utilized for between-group comparisons.
[c]M(IQR) description, and Mann–Whitney U-test is applied for group comparisons.

Correlation analysis of the latency period between CRP and ERP in various sleep efficiency groups

We performed additional experimental procedures to evaluate the consistency of our findings under different conditions. As shown in Table 3, after accounting for potential factors that may have an impact, controlling for age, gender, BMI, education level, smoking habits, alcohol consumption, antidepressant usage, and use of benzodiazepine medication, in individuals with low sleep efficiency MDD, the higher CRP levels were associated with increased levels of P3b latency [β with 95% CI: 15.202 (6.506, 23.897), P = 0.001], while this association was not observed in the normal sleep efficiency group (β = 0.984, P = 0.841).

Mediation by C-reactive protein

Additional comprehensive analysis is warranted; we employed a mediation model to examine the involvement of CRP in mediating the relationship between SE and P3b latency in individuals diagnosed with MDD. Our findings show that CRP has a significant mediating effect on the duration of P3b latency associated with SE (indirect effect = -28.3557, 95% CI: -70.7087, -1.1506; see Figure 3).

Discussion

This study aimed to investigate the relationship between sleep efficiency, inflammation, and changes in cognitive function in patients with MDD. The study provides a comprehensive analysis of the associations of SE and serum CRP levels with ERP latency indicators. The findings indicated that alterations in sleep quality among MDD patients were linked to levels of serum CRP and P3b latency. Furthermore, a significant correlation was observed between serum CRP levels and ERP latency, specifically in patients with low sleep efficiency. Mediation analysis for patients with MDD suggested that sleep efficiency may be related to the P3b latency mediated by higher CRP. Our findings suggest a potential bidirectional relationship between elevated inflammatory markers, diminished sleep quality, and cognitive dysfunction in MDD

TABLE 2 Comparison of ERP-related indexes in MDD patients (n = 101).

ERP related indexes	SE ≥ 90% (n 45)	SE < 90% (n 56)	t	F	P
MMN (ms)	248.62 ± 34.98	248.41 ± 32.75	0.03	0	0.989
N1 (ms)	103.91 ± 18.27	103.88 ± 21.45	0.01	0.06	0.801
P2 (ms)	180.36 ± 18.67	184.08 ± 22.19	-0.90	0.68	0.411
N2 (ms)	228.42 ± 27.78	239.16 ± 26.18	-1.99*	4.50*	0.036
P3a (ms)	280.15 ± 24.63	292.27 ± 26.14	-2.38*	6.35*	0.013
P3b (ms)	336.46 ± 24.45	356.91 ± 27.32	-3.92**	15.22**	<0.001

The t-value represents the statistic of the t-test conducted between the two groups, while the F-value represents the statistic of analysis of covariance (ANCOVA) performed between the two groups after adjusting for age and BMI. Significance levels are denoted as *P <0.05 and **P <0.01.

patients. However, the observational nature of this study limits our ability to establish causal relationships.

Patients suffering from MDD regularly experience difficulties with sleep (53, 54), although not all individuals exhibit a noticeable reduction in sleep efficiency. Recent studies have indicated that self-reported sleep appeared to reflect the sleep misperception commonly presented in persons with depressive and anxiety symptoms (55, 56). The results of retrospective self-report questionnaires may be influenced by potential bias and patients' cognitive limitations as well as their pessimistic attitudes. Recently, objective assessment tools have received great attention and popularity in improving the detection and prevention of depression. Currently, PSG is considered the gold standard for sleep assessment tools (26, 27, 57). Additionally, receiving feedback from PSG results can assist in tailoring therapy specifically aimed at addressing sleep issues for the patients. Event-related potentials can overcome issues related to motivation and attention factors during measurement (17, 21, 58). The latest analysis suggests that P300, as a crucial component of the ERP, has indeed shown a significant clinical value in the study of cognitive dysfunction in MDD (8, 17).

Our findings support previous evidence (24, 36, 59) that MDD patients exhibit a negative correlation between sleep and cognitive dysfunction. Clinically, ERP is commonly utilized as a reliable indicator for evaluating the executive cognitive functions of patients, such as planning, decision-making, and problem-solving abilities (8, 27, 60). Consistent with previous studies (24, 48), there is a significant link between reduced sleep quality and impaired executive function. Low sleep efficiency struggles to maintain focus

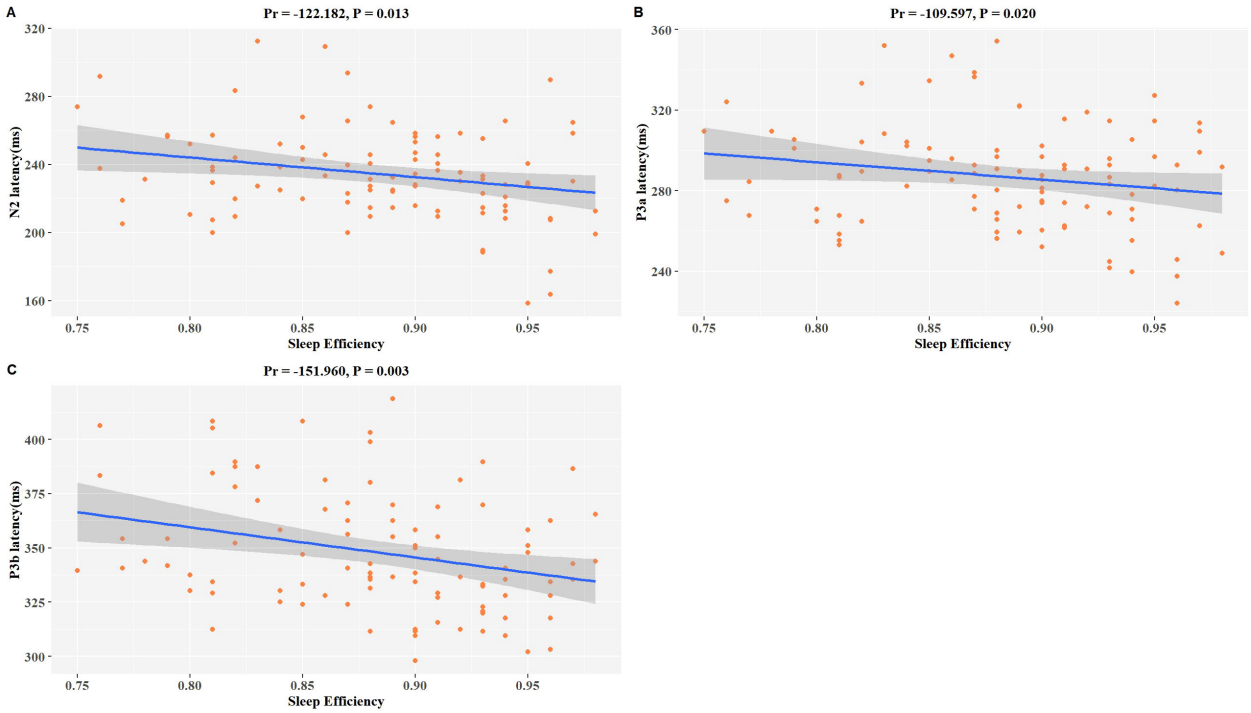

FIGURE 1
Associations between SE and P3b latency in MDD patients. The model was controlled for age, gender, BMI, education level, smoking habits, alcohol consumption, antidepressant usage, and use of benzodiazepine medication. **(A–C)** Scatter plots show a negative correlation trend between SE and changes in N2 latency, P3a latency, and P3b latency. The downward slope indicates negative correlations. pr is the partial correlation coefficient.

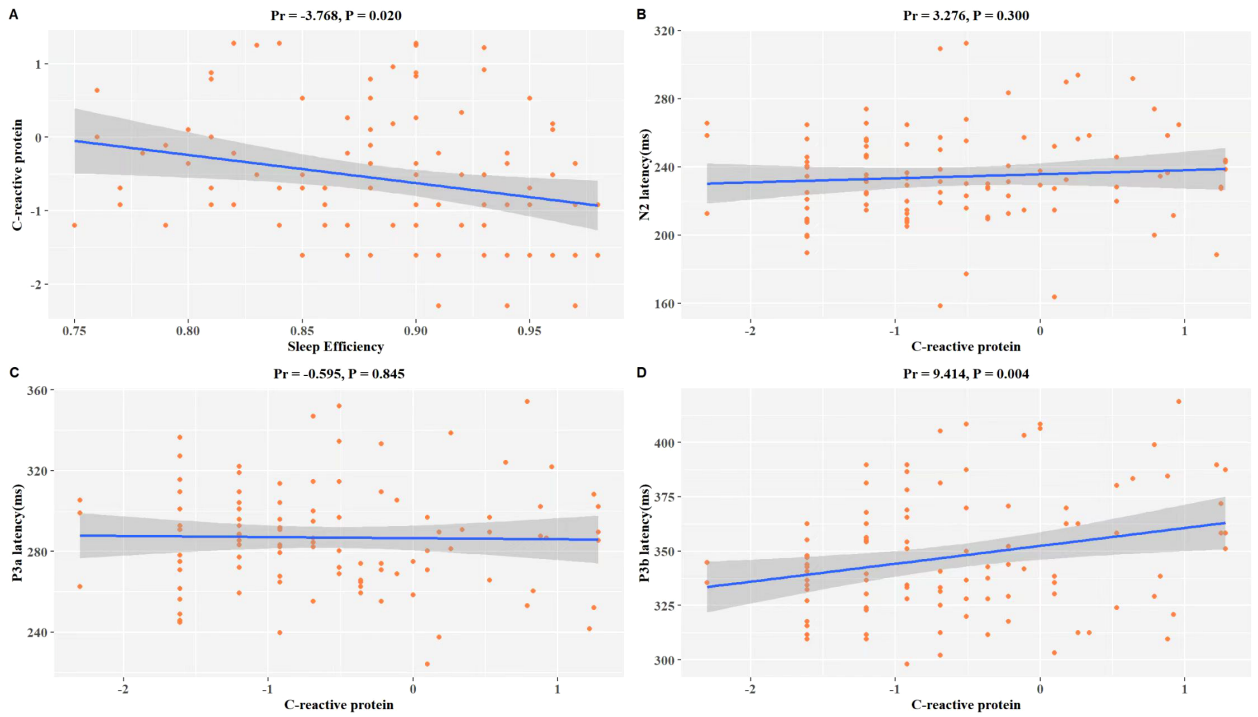

FIGURE 2
Association of LnCRP and sleep quality, and P300 latency of MDD. The model was controlled for age, gender, BMI, education level, smoking habits, alcohol consumption, antidepressant usage, and use of benzodiazepine medication. **(A)** Scatterplot of the relationship between LnCRP and SE. **(B)** Scatterplot of the relationship between LnCRP and N2 latency. **(C)** Scatterplot of the relationship between LnCRP and P3a latency. **(D)** Scatterplot of the relationship between LnCRP and P3b latency. Pr is the partial correlation coefficient. LnCRP and CRP were transformed to natural logarithms in the analysis.

for extended periods and is distracted by external stimuli, which can hinder their ability to learn and perform tasks effectively daily (61, 62). Moreover, difficulties in memory capacity can cause significant inconvenience in both personal and professional aspects of life (5, 40, 63). Getting enough sleep plays a crucial role in improving cognitive function for patients with MDD (9, 26, 36). Research in the field of mood disorders consistently shows that sleep affects cognitive function regulation through various ways and pathways (8, 9, 26), poor sleep quality exacerbates cognitive decline, and early intervention can improve patient prognosis and cognitive recovery (7, 10). Our study offers a fresh perspective: improving the sleep quality of people with MDD could be a potential strategy to reduce their cognitive decline.

Poor sleep quality not only directly affects cognitive function but also influences inflammatory factor levels. Our findings demonstrate that reduced sleep efficiency is associated with elevated serum C-reactive protein (CRP) levels. Impaired sleep may contribute to increased inflammatory markers (59, 60), and prolonged inflammatory responses could play a role in the onset and progression of mood disorders (2, 27, 35). In patients with MDD, heightened levels of peripheral inflammatory markers may correlate with the severity of specific clinical symptoms (28, 61. Accumulating evidence shows that sleep quality affects inflammatory factor regulation, potentially leading to health issues (30–32). In short, these results suggest that sleep disruptions could lead to increased levels of inflammation

TABLE 3 Comparison of P300-related indexes and CRP stratified by SE.

Variable	SE ≥ 90% (*n* 45)		SE < 90% (*n* 56)	
	β (95% CI)	*P*	*β* (95% CI)	*P*
N2 (ms)	-2.320 (-12.432, 7.792)	0.644	3.958 (-5.338, 13.253)	0.396
P3a (ms)	-6.437 (-14.899, 2.026)	0.132	2.960 (-6.017, 11.937)	0.510
P3b (ms)	0.984 (-8.919, 10.888)	0.841	15.202 (6.506, 23.8979	0.001**

The *t*-value represents the statistic of the *t*-test conducted between the two groups, while the *F*-value represents the statistic of analysis of covariance (ANCOVA) performed between the two groups after adjusting for age, gender, BMI, education level, smoking habits, alcohol consumption, antidepressant usage, and use of benzodiazepine medication. Significance levels are denoted as *P <0.05 and **P <0.01.

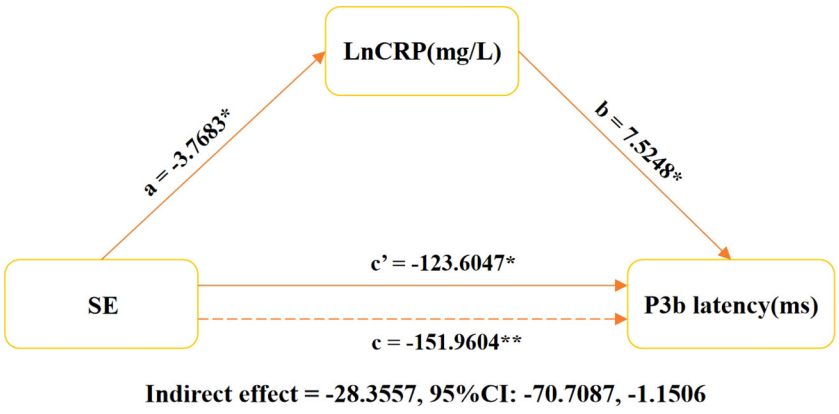

FIGURE 3
Relationship between latency of LnCRP, SE, and P3b in MDD patients. The graph illustrates the results of a mediation analysis on the relationship between sleep efficiency (SE), log-transformed C-reactive protein (CRP), and P3b latency. It includes estimates for mediated (a × b), direct (c'), and total effects, with LnCRP as the mediator. The models were controlled for age, gender, BMI, education level, smoking habits, alcohol consumption, antidepressant usage, and use of benzodiazepines medication. *$P < 0.05$, **$P < 0.001$. SE, sleep efficiency; P3b latency, latency of P3b component in event-related potentials. LnCRP and CRP were transformed to natural logarithms in the analysis. pr, partial coefficient; CI, confidence interval.

markers. Note the significant correlation between sleep efficiency in patients with MDD and serum levels of CRP, which aligns with previous research demonstrating the impact of sleep disturbances on elevated inflammatory markers in animal models (64, 65). Our results align with prior studies indicating a possible link between poor sleep quality and increased inflammation in individuals experiencing depression (66, 67). The somatic symptoms of MDD may have a stronger link to inflammatory responses than affective symptoms (35, 68, 69), and improving sleep quality can reduce systemic inflammation in patients with MDD (67, 70). Low-level systemic inflammation may be involved in various neuropsychiatric disorders that affect mood and cognitive function (30, 39, 40). Our study discovered that the association between inflammation and impaired cognitive function in individuals with MDD aligns with earlier research results (30, 31, 39, 67, 71). The presented evidence illuminates the intricate interplay of sleep, biomarker activation, and cognitive performance in patients with MDD. Research has found that the decline of cognitive and executive functions among individuals with obstructive sleep apnea (OSA) is associated with inflammation, but this association could be alleviated by reducing serum CRP (72, 73). Reversing inflammation could be a potential strategy to address cognitive decline with MDD.

Previous research on the neurobiology of MDD accompanied by sleep disturbances has mainly focused on factors such as metabolomics, changes in hormone levels, alterations in neurotransmitters, genetics, and epigenetics. Limited investigation has been conducted on the associations among serum CRP levels (a marker of inflammation) and both sleep quality and cognitive function (74, 75). Various biomarkers exert their effects through distinct signaling pathways, leading to diverse impacts on the regulation of neurocognitive function (76, 77). This variation may result from the varying levels of cognitive impairment among the subjects. Sleep regulates gene expression in the sympathetic nervous system and hypothalamic–pituitary–adrenal axis, leading to fluctuations in inflammation levels that affect cognitive function (30, 37, 78). Certain research examines the connection between sleep, inflammation, and cognitive function in patients with MDD as well as the effects of atypical sleep duration on response time and visual memory (30, 39, 54, 79). Previous studies have found that changes in white blood cells can affect cognitive function during sleep (71), and there is a significant association between systemic inflammation and nighttime awakening and dementia occurrence (74). Consistent with our findings, MDD with lower SE correlates with higher CRP levels, which may be related to the severity of cognitive impairment (74, 79). Serum CRP levels are correlated with SE and neurocognitive function. Among them, CRP may mediate the association between SE and impaired cognitive function. The improvement of SE may help reduce the level of serum CRP, thereby helping to prevent adverse neurocognitive changes.

Consistent with other studies, one of the main reasons for the poor prognosis of patients with MDD cognitive dysfunction is their difficulty in recovery, and currently, antidepressant drugs have limited ability to improve cognitive function. Our study has identified distinct alterations in cognitive function and levels of the inflammatory marker CRP in individuals with major depressive disorder (MDD) and poor sleep quality. Additionally, our study further elucidates the sequential relationships among sleep, CRP, and cognition, highlighting their close interdependence and emphasizing the complexity of connections in the prognosis of MDD. These results shed light on potential biological pathways linking sleep and cognitive function, offering valuable insights for the early detection and management of cognitive impairment in patients with depression.

Strengths and limitations

Previous studies hint at a connection between sleep efficiency (SE) and cognitive function, and our research indicates that CRP plays a key role in this link. We utilized objective measurements for

SE and event-related potential P300 indicators. For individuals with MDD (8, 17, 58), event-related potential measurements are more sensitive in evaluating cognitive function and detecting differences compared to scale assessments, enhancing the reliability and accuracy of our data. Future research should explore the causes and directions of these connections. Through longitudinal studies, the immunological mechanisms behind neurocognitive decline in individuals with MDD will be clarified.

This approach shows promise to address cognitive impairment in MDD; however, there is currently limited research on this topic within the field of MDD. There are, however, certain limitations to this study. As it is a cross-sectional study lacking functional intercept indicators, it is impossible to ascertain whether CRP mediates the causal relationship between sleep efficiency and impaired cognitive function. Consequently, longitudinal studies are necessary to further validate the role of CRP as a mediating variable, which still requires improvement. The small sample size may restrict the detection of changes in serum CRP levels and hinder the identification of potential associations among depression, cognition, and sleep. Additionally, potential confounding factors such as disease episode frequency, illness duration, and treatment history were not fully accounted for, which may influence the observed relationships. Future studies should further refine the collection and analysis of such clinical variables. Due to the global health crisis at that time and ethical considerations, we are unable to recruit a normal control group for PSG and ERP data collection in the ward. Future research should consider incorporating a robust control cohort. While polysomnography is utilized to mitigate the first-night effect in clinical practice, its effectiveness for normal controls in a hospital setting is limited (54, 61, 71). Subsequent studies could investigate alternative sleep measurement methods, such as activity monitoring. A larger sample size is essential for more precise outcomes and reliable data for clinical prognosis.

Conclusion

We found a robust association of the poorer sleep quality with the higher levels of CRP and cognitive function decline in patients with MDD. The levels of inflammatory factors may mediate the relationship between sleep efficiency and cognitive decline. Improved sleep quality and anti-inflammatory agents or diet (micronutrient supplementation) could prevent cognitive decline in patients with MDD. Further randomized controlled intervention trials are needed.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by the Ethics Committee of Hefei Fourth People's Hospital. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

LF: Data curation, Writing – original draft. PW: Writing – original draft. TX: Data curation, Writing – review & editing. SD: Investigation, Writing – review & editing. TW: Writing – review & editing. JZ: Writing – review & editing. AZ: Writing – review & editing. PZ: Conceptualization, Data curation, Formal Analysis, Writing – review & editing. DZ: Conceptualization, Funding acquisition, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Exploring the relationship and shared mechanisms of major depressive disorder and diabetic kidney disease: a comprehensive clinical and genetic analysis

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Introduction: Major depressive disorder (MDD) is a common comorbidity in diabetes mellitus (DM), while diabetic kidney disease (DKD) represents a severe complication of DM. However, the clinical and genetic associations between MDD and DKD remain unclear. This study aimed to investigate their shared biomarkers, molecular pathways, and immune features.

Methods: We analyzed data from the National Health and Nutrition Examination Survey (NHANES, 2005–2018) to assess the association between MDD and DKD. Genetic correlation was evaluated using linkage disequilibrium score regression (LDSC), and causality was tested with Mendelian randomization (MR). Gene expression datasets were integrated to identify crosstalk genes, followed by protein–protein interaction (PPI) analysis to detect hub genes. Diagnostic performance was validated using least absolute shrinkage and selection operator (LASSO) regression and receiver operating characteristic (ROC) curves. Immune infiltration was assessed, and potential therapeutic compounds were predicted through connectivity map (cMAP) analysis and molecular docking.

Results: Clinical analysis revealed a significant association between MDD and DKD (OR = 1.45, 95% CI: 1.28–1.64). LDSC indicated a significant genetic correlation (r = 0.2153, P = 0.008), although MR analysis did not support a causal relationship. A total of 83 crosstalk genes were identified, primarily enriched in inflammation and immune regulation pathways. PPI analysis highlighted eight hub genes, with CD163 and KLRB1 emerging as promising shared diagnostic biomarkers. Validation using LASSO and ROC confirmed their diagnostic potential. Immune infiltration analysis revealed shared immune cell alterations. Furthermore, cMAP analysis and molecular docking suggested rucaparib and levocetirizine as candidate therapeutic agents.

Discussion: Our findings demonstrate a genetic and immunological link between MDD and DKD. CD163 and KLRB1 may serve as potential biomarkers and therapeutic targets, offering new insights into the shared mechanisms and treatment strategies for comorbid MDD and DKD.

KEYWORDS

major depressive disorder, diabetic kidney disease, NHANES, genetic correlation, transcriptomic analysis

1 Introduction

Diabetic kidney disease (DKD) is a clinical manifestation of the kidneys in diabetic patients characterized by proteinuria, hypertension, and a progressive decline in renal function. DKD is a frequent microvascular complication arising from diabetes mellitus (DM), affecting approximately 30% to 40% of DM patients (1, 2). Along with the increasing prevalence of DM globally, the incidence of DKD is also on the rise. DKD is a primary contributor to chronic kidney disease and renal failure, significantly impacting patients' quality of life and prognosis, and it may even lead to death (2). Major depressive disorder (MDD) ranks among the prevalent mental disorders characterized by enduring feelings of sadness, reduced appetite, decreased interest in activities, hopelessness, sleep disorder, and even suicidal behavior in severe cases (3). The prevalence of MDD has rapidly increased worldwide in recent years, with more than 700,000 individuals committing suicide due to MDD each year, thereby imposing a heavy burden on individuals and society (4).

Compared to the general population, individuals with DM have twice the likelihood of experiencing depression and anxiety disorders. Diabetes-related complications, including DKD, are closely correlated with depression (5, 6). Cohort studies have shown that DKD patients with depression progress to end-stage renal disease at a rapid rate (7). Similarly, patients with DKD typically have more symptoms of depression and anxiety, often resulting in unfavorable clinical outcomes, such as accelerated renal function decline, increased hospitalizations, elevated mortality rates, and poor quality of life (8, 9). The pathophysiological mechanisms related to MDD include dysregulation of the hypothalamic–pituitary–adrenal-immune axis and activation of proinflammatory cytokines, which may lead to insulin resistance and heighten the risk of developing DM and its associated complications (10). A meta-analysis has indicated a bidirectional relationship between MDD and DKD, with DKD potentially predicting MDD and MDD serving as an indicator of DKD (11).

However, despite accumulating epidemiological evidence on the association between MDD and DKD, the underlying molecular and genetic mechanisms linking the two diseases remain largely unexplored. In particular, there is a lack of studies identifying key shared genes or pathways involved in this comorbidity. Further research is warranted to explore the relationship between MDD and DKD, particularly concerning cellular and molecular mechanisms. The present study employed bioinformatics techniques to identify genes involved in the crosstalk between MDD and DKD, revealing the potential mechanisms underlying the interactions between these two diseases and predicting small molecule compounds with therapeutic potential.

2 Materials and methods

This study utilized repeated cross-sectional data from the National Health and Nutrition Examination Survey (NHANES) cycles between 2005 and 2018, combined with genome-wide association study (GWAS) data, to investigate the genetic correlation and causal relationship between MDD and DKD using linkage disequilibrium score regression (LDSC) and bidirectional Mendelian randomization (MR). Differentially expressed genes were identified and subjected to functional enrichment, protein-protein interaction (PPI) network construction, and least absolute shrinkage and selection operator (LASSO) regression to select key biomarkers. Potential therapeutic drugs were screened via the Connectivity Map (cMAP) database and validated through molecular docking to explore drug-target interactions, aiming to elucidate shared mechanisms and therapeutic targets for both diseases (Figure 1).

Abbreviations: MDD, Major Depressive Disorder; DM, Diabetes Mellitus; DKD, Diabetic Kidney Disease; NHANES, National Health and Nutrition Examination Survey; LDSC, Linkage Disequilibrium Score Regression; MR, Mendelian Randomization; PPI, Protein-Protein Interaction; LASSO, Least Absolute Shrinkage and Selection Operator; ROC, Receiver Operating Characteristic; CMap, Connectivity Map; OGTT, Oral Glucose Tolerance Test; HbA1c, Glycated Hemoglobin; UACR, Urinary Albumin-to-Creatinine Ratio; Egfr, Estimated Glomerular Filtration Rate; PIR, Poverty Income Ratio; BMI, Body Mass Index; CHD, Coronary Heart Disease; GEO, Gene Expression Omnibus; DEGs, differentially expressed genes; OR, Odds Ratio; GO, Gene Ontology; BP, Biological Process; CC, Cellular Component; MF, Molecular Function; KEGG, Kyoto Encyclopedia of Genes and Genomes; AUC , Area Under the Curve; CI, Confidence Interval; NK, Natural Killer; IL-1β, Interleukin-1β; TNF-α, Tumor Necrosis Factor-α; IL-6, Interleukin-6; PARP, Poly (ADP-ribose) Polymerase.

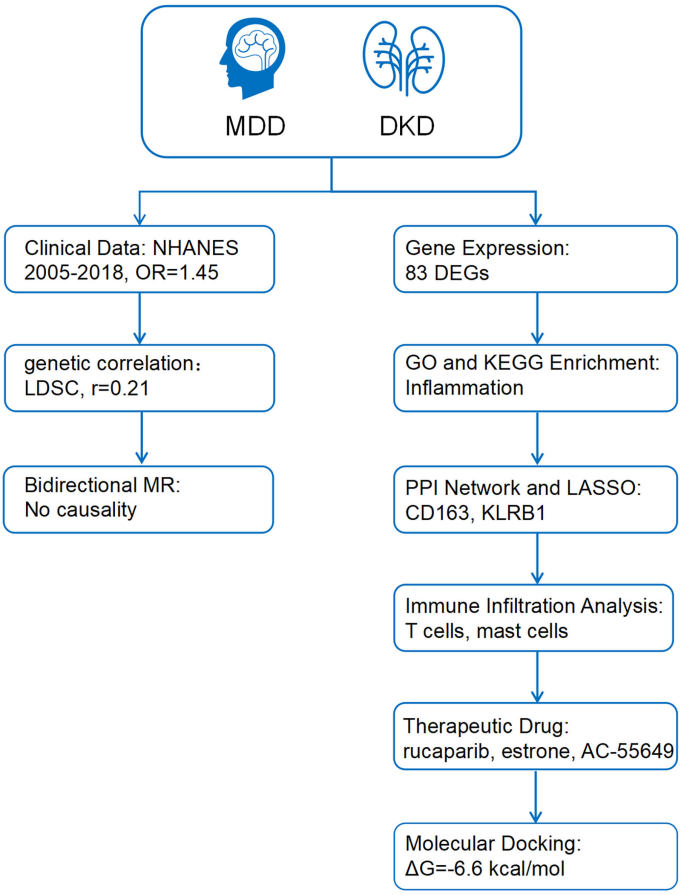

FIGURE 1
The working flow chart of this study. MDD, Major depressive disorder; DKD, diabetic kidney disease; NHANES, National Health and Nutrition Examination Survey; LDSC, Linkage Disequilibrium Score Regression; MR, Mendelian randomization; DEGs, differentially expressed genes; PPI, Protein-protein interaction; LASSO, least absolute shrinkage and selection operator.

2.1 Data collection and processing

The study utilized repeated cross-sectional data from NHANES cycles conducted between 2005 and 2018. NHANES is a nationwide survey that provides comprehensive health and nutrition data from a representative sample of the non-institutionalized U.S. population through complex, multistage sampling methods. The diagnostic criteria for diabetes were as follows: a) a previous diagnosis reported by a healthcare professional; b) fasting plasma glucose ≥7.0 mmol/L; c) glycated hemoglobin (HbA1c) ≥6.5%; d) 2-hour plasma glucose level ≥11.1 mmol/L during an Oral Glucose Tolerance Test (OGTT); or e) the use of diabetes medications or insulin (12, 13). According to the KDIGO 2021 Guidelines, CKD was defined as having a urinary albumin-to-creatinine ratio (UACR) > 30 mg/g and/or an estimated glomerular filtration rate (eGFR) < 60 mL/min/1.73 m^2 (14). DKD was defined as CKD combined with diabetes mellitus. Participants with a total PHQ-9 score of ≥ 10 were considered to have MDD. In addition, age, gender, race/ethnicity, education level, poverty income ratio (PIR), marital status, smoking status, body mass index (BMI), blood pressure, hypertension, high cholesterol, and coronary heart disease (CHD) were included as covariates. The final sample size for this study was 15,574 after systematic exclusion (Supplementary Figure 1).

The GWAS data required for LDSC and MR analyses were sourced from public databases (Supplementary Table 1). From these data, 157 genetic instruments were identified for assessing MDD and 10 for evaluating DKD.

Gene expression profiling datasets associated with MDD and DKD were obtained from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/). The MDD dataset, GSE98793, which utilizes the GPL570 platform (Affymetrix Human Genome U133_Plus2.0), consists of 192 samples, including 128 samples from MDD patients and 64 samples from healthy control individuals. The DKD dataset, GSE30122, encompasses three datasets that are all based on the GPL570 platform (Affymetrix Human Genome U133A 2.0), and it comprises 69 samples, including 50 DKD patients and 19 healthy control individuals.

2.2 Statistical analysis

Due to the skewed distribution of the data, categorical variables are presented as frequencies (percentages), and continuous variables are presented as medians (interquartile ranges). The Chi-square test or Mann-Whitney U test was used to assess

differences in DKD and MDD between exposed and unexposed groups. Logistic regression models were employed to calculate the odds ratios (ORs) for DKD and MDD. Subsequently, multivariable regression analysis was conducted to adjust for the effects of covariates, yielding adjusted odds ratios.

Data were weighted to produce accurate estimates that reflect the non-institutionalized civilian population of the United States. Statistical analyses were conducted using the Survey package in R software (version 4.2.3). A two-sided $p < 0.05$ was considered statistically significant.

2.3 Genetic correlation analysis

LDSC analysis was performed to assess the genetic correlation between MDD and DKD using the software available at https://github.com/bulik/ldsc (15). Subsequently, bidirectional two-sample MR analysis was conducted with DKD and MDD as exposure and outcome variables. Sensitivity and heterogeneity tests were also conducted to validate the MR findings.

2.4 Analysis of differentially expressed genes

All operations were conducted in R (version 4.2.3). After preprocessing and normalizing the data, the limma package in R was used to identify DEGs. DEGs with a corrected $p < 0.05$ and $|\log FC| \geq 0.5$ in the GSE30122 dataset and with a corrected $p < 0.05$ and $|\log FC| \geq 0.1$ in the GSE98793 dataset were screened (16, 17). The more lenient threshold in the GSE98793 dataset was adopted to avoid overlooking potentially important DEGs with modest expression changes. Clustered heatmaps and volcano plots of the DEGs were generated using the pheatmap and ggplot2 packages in R, respectively. The ggVenn package in R was used to create Venn diagrams to identify genes involved in crosstalk between MDD and DKD for further analysis.

2.5 GO and KEGG enrichment analyses

Gene Ontology (GO) enrichment analysis is a structured, computerized approach aimed at elucidating the functions of genes and gene products, encompassing biological processes (BP), cellular components (CC), and molecular functions (MF). The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis serves as a widely utilized enrichment tool to unveil biochemical mechanisms and functions (18). The identified crosstalk genes were subjected to GO and KEGG functional enrichment analyses, employing the clusterProfiler package in R. The ggplot2 and ggrepel packages in R were used to visualize the results.

2.6 Construction of the PPI network and identification of hub genes

The STRING database (https://string-db.org) is commonly utilized for constructing PPI networks (19). The screened crosstalk genes were imported into the STRING database to construct a PPI network, which had combined scores exceeding 0.4. The network was visualized using Cytoscape version 3.9.1, Cytoscape Consortium. Hub genes were identified by the Cytohubba plugin and MCODE algorithm.

2.7 Identification of biomarkers using LASSO analysis

LASSO analysis is a regression technique designed to enhance prediction accuracy by identifying variables with strong predictive power and low correlation from high-dimensional data (20). The glmnet package in R was used to perform LASSO regression, which identified the influential predictive factors among the hub genes that may serve as diagnostic biomarkers for MDD and DKD.

2.8 Expression levels and diagnostic value of candidate biomarkers

The ggplot2 package in R was used to create boxplots to assess biomarker expression levels ($p < 0.05$). The pROC package in R was used to compute the area under the curve (AUC) of receiver operating characteristic (ROC) curves to assess the validity of potential shared diagnostic biomarkers in the GSE98793 and GSE30122 datasets.

2.9 Immune infiltration analysis

CIBERSORTx (https://cibersortx.stanford.edu/) is an online platform for immune infiltration analysis, and it was used to explore the differences in the distribution of immune cells between patients with both MDD and DKD and healthy individuals. Finally, Spearman rank correlation analysis was employed to assess the correlation between the expression levels of potential shared diagnostic biomarkers and the abundance of infiltrating immune cells, with a significance threshold set at $p < 0.05$.

2.10 cMAP analysis and molecular docking

cMAP (https://clue.io/) is a gene expression profiling database that employs gene expression signature interventions to unveil connections among drugs, genes, and diseases, aiding in the screening of potential drug candidates (21). In the present study, co-upregulated DEGs related to MDD and DKD were uploaded to the cMAP database to identify potential therapeutic drugs. The top 10 drug candidates with the most significant negative scores were selected as potential therapeutics for MDD and DKD.

To evaluate the binding affinity between the aforementioned small-molecule drugs and biomarkers, molecular docking analysis was conducted. The three-dimensional structures of the target proteins were retrieved from the RCSB Protein Data Bank

TABLE 1 Baseline characteristics and OR of participants by DKD levels in NHANES (2005–2018).

Variables	Total (n = 15574)	Non-DKD (n = 11928)	DKD (n = 3646)	P value	OR (95%CI)	Adjusted OR (95%CI)
MDD				< 0.0001		
No	14203 (92.33%)	10968 (92.87%)	3235 (89.83%)		Reference	Reference
Yes	1371 (7.67%)	960 (7.13%)	411 (10.17%)		1.45 (1.28, 1.64)	1.24 (1.07, 1.42)
Age(years)	50.00 (37.00, 63.00)	48.00 (35.00, 59.00)	65.00 (53.00, 75.00)	< 0.0001	1.06 (1.06, 1.07)	1.05(1.04,1.05)
PIR	3.11 (1.57, 5.00)	3.30 (1.65, 5.00)	2.40 (1.30, 4.29)	< 0.0001	0.87 (0.85, 0.89)	0.89 (0.87, 0.92)
BMI(kg/m2)	28.70 (24.80, 33.50)	28.40 (24.59, 33.10)	30.20 (26.00, 35.50)	< 0.0001	1.03 (1.02, 1.03)	1.02 (1.02, 1.03)
ASBP(mmHg)	120.67 (111.33, 132.00)	119.33 (110.00, 129.33)	130.67 (116.67, 146.67)	< 0.0001	1.03 (1.03, 1.04)	1.01 (1.01, 1.01)
ADBP(mmHg)	70.67 (64.00, 77.33)	70.67 (64.00, 77.33)	68.67 (59.33, 77.33)	< 0.0001	0.99 (0.98, 0.99)	0.99 (0.99, 0.99)
Gender				0.0003		
Male	7711 (49.06%)	5910 (49.97%)	1801 (44.83%)		Reference	Reference
Female	7863 (50.94%)	6018 (50.03%)	1845 (55.17%)		1.01 (0.93, 1.08)	0.98 (0.89, 1.07)
Race				< 0.0001		
Mexican American	2208 (7.76%)	1739 (7.89%)	469 (7.15%)		Reference	Reference
Non-Hispanic White	6681 (68.82%)	5074 (69.08%)	1607 (67.62%)		1.17 (1.05, 1.32)	0.93 (0.81, 1.07)
Non-Hispanic Black	3259 (10.17%)	2281 (9.28%)	978 (14.32%)		1.59 (1.40, 1.80)	1.36 (1.18, 1.58)
Other Hispanic	1472 (5.35%)	1213 (5.63%)	259 (4.02%)		0.76 (0.65, 0.89)	0.68 (0.57, 0.82)
Other Race	1954 (7.90%)	1621 (13.59%)	333 (6.90%)		0.79 (0.67, 0.94)	1.04 (0.87, 1.24)
Education level				< 0.0001		
Below high school	3565 (14.32%)	2478 (12.92%)	1087 (20.84%)		Reference	Reference
High School or above	12009 (85.68%)	9450 (87.08%)	2559 (79.16%)		0.62 (0.57, 0.67)	0.96 (0.86, 1.06)
Marital status				< 0.0001		
No	5967 (33.99%)	4323 (32.58%)	1644 (40.57%)		Reference	Reference
Yes	9607 (66.01%)	7605 (67.42%)	2002 (59.43%)		0.69(0.64, 0.75)	0.83 (0.76, 0.91)
Smoke				0.0002		
No	8548 (54.82%)	6724 (55.73%)	1824 (50.58%)		Reference	Reference
Yes	7026 (45.18%)	5204 (44.27%)	1822 (49.42%)		1.29 (1.20, 1.39)	0.98 (0.90, 1.07)
Hyptersion				< 0.0001		

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TABLE 1 Continued

Variables	Total (n = 15574)	Non-DKD (n = 11928)	DKD (n = 3646)	P value	OR (95%CI)	Adjusted OR (95%CI)
No	7883 (56.67%)	7037 (63.22%)	846 (26.13%)		Reference	Reference
Yes	7691 (43.33%)	4891 (36.78%)	2800 (73.87%)		4.76 (4.37, 5.18)	1.81 (1.63, 2.01)
High cholesterol level				< 0.0001		
No	9149 (60.63%)	7492 (63.63%)	1657 (46.62%)		Reference	Reference
Yes	6425 (39.37%)	4436 (36.37%)	1989 (53.38%)		2.03 (1.88, 2.19)	1.03 (0.95, 1.13)
CHD				< 0.0001		
No	14727 (95.30%)	11523 (96.81%)	3204 (88.25%)		Reference	Reference
Yes	847 (4.70%)	405 (3.19%)	442 (11.75%)		3.93 (3.41, 4.52)	1.78 (1.52, 2.07)

MDD, Major depressive disorder; DKD, diabetic kidney disease; PIR, poverty income ratio; BMI, body mass index; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; CHD, coronary heart disease.

(https://www.rcsb.org/) , and PyMOL software (version 2.5.0) was used to remove water molecules, ligands, and other modifications (22, 23). The 3D structures of the small molecules were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) , followed by hydrogen addition and charge assignment. Finally, molecular docking was performed using AutoDock Vina (version 1.1.2), and binding sites with binding energies lower than −5.0 kcal/mol were considered to indicate stable interactions.

3 Results

3.1 Relationship between MDD and DKD

Tables 1, 2 present the baseline characteristics of the patients and the results of the logistic regression analysis for DKD and MDD. The results showed that MDD was significantly associated with an increased risk of DKD. In the univariate logistic regression analysis, the prevalence of MDD was higher in the DKD group than in the non-DKD group [OR = 1.45, 95% confidence interval (CI), 1.28-1.64]. The association remained significant after adjustment for covariates (adjusted OR = 1.24, 95% CI, 1.07-1.42).

3.2 Genetic correlation

LDSC analysis revealed a significant genetic correlation between MDD and DKD, with a correlation coefficient of 0.2153 (p = 0.008) (Supplementary Table 2). Although the MR analysis indicated no causal relationship between the two diseases (Supplementary Figure 2), this result was supported by sensitivity tests (Supplementary Table 3).

3.3 Identification of crosstalk genes for MDD and DKD

After data preprocessing, a total of 1128 DEGs were identified from the GSE98793 dataset, including 518 upregulated genes and 611 downregulated genes (Figures 2A, C). From the GSE30122 dataset, a total of 828 DEGs were identified, encompassing 266 upregulated genes and 645 downregulated genes (Figures 2B, D). Altogether, 83 genes related to MDD and DKD crosstalk were identified by Venn diagrams (Figure 2E), of which 12 DEGs were commonly upregulated (ZNF91, TGFBR3, PCDH9, FGF9, CD83, COL4A3, P3H2, KANK3, ZBTB10, MID2, RABL3, and WNT10B).

3.4 GO and KEGG enrichment analyses of crosstalk genes

The 83 crosstalk genes were subjected to GO enrichment analysis, and a total of 374 GO terms were obtained, comprising 301 BP terms, 26 CC terms, and 47 MF terms (Figure 2F). Regarding the BP terms, the genes related to crosstalk were primarily enriched in epithelial cell

TABLE 2 Baseline characteristics and OR of participants by MDD levels in NHANES (2005–2018).

Variables	Total (n = 15574)	Non-MDD (n = 14203)	MDD (n = 1371)	P value	OR(95%CI)	Adjusted OR(95%CI)
DKD				< 0.0001		
No	11928 (82.35%)	10968 (82.83%)	960 (76.59%)		Reference	Reference
Yes	3646 (17.65%)	3235 (17.17%)	411 (23.41%)		1.45 (1.28, 1.64)	1.19 (1.03, 1.36)
Age(years)	50.00 (37.00, 63.00)	50.00 (37.00, 63.00)	51.00 (39.00, 62.00)	0.25	1.00 (1.00, 1.01)	0.99 (0.99, 1.00)
PIR	3.11 (1.57, 5.00)	3.25 (1.68, 5.00)	1.64 (0.91, 3.20)	< 0.0001	0.67 (0.64, 0.70)	0.73 (0.70, 0.77)
BMI(kg/m2)	28.70 (24.80, 33.50)	28.50 (24.80, 33.23)	30.90 (25.60, 36.00)	< 0.0001	1.04 (1.03, 1.05)	1.03 (1.02, 1.04)
ASBP(mmHg)	120.67 (111.33, 132.00)	120.67 (111.33, 132.00)	120.67 (111.33, 132.67)	0.41	1.00 (1.00, 1.00)	0.99 (0.99, 0.99)
ADBP(mmHg)	70.67 (64.00, 77.33)	70.67 (63.33, 77.33)	70.00 (64.00, 78.00)	0.15	1.00 (1.00, 1.01)	1.01 (1.01, 1.02)
Gender				< 0.0001		
Male	7711 (49.06%)	7180 (49.98%)	531 (37.96%)		Reference	Reference
Female	7863 (50.94%)	7023 (50.02%)	840 (62.04%)		1.61 (1.45, 1.82)	1.67 (1.47, 1.89)
Race				0.008		
Mexican American	2208 (7.76%)	2028 (7.86%)	180 (6.50%)		Reference	Reference
Non-Hispanic White	6681 (68.82%)	6069 (69.02%)	612 (66.39%)		1.14 (0.96, 1.35)	1.38 (1.14, 1.67)
Non-Hispanic Black	3259 (10.17%)	2964 (9.96%)	295 (12.68%)		1.12 (0.92, 1.36)	1.06 (0.86, 1.31)
Other Hispanic	1472 (5.35%)	1310 (5.24%)	162 (6.65%)		1.39 (1.11, 1.74)	1.48 (1.17, 1.86)
Other Race	1954 (7.90%)	1832 (7.92%)	122 (7.78%)		0.75 (0.59, 0.95)	1.18 (0.92, 1.52)
Education level				< 0.0001		
Below high school	3565 (14.32%)	3094 (13.49%)	471 (24.30%)		Reference	Reference
High School or above	12009 (85.68%)	11109 (86.51%)	900 (75.70%)		0.53 (0.47, 0.60)	0.71 (0.62, 0.81)
Marital status				< 0.0001		
No	5967 (33.99%)	5229 (32.69%)	738 (49.68%)		Reference	Reference
Yes	9607 (66.01%)	8974 (67.31%)	633 (50.32%)		0.50 (0.45, 0.56)	0.65 (0.57, 0.73)
Smoke				< 0.0001		
No	8548 (54.82%)	8011 (56.30%)	537 (37.06%)		Reference	Reference
Yes	7026 (45.18%)	6192 (43.70%)	834 (62.94%)		2.01 (1.79, 2.25)	1.89 (1.67, 2.13)
Hyptersion				< 0.0001		

(Continued)

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TABLE 2 Continued

Variables	Total (n = 15574)	Non-MDD (n = 14203)	MDD (n = 1371)	P value	OR(95%CI)	Adjusted OR(95%CI)
No	7883 (56.67%)	7337 (57.61%)	546 (45.38%)		Reference	Reference
Yes	7691 (43.33%)	6866 (42.39%)	825 (54.62%)		1.61 (1.44, 1.81)	1.39 (1.20, 1.61)
High cholesterol level				< 0.0001		
No	9149 (60.63%)	8467 (61.24%)	682 (53.31%)		Reference	Reference
Yes	6425 (39.37%)	5736 (38.76%)	689 (46.69%)		1.49 (1.33, 1.67)	1.39 (1.22, 1.58)
CHD				0.0004		
No	14727 (95.30%)	13464 (95.54%)	739 (92.31%)		Reference	Reference
Yes	847 (4.70%)	1263 (4.46%)	108 (7.69%)		1.56 (1.26, 1.91)	1.33 (1.06, 1.67)

proliferation (GO:0050673), positive regulation of cell adhesion (GO:0045785), regulation of epithelial cell proliferation (GO:0050678), cell chemotaxis (GO:0060326), and the immune response-regulating signaling pathway (GO:0002764). For the CC terms, enrichment was observed in secretory granule lumen (GO:0034774), cytoplasmic vesicle lumen (GO:0060205), vesicle lumen (GO:0031983), collagen-containing extracellular matrix (GO:0062023), and the external side of the plasma membrane (GO:0009897). Finally, for the MF terms, enrichment was observed for glycosaminoglycan binding (GO:0005539), endopeptidase activity (GO:0004175), serine-type endopeptidase activity (GO:0004252), serine-type peptidase activity (GO:0008236), and serine hydrolase activity (GO:0017171).

KEGG analysis revealed enrichment of crosstalk genes in 565 pathways, predominantly involving the PI3K/Akt signaling pathway, Hippo signaling pathway, and pathways related to proteoglycans in cancer, gastric cancer, and human papillomavirus infection (Figure 2G).

3.5 Construction of the PPI network and identification of hub genes

Based on the 83 crosstalk genes, the PPI network created using the STRING database comprised 88 nodes and 316 edges. A network diagram was constructed with Cytoscape software. Cytohubba was utilized to further screen the hub genes, and the top 10 genes were selected based on node degree ranking. In addition, a key module was extracted via the MCODE plugin, and the intersection of the two results (Figure 3A) identified the following eight hub genes: CXCR6, GZMA, CD163, KLRB1, GZMK, CCR5, CD3D, and CD8A.

3.6 Selection of biomarkers and validation of diagnostic value

LASSO regression analysis was subsequently conducted to identify potential shared diagnostic genes. Three out of eight hub genes were identified in both the GSE98793 dataset and the GSE30122 dataset (Figure 3B). Ultimately, two overlapping hub genes, namely, CD163 and KLRB1, emerged as the most promising shared diagnostic biomarkers for both MDD and DKD (Figure 3C).

Figure 3D illustrates the expression levels of the two diagnostic biomarkers in MDD and DKD. CD163 is upregulated in both diseases, while KLRB1 is upregulated in MDD but downregulated in DKD. Additionally, the sensitivity and specificity of the diagnostic biomarkers were evaluated. In the GSE30122 dataset, both diagnostic biomarkers CD163 (AUC = 0.909) and KLRB1 (AUC = 0.827), demonstrated good diagnostic value. In the GSE98793 dataset, the two biomarkers showed higher diagnostic value (CD163, AUC = 0.611; and KLRB1, AUC = 0.652) (Figure 3E). The results showed that both diagnostic biomarkers had significant diagnostic value in disease classification, but the predictive performance in the DKD dataset was better than that in the MDD dataset.

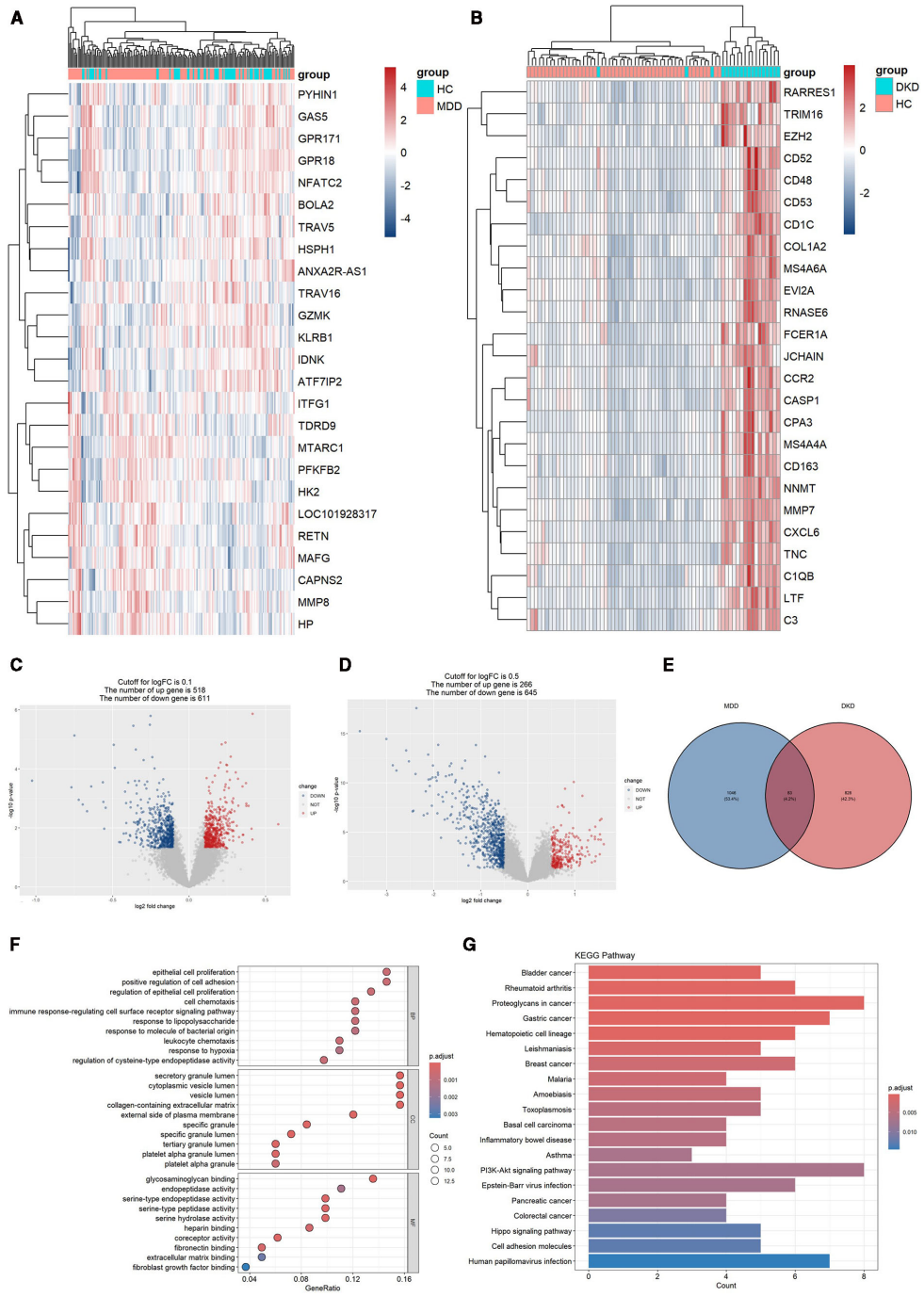

FIGURE 2
DEG expression in the two datasets and functional enrichment analyses of the crosstalk genes. **(A)** Heatmap of the top 25 DEGs in the GSE98793 dataset. **(B)** Heatmap of the top 25 DEGs in the GSE30122 dataset. **(C)** Volcano plot of DEGs in the GSE98793 dataset. **(D)** Volcano plot of DEGs in the GSE30122 dataset. **(E)** Identification of 83 genes related to crosstalk between the DEGs of MDD and DKD. **(F)** GO analysis of the crosstalk genes. **(G)** KEGG pathway enrichment analysis of the crosstalk gene.

3.7 Immune cell infiltration in MDD and DKD

To further explore the immune status in MDD and DKD, the percentage of 22 immune cells in each sample was calculated by the CIBERSORT algorithm. Figures 4A, D show the infiltration of 22 immune cell types in the GSE98793 and GSE30122 datasets, respectively. In the GSE98793 dataset, only resting CD4+ memory T cells, activated memory CD4+ T cells, and monocytes exhibited significant infiltration in MDD samples (Figure 4B). In the GSE30122 dataset, memory B cells, plasma cells, γδ T cells, resting natural killer cells, M1 macrophages, M2 macrophages, and resting mast cells exhibited significant infiltration in DKD (Figure 4E). These results suggested that both MDD and DKD

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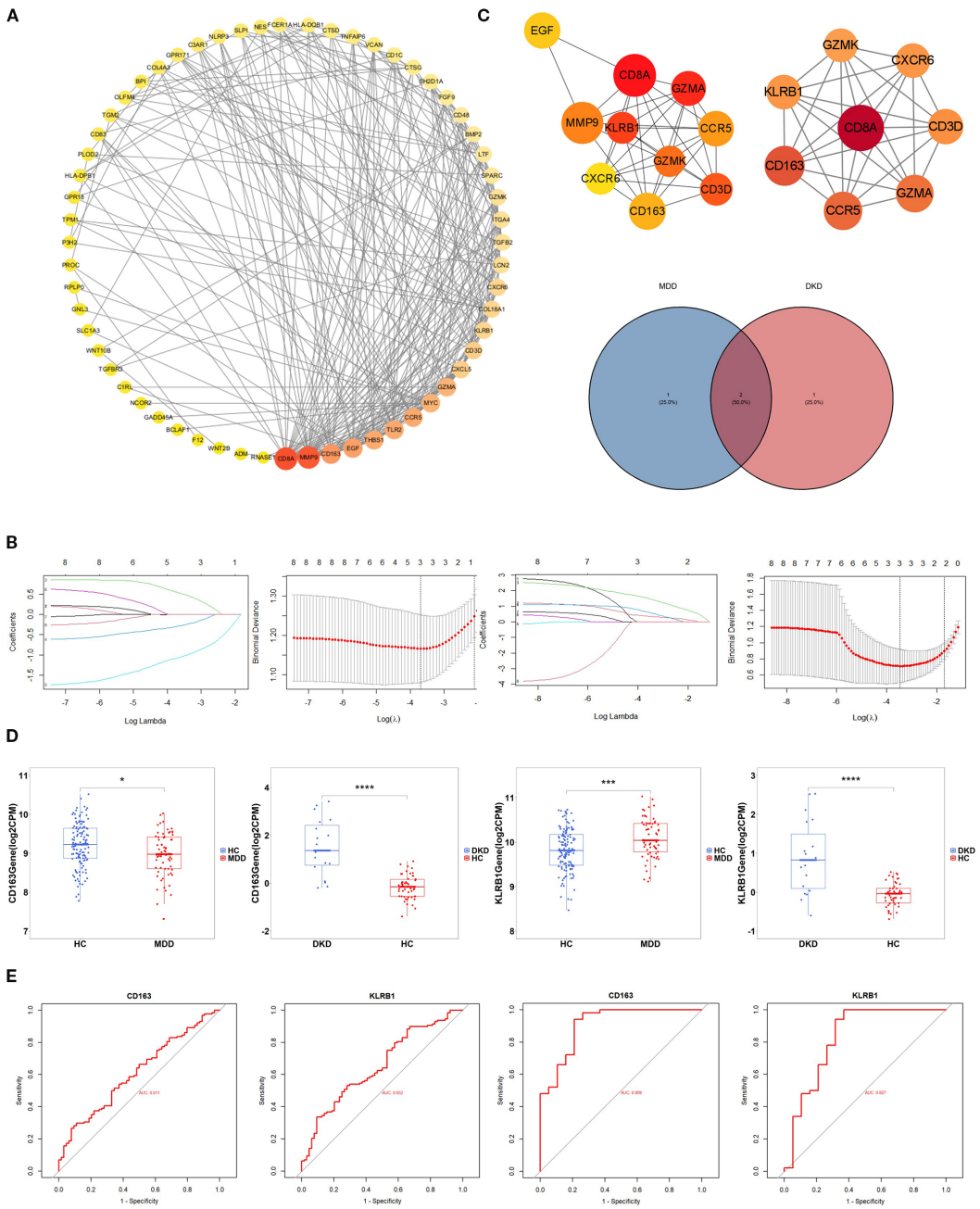


FIGURE 3
Identification of hub genes. **(A)** PPI network diagram of crosstalk genes. Interaction network of the hub genes identified by Cytohubba.Hub genes extracted by Cytohubba and MCODE. The weight of a hub gene across the network increases with the hue of the gene. **(B)** Distribution of coefficients and coefficient profiles of variables in LASSO regression models in MDD and DKD. **(C)** Venn diagram showing the potential shared diagnostic biomarkers for MDD and DKD. **(D)** Detection of the expression levels of the two potential shared diagnostic biomarkers in MDD and DKD. **(E)** ROC curves of the two potential shared diagnostic biomarkers for MDD (left) and DKD (right). The symbols represent significance levels as follows: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

patients exhibit immune activation. Although both diseases involve immune activation, the proportions of significantly infiltrating immune cells differed. Additionally, significant correlations were identified for CD163 and KLRB1 expression levels with the infiltration levels of multiple immune cells in both the MDD and DKD samples (Figures 4C, F).

3.8 Identification of small molecule compounds and molecular docking for MDD and DKD

The common upregulated crosstalk genes identified in the GSE98793 and GSE30122 datasets were imported into the cMAP

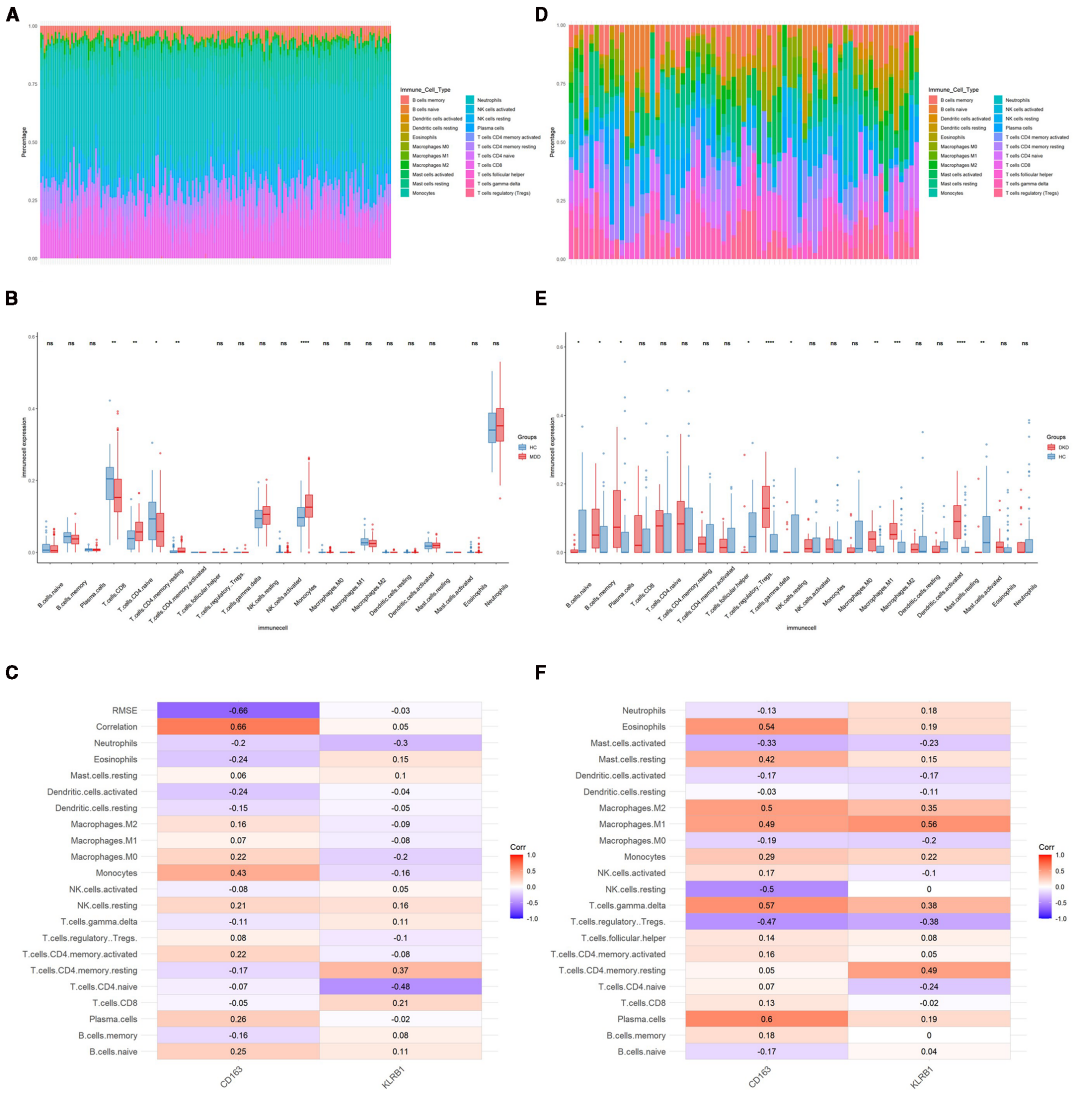


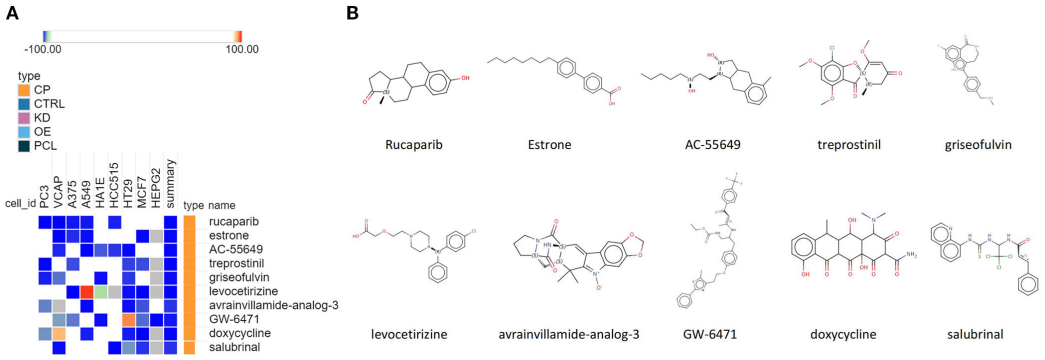
FIGURE 4
Identification of immune cells in MDD and DKD. **(A)** Immune cell infiltration map in the GSE9879 dataset. **(B)** Box plot showing the comparison of 22 types of immune cells between MDD patients and healthy control individuals. **(C)** Heatmap showing the correlations between common immune cells and potential shared diagnostic biomarkers in the GSE98793 dataset. **(D)** Immune cell infiltration map in the GSE30122 dataset. **(E)** Box plot showing the comparison of 22 types of immune cells between DKD patients and healthy controls. **(F)** Heatmap showing the correlations between common immune cells and potential shared diagnostic biomarkers in the GSE30122 dataset. *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001; ns, not significant.

database to search for small molecule compounds capable of reversing the expression of pathogenic genes associated with MDD and DKD. The top 10 compounds with the highest negative scores included rucaparib, estrone, AC-55649, treprostinil, griseofulvin, levocetirizine, avrainvillamide-analog-3, GW-6471, doxycycline, and salubrinal, which are considered potential therapeutic agents (Figure 5A). The targeting pathways and chemical structures of these 10 compounds are shown in Figures 5B, C.

Molecular docking results showed that the binding energies of CD163 with rucaparib and levocetirizine were –6.26 and –6.60 kcal/mol, respectively. KLRB1 exhibited binding energies lower than –5.00 kcal/mol with rucaparib, estrone, AC-55649, griseofulvin, levocetirizine, and salubrinal, with the lowest binding energy observed for levocetirizine at –6.09 kcal/mol (Figure 6). Detailed information on the binding energies, key binding sites, and number of hydrogen bonds between each small molecule and target protein is provided in Supplementary Table 4.

4 Discussion

Previous studies have demonstrated a bidirectional association between MDD and DM, with Type 2 DM being linked to a 24% higher prevalence of MDD, and MDD showing a 60% higher incidence in individuals with Type 2 DM (24, 25). Research on the comorbidity of DM and MDD has also been increasing annually. Fang et al. confirmed a mutually influential relationship



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Name	Description	ID	Score
rucaparib	PARP inhibitor	BRD-K88560311	-99.37
estrone	estrone estrogen receptor agonist, estrogenic hormone	BRD-A37959677	-99.01
AC-55649	RAR agonist, retinoid receptor agonist	BRD-K9317058	-98.38
treprostinil	treprostinil prostacyclin analog	BRD-A67438293	-98.28
griseofulvin	microtubule inhibitor, microtubule stabilizing agent, mitosis inhibitor	BRD-K08273968	-97.43
levocetirizine	histamine receptor antagonist, interleukin expression inhibitor, tumor necrosis factor expression inhibitor	BRD-K27184429	-97.31
Avrainvillamide-analog-3	nucleophosmin inhibitor	BRD-K39569857	-97.07
GW-6471	PPAR receptor antagonist	BRD-A72596465	-96.80
doxycycline	metalloproteinase inhibitor, 30S ribosomal protein inhibitor, matrix metalloprotease inhibitor, protein arginine deiminase inhibitor	BRD-A88774919	-96.65
salubrinal	eukaryotic translation initiation factor inhibitor, GlyT-1 inhibitor	BRD-A77299732	-96.61

FIGURE 5
Identification of potential small molecule compounds for the treatment of MDD and DKD via cMAP analysis. **(A)** Heatmap showing the top 10 small molecule compounds with the most significantly negative enrichment scores. **(B)** The chemical structures of the 10 small molecule compounds are shown. **(C)** Descriptions of the top 10 compounds.

between MDD and DKD (11). The pathogenesis of MDD is associated with immune system dysregulation, and enhanced expression of inflammatory markers (IL-6, C-reactive protein, and TNF-α) increases the risk of DKD (26, 27). In addition, MDD increases the activity of the hypothalamic-pituitary-adrenal axis, sympathetic nervous system, stress hormones, and cortisol. These factors stimulate glucose production, lipolysis, and free fatty acid cycling but decrease insulin secretion and sensitivity, potentially resulting in an elevated risk of developing DKD (28). Although the bidirectional relationship between DKD and MDD has been acknowledged, the underlying mechanisms remain poorly understood. Our cross-sectional analysis revealed a significant association between DKD and MDD, and genetic analyses indicated a degree of genetic correlation between the two conditions. However, there was no clear evidence supporting a causal relationship, which may be influenced by unmeasured confounders such as lifestyle or medication factors. These findings suggest a potential shared genetic background rather than a direct causal pathway, underscoring the complexity of the relationship. Therefore, we hypothesized that DKD and MDD share common differential genes and biological pathways, which we proceeded to investigate.

The present study identified 83 genes associated with MDD and DKD through differential expression analysis of the GSE98793 and GSE30122 datasets. Enrichment analysis discovered a significant presence of the PI3K/Akt signaling pathway and Hippo signaling pathway in MDD and DKD. Eight hub genes, namely, CXCR6, GZMA, CD163, KLRB1, GZMK, CCR5, CD3D, and CD8A, were identified from the PPI network, all of which are closely related to MDD and DKD. In addition, LASSO analysis was used to identify two hub genes, namely, CD163 and KLRB1, as potential shared diagnostic biomarkers, and their diagnostic value was validated, confirming their diagnostic significance in both diseases. Immune infiltration analysis using the DEGs of MDD and DKD was performed. T cells and monocytes exhibited significant infiltration in MDD, while various immune cells, including B cells, macrophages, and mast cells, exhibited significant infiltration in DKD. Finally, drug prediction was performed on the genes involved in the crosstalk between MDD and DKD, identifying multiple small molecule compounds as potential therapeutic drugs for MDD and DKD.

Enrichment analysis revealed that the comorbidity of MDD with DKD was primarily associated with the PI3K/Akt and Hippo signaling pathways. Additionally, the comorbidity of MDD with DKD was closely related to various cancers, such as bladder cancer, gastric cancer, and breast cancer, as well as autoimmune diseases, such as rheumatoid arthritis. Thus, the comorbidity of MDD and DKD may share common biological mechanisms with certain

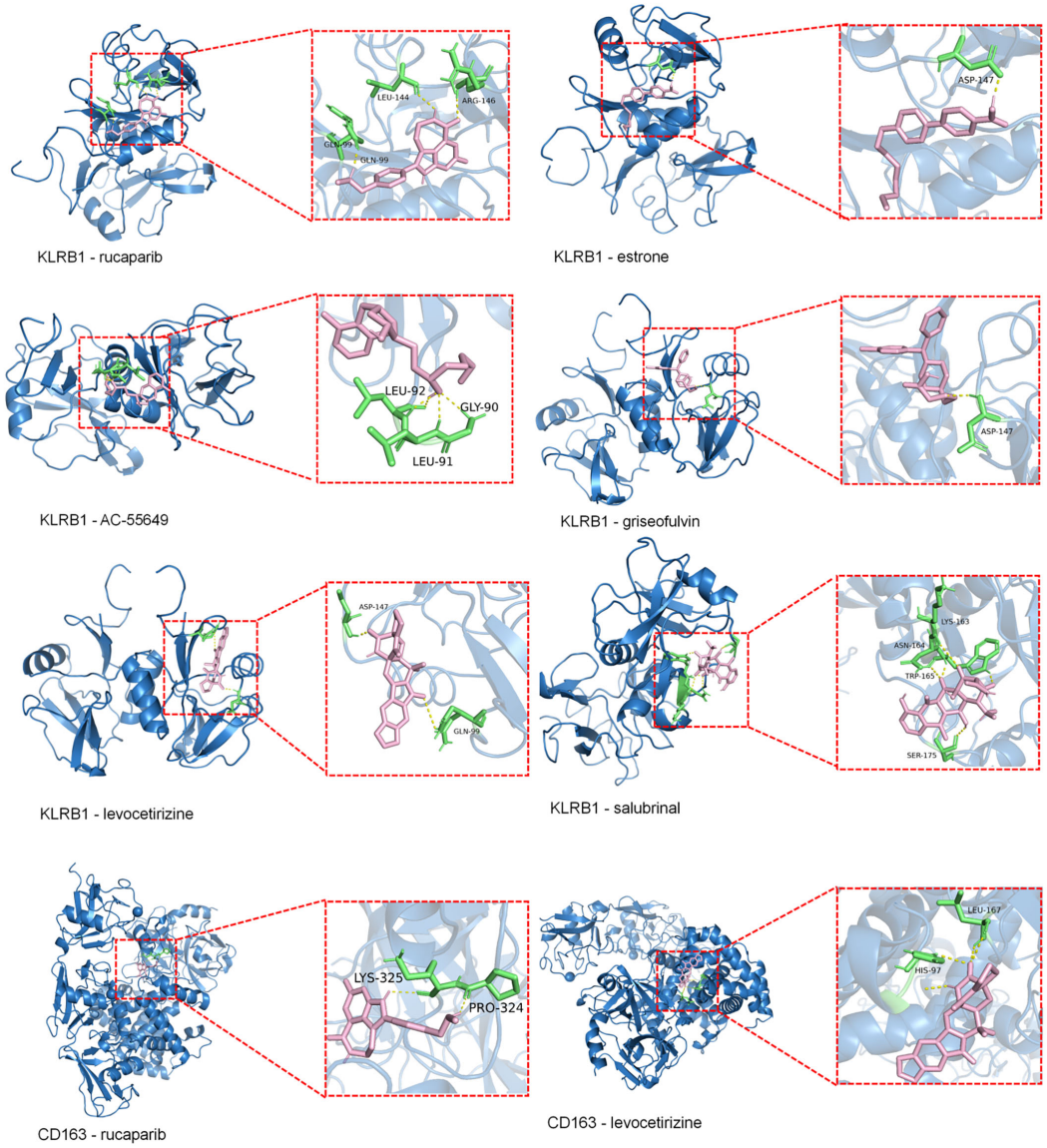

FIGURE 6
Molecular docking of KLRB1 and CD36 with small molecules (Binding Energy > −5 kcal/mol). Blue represents the macromolecular protein structure, green indicates the key amino acid residues involved in binding, pink denotes the small-molecule ligands, and yellow lines highlight the hydrogen bonds formed between the ligands and the protein residues.

cancers. The abnormal activation of these two signaling pathways in MDD and DKD may be linked to an elevated risk of cancer, indicating a potential interplay or cross-impact between them. The PI3K/Akt signaling pathway is a critical cellular signaling cascade that is pivotal for numerous biological processes, including cell survival, proliferation, differentiation, and metabolism (29). It has been suggested that the activity of the PI3K/AKT pathway may be inhibited in diabetic states, which can lead to a series of pathophysiological alterations, including increased cell apoptosis, enhanced oxidative stress, cell proliferation, and inflammatory responses (30). Further study of the mechanism of the PI3K/AKT pathway in DKD will enhance the understanding of the disease pathogenesis and lay a theoretical groundwork for the development of novel therapeutic strategies.

mTOR is a key downstream effector of the PI3K/AKT signaling pathway and plays a central role in regulating cell growth, metabolism, and autophagy. Abnormal activation or inhibition of mTOR is closely associated with the development of various diseases. In Alzheimer's disease, excessive activation of mTOR may inhibit autophagy, leading to the accumulation of abnormal proteins, which is thought to contribute to the disease's pathogenesis (31). In contrast, studies suggest that in patients with major depressive disorder, mTOR signaling activity may be suppressed, and activation of this pathway has been associated with antidepressant effects (32). Lima et al. reported that valproic acid (VPA) has antidepressant effects, which may be associated with the modulation of the PI3K/Akt/mTOR signaling pathway (33). The present findings provide important insight for exploring new targets

for the treatment of MDD. Additionally, the present study provides a theoretical basis for the clinical application of PI3K/Akt/mTOR pathway modulators as antidepressant medications.

The Hippo pathway regulates cell proliferation, apoptosis, and organ size, thereby maintaining tissue and organ homeostasis, and the main components of the Hippo pathway include MST1/2, LATS1/2, and their substrates. The Hippo pathway was initially discovered in Drosophila and has since been extensively studied in mammals (34). The Hippo pathway is an important cellular signaling pathway that may contribute to the development and progression of DKD, and related studies are ongoing. As a downstream effector of the Hippo signaling pathway, YAP promotes renal interstitial fibrosis in DKD, and high expression of YAP is correlated with increased systolic blood pressure, blood urea nitrogen, and creatinine, as well as with the progression of DKD staging and DKD pathological classification. Inhibiting YAP activity may slow the progression of DKD (34, 35). Therefore, targeting the Hippo signaling pathway may be a therapeutic strategy for DKD. There is no conclusive evidence linking the Hippo pathway to MDD occurrence and progression. However, previous studies on immunological characteristics have revealed the involvement of MST1/2 in regulating lymphocyte adhesion, migration, and CD4+ antigen recognition (36), which aligns with the present immune infiltration analysis, demonstrating that CD4+ T cells were significantly infiltrated in MDD, suggesting that the Hippo pathway may have a potential biological link to MDD. Currently, direct clinical and experimental data confirming the association between MDD and the Hippo signaling pathway are lacking. Thus, additional research is warranted to elucidate the mechanisms of the Hippo signaling pathway in MDD and its potential value as a therapeutic target for MDD.

Through the PPI network, eight hub genes that are closely associated with the immune system and inflammation regulation were identified. Among them, CD163, CCR5, CD3D, KLRB1, and CD8A serve as surface markers of immune cells and play a role in regulating immune responses. GZMA and GZMK encode proteases found in natural killer cells that are involved in cytotoxicity and the modulation of inflammatory responses (37). CXCR6, also known as CD186, is a chemokine receptor that is mainly expressed in immune cells, especially in activated T cells, natural killer cells, macrophages, and dendritic cells. CXCR6 participates in the immune response within the body, the inflammatory response, tissue cell migration, and tumor immunity (38). These eight hub genes share commonalities in regulating the immune system and inflammation, suggesting their pivotal roles in the pathogenesis of both MDD and DKD. These shared characteristics may explain their identification as relevant genes in both MDD and DKD.

Among the hub genes, CD163 and KLRB1 were identified as potential shared diagnostic biomarkers for MDD and DKD according to LASSO analysis. On the surface of macrophages, CD163 is a widely expressed receptor protein that serves as a marker for monocytes and tissue macrophages. CD163 participates in immune regulation by binding and clearing hemoglobin, regulating cytokine production and release, and modulating inflammatory responses. Furthermore, changes in serum CD163 levels are closely associated with disease status and inflammation severity, suggesting that CD163 is a potential biomarker of inflammation for disease diagnosis and monitoring (39). Research has shown that in patients with DM, the CD163 expression level in monocytes is negatively correlated with the type and severity of diabetic complications (40). However, another study has indicated that glomerular CD163+ macrophages are positively associated with DKD grade, interstitial fibrosis, tubular atrophy, and glomerulosclerosis (41). In 2017, Samuelsson et al. confirmed that CD163 is a promising early diagnostic biomarker for DKD (42). Similarly, Wang et al. corroborated this finding, aligning with the results of the present study (43).

KLRB1, also known as CD161, is a cell surface molecule belonging to the C-type lectin receptor family. KLRB1 is a transmembrane protein widely expressed in humans and other mammals. KLRB1 plays a pivotal regulatory role in the immune system and is particularly associated with natural killer (NK) cells and certain subsets of T cells (44). Currently, there are few studies on the association of KLRB1 with MDD and DKD, but its possible involvement in the immune system to regulate biological processes, such as the inflammatory response, autoimmune diseases, and antitumor immunity, may be relevant to the development of MDD and DKD. Therefore, further investigation of KLRB1 may aid in enhancing the understanding of the regulatory mechanisms of the immune system and provide new targets and strategies for the treatment of both diseases.

In addition, CD163 and KLRB1 are closely associated with tumor development. CD163 macrophages are abundant in the tumor microenvironment, and CD163 has been utilized for identifying tumor-associated macrophages in malignant diseases. For example, increased numbers of CD163+ macrophages and CD163+ gastric cancer cells are correlated with gastric tumor invasion and poor prognosis (45). Cheng et al. explored the relationship between KLRB1 and pancancer, and they reported that KLRB1 may impact tumor immunity by modulating the levels of infiltrating immune cells, particularly macrophages and lymphocytes, and that KLRB1 acts as a protective gene in the majority of cancers (46). The enrichment analysis in the present study revealed that MDD and DKD were closely related to various tumors, which may be associated with the molecular mechanisms of CD163 and KLRB1. Future studies will explore the mechanism of CD163 and KLRB1 in patients with DKD combined with MDD to offer novel insights into the clinical diagnosis and treatment of this disease.

As mentioned above, the immune mechanisms of MDD and DKD are pivotal in the onset and progression of these diseases. An increased inflammatory response, activation of immune cells, and neuroimmune interactions may be common immune mechanisms in both diseases. In MDD patients, immune cells, such as T cells, macrophages, and monocytes, may be in an activated state, and their number and activity may increase (47). In contrast, in DKD patients, immune cells, such as macrophages, dendritic cells, lymphocytes, mast cells, and neutrophils, are involved in the genesis and development of DKD (48). Immune cells produce various inflammatory factors, such as interleukin-1β (IL-1β),

tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6). Moreover, abnormal production of these inflammatory factors may affect neuronal activity, neurotransmitter levels, and neuroplasticity, leading to depressive symptoms (49). Moreover, the aforementioned inflammatory factors have been shown to exert a pivotal influence on DKD (48). The immune cell infiltration analysis findings in the present study align with previous research findings. CD4+ T cells and monocytes significantly infiltrated MDD patients, while various immune cells, such as B cells, macrophages, and mast cells, significantly infiltrated DKD patients. The two potential diagnostic biomarkers identified in the present study, namely, CD163 and KLRB1, are widely expressed in various immune cells, such as monocytes, macrophages, and lymphocytes, and they contribute to the pathogenesis and progression of diseases by modulating inflammation and immune responses.

In the present study, 12 commonly upregulated crosstalk genes in MDD and DKD were imported into the cMAP database, which identified 10 small molecule compounds (rucaparib, estrone, AC-55649, treprostinil, griseofulvin, levocetirizine, avrainvillamide-analog-3, GW-6471, doxycycline, and salubrinal) as potential therapeutic agents. cMAP analysis revealed that rucaparib had the most significant negative enrichment score, indicating that it effectively influences the expression of pathogenic genes associated with the comorbidity of MDD and DKD. Rucaparib is a poly (ADP-ribose) polymerase (PARP) inhibitor primarily used for the treatment of metastatic breast cancer patients with BRCA1 or BRCA2 mutations. Studies have also shown the inhibitory effect of rucaparib on diseases, such as ovarian cancer and prostate cancer (50). There are no definitive studies demonstrating a role for rucaparib in DKD or MDD. However, evidence suggests that PARP is involved in inflammation and metabolic regulation. Overactivation of PARP may lead to pathophysiological processes, such as excessive increases in the inflammatory response, apoptosis, and metabolic abnormalities (51). PARP inhibitors have the potential to alleviate inflammation and metabolic disorders by inhibiting PARP activity. PARP inhibitors have been demonstrated to significantly reduce the development of nephropathy caused by DM, as well as reduce oxidative stress levels, inhibit inflammatory responses, and alleviate renal fibrosis (52). In addition, the expression level of PARP1 is significantly elevated in MDD patients, and it decreases after electroconvulsive therapy (53). Therefore, PARP inhibitors have a theoretical basis for the treatment of DKD combined with MDD, and they may become a potential strategy for disease treatment. Additionally, other small molecules, such as histamine receptor inhibitors, interleukin expression inhibitors, NPM1 protein inhibitors, and PPAR receptor antagonists, are closely related to inflammation regulation. These compounds may hold promise for the treatment of MDD, DKD, and other inflammation-related diseases.

The present study had several limitations. Although CD163 and KLRB1 were identified as diagnostic biomarkers using bioinformatics methods, the lack of comprehensive validation and analysis of clinical samples may affect their reliability in clinical applications, thus requiring further experimental support. Additionally, enrichment analysis and drug prediction were based solely on gene expression data analysis, and further experimental validation and functional studies are required to confirm the biological significance and mechanism of these findings. Furthermore, due to the lack of lifetime diagnostic information and diabetes subtyping in the NHANES database, our definitions of MDD and diabetes have certain limitations, which should be addressed in future studies by incorporating clinical classifications and expert consultation. Finally, the NHANES dataset does not provide explicit information on type 1 and type 2 diabetes, which limits our ability to conduct subtype-specific analyses; future studies are encouraged to incorporate clinical classification for greater precision.

5 Conclusion

The present study indicated that CD163 and KLRB1 are potential shared diagnostic biomarkers for MDD and DKD, and it revealed the underlying biological processes common to both diseases. These findings provide important clues for future studies and are expected to provide new targets and strategies for the diagnosis and treatment of MDD and DKD.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

Author contributions

H-xL: Data curation, Conceptualization, Validation, Writing – original draft. T-tW: Software, Formal Analysis, Writing – original draft. Y-dC: Validation, Writing – original draft. C-hQ: Writing – original draft, Conceptualization, Data curation. S-kF: Formal Analysis, Validation, Writing – review & editing. X-wW: Data curation, Writing – review & editing, Software. X-hW: Validation, Conceptualization, Writing – original draft. W-xS: Funding acquisition, Supervision, Writing – review & editing. PS: Writing – review & editing, Supervision, Visualization, Funding acquisition.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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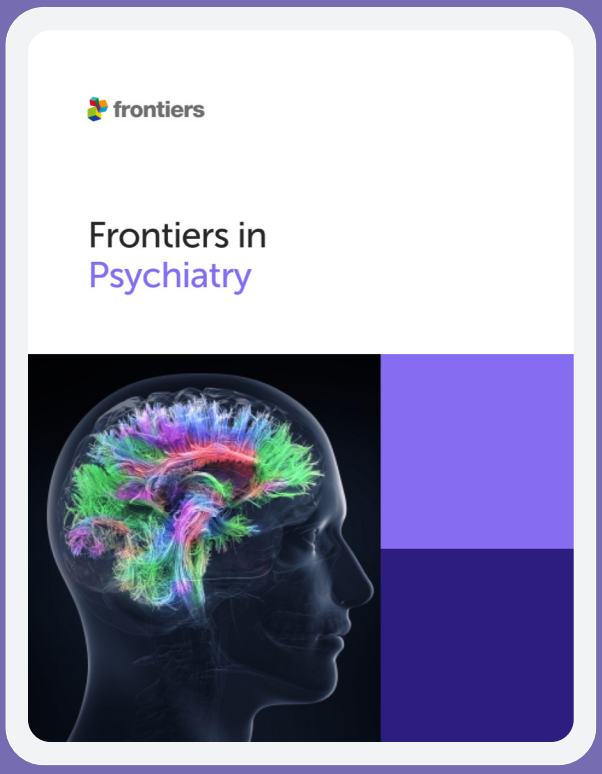

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