# NOVEL APPROACHES TO IMPROVE DETECTION, DIFFERENTIATION AND TREATMENT IN MOOD DISORDERS

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# NOVEL APPROACHES TO IMPROVE DETECTION, DIFFERENTIATION AND TREATMENT IN MOOD DISORDERS

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EDITORIAL
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# Editorial: Novel Approaches to Improve Detection, Differentiation and Treatment in Mood Disorders

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## Editorial of the Research Article

### Novel Approaches to Improve Detection, Differentiation and Treatment in Mood Disorders

Mood disorders are a group of common conditions with uncertain disease mechanisms and unsatisfactory treatment outcomes which significantly affect quality of life (1). Refining our understanding of the aetiology of these conditions and improving treatment response are essential next steps to improve outcome. This topic includes research which aims at detection, characterisation and differentiation within mood disorders, with a particular focus on aetiological mechanisms and pathways to improve treatment response.

Eight articles published in this topic originated from China (approximately 70%), one from Taiwan, one from South Korea and one from Europe/North America. We hope that the studies presented in this topic contribute to the quest of exploring novel approached to increase precision in the identification and treatment of mood disorders.

Cognitive changes in mood disorders are becoming increasingly important in relation to mechanisms of disease onset and progression and as potential targets for personalised interventions (2–4). In this topic Ronold et al. assessed cognitive function and depressive symptoms in unipolar major depression 5 years after the first diagnosis. Authors identified persistence of abnormalities in executive functions that might be independent of depressive symptoms. In synergy, Chen W-Y. et al. investigated the cognitive function of patients with bipolar disorder type I. In their retrospective analysis of around 6.5 years, the authors found that bulk of episodes with psychotic features was an independent risk factor for cognitive decline with a steeper decline after age of 42. These studies taken together show that cognitive dysfunction within mood disorders might differ in trajectory requiring a diversified approach to optimise prevention and treatment.

In relation to the importance of early detection and preventative approaches, Chen X. et al. describe MoodBox, an internet-based psychological intervention designed to improve the psychological well-being of individuals experiencing subclinical depressive symptoms aiming at reducing transition into major depression. The intervention, to be tested in a multicentre, randomised, non-blinded, superiority study intends to compare MoodBox with an online psychoeducation programme and a naturalistic observational group for 8 weeks with a further 1 year follow-up phase.

There is interest in identifying potential novel therapeutic targets in major depression. Psilocybin is a novel approach currently under investigation for treatment refractory major

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depression which benefits from psychological assistance (5). The training that therapists receive to support this intervention is important. Tai et al. describe a new manualized evidence-based psychotherapeutic approach to assist psilocybin therapy. The intervention, developed in partnership with different mental health researchers, practitioners and experts across the US, Canada and Europe is approved by the US Food and Drug Administration. Li et al. describe the role of peripheral facial injection of botulinum neurotoxin type A in major depression. Preclinical models suggest that anti-depressant effects might be associated with up-regulation of serotonin levels and expression of brain-derived neurotrophic factor in the hippocampus. Authors conclude that with limitations, clinical data are encouraging and suggest that this therapy is a potential effective and safe intervention for the management of depression. Song et al. investigated antithrombin III (ATIII) as a potential biomarker for the evaluation and prediction of treatment response in major depression. The authors randomised patients to treatment with occipital repetitive transcranial magnetic stimulation (rTMS) or sham treatment for 5 days. The results revealed a reduction in ATIII after occipital rTMS in depressed patients and a relationship between change in ATIII and therapeutic response to occipital rTMS.

Studies evaluating differentiation within mood disorders and between mood disorders and other mental health conditions are particularly important to improve disease specificity at the point of diagnosis and treatment. Ge et al. described a network analysis method to map the presence of anxiety symptoms in individuals with major depression to potentially increase diagnostic precision and target interventions to reduce the occurrence of treatment resistance. Results revealed eight co-occurring symptoms in the network structure. Dong et al. investigated differences in gut microbiota between representative cases of major depressive disorder and general anxiety disorder. The study elucidated a gut-microbiome signature associated with these two conditions that might facilitate differential diagnosis and targeted therapeutic interventions.

In relation to developing cost effective approaches which provide innovative and simple solutions for diagnosis or help to identify those in need of supportive measure or predictors of treatment response, there are several new technologies which have recently been adopted in mental health including computer-based applications (6). Lee et al. provide a literature review of the use of wearable devices and sensors in patients with depression and discuss issues regarding utility, reliability, users' perspectives and privacy. Wang et al. investigated the association between depression and gait characteristics with the aim to assist the diagnosis of depression by using support vector machine algorithms. The most efficient model they described used time- and frequency-domain features with a very high specificity, suggesting that depression could be effectively recognized through gait analysis.

Finally, the article by Qian et al. investigated the role of chromatin remodelling which included histone acetylation in an animal model of major depression. The authors showed an association between depressive-like behaviours induced by chronic social defeat stress in mice and a decrease in the class II histone deacetylase HDAC7 in the nucleus accumbens. The works suggests that HDAC7 might be a promising therapeutic target for depression.

The work published in this issue adds to recent discoveries in mood disorders and contribute to improve aetiological and detection pathways which offer new opportunities for developing novel treatments (7). Some of the most interesting recent advances in mood disorders include the realisation that epigenetic markers can transmit across generations (8), the validation and consistent replication of structural and functional neuroimaging changes in mood disorders (9–11), new developments in the techniques to analyse co-morbidities at brain level (12), the development of multi-essays serological tests to integrate biological markers with clinical phenotypes (13). Esketamine and more recently Lumateperone are among the most novel approved new treatments for mood disorders (14, 15), whereas esmethadone (16) and new neuromodulation techniques such as transcranial alternating and direct current stimulation (17, 18) are some of the new approaches under consideration.

In conclusion although mood disorders are complex condition which offer considerable challenges at different levels from the recruitment of research participants (19) to limited knowledge at brain level (20), our understanding of aberrant processes is expanding and new treatments are increreasingly becoming available.

## AUTHOR CONTRIBUTIONS

KA, AH-V, and ES contributed to writing of the first and subsequent drafts. DA conceived the idea, contributed to the writing of the manuscript, and supervised the work. All the authors approved the final version of the manuscript.

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# A Longitudinal 5-Year Follow-Up Study of Cognitive Function After First Episode Major Depressive Disorder: Exploring State, Scar and Trait Effects

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Major depression (MDD) is associated with cognitive deficits in processing speed and executive function (EF) following first episode (FE). It is unclear whether deficits are state or trait related. Studies following FE MDD over longer periods are lacking, making it uncertain how cognition and symptoms develop after the initial episode. The present study assessed cognitive function and symptoms 5 years following FE MDD. In addition, the study explored relationships between MDD symptoms, rumination, and cognitive deficits with regards to the trait, state, and scar perspective. Twenty-three participants with previous FE MDD, and 20 matched control participants were compared on Delis-Kaplan Executive Function System measures of processing speed and EF, in a 5-year longitudinal follow-up study. Correlations between current symptoms- and history of MDD, rumination, cognition were investigated. Findings indicated that cognitive deficits persisted with no clear signs of exacerbation after initial episode. Inhibition appeared independent of current and previous symptoms of depression. Processing speed was related to depressive- symptoms and rumination. In conclusion, results indicated persisting, stable deficits in both EFs and processing speed. Findings further suggest that depressive symptoms could be related to deficits in processing speed, indicating state effects. There was limited support for worsening of cognition after initial episode. Some aspects of EF like Inhibition could show persistent deficits independent of depressive symptoms indicating trait effects.

**Keywords: major depression and executive dysfunction, first episode major depressive disorder, processing speed, risk factors, rumination, state, trait, scar**

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## INTRODUCTION

Major Depressive Disorder (MDD) is one of the most prevalent and taxing disorders worldwide (1). Recurrence following first episode MDD are of particular concern (2), with estimate rates up to 90% in health care seeking individuals (3). Recurrence leaves patients with higher disability, lower quality of life, affecting everyday functioning (4, 5), and could be incremental (6, 7).

Residual symptoms are important risk factors in recurrent MDD (8). Cognitive deficits persisting in remission (9–11), could contribute to relapse, recurrence, and impaired daily functioning in MDD (12–14). However, the literature is mixed regarding whether deficits in executive- (15–17), or lower cognitive functions persist in remission (11, 18). Hierarchical organization of neuropsychological function implies that lower processing tasks are the foundation of higher cognitive functions like EF (19). Thus, separating EF from processing speed, seems to be important when investigating cognitive deficits following MDD (20, 21). How EF and processing speed relate to other residual symptoms and risk factors in remission from MDD is uncertain (22, 23), however.

Not everyone with MDD shows cognitive deficits (24). Differences in risk factors for cognitive deficits could help explain this, and include: depression status (9, 10, 15, 25), depressive symptoms (26), number- and length of episodes (11, 27), comorbid disorders (19, 20, 28, 29), rumination (30). In addition, comorbidity (31), and rumination [for a recent review see (32)], have been associated with a more severe course of illness. According to Allot et al. (22) development of both MDD and EF occur in parallel in adolescence and early adulthood. Thus, following a group of young adults from FE MDD reduces risk factors and moderators like age (33, 34). Moreover, longitudinal studies investigating FE MDD over longer periods in remission are lacking (23), precluding how cognition develops following FE MDD.

Many central issues regarding the neurocognitive profile in MDD can be illustrated by the state, trait and scar debate [for a discussion see (23, 35)]. *States* can be understood as deficits caused by-, and fluctuating with-, depressive symptoms. There are mixed findings regarding state effects on EF (11, 17, 26). Findings are also mixed regarding processing speed (11, 25), but most authors seem to find a relationship to depressive symptoms or status (15, 26, 36, 37). *Scars* are neurobiological changes due to previous depression or environmental stressors. Scarring could include length, number, and severity of MDD, resulting in exacerbated cognitive impairment (22). Neurobiological changes could also increase risk for further episodes of MDD (7). In addition to this, common treatments could mediate changes and further alter neurocognitive function (25, 38). Scarring effects could be investigated through the relationship between previous MDD duration and symptom severity, and later cognitive function (22). Semkovska et al. (11) found number of episodes negatively influenced attention, processing speed, verbal fluency, and task shifting, supporting scar effects. When manifested as *traits,* impairments are independent of scars and current symptom states, predating FE MDD. There seems to be most agreement on persistent deficits in EFs (15, 25, 36, 39), with mixed findings regarding verbal fluency (15, 25, 36, 39).

The current study was a 5 year longitudinal study, investigating EF, inhibition, working memory/mental flexibility, and verbal fluency, in addition to motor- and processing speed. Previous studies investigating the FE group found deficits in EFs and processing speed in the acute phase- (40), and 1 year following FE MDD (41). (41) found lasting impairments in the EF tasks Inhibition/Switching and verbal fluency. The current study investigated if deficits and symptoms persisted or normalized, after 5-years. In addition, the trait, state, and scar perspective were utilized in exploring the findings. To the authors knowledge, this is the first study to measure cognition in a group with FE MDD over 5 years. Consequently, the study could contribute to an increased understanding of the longitudinal development of cognitive residual symptoms and course of illness following FE MDD. The following hypotheses were investigated:

1) We predict that cognitive deficits persist after 5 years, and that a group with previous FE MDD will differ from a matched control-group, on tests measuring both processing speed and EFs.

2) It is expected that cognitive deficits and rumination related to depressive symptoms at time of assessment could represent state effects. Cognitive deficits and symptoms related previous length and strength of depression, worsening over time, could represent scar effects. Cognitive deficits that are relatively stable and independent of current- and previous symptoms of depression, could represent traits. EF is suspected to be relatively independent of state and scar effects, and a relationship between EF and rumination is expected. Processing- and motor speed are suspected to be influenced by depressive state and scar effects.

# MATERIALS AND METHODS

## Design

This longitudinal 5-year case control follow-up study examined a group with FE MDD and matched controls. There were three points of assessment: Participants were assessed at baseline in the acute phase of MDD (T1), after 1 year (T2), and after 5 years (T3). For additional information, see **Figure 1**.

## Recruitment and Participant Flow

Participants in the depression group (DG) were recruited from primary healthcare and student healthcare services in Bergen, Norway. Participants were informed about the ongoing study through the cooperation of physicians and psychologists in primary- and university healthcare clinics. A study coordinator contacted interested patients that met the inclusion criteria at T1. Initial inclusion criteria were current FE depression of a moderate- to severe degree, indicated by a score of $\geq$20 on the Montgomery Åsberg Depression Rating Scale [MADRS; (42)]. Initial exclusion criteria for the DG were earlier history-, treatment-, or diagnosis of depression. Exclusion criteria for all participants in the DG from T1, T2, and T3 were the following: Psychosis, electroconvulsive treatment, alcohol- or substance abuse, brain damage, neurological- or severe somatic disorder. A trained psychologist assessed exclusion criteria at each time point by a structured questionnaire (T1, T2, and T3), and The Norwegian version of Mini-International Psychiatric structural interview [MINI; (43)] for the DG (T1 and T2). A control group was recruited, individually matched on the following variables: Sex, age, and years of

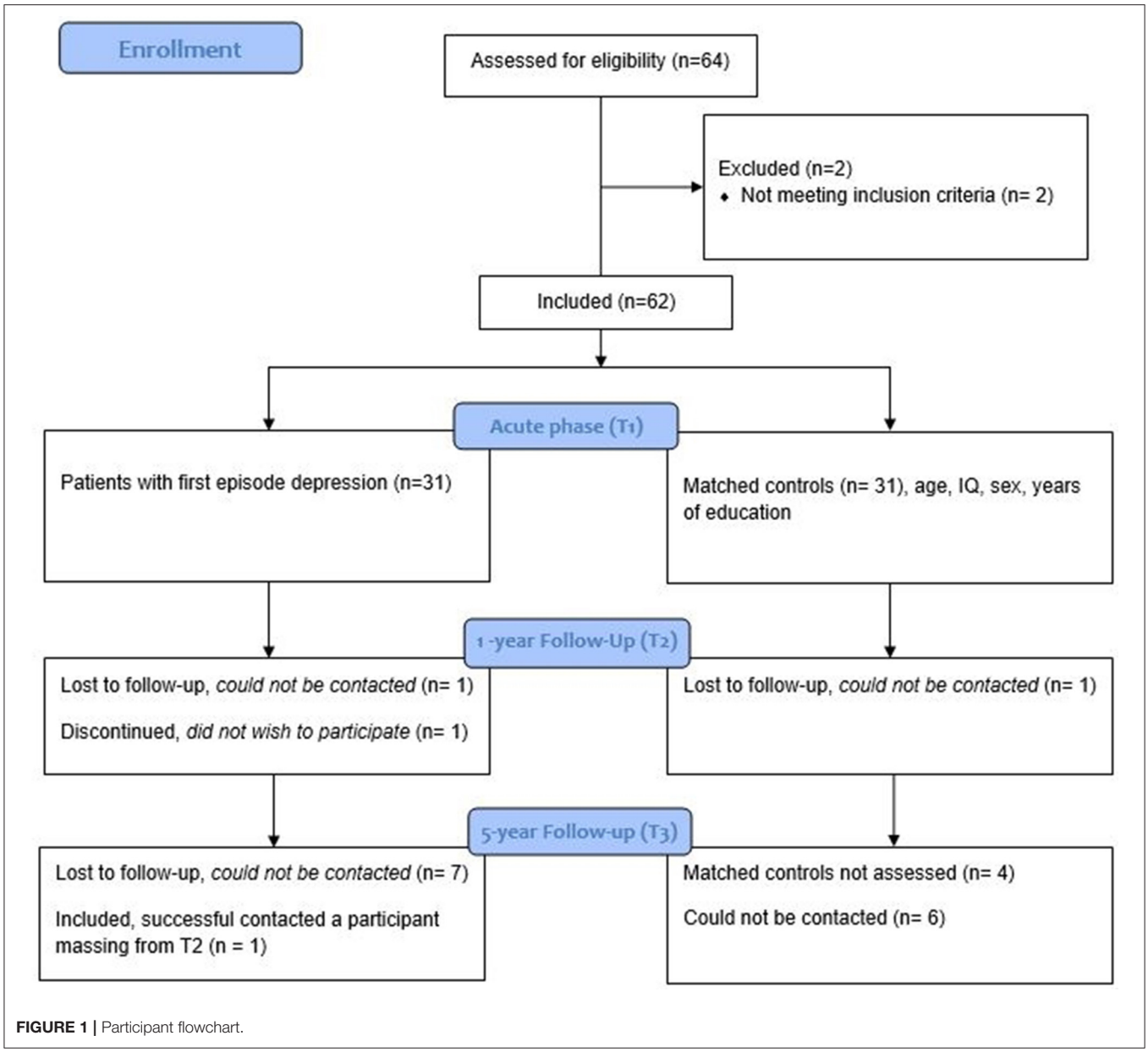


**FIGURE 1 |** Participant flowchart.

education (±2 years), and the matching was valid at T3 (see **Table 1**). Controls were recruited at the University of Bergen and through colleges at the Department of Biological and Medical Psychology. Exclusion criteria for controls were: History of any mental disorder, alcohol- or substance abuse, brain damage, neurological- or severe somatic disorder, measured by a structured questionnaire designed for the study (at T1, T2, and T3). All participants were invited to a 1-year follow up assessment (T2) at T1. At the 5-year follow up assessment (T3), participants were contacted by mail and invited to take part in the study. Dropouts were largely participants that the study was unable to contact due to expired contact information, and participants that had moved away (see **Figure 1**).

## Clinical Assessments and Rumination Measures

Clinical assessments were made by trained psychologists. MINI was used to assess history of psychiatric disorders in the DG at T1 (version 5.0), and at T3 (version 6.0). All in the DG met DSM-IV criteria for MDD as measured by MINI. Depressive symptoms were measured by MADRS at all time points. At T3 a retrospective assessment of relapse-, recurrence, and duration of MDD was done using the National Institute of Mental Health's Life Chart Method [NIMH LCM; (44)]. The LCM was used to measure relapse and asses months of depression in the DG since T2. Relapse was defined as reporting one or more depressive episode since T2. Structured interviews were done in the DG assessing exclusion criteria, the use of psychotropic medication

and psychological treatment. Controls were not assessed by clinical interviews or MADRS, as psychiatric disorders were exclusion criteria in this group.

Both the DG and controls completed self-report assessments of depressive- and neurotic rumination at both T2 and T3. Depressive rumination was measured by the Norwegian version of the Ruminative Responses Scale [RRS; (45)], a 22 item four point likert questionnaire that measures ruminative responses to depressive mood. The Norwegian version of the 12 item five point likert rumination subscale of Rumination-Reflection Questionnaire (RRQ) measured rumination independently of depressive mood. This form of self-rumination is associated with the personality trait neuroticism (46, 47). At T2 RRS was only administered to the DG. RRQ was administered in both groups at T2 and T3 (see **Table 1**).

## Clinical Profile

Participants differed in how their course of illness developed following FE MDD. At T3 symptom severity as measure by MADRS ranged from not depressed <12 ($n$ = 16), to mild- 12–22 ($n$ = 5), and moderate symptoms 23–29 ($n$ = 2) as indicated by the Norwegian MADRS manual (48). Seventeen patients had one or more episodes of relapse (74%) between T2 and T3. Almost half of the group ($n$ = 11) had histories of comorbid psychiatric symptoms as assessed by MINI at T1 and T3, hereunder: Generalized Anxiety Disorder, Panic Disorder With- and Without Agoraphobia, Agoraphobia, Obsessive Compulsive Disorder, and Social Phobia. There were no instances of exclusion criteria as assessed by MINI; neither Psychotic Disorder nor Alcohol- or Substance Abuse. At T3 four patients were using psychotropic medication: One was currently using a Selective Serotonin Reuptake Inhibitor medication (SSRI: Escitalopram), while two were using Selective Serotonin-Noradrenalin Reuptake Inhibitors (SNRI: Venlafaxine). Finally, one participant was prescribed a sedative (Chlorprothixene: Truxal). The same patients were also receiving psychological treatment and outpatient psychiatric follow up. One patient was currently in psychotherapy, while three were in contact with the District Psychiatric Center for outpatient follow up of psychotropic mediation and/or psychotherapy (see **Table 1** for more clinical characteristics).

**TABLE 1 |** Demographics and clinical measures.

| Demographics and clinical measures for T3 groups (M/F) | Depression group $n$ = 23 (11/12) | Control group $n$ = 20 (9/11) |
|---|---|---|
| | M (SD) | M (SD) |
| Age | 30.34 (5.74) | 30.45 (6.09) |
| Education | 15.34 (2.34) | 16.6 (2.01) |
| IQ[a] | 118.65 (8.47) | 119.7 (8.27) |
| Age of onset MDD | 25.61 (5.73) | |
| T2 RRS[b] | 45.49 (11.89) $n$ = 22 | |
| T3 RRS*** | 48.43 (13.31) | 30.15 (9.74) |
| T2 RRQ*** | 44.90 (8.99) $n$ = 20 | 30.26 (7.26) $n$ = 19 |
| T3 RRQ*** | 42.13 (12.52) | 32.40 (8.02) |
| T1 MADRS[b] | 24.43 (3.8) | |
| T2 MADRS[b] | 10.27 (5.64) $n$ = 22 | |
| T3 MADRS[b] | 8.87 (8.13) | |
| Months depressed since T2 | 12.60 (14.45) $n$= 21 | |

*M, Mean; SD, Standard Deviation; M/F, Males/Females; n, Number of participants; IQ, Intelligence Quotient. [a]measured at inclusion [***]Significantly different between groups $p < 0.001$, RRS, Ruminative Responses Scale; RRQ, Rumination-Reflection Questionnaire (Rumination subscale); MADRS, Montgomery Aasberg Depression Rating Scale; [b]CG were not assessed.*

## Ethics and Compensation

All participants gave informed consent to participate in the study. Participants received a gift card valued at 400 Norwegian Kroner (∼50 United States Dollars) for their participation. The study was approved by the Regional Committee for Medical and Health Research Ethics and was performed in accordance with the World Medical Associations Declaration of Helsinki regarding Ethical Principles for Medical Research Involving Human Subjects (49).

## Neuropsychological Assessment

Neuropsychological testing took place at the University outpatient clinic for neuropsychology at all points of assessment. Experienced test technicians did the neuropsychological assessment. IQ was measured at inclusion (T1) by Wechsler Abbreviated Scale of Intelligence [WASI; (50)]. Participants completed a battery of standardized neuropsychological tests at all time points. EF and processing speed were assessed by Delis et al. (51) Delis-Kaplan Executive Function System (D-KEFS). Three subtests from the battery were investigated: The Color Word Interference Test (CWIT) measuring processing speed, inhibition, switching and general EF (21), the Verbal Fluency Test (VFT), and the Trail Making Test (TMT) measuring motor- and processing speed, as well as switching (52). See **Supplementary Materials 1** for description of tasks.

Contrast-, composite-, and error scores were computed based on descriptions in the D-KEFS manual (51), and previous studies (41). Contrast scores were made to separate EFs from processing speed, subtracting the Color Naming and Word Reading conditions from the Inhibition- [Inhibition Contrast = Inhibition – (Color Naming + Word Reading)/2], and Inhibition/Switching conditions [Inhibition/Switching Contrast = Inhibition/Switching – (Color Naming + Word Reading)/2]. An EF composite score was for the CWIT overall by separating the processing speed- from the EF conditions [CWIT Executive function composite = (Inhibition + Inhibition/Switching)/2 – (Color Naming + Word Reading)/2]. To separate processing speed and lower cognitive processes from EF, contrast scores for TMT Number-Letter Switching were calculated [Contrast Number-Letter Switching = Number-Letter Switching – (Visual Scanning + Letter Sequencing + Number Sequencing + Motor Speed)/4]. Error scores were pooled for the different D-KEFS tests. In addition, CWIT error scores were pooled to represent executive dysfunction in the Inhibition and Inhibition/Switching conditions, and processing deficits in the Color Naming and Word Reading conditions.

## Data Scoring and Analysis

All statistical analyses were performed in Statistical Package for the Social Sciences (SPSS version 25). Raw scores, that consisted of seconds to complete task (CWIT, TMT, high score = poor performance), and words generated per minute (VFT, high score = good performance), were used for the neuropsychological tests. Variables were plotted and checked for linearity and outliers. Outliers were inspected and determined to represent real scores and not errors. Normality was assessed using Kolmogorov-Smirnov test of normality and non-parametric tests were used when assumptions were violated. Cohen (53) was used to describe effect sizes as small, medium and large.

### Differences Between the DG and Controls in Cognitive Function Over Time

Mixed between-within subjects ANOVA was used to calculate differences between groups in cognitive function and change over the three points of assessment (Group × Time × D-KEFS conditions). Box's test of equality of Error Variance and Mauchly's Test of Sphericity was performed and Multivariate statistics reported when the latter assumption was violated. Levenes test of Equality of Error Variances was performed for all ANOVA analyses, and Welch values given when this assumption is violated. One participant in the DG was only available at T1 and T3 and was thus missing from the Mixed between-within subjects ANOVA, and other analyses containing data from T2 (see **Figure 1**). Groups at T3 were compared by one-way analysis of variance (ANOVA) on matched variables and clinical measures. Mann Whitney *U*-tests were used to assess differences between groups on non-parametric data, and independent samples chi square tested for categorical variables. Change scores were calculated by subtracting D-KEFS scores at T2 from D-KEFS scores at T3. Negative values implied decreased performance over time (with the exception of VFT, were the opposite was the case). Paired sample *t*-tests were used to assess changes on D-KEFS by comparing scores from T2 to scores from T3 in the DG, and the control group.

### Separating EF and Processing Speed

One-way ANOVA analyses were used to investigate differences between groups on the different D-KEFS conditions and contrast scores at T3. Man Whitney *U*-tests were used to assess error scores and the CWIT EF composite score. Effect size measures ($\eta^2$) for Man Whitney *U*-tests were calculated through the following formula ($\eta^2 = Z^2/N\text{-}1$).

### Traits, States, and Scars, Relationships Between Depression, Rumination, and Cognitive Function

Bivariate correlation coefficients were calculated to explore relationships at T3. The relationship between symptoms of MADRS, RRS, RRQ, and D-KEFS scores was investigated. Spearman's Rho was used as a non-parametric alternative to Pearson's correlation coefficients when assumptions for the latter were not met. To separate depressive state (MADRS T3) from scar effects, an explorative composite score consisting of a standardization (Computed in SPSS) of number of months depressed (*Z*-score months depressed), combined with standardized MADRS scores *before* T3 (T1, T2) were calculated: Scar composite = (ZMADRS T1 + ZMADRS T2 + Zmonths depressed)/3.

# RESULTS

## Matching of Groups

Groups did not differ significantly ($p > 0.05$) on any of the matched variables sex, age, and years of education, nor in IQ (see **Table 1** for means and frequencies).

## Differences Between the DG and Controls in Cognitive Function Over Time

Mixed between-within subjects ANOVA found a significant interaction effect for Time × Condition in all D-KEFS tests (see **Table 2**). Means indicated that scores from T1 improved. A lack of a Time × Condition × Group interaction supported that improvements did not differ between groups, and that

**TABLE 2 |** Cognitive differences between groups over time.

| | | | Main effects | | | Interaction effects | | |
|---|---|---|---|---|---|---|---|---|
| | | Group | Condition | Time | Time × group | Time × condition | Condition × group | Time × cond. × group |
| CWIT | Wilk's λ | | 0.035 | 0.501 | 0.918 | 0.594 | 0.869 | 0.910 |
| | $F_{(df)}$ | 13.55 (1, 40) | 347.96 (3, 38) | 19.34 (2, 39) | 1.75 (2, 39) | 3.99 (6, 35) | 1.906 (3, 38) | 0.58 (6, 35) |
| | Partial eta-sq. | 0.253 | 0.965 | 0.131 | 0.082 | 0.406 | 0.131 | 0.090 |
| | F-sig. | $p = 0.001$ | $p < 0.001$ | $p < 0.001$ | $p = 0.188$ | $p = 0.004$ | $p = 0.131$ | $p = 0.74$ |
| VFT | Wilk's λ | | 0.035 | 0.772 | 0.968 | 0.759 | 0.918 | 0.971 |
| | $F_{(df)}$ | 3.48 (1, 40) | 534.53 (2, 39) | 5.57 (2, 39) | 0.653 (2, 39) | 2.93 (4, 37) | 1.751 (2, 39) | 0.28 (4, 37) |
| | Partial eta-sq. | 0.08 | 0.965 | 0.228 | 0.032 | 0.241 | 0.082 | 0.029 |
| | F-sig. | $p = 0.069$ | $p < 0.001$ | $p = 0.006$ | $p = 0.526$ | $p < 0.05$ | $p = 0.19$ | $p = 0.89$ |
| TMT | Wilk's λ | | 0.085 | 0.559 | 0.953 | 0.446 | 0.791 | 0.898 |
| | $F_{(df)}$ | 6.53 (1, 40) | 99.53 (4, 37) | 15.41 (4, 39) | 0.953 (2, 39) | 5.12 (8, 33) | 2.45 (4, 37) | 0.469 (8, 33) |
| | Partial eta-sq. | 0.140 | 0.915 | 0.441 | 0.047 | 0.554 | 0.209 | 0.102 |
| | F-sig. | $p = 0.015$ | $p < 0.001$ | $p < 0.001$ | $p = 0.39$ | $p < 0.001$ | $p = 0.063$ | $p = 0.87$ |

*CWIT, Color Word Interference Test; VFT, Verbal Fluency Test; TMT, Trail Making Test; (df), degrees of freedom; Partial Eta-sq., Partial Eta Squared.*

**TABLE 3 |** Cognitve performance at T3.

| Groups at T3 (M/F) | Depression group $n = 23$ (11/12) | | Control group $n = 20$ (9/11) | | Statistics | | |
|---|---|---|---|---|---|---|---|
| **D-KEFS measure** | **M (SD)** | **Change score from T2 $n = 22$** | **M (SD)** | **Change score from T2** | $F_{(1,41)}$ | $p$ | **eta sq.** |
| Color word interference test | | | | | | | |
| Color naming | 29.87 (4.68) | −0.55 | 26 (4.63) | 0.5 | **7.37 | <0.01 | 0.152 |
| Word reading | 22.52 (3.3) | 0.5 | 18.9 (2.43) | 0.95 | ***16.37 | <0.000 | 0.285 |
| Inhibition | 48.04 (8.28) | −0.95 | 41 (5.91) | 0.1 | ***10.02 | <0.001 | 0.196 |
| Inhibition/switching | 56.13 (7.52) | −0.41 | 48.65 (9.58) | −1.05 | ***8.22 | <0.001 | 0.167 |
| Inhibition contrast | 21.85 (6.38) | −0.52 | 18.55 (5.57) | −0.625 | 3.13 | 0.084 | 0.071 |
| Inhibition/switching contrast | 29.93 (5.64) | −0.52 | 26.2 (8.29) | −1.78 | 3.05 | 0.088 | 0.069 |
| Verbal fluency | | | | | | | |
| Letter fluency$^{hs}$ | 50.78 (11.48) | −1.27 | 55.2 (10.51) | −0.4 | 1.71 | 0.198 | 0.040 |
| Category fluency$^{hs}$ | 48.48 (10.18) | 0.27 | 51.75 (9.68) | 3.1 | 1.16 | 0.288 | 0.027 |
| Category switching$^{hs}$ | 14.96 (2.2) | 0.32 | 15.4 (2.32) | 0.65 | 0.41 | 0.527 | 0.010 |
| Trail making test | | | | | | | |
| Visual scanning | 17.91 (4.5) | 0.5 | 15.25 (2.88) | 1 | *5.15 | 0.029 | 0.112 |
| Number sequencing | 24.04 (8.47) | 1.95 | 17.3 (4.92) | 2.55* | **9.78 | 0.003 | 0.193 |
| Letter sequencing | 24.17 (8.95) | −1.5 | 17.2 (3.37) | 2.55 | **10.78 | 0.002 | 0.203 |
| Number letter switching | 62.96 (18.95) | 0.045 | 50.1 (15.26) | 2.65 | *5.88 | 0.02 | 0.126 |
| Motor speed | 20.13 (9.68) | −0.86 | 16.8 (5.02) | 1.45 | 1.91 | 0.174 | 0.045 |
| Contrast number letter switching | 41.39 (15.33) | 0.02 | 33.46 (14.43) | 0.76 | 3.02 | 0.09 | 0.069 |

*M/F, Males/Females; n, Number of participants; M, Mean (seconds to complete task, except for verbal fluency); SD, Standard Deviation; Df, Degrees of freedom $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{hs}$high score indicate high performance in these conditions (words generated).*

both processing speed and EF improved similarly over time. In addition, there was a significant main effect of group on the CWIT and TMT, while the VFT only approached significance, with a medium effect size. Overall, groups showed similar improvements, but differed in test performance.

One-way ANOVA tests indicated no significant differences between groups on change scores from T2 to T3. This was supported by paired sample *t*-tests on D-KEFS scores from T2 to T3, that showed there were no significant improvements in scores, with the exception of controls improving on TMT Number Sequencing from T2 (see **Table 3**). In sum, this supported that the Time × Condition interaction above was due to changes from T1 to T2, not from T2 to T3. Cognitive deficits persisted after 5 years and were relatively stable following the acute phase of FE MDD.

One-way ANOVA tests investigated which of the cognitive functions measured by D-KEFS, processing speed or EF, showed largest differences between groups (see **Table 3**). The DG performed significantly poorer than controls in all the conditions of the CWIT at T3. The contrast scores for Inhibition and Inhibition/Switching were not significantly different, although differences showed moderate effect sizes. There were significant differences in all the conditions of the TMT, except Motor Speed. The Number-Letter Switching contrast score approached significance, with a moderate effect size. There were no significant differences in the VFT conditions with small effect sizes. Groups significantly differed in conditions measuring both EF and processing speed, but not motor speed. Contrast scores were not significantly different.

## Separating EF and Processing Speed

A significant difference between the groups was found on a composite score for Inhibition and Inhibition/Switching, with the DG performing poorer ($M = 25.89$, $Mdn = 27.5$, $n = 23$) than controls ($M = 22.38$ $Mdn = 22.75$, $n = 20$) $U = 146.5$, $p = 0.023$, $\eta^2 = 0.099$. Error scores from the two processing speed conditions were also compared to the executive conditions. Errors in the processing speed conditions differed with the DG making more errors ($M = 0.87$, $Mdn = 1$), and controls fewer ($M = 0.25$, $Mdn = 0$), Mann Whitney $U = 150.5$, $p = 0.023$, $\eta^2 = 0.123$. The DG made more errors in the executive conditions ($M = 2.13$, $Mdn = 2$), compared to controls ($M = 0.85$, $Mdn = 0.50$), Mann Whitney $U = 92$, $p = 0.001$, $\eta^2 = 0.286$. No significant differences in error scores appeared in either VFT or TMT. Differences in composite score and errors in the EF conditions of CWIT, supported deficits in EF when controlling for processing speed.

## Course of Illness

Overall, symptoms of depression and rumination were relatively stable in the DG after T1 (see **Table 1**). The standard deviation of MADRS and months depressed at T3 suggested increased variance of depression in the DG, however. This could reflect a polarization of depressive symptoms in the group. Comorbid disorders increased in the group. At T1 only (16%) had a history of comorbid disorders (one of these dropped out). At T3 (48%) had a history of comorbid disorders. McNemar's test showed that increased comorbidity was significantly greater than chance from

**TABLE 4 |** Relationships between depressive symptoms, rumination, and cognitive tests.

| | Relationships between symptoms and cognitive tests | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | MADRS | 1 | | | | | | | | | | | | | | | | | |
| 2 | RRS | **0.541**** | 1 | | | | | | | | | | | | | | | | |
| 3 | RRQ | 0.305 | 0.810** | 1 | | | | | | | | | | | | | | | |
| 4 | CWIT: color naming | 0.341 | 0.174 | 0.141 | 1 | | | | | | | | | | | | | | |
| 5 | CWIT: word reading | **0.528**** | **0.426*** | 0.155 | 0.512* | 1 | | | | | | | | | | | | | |
| 6 | CWIT: inhibition | 0.049 | 0.037 | 0.217 | 0.671** | 0.313 | 1 | | | | | | | | | | | | |
| 7 | CWIT: inhibition/switching | 0.363 | 0.241 | 0.242 | 0.656** | 0.581** | 0.357 | 1 | | | | | | | | | | | |
| 8 | CWIT: inhibition contrast | −0.164 | −0.076 | 0.223 | 0.398 | −0.003 | 0.877** | 0.014 | 1 | | | | | | | | | | |
| 9 | CWIT: Inh/switching contrast | 0.182 | 0.104 | 0.169 | 0.266 | 0.222 | 0.044 | 0.847** | −0.224 | 1 | | | | | | | | | |
| 10 | VFT: letter fluency | −0.063 | 0.203 | 0.252 | 0.135 | −0.236 | 0.147 | 0.09 | 0.227 | 0.152 | 1 | | | | | | | | |
| 11 | VFT: category fluency | **−0.424*** | −0.362 | −0.101 | −0.022 | −0.500* | 0.058 | −0.02 | 0.204 | 0.09 | 0.578** | 1 | | | | | | | |
| 12 | VFT: category switching | −0.26 | −0.231 | 0.164 | 0.03 | −0.285 | 0.35 | −0.127 | 0.518* | −0.159 | 0.144 | 0.591** | 1 | | | | | | |
| 13 | TMT: visual scanning | **0.551**** | 0.137 | 0.053 | 0.548** | 0.464* | 0.388 | 0.649** | 0.088 | 0.430* | −0.243 | −0.247 | −0.168 | 1 | | | | | |
| 14 | TMT: number sequencing | **0.521*** | 0.264 | 0.088 | 0.509* | 0.457* | 0.335 | 0.591** | 0.032 | 0.420* | 0.183 | −0.121 | −0.167 | 0.667** | 1 | | | | |
| 15 | TMT: letter sequencing | **0.545**** | 0.171 | 0.034 | 0.560** | 0.387 | 0.15 | 0.443* | −0.025 | 0.199 | 0.002 | −0.029 | 0.119 | 0.550** | 0.457* | 1 | | | |
| 16 | TMT: number-letter switching | **0.489*** | −0.088 | −0.246 | 0.580** | 0.244 | 0.331 | 0.376 | 0.1 | 0.221 | 0.055 | 0.025 | 0.024 | 0.538** | 0.662** | 0.681** | 1 | | |
| 17 | TMT: motor speed | 0.239 | 0.096 | 0.009 | 0.369 | 0.539** | 0.178 | 0.550** | −0.165 | 0.434* | −0.292 | −0.275 | −0.357 | 0.470* | 0.402 | 0.186 | 0.31 | 1 | |
| 18 | TMT: N-L switching contrast | 0.308 | −0.192 | −0.344 | 0.487* | 0.09 | 0.301 | 0.157 | 0.154 | 0.031 | 0.079 | 0.097 | 0.083 | 0.34 | 0.472* | 0.566** | 0.946** | 0.124 | 1 |

**p < 0.05 (2-sided), **p < 0.01 (2-sided), MADRS, Montgomery Aasberg Depression Rating Scale; RRS, Ruminative Responses Scale; RRQ, Rumination-Reflection Questionnaire (Rumination subscale); CWIT, Color word interference test; VFT, Verbal Fluency Test; TMT, Trail Making Test.*
*Bold values represent potential state effects.*

T1 to T3, $p = 0.039$ (2-sided), in the DG. This could indicate considerable heterogeneity regarding course of illness after T1.

## Relationships Between Symptoms and Cognitive Function

Spearman's Rho was calculated to explore the relationship between depressive symptoms and D-KEFS scores at T3 (see **Table 4**). MADRS showed small to large correlations with CWIT scores. Word Reading showed the largest relationship to MADRS. Relationships between EF (CWIT contrast and composite scores) and T3 MADRS score was small. There were small to medium relationships between CWIT and depressive rumination, showing similar, but smaller relationships than MADRS. MADRS showed a large relationship to depressive rumination supporting this. The CWIT processing speed measures showed small correlations to neurotic rumination. RRQ showed a moderate relationship to Inhibition/Switching, $r = 0.34$ $n = 23$ $p = 0.112$ (2-sided), although this relationship was smaller on the contrast score $r = 0.303$ $n = 23$ $p = 0.116$ (2-sided). CWIT processing speed measures showed the largest relationships to depressive- symptoms and rumination. Neurotic rumination differed, showing only a moderate relationship to Inhibition/Switching.

## State Effects VFT and TMT

T3 MADRS showed small to medium relationships to VFT. Category Fluency showed the largest correlation (see **Table 4**). There were similar but smaller relationships to RRS, and only small relationships to neurotic rumination. MADRS showed small to large correlations with TMT. Most of the processing speed measures showed large relationships to MADRS. TMT Letter Sequencing showed the largest association with MADRS. TMT showed mostly small relationships to both types of rumination. One exception was for the TMT Number-Letter Switching contrast score, that showed a negative moderate relationship with RRQ $r = -0.3$ $n = 23$ $p = 0.108$ (2-sided). Category fluency and the processing speed measures of TMT showed strongest relationships to depressive symptoms. Neurotic rumination showed a moderate negative relationship to Number-Letter Switching contrast score.

## Scar and Trait Effects

The scar composite score was used to investigate how previous history of depression related to D-KEFS scores and rumination at T3. The scar composite showed small correlations to all of the CWIT scores except for the CWIT Inhibition/Switching condition, with a moderate correlation $r = 0.439$ $n = 23$, $p = 0.036$ (2-sided), and an even stronger relationship to the Inhibition/Switching contrast score $r = 0.486$ $n = 23$, $p = 0.019$ (2-sided), indicating that this relationship could not be explained by processing speed. The scar composite showed a large correlation to RRQ at T3 $r = 0.562$, $n = 23$, $p = 0.005$ (2-sided), higher than to current depressive symptoms (see **Table 4**), which could suggest that neurotic rumination show larger relationship to MDD over time than current depressive symptoms. Differences between the DG and controls in CWIT error scores were new, and could thus represent a scar effect. Error scores on the CWIT, especially errors in the EF conditions, showed weak relationships to the scar composite, $rho = -0.064$ $n = 23$, $p = 0.773$ (2-sided) in addition to MADRS at T3 $rho = -0.073$ $n = 23$, $p = 0.742$ (2-sided), however. The scar composite was related to Inhibition/Switching and neurotic rumination at T3, but not new differences between groups CWIT error scores.

## Traits, Stable Differences Between DG and Controls That Are Unrelated to State and Scar Measures

The CWIT Inhibition contrast score showed small correlations to both state- $rho = -0.164$, $n = 23$, $p = 0.454$ (2-sided), and scar measures of depression, $rho = -0.069$ $n = 23$, $p = 0.753$ (2-sided). It did, however, show a medium correlation to error scores in the executive condition, $rho = 0.451$, $n = 23$, $p = 0.031$, thus EF errors could be related to a trait EF/Inhibition impairment independent of state- and scar effects. CWIT Inhibition score differed between the DG and controls at both T1, T2 and T3, and show negligible relationships to both the scar composite $r = 0.059$ $n = 23$, $p = 0.788$, and MADRS at T3 $rho = 0.049$ $n = 23$, $p = 0.825$ (2-sided), and a comparable relationship to error scores in the EF conditions $rho = 0.382$ $n = 23$, $p = 0.072$ (2-sided). In conclusion, there is some support for stable deficits in CWIT Inhibition that could be independent of state- and scar effects, which thus could represent a cognitive trait deficit in MDD.

# DISCUSSION

The main aim of the present study was to investigate cognitive residual symptoms in the first longitudinal 5-year follow up study of FE MDD. In addition, relationships between current- and previous depression, current rumination, and cognitive deficits were also explored. It was expected that stable deficits, unrelated to current and previous MDD history, could represent traits.

## Persisting Deficits

The first hypothesis predicted that cognitive deficits would persist after 5 years. This hypothesis was supported. Results suggested that there are broad, relatively stable deficits on most of the cognitive measures. Stable differences are in line with several reviews and meta-analyses showing that cognitive deficits persist in remission (9, 11, 15, 36). However, this is the first study to show deficits 5 years following FE MDD. Importantly, there were no indication of significant cognitive decline after initial episode, and therefore little support for a worsening of cognition during the 5 years, although the study could be underpowered to detect small changes.

## Deficits in EF and Processing Speed

Current findings support cognitive deficits in *both* EF and processing speed, in line with the first hypothesis. Although there were larger effect sizes for differences in the latter, there was also moderate effects for differences in EF contrast scores controlling for processing speed. The lack of significant differences in motor speed, also suggest that motor slowing is not sufficient to explain differences on the tests (16, 21). Persisting deficits in EF, even when controlling for processing speed, is mostly supported. This could be contrary to Semkovska et al. (11), were the authors suggest that executive dysfunction is due to deficits in processing speed. Deficits in processing speed showed the largest effects, however.

## Course of Illness and Cognition

**Table 1** indicated that the DG differed in their rate of depression following first episode (large standard deviations), which could influence results. In addition, the increase in comorbidity could influence cognitive function and has been shown to have a relationship to processing speed (20), but not inhibition (54). Of note, there was no indication of significant worsening in cognitive functions after T1, and therefore limited evidence to support cognitive exacerbation from the increase in comorbidity. The study could be underpowered to detect this, however. The increase could illustrate the need for longer follow up times in clinical studies, as comorbidity commonly increase with increasing follow up time (31). The relatively high depressive rumination could indicate that rumination represent risk factor in remission from MDD (32, 55, 56). Residual symptoms like rumination, and risk factors like comorbid disorders, could thus be of importance when planning treatment and prevention strategies. The majority of patients had undergone psychological- and/or pharmaceutical treatments, that could have influenced neurocognitive function (25, 38). However, some authors have suggested that at least some cognitive deficits persist despite "successful" treatment (39), as indicated the current study, and thus new interventions targeting cognitive functions seem warranted.

## Are Deficits Associated With Depressive Symptoms States, Scars, or Traits?

There was mixed support for the hypothesized state, scar, and trait effects. Preliminary results supported the hypothesis that processing speed deficits are influenced by state effects. In addition, this finding is in accordance with meta-analyses and reviews showing relationships between MDD and processing speed deficits (26, 37). Similar, albeit weaker, relationships were found between depressive symptoms, depressive rumination, and cognitive tests. This could suggest that the relationship

between depressive rumination and cognitive function is due to depressive symptoms in the current sample. The small relationships between depressive rumination and EF went contrary to our expectations. Recent meta-analyses, however, support small relationships between EF and rumination (57–59). Of note, neurotic rumination at T3 showed different associations to cognitive tests, and stronger relationships to EF, compared to depressive rumination. Inhibition and switching were related to neurotic rumination, in support of our hypothesis. Switching, an EF, somewhat unexpectedly showed a moderate negative relationship to neurotic rumination, although some studies have found relationships between rumination and better scores on some aspects of EF (60). Alternatively, this could be a spurious relationship. In conclusion, measuring different forms of rumination (61), like neurotic rumination, could probably further elucidate on the relationship between rumination and cognition.

The exploratory scar composite score showed a relationship to Inhibition/Switching. There was no relationship between the scar composite and contrast score for Inhibition. This could indicate a scar effect on mental flexibility, although this finding must be taken with caution, as there was no clear indication for a worsening of cognitive functions over time. Thus, there is limited support for the scar hypothesis in the present study. Age and follow-up time could explain this however: 5-years might be too short, and participants to young, for a scaring effect to appear. Neuropsychological exacerbation caused by depression could probably be more apparent with increasing age (33). Semkovska et al. (11) found evidence for exacerbation of cognition with number of depressive episodes, although this finding could be influenced by age as well. However, it is hard to conclude about scar effects without measuring cognitive functions before onset of FE MDD.

Of note, neurotic rumination correlated with both the scar composite and Inhibition/Switching. Surprisingly, the relationship between neurotic rumination and history of depression, was higher than that to current depressive symptoms. This could suggest that neurotic rumination is a risk factor for-, or at least associated with MDD history. It could be that neuroticism/rumination and Inhibition/Switching are a part of risk factors for MDD over time. The former could support emerging perspectives for understanding mental illness that focus on neuroticism like the p-factor model (62), while the latter is supported by Schmid and Hammar (41), that found relationships between Inhibition/Switching and relapse and recurrence in a FE sample. Differences between the DG and controls in CWIT error scores are new and could thus represent a scar effect, but was not related to the scar composite. The scar composite is a novel construct based on theoretical assumptions [see Figure 1 in (22)], and might imperfectly capture the nature of the depressive history in our sample, however. In conclusion, there is insufficient evidence in the current paper to conclude regarding the scar hypothesis. Finally, neurotic rumination and Inhibition/Switching could be related to history of depression.

Inhibition, although significantly different between the DG and controls during the 5 years, did not show any sizable or significant relationships to history- or symptoms of depression nor rumination. All this could indicate that inhibition represent a trait and a cognitive risk factor in a group with recurrent depression. The EF function of Inhibition is recognized in other longitudinal studies as a stable deficit in MDD (63–65), in addition to several meta-analyses (9, 17, 36, 37). Inhibition is also the function most strongly associated with the unity EF factor (66), which could point toward a general persisting EF impairment as a trait associated with recurrent MDD.

## Strengths and Limitations

This was the first study to investigate cognition in FE MDD after 5 years. Thus, the study could contribute with a unique perspective on the development of MDD. The current study is important due to the considerable length of follow-up time making it able to assess change and stability in cognitive function in relation to symptoms and course of illness. The thorough neuropsychological testing enabled differentiation between processing speed and EF. In addition, symptoms were measured at different time points, making it possible to investigate longitudinal relationships between cognition and symptoms. Furthermore, the study points to several variables which are relevant for further research, like persisting cognitive deficits (neurotic) rumination, and increased rate of comorbidity following the FE MDD. The increase in comorbidity, however, entails that the study did not asses MDD alone, but also comorbid disorders. This might more accurately reflect the common courses of illness and thus enhance the ecological validity of the current study, but also potentially confound results as discussed above and below. Future studies with larger samples should investigate how risk factors like comorbidity, rumination, relapse, and different treatments mediate and moderate cognition and course of illness in MDD.

Despite some strengths, the study also had major limitations. The results were from a small sample, and a selected group. All participants in the DG were outpatients. IQ was in the average to above average range which could mask deficits. However, the DG and controls showed comparable IQ scores and the groups did not differ on matched variables. MINI does not measure personality pathology (other than antisocial personality disorder) which could have been present- and influenced results. In addition, comorbidity, depressive symptoms, and treatment effects, could have confounded results. Dropout was also considerable. Interestingly, dropout was higher in the control group, which could suggest that clinically unrelated factors played a part in this. Many participants were students that moved away after completing university. The lack of clinical assessments of the controls could be viewed as a major limitation. Symptoms in the control group could have influenced results, but the relatively low rumination scores at T3 (see **Table 1**) suggested that this was not a major issue, however. There were also issues regarding measurements and sample size. Given the long follow up time, the assessments of months depressed could be influenced by subjective memory and is probably not completely accurate, which could have influenced the novel scar composite. Due to increased type II error rate Bonferroni adjustments were not made to significance levels. This could

have increased false positive findings. In addition, the study could be underpowered to detect small changes over the 5 years, which could have resulted in the lack of support for scarring effects. In addition, correlations and other effect sizes might be unstable and inflated due to the small sample (67), and should be interpreted with caution. Results should be considered preliminary and should be replicated in larger samples [for a discussion see (68)]. Also, importantly, correlation does not imply causation, thus the current study cannot say anything about the direction of the relationship between symptoms and cognition. Future studies should longitudinally investigate risk factors in larger samples, making it possible to use more complex statistics to causally model relationships between variables, like in structural equation models. Furthermore, several measures of EFs should be included to facilitate composite scores to more accurately capture the diversity functions of EF and their relationships to symptoms, risk factors, and treatments. Finally, to best inform on the state, trait, and scar debate, prospective longitudinal studies should be done, measuring cognition before the onset of MDD, and thus asses predisposing traits, and potentially scarring effects of FE MDD not captured by the current study.

## CONCLUSION

The present study indicated that a group of former FE MDD patients showed lasting, stable, deficits in cognition compared to a healthy matched control group after 5 years. There were deficits in both processing speed and EF. Findings suggest that processing speed are related to depressive symptoms indicating state effects. There was no clear worsening of cognitive function. Some aspects of EF like Inhibition showed persistent deficits independent of depressive symptom state, indicating trait effects. The study underscores the importance of persisting cognitive residual symptoms following FE MDD, and the need to adapt treatments and prevention strategies targeting cognitive functioning.

## 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 human participants were reviewed and approved by the Regional Committee for Medical Research Ethics and the Norwegian Data Inspectorate approved the study. The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

ER: collection and analysis of data, writing manuscript draft, tables, and figures. KO: writing and editing. ÅH: study PI, writing and editing. All authors contributed to the article and approved the submitted version.

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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.2020.575867/full#supplementary-material

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STUDY PROTOCOL
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# Efficacy of an Internet-Based Intervention for Subclinical Depression (MoodBox) in China: Study Protocol for a Randomized Controlled Trial

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**Background:** Subclinical depression is a prevalent mental health problem and increases the incidence of the onset of major mood disorders, such as major depressive disorder (MDD). Psychological interventions have been proved to be effective for reducing depressive symptoms for people with subclinical depression and can prevent the onset of MDD. However, people have limited access to face-to-face psychotherapy. Internet-based psychological intervention is an alternative treatment option. The aim of the study is to evaluate the efficacy of MoodBox, an online psychological intervention program, for subclinical depression.

**Methods:** This study is a multicenter, randomized, controlled, non-blinded superiority study with three parallel groups. A total of 435 first-year university students with subclinical depression will be recruited. Eligible participants will be randomly assigned to the MoodBox group, the online psychoeducation group, and the naturalistic observation group at a ratio of 1:1:1. The intervention period is 8 weeks, and participants will be continuously followed up for 1 year. The primary outcome of the study is the efficacy of the intervention, defined as measured by the Patient Health Questionnaire (PHQ-9).

**Discussion:** This is the first study to innovatively develop and test an intervention to improve psychological well-being and decrease the incidence of MDD in a subclinical depression population in China. Once proven effective and acceptable, MoodBox could be potentially integrated into the routine clinical service to facilitate the management for people with subclinical depression.

**Clinical Trial Registration:** The trial is registered with the Chinese Clinical Trial Registry on 21 July 2020 (No. ChiCTR2000034826).

**Keywords: subclinical depression, internet-based, digital intervention, psychotherapy, prevention, RCT - randomized controlled trial**

# INTRODUCTION

Subclinical depression, also known as subthreshold depression, subsyndromal depression, or minor depression, is considered to be a condition that does not meet the diagnostic criteria for depression but present with at least two and no more than four depressive symptoms, which must include one of the core symptoms of major depressive disorder (MDD) (i.e., depressed mood or anhedonia) and last a duration for at least 2 weeks (1–3). The prevalence of subclinical depression ranges from 1.3 to 17% in primary care and from 1.4 to 17.2% in community settings (2). Subclinical depression has detrimental effects on psychosocial function and quality of life, increases service utilization, and carries a high risk of developing into a full-blown depression (4, 5). Furthermore, a meta-analysis indicated that the mortality rates in subclinical depression are comparable with those in MDD (6).

Although depressive disorders are the most frequent consequence of subclinical depression, it can also lead to other mood disorders. From the clinical staging approach, subclinical depression could be the prodromal stage for a variety of mental health problems (7). However, studies on subclinical depression mostly focus on preventing the onset of depression, and other mood disorders are commonly overlooked (8, 9). Moreover, in light that depressive episode is usually the first episode of bipolar disorder (10), especially in young age, the reduction of depressive symptoms is not equivalent to remission of subclinical depression; alternatively, it may be a sign for the development of mania or hypomania. Therefore, a comprehensive assessment of mood events is necessary for subclinical depression.

Psychotherapy is effective in reducing depressive symptoms for people with subclinical depression and can prevent the onset of MDD (11). Although medication is recommended to treat depression, no evidence has supported the use of medication for subclinical depression (12). Cognitive behavioral therapy (CBT), interpersonal psychotherapy (IPT), problem-solving therapy (PST), and internet-based psychotherapy are primary choices for treating subclinical depression and preventing depression (13). However, traditional psychotherapy (i.e., face-to-face therapy carried out by a therapist) is not suitable for the preventive use in a large population, due to the limited number of qualified therapists and high costs (14–16). Moreover, in China, currently, mental health service is prioritized for people who meet the clinical threshold, and people with subclinical conditions are largely underserved (17).

Internet-based psychotherapy has the advantage of being accessible at any time and place, people can work in their own pace, and at-risk individuals can be reached more timely as compared with traditional face-to-face approach (18). Therefore, an effective internet-based psychological intervention has the potential to meet the need for the underserved population. Although internet-based interventions have been proved effective in western countries (18–20), to date, in China, no internet-based psychological intervention for subclinical depression has been developed and tested. To address the gap, informed by evidence-based psychological interventions for subclinical depression (e.g., CBT and IPT), we developed an internet-based psychological intervention program, named MoodBox, for people with subclinical depression, and we carried out a randomized controlled trial (RCT) to test the efficacy of the program. The primary aim of the study is to evaluate the efficacy of MoodBox for subclinical depression. The secondary aims are to evaluate the feasibility, usability, acceptability, and safety of MoodBox. To the authors' knowledge, MoodBox is the first internet-based intervention for subclinical depression in China.

# METHODS

## Study Design

The study is a multicenter, randomized, controlled, non-blinded superiority study with three parallel groups. People with subclinical depression will be invited to participate in the study. Eligible participants will be randomly assigned to one of the three groups—the MoodBox group, the online psychoeducation group, and the naturalistic observation group—at a ratio of 1:1:1. By designing a three-arm study and choosing online psychoeducation and naturalistic observation as comparators, we aim to minimize the influence of digital placebo effect and the natural remission of subclinical depression. The intervention period of the study is 8 weeks. Following the completion of the intervention period, the participants will be continuously followed up for 1 year. All assessments will be conducted remotely using a social media app (WeChat) to send out notifications to participants. At each follow-up time window, a notification will be sent out via the WeChat public account to remind the participant to fill out a set of self-report scales. The participants will be asked to report general information, symptom severity, social functioning, childhood traumatic experiences, parenting experiences, attachment, and coping style at the baseline and to report their symptom severity and social functioning at each follow-up time point. After the baseline assessment, the participants will be assessed at 2, 4, and 6 weeks after they started to receive the intervention and a post-intervention assessment at 8 weeks. After that, participants will enter the follow-up phase and will be assessed at 12-week, 6-month, 9-month, and 12-month follow-up time points. See **Figure 1** for a flowchart demonstrating the study procedure in detail. The study has been registered as a clinical trial on the Chinese Clinical Registry website (Registration number: ChiCTR2000034826).

## Participation Eligibility

The eligibility of participants will be assessed in accordance with the following inclusion and exclusion criteria by psychiatrists through clinical interview and relative scales. The inclusion criteria are (1) age 18 years or older, no restriction for gender; (2) the score of two to four items on the Patient Health Questionnaire (PHQ-9) scale $\geq 2$ (must include the first or second item, which are the indicator for core symptoms of depression) and a total score $\leq 9$; (3) not meeting diagnostic criteria for any mental disorders according to the *Diagnostic and Statistical Manual of Mental Disorders* (Fourth Edition) (DSM-IV); and (4) being able to give consent. The exclusion criteria are (1) having severe and

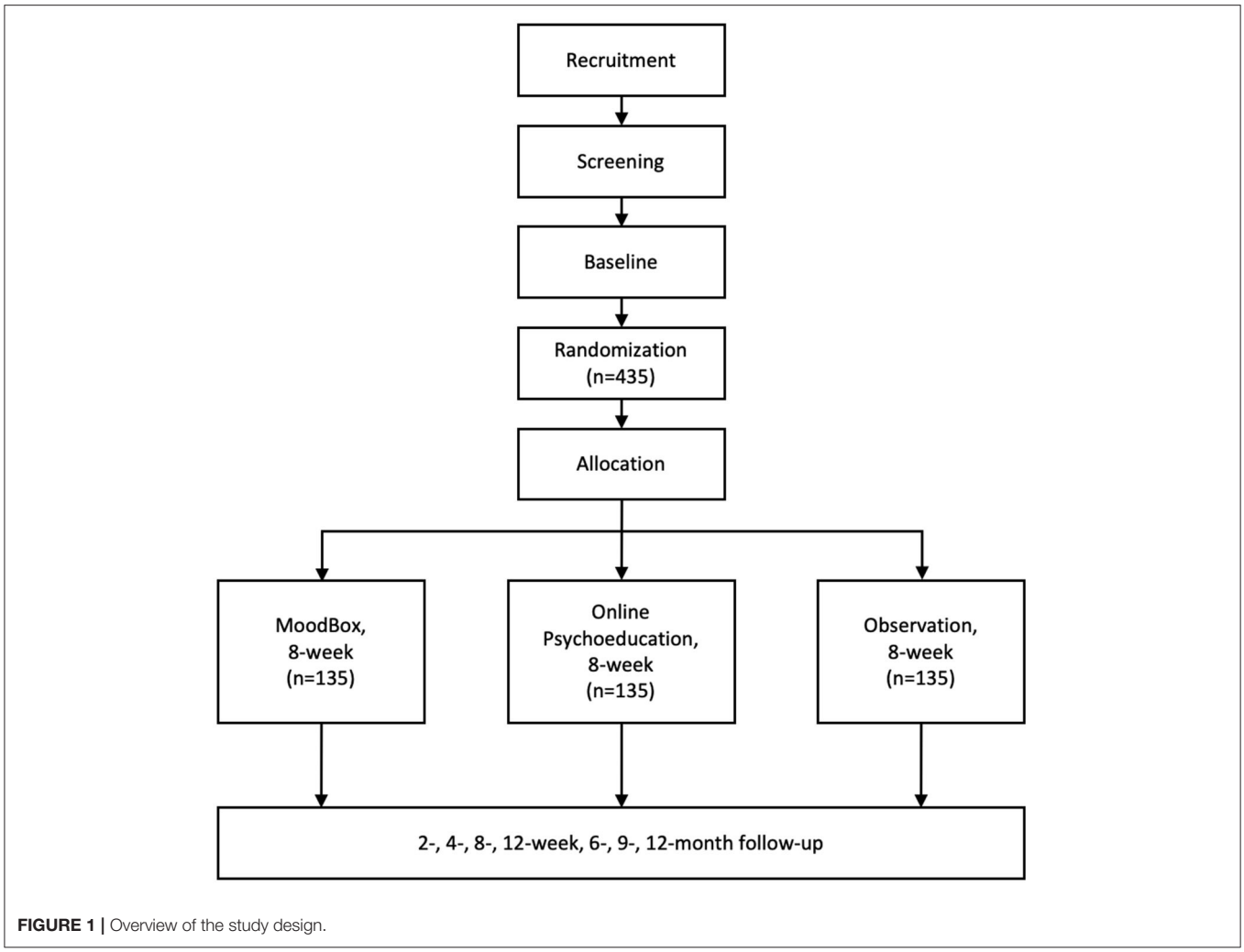


**FIGURE 1 |** Overview of the study design.

unstable physical illness; (2) having organic brain damage; or (3) being alcohol or drug dependent. A psychiatrist will be using Mini-International Neuropsychiatric Interview (MINI) to rule out any mental disorders. The exclusion criteria will be assessed through clinical interview by completing a screening checklist of the study.

## Sample Size

Four hundred thirty-five participants are intended to enroll and will be randomized with a ratio of 1:1:1. The estimated sample size provides 80% power (two sided $\alpha = 0.025$, considering a multiple comparison) to detect difference in the primary outcome and the proportion of participants without subclinical depression between the MoodBox group with the online psychoeducation group and the observation group in a survival analysis (assuming the minimum difference is 14%) after 8 weeks. Taking into account a predicted 20% attrition for this comparison, at least 145 participants would be needed in each group. The calculation was based on a meta-analysis that reported clinical difference between the internet-based intervention group and psychoeducation group (9), and the calculating methods were in accordance with Chow et al.'s instruction (21).

## Recruitment and Randomization

The study is a multicenter, three-arm, parallel RCT. The study will be conducted at three universities in Beijing, China. First-year students who are waiting for the regular physical examination will be invited by research staff to complete a brief screening questionnaire to assess their eligibility. Students who meet the eligibility criteria will be provided with the participant information sheet and the consent form of the study. If the participants were interested in participating in the study, research staff would give a more comprehensive face-to-face explanation of the study in a private consultation room. Written consent will be required before the participant formally enrolls in the study. Eligible participants will be randomly assigned to three groups—the MoodBox group, the online psychoeducation group, or the observation group—with a ratio of 1:1:1. Randomization will take place after the completion of baseline assessment using a randomization code, which will be developed in SAS 9.4 (SAS

**TABLE 1 |** The content of the MoodBox program.

| Themes | Contents | Session |
|---|---|---|
| Introduction | What is depression? | Session 1 |
| | What is MoodBox? | Session 1 |
| | Introduction of the process of MoodBox | Session 1 |
| | Symptom assessment | Session 1 |
| Cognitive intervention | Learning automatic thoughts | Sessions 2–6 |
| | Identify distortion thoughts | Sessions 2–6 |
| | Practicing cognitive intervention techniques | Sessions 7–10 |
| | Review and testing | Session 11 |
| Interpersonal intervention | Interpersonal problem diagnoses | Session 12 |
| | Learning interpersonal theory | Session 13 |
| | Practicing interpersonal intervention | Sessions 14–17 |
| | Review and testing | Session 18 |
| Relaxation | Mindfulness meditation | Sessions 2–10 and Sessions 12–17 |
| | Relaxation music | Sessions 2–6 |
| Review and termination | | Sessions 19–20 |

Institute Inc., Cary, NC, USA) by a statistician who is not involved in the study.

## Intervention

### MoodBox Group

The MoodBox group will receive an 8-week internet-based psychotherapy program. The program is a web-based psychological intervention informed by evidence-based psychological interventions, including CBT, IPT, and mindfulness meditation. The program consists of four core modules, which are symptom assessment, cognitive therapy, interpersonal therapy, and relaxation. There are 20 sessions in total, and each session takes approximately 30 min to complete. Participants are expected to complete a minimum of two sessions per week. After finishing each session, participants will receive a virtual trophy in the program as a reward. Participants will be assigned with a user account and receive training of the program at the first visit.

An outline of a brief description of the internet-based intervention program contents can be found in **Table 1**. The first module (session 1) of MoodBox is an introduction of the program that provides a comprehensive explanation of depression and the program, and there is an assessment of depression using PHQ-9 at the end of this session. The second module is cognitive intervention (sessions 2–11), which is based on cognitive reconstruction technique of CBT. This module will illustrate by animation the different types of automatic thoughts that are related to depression. After the illustration, participants will be required to identify their own automatic thoughts and to practice modifying the automatic thoughts by doing exercises in the program. The third module is interpersonal intervention (sessions 12–18). This module is based on IPT theory and focuses on interpersonal problems related to depression. The fourth module is relaxation, which includes mindfulness meditation and relaxation music. This module is not stand-alone; instead, it was embedded in other modules and would be presented after finishing each session. The final module is review and termination (sessions 19 and 20). In this module, participants will review all the previous sessions and will be given advice for their future life.

### Online Psychoeducation Group

For the online psychoeducation group, a psychoeducation website will be provided. The website will deliver psychoeducation that includes general information about depression and tips for managing depressive moods. However, the psychoeducation website does not contain any therapeutic contents. Participants will be asked to visit the website twice a week during the 8-week intervention period and will be followed up continuously for 1 year.

### Naturalistic Observation Group

Participants in the observation group will only receive regular follow-up at the same visit time point with the MoodBox group and will be followed up continuously for 1 year.

## Outcome Measures and Data Collection Procedure

The follow-up procedure has been summarized in **Table 2**, including the outcome measures and the assessment time points of the study. Other information includes demographics information, medical history, and current treatment plan, which will be collected by questionnaires via WeChat public account. The adverse events will be assessed by participants' self-report and documented by the research team.

### Primary Outcome

The primary outcome is the efficacy of the intervention measured by the PHQ-9 (22). PHQ-9 is a nine-item reliable and valid self-report depression severity screening and diagnostic tool based on the DSM-IV criteria (23, 24). Response options are "not at all," "several days," "more than half the days," and "nearly every day," scored as 0, 1, 2, and 3, respectively (total score range from 0 to 27). In the current study, efficacy is defined as the participants score $\geq 2$ on less than two items on the PHQ-9 scale at post-treatment assessment. PHQ-9 has demonstrated high reliability and validity in the Chinese population, with Cronbach's alpha for the internal consistency reliability being 0.86, the correlation coefficient for the 2-week test–retest of the total score being 0.86, and positive correlation with the Self-Rating Depression Scale (SDS; $r = 0.29$, $p < 0.001$) and the 36-item Short Form Health Survey (SF-36; correlation coefficients ranged from $-0.11$ to $-0.47$, $p < 0.001$) (25).

**TABLE 2 |** Measures and visit time point of outcome assessment.

| Visit time point | Visit 1 (−7 to 0 days) | Visit 2 (day 0) | Visit 3 (14 ± 2 days) | Visit 4 (28 ± 2 days) | Visit 5 (42 ± 2 days) | Visit 6 (56 ± 7 days) | Visit 7 (84 ± 7 days) | Visit 8 (6 months ± 14 days) | Visit 9 (9 months ± 14 days) | Visit 10 (12 months ± 14 days) |
|---|---|---|---|---|---|---|---|---|---|---|
| Informed consent | × | | | | | | | | | |
| Patient general demographics | × | | | | | | | | | |
| Medical history | × | | | | | | | | | |
| Physical and nervous system examination | × | | | | | | | | | × |
| DSM-IV diagnosis | × | | | | | | | | | × |
| Inclusion and exclusion criteria | × | × | | | | | | | | |
| General information collection form | | × | × | × | × | × | × | × | × | × |
| PHQ-9 | × | × | × | × | × | × | × | × | × | × |
| GAD-7 | | × | × | × | × | × | × | × | × | × |
| ASRM | | × | × | × | × | × | × | × | × | × |
| LES | | × | × | × | × | × | × | × | × | × |
| CTQ | | × | | | | | | | | |
| IPPA | | × | | | | | | | | |
| EMBU | | × | | | | | | | | |
| CSQ | | × | | | | | | | | |
| Treatment status | | × | × | × | × | × | × | × | × | × |
| Adverse events | × | × | × | × | × | × | × | × | × | × |
| Adherence to intervention | | | × | × | × | × | | | | |
| Satisfaction and acceptability for the intervention | | | × | × | × | × | | | | |

*DSM-IV, Diagnostic and Statistical Manual of Mental Disorders (Fourth Edition); PHQ-9, Patient Health Questionnaire; GAD-7, Generalized Anxiety Disorder-7; ASRM, Altman Self-Rating Mania Scale; LES, Life Event Scale; CTQ, Childhood Trauma Questionnaire; IPPA, Inventory of Parent and Peer Attachment; CSQ, Coping Style Questionnaire.*

### Secondary Outcomes

#### *Onset of Mood Events*

The incidence of mood events of participants in each group (MoodBox group, online psychoeducation group, and naturalistic observation group) during the follow-up period will be assessed by (a) depressive episode: meet the diagnosis of a depressive episode as measured by PHQ-9 diagnostic algorithm or according to the DSM-IV diagnostic criteria; (b) hypomania or manic episode: the Altman Self-Rating Mania Scale (ASRM) scale total score $\geq$ 5 or meet the diagnosis of hypomania or manic episode based on DSM-IV diagnostic criteria.

ASRM is a five-item mania self-rating scale for assessing the severity of mania or hypomania (26). A cutoff score of 5 or higher indicates a high probability of a manic or hypomanic condition (based on a sensitivity rating of 85.5% and a specificity rating of 87.3%).

#### *Response Rate*

The response rate is defined as the proportion of participants who do not meet the criteria of subclinical depression of each study group during the follow-up period. The efficacy of the continuous intervention is defined as the participants showed a decrease of depression symptoms measured by PHQ-9 (less than two items of the scale score $\geq$ 2 and the total score $<$ 5) for all the post-treatment follow-up assessments after completing 8 weeks' intervention.

#### *Participant Adherence to the Intervention*

The adherence of participants to the assigned intervention will be measured by the usage of the intervention website. Specifically, we will measure the frequency of log-in, duration of website use, and completeness of homework.

#### *Participant Satisfaction and Acceptability for the Intervention*

The satisfaction and acceptability of the intervention will be assessed by questionnaires at the end of the intervention. In the questionnaire, the researchers asked about "how easy is it for you to understand the content?," "how easy is it for you to use the program?," "do you feel the program can help you manage your mental difficulties?," "do you want to use the program in the future?," and "will you recommend the program to other people?." The participants will be asked to answer each question in a scale of 1–5. Qualitative interviews will also be conducted with participants who are willing to participate.

#### *Contributing Factors of Subclinical Depression*

In order to identify the contributing factors of subclinical depression, psychosocial outcomes including anxiety, life events, attachment style, coping style, and parenting style will also be assessed, which will be measured by the Generalized Anxiety Scale (GAD)-7, the Life Event Scale (LES), Childhood Trauma Questionnaire (CTQ), the Inventory of Parent and Peer Attachment (IPPA), Egna Minnen Beträffande Uppfostran ("own memories of parental rearing practices in childhood," EMBU), and Coping Style Questionnaire (CSQ), relatively. The Generalized Anxiety Disorder-7 (GAD-7) is a seven-item self-report scale for assessing the severity of anxiety. Each item is scored from 0 to 3, with a total score of 21 (27). The Chinese version of the scale established good reliability (Cronbach's $\alpha = 0.90$) (28). Negative life events will be measured by LES, a 48-item self-report scale to measure positive and negative life events (29). The scale measures events regarding serious illness, housing, relationships and social difficulties, relationship breakdowns, unemployment, and financial crisis. Childhood traumatic experiences will be assessed by CTQ (30). The CTQ consists of 28 items, with each item scored from 1 to 5 with a total score of 125. Attachment will be measured using IPPA, a 25-item scale consisting of two core components: parent attachments and peer attachments (31). The EMBU is used to measure perceived parents' raring behaviors (32), with a questionnaire comprising 81 questions and 15 subscales covering raring behaviors such as overinvolvement, affection, overprotectiveness, guilt engendering, and rejection. Coping strategies will be assessed using CSQ (33). The CSQ consists of 20 items to assess different ways of coping, with each item having four response options: "never used," "occasionally used," "sometimes used," and "often used."

## Statistical Consideration

Data management and statistical analysis will be conducted using SAS9.4 (SAS Institute Inc., Cary, NC, USA). The sample characteristics would be compared using chi-square test for categorical variables or using Fisher's Z test when needed; ANOVA tests for normal distributed variables; and Mann–Whitney's U test for non-normal distributed variables. Analysis of efficacy will be based on an intent-to-treat principle, comprising all participants randomized regardless of the treatment group. The Kaplan–Meier survival analysis will be used to calculate the estimated time from baseline to response during 8 weeks' follow-up.

The Cox proportional hazard regression model using the Breslow method will be used to compare estimated time to response between groups, with control for covariates such as age, gender, years of education, and disease-related conditions; also we would use the exact method as sensitive analysis in case of tied survival data. Considering the long interval between visits after week 8, we will also use maximum likelihood estimation for Cox hazard regression model following Shen's work (34) to control left interval truncated data. Secondary analyses will be performed to assess changes from baseline to the endpoint of the study on scores of scales, using mixed-effects model for repeated-measures analysis (MMRM). A $p$-value $< 0.05$ is considered as statistically significant. The Bonferroni method will be used to control multiple comparisons of type I error inflation.

# DISCUSSION

Although recommendations have been made to manage subclinical depression (35, 36), most people with subclinical depression do not receive any treatment (14–16), even though the risk of subclinical depression turning into a full-blown depressive disorder is high. In recent years, in China, an increasing

number of mental health professionals recommend people with subclinical depression to receive psychological interventions. However, because the number of qualified psychotherapists is limited, there is a significant lack of accessibility for people with subclinical depression in China (16). Internet-based interventions are suitable for resource-limited settings with constrained mental health services and have the potential to be scaled up, which in turn will be a solution for scalable subclinical intervention.

MoodBox combined different evidence-based psychological interventions that are established as being effective to reduce depressive symptoms, including CBT, IPT, and mindfulness meditation. The theoretical background of existing internet-based psychological interventions is primarily based on CBT. By combining multiple evidence-based interventions, MoodBox offers a more comprehensive intervention and provides more treatment options. Due to the diverse symptom presentation of people with subclinical depression, a comprehensive intervention has the potential to cover a broader range of symptoms, which in turn may have a positive impact on treatment adherence, satisfaction, and acceptability. Second, we measured mood events to provide a comprehensive understanding of the reduction of subclinical depression on preventing mood disorders. Previous research mainly focused on the efficacy of preventing depression, without paying attention to the outcome of other mental health problems. Subclinical depression is not only an early sign of major depression but also an early sign of other mental health problems, such as bipolar disorder. There is a necessity to understand how the reduction of subclinical depression can contribute to the prevention of other mood events. Another strength of the study is the use of WeChat to send out notifications to participants for follow-up information, and the implementation of the electronic data capture (EDC) system for data management. WeChat is a messaging and social media app that has been wieldy used in China, especially for adolescents. There are over one billion active monthly users of WeChat. Therefore, sending follow-up notification using WeChat is an efficient method to manage follow-up visit remotely. Additionally, participants can complete scales on their phone using the link sent via WeChat, which will allow the data directly to enter the EDC database and mitigate any error that may be caused by entering data manually. This new follow-up method can improve the quality of the trial by enacting higher requirements for quality control. Additional, ethical issues and protecting subjects' privacy are also important during the follow-up by WeChat platform, as all the research staff will sign a confidentiality agreement and operate in strict compliance with the program provisions and ethical requirements.

There are some limitations of the study. First, all participants will be recruited from first-year university students in Beijing; thus, the sample might not be representative of the subclinical depression population in China. However, in light of the findings that the onset age of depression is during teenage years (37), adolescents and young adults are a high-risk population for subclinical depression. Moreover, university students from the three universities were recruited across the whole country; therefore, it is representative to a certain extent. Second, although the length of treatment and follow-up period (i.e., after the 8-week intervention, participants will be followed up for 1 year) is sufficient to demonstrate treatment effect, due to the relatively short follow-up period, the long-term effect of the intervention cannot be evaluated in this study.

To our knowledge, this is the first study to innovatively develop and test an intervention to improve psychological well-being and decrease the incidence of MDD in subclinical depression population. Once proven effective and acceptable, MoodBox could be potentially integrated into the routine clinical service to facilitate the management for people with subclinical depression.

## ETHICS STATEMENT

The study will be performed according to the ethical standards stated in the Declaration of Helsinki and its subsequent updates. All participants will be provided with an overview of the study's aims and characteristics summarized in the Informed Consent Form (ICF) and will be obtained written informed consent. The voluntary character of the study will be specified, indicating that withdrawal from the study is permitted at any time, without interfering with the usual treatment. The Ethics Committee Board of Beijing Anding Hospital approved the study protocol on 4 January 2020 (No. 202006FS-2).

## AUTHOR CONTRIBUTIONS

GW secured grant funding and supervised the study design. XC designed the study and drafted the manuscript. XZha and XZhu reviewed and edited the manuscript. All authors contributed to the article and approved the submitted version.

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# Depressive-Like Behaviors Induced by Chronic Social Defeat Stress Are Associated With HDAC7 Reduction in the Nucleus Accumbens

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Persistent symptoms of depression indicate the adaptive involvement of stable molecules in the brain that may be manifested at the level of chromatin remodeling, such as histone acetylation. Former studies have identified alterations in histone acetylation and deacetylation in several animal models about depression. However, the specific histone deacetylases related with depression are needed to be explored. Here, social avoidance behaviors, anxiety-, and depression-like behaviors were all found in mice suffered from chronic social defeat stress. Moreover, we also discovered that the amount of the class II histone deacetylase, HDAC7 rather than HDAC2, was significantly decreased in the nucleus accumbens of defeated mice, which suggested that HDAC7 might be a crucial histone deacetylase in a chronic social defeat stress model. Our data showed that the depressive-like behaviors induced by chronic social defeat stress were associated with HDAC7 reduction in nucleus accumbens. HDAC7 might be a promising therapeutic target for depression.

**Keywords: chronic social defeat stress, depression, HDAC7, nucleus accumbens (NAc), epigenetic**

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## SUMMARY BOX

**What is already known?** Persistent symptoms of depression may be manifested as changes in chromatin remodeling.

**What are the new findings?** Our data show that the depressive-like behaviors induced by chronic social defeat stress were associated with HDAC7 reduction in nucleus accumbens.

**What do the new findings imply?** HDAC7 may be a promising therapeutic target for depression.

## INTRODUCTION

According to a global report published by WHO in 2017, ∼322 million people worldwide suffer from depression, of which nearly half live in the South-East Asian and Western Pacific regions (1). Depression is believed to have a close correlation with genetic material involving aberrant changes in gene expression (2, 3). Accumulating evidence has indicated that histone acetylases and deacetylases dynamically regulate epigenetic mechanisms, such as histone acetylation (4, 5).

In a rodent model of depression caused by chronic social defeat stress, depressive symptoms are improved by the inhibitors of class II histone deacetylase, which will contribute to changes in gene expression related to depression similar to fluoxetine (6), thereby suggesting that class II HDACs may be a promising therapeutic target for depression.

In epigenetic mechanisms that may be associated with psychiatric illness, one of the most appealing features is changes of chromosomal structures and expressional alterations of specific genes under environmental stimuli (7, 8). Histone deacetylases (HDACs) are one type of chromatin-modifying enzymes used for the elaborate process of epigenetic regulation, therefore they could effectively link environmental stimuli with alteration of gene expression (9). HDACs are comprised of four classes, of which class I, class II, and class III are distinct based on their homology with yeast genes *rpd3*, *hda1*, and *sir2*, respectively; class IV includes only HDAC11 (10). HDAC inhibitor infusion into the nucleus accumbens (NAc), hippocampus, and cortex can elicit antidepressant-like responses, which supports the functional role and potential utility of such inhibitors in the treatment of depression (11–13). Moreover, treatment of mice with the inhibitor of class I and II HDAC (MS-275) reverses the effects of chronic social defeat stress on gene expression in the NAc, with a striking similarity to the function of the standard antidepressant, fluoxetine (14, 15). Despite the antidepressant effect of MS275 being confirmed, there is no direct evidence regarding which kind of HDACs as a form of essential mediators plays an important part in the anti-depressant process (16).

NAc has been demonstrated to be involved in the development of depressive disorders and the regulation of antidepressant action (17, 18). In particular, elevated levels of brain-derived neurotrophic factor (BDNF) in the NAc are associated with depressive behaviors, in which BDNF exerts an antidepressant role in the hippocampus (19). Moreover, when inhibitors of HDAC are directly infused into several brain regions including the medial prefrontal cortex, ventral hippocampus, NAc, or amygdala, they produce an antidepressant effect (20, 21).

HDAC7, an essential member of class II HDACs, is widely expressed in the central nervous system (22). Limited studies have been conducted on the function of HDAC7 in depression. Therefore, we investigated whether HDAC7 in the NAc is involved in the occurrence of depressive-like behaviors. Here, we specifically focused on the altered expression of HDAC7 in the NAc of mice who suffered from chronic social defeat stress.

# METHODS

## Animals

Male C57BL/6J mice aged 6–8 weeks weighing 23–25 g and male CD-1 retired mice aged 8–9 months were purchased from Charles River (Wilmington, MA). All mice were housed in standard cages at controlled temperature (22 ± 2°C) under diurnal conditions (12 h light/dark cycle). Food and water were available *ad libitum*. All handling procedures for animals were conducted following the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the institutional animal care and use committee of Shandong University.

## Chronic Social Defeat Stress

Chronic social defeat stress was induced as previously described (23) with modifications according to the experimental requirements. C57BL/6J mice were continuously subjected to social defeat stress for 10 days. CD1 retired breeders were used as aggressors in the experiment if they met the following criteria: Firstly, the CD1 resident aggressor must attack in at least two consecutive sessions during three consecutive screening sessions lasting for 180 s; Secondly, the attack latency recorded for each session must be <60 s. Each mouse was introduced into the homecage of a stranger CD1 resident aggressor every day and was defeated physically for 10 min. After 10 min of physical interaction, the residents and intruders were separated by perforated plexiglass to allow sensory contact in the following duration of 24 h. Every day, each mouse was introduced into a new homecage of a resident CD1 aggressor. The animals in the control group were housed in pairs on each side of a perforated plexiglass partition and were handled in the same manner as those in the experimental groups every day.

## Behavioral Procedures

Fourteen mice were subjected to social approach-avoidance test, open field test, elevated plus maze (EPM) test, tail suspension test, and forced swimming test according to the following procedures. After 5 days of the behavioral tests, mice were killed after the behavioral tests, and brain tissues were collected for western blot assay.

### Open Field Test

An open field test was conducted as a previously described report from our laboratory with modifications (24). The spontaneous motivation ability and anxiety-like behavior were assessed in the open field test. The area of tan open field test consisted of a 40 cm × 40 cm area divided into central (20 cm × 20 cm) and peripheral regions by black metal walls with height at 35 cm. Mice could acclimatize themselves to the test room for 1 h before the experiment. During the test, mice were placed at the center region of the field, and their behaviors were recorded for 10 min. A video-tracking system (Smart 3.0, Harvard apparatus, Holliston, MA, USA) was used to score the traveled distance between the central and peripheral areas for the assessment of spontaneous locomotor activity. The number of entries to the central zone, latency to leave the central zone, and time stayed in the central zone were all recorded to evaluate anxiety-like behaviors.

### EPM Test

As previously described (25) with modifications, the EPM was comprised of two open arms (30 cm × 5 cm) with a small raised lip (0.5 cm), two enclosed arms (30 cm × 5 cm), and a central platform (5 cm × 5 cm) at 38.5 cm above the ground. During a 5-min session, each mouse was initially placed on the center platform and facing an open arm. The time stayed in open arms and the number of entries into the open arms was scored using a video-tracking system (Smart 3.0).

### Social Approach-Avoidance Test

The approach-avoidance behaviors of experimental mice toward an unfamiliar social target (CD1 mouse) were carried out as previously described (26) with modifications. The arena was a black metal open field (40 cm × 40 cm) placed in complete darkness. A camera equipped with an infrared filter and lights was used to perform the video recording. Each experimental mouse was introduced into the open field and its trajectory was continuously recorded for two 2.5-min sessions with a 30-s duration for the mouse to rest in its home cage. In the first session ("no target"), an empty mesh cage made of plastic (10 cm × 6.5 cm) without a target was located at one end of the open field. During the second session ("target"), an unfamiliar CD1 male mouse was introduced into the mesh cage as a social target animal with other same conditions. Between the two sessions, the experimental mouse was removed from the arena and was placed back into its home cage for ∼1 min. The video-tracking data from both the "no target" and "target" conditions were used to determine the total distance moved. The time spent by the experimental mouse in the "interaction zone" (a corridor with a width of 8 cm surrounding the mesh cage) and the "corners" (two square areas opposite to the location of the target cage) was recorded using a video-tracking system (Smart 3.0).

### Forced Swimming Test

A forced swimming test was performed as previously described in a published study from our laboratory (27). Briefly, C57BL/6J mice were placed into a glass cylinder (25 cm in height, 10 cm in diameter) filled with 22°C water up to a height of 18 cm. The percentage of time spent in an immobile state was determined in a testing period of 6 min. Immobility was defined as the absence of struggling, immobile posture, and floating on the water. Swimming was defined as intensive use of the forepaws for moving forward to the center or along the sides of the cylinder. The body was usually oriented parallel to the sides of the cylinder. Climbing was defined as active pawing of the cylinder wall and lifting of the paws above the water surface. The body was oriented with the head toward the wall, which faced perpendicularly to the side of the cylinder.

### Tail Suspension Test

The tail-suspension test is a mouse behavioral test useful in assessing antidepressant-like activity (28). As previously described (29) with modifications, mice were suspended upside down, and a metal bar was used to tape their tails at the position of 1 cm from the tip. Their heads were held 35 cm above the ground, and a camera was used to record immobility inthe active period of 6 min. Immobility was defined as no movement of the limbs and tails. Mice that climbed up their tails were excluded from the experiment.

## Brain Tissue Collection

After 5 days of behavioral experiments, the brain, including NAc, cerebral cortex, and dorsal hippocampus were dissected, frozen, and sliced using the freezing microtome (Braintree Scientific, Braintree, MA). Brain slices were stored at −80°C until use.

## Western Blot Assay

The extraction of tissue protein from NAc was performed as previously described (21, 22). A bullet Blender Homogenizer (Nextadvance) was used to homogenize dissected brain tissue in ice-cold RIPA buffer (50 mM Tris, 150 mM NaCl, 0.1% SDS, 0.5% sodium deoxycholate, 1% IGEPAL CA-630, pH 7.4) supplemented with protease inhibitors (Complete, Roche). Extracts were centrifuged at 14,000 g for 20 min at 4°C, and then cellular proteins were collected. The concentration of total cellular proteins was determined by a standard BCA assay before added in a loading buffer at 100°C for 5 min. Then, 80 μg of lysate proteins was separated by SDS-PAGE gels and then transferred to a polyvinylidene fluoride (PVDF) membrane (Roche, Swiss). Anti-HDAC1 (Sigma, 1:1,000), anti-HDAC2 (Sigma, 1:1,000), anti-HDAC6 (Sigma, 1:1,000), anti-HDAC7 (Sigma, 1:1,000), and anti-β actin from rabbit were used as primary antibodies. Horseradish peroxidase (HRP)-conjugated goat anti-rabbit secondary antibodies (Calbiochem, 1:1,000) were used to label the rabbit-derived primary antibodies. The images of protein bands were visualized by enhanced chemiluminescence (ECL, Pierce). Densitometry analysis of the bands was performed using Quantity One (version 4.6.2, Bio-Rad).

## RNA Extraction and Quantitative PCR (qPCR)

Total RNA was extracted from NAc, cerebral cortex, and dorsal hippocampus using TRIzol-A$^+$ RNA isolation reagent (TIANGEN) according to the manufacturer's protocol. cDNA was then synthesized using the ReverTra Ace qPCR RT Kit (catalog #FSQ-101; TOYOBO). Afterward, real-time qPCR was performed using an SYBR Green PCR kit (Applied Biosystem, USA). The primers were as follows: HDAC7, 5′-GCCTCCATC GACCACTTAACC-3′ (forward) and 5′-CGAGGGTATCTGTC GCAGTC-3′ (reverse); and β-actin, 5′-CGTTGACATCCGTAA AGACCTC-3′ (forward) and 5′-CCACCGATCCACACAGAG TAC-3′ (reverse). β-actin was used as an internal control. The relative expression of HDAC7 mRNA was calculated using the $2^{-\Delta\Delta CT}$ method as previously described (30).

## Statistical Analysis

All data were collected from at least three independent experiments and presented as mean ± S.E.M. Comparisons of data from various behavioral tests and western blot assay between defeated and control groups were evaluated via two independent samples *t*-test. Data among multiple groups were analyzed using one-way analysis of variance (ANOVA) with the least significant difference test for pairwise comparison. A *P*-value of 0.05 was considered to be the critical cutoff value of statistical significance. All data analyses were carried out using the SPSS statistical program version 18.0 (SPSS Inc., Chicago, IL, USA).

# RESULTS

## Chronic Social Defeat Stress Successfully Induced Social Avoidance Behaviors

The social approach of experimental mice toward an unfamiliar social target (CD1 mouse) enclosed in a plastic mesh cage was

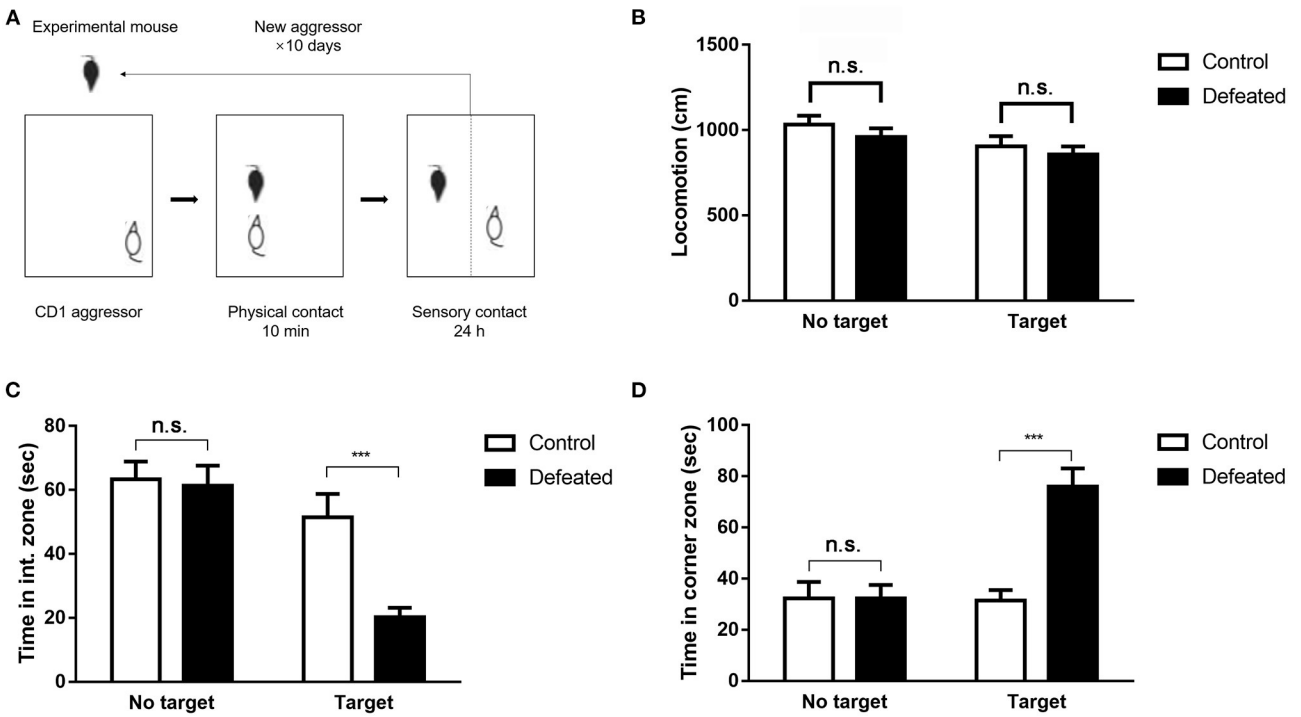


**FIGURE 1 |** Chronic social defeat stress-induced persistent social aversion in mice. **(A)** The paradigm of chronic social defeat stress. **(B–D)** A social target decreased the time spent in the interaction zone compared with that in the control mice, and increased the time spent in the corner zone after social defeat. Chronic social defeat stress did not affect total locomotion of the experimental mice. Data are expressed as means ± S.E.M ($n = 14$ per group). Data among multiple groups were analyzed using one-way analysis of variance (ANOVA) with the least significant difference test for pairwise comparison. $^{***}P < 0.001$ compared to control.

monitored by a video-tracking system (**Figure 1A**). As expected, undefeated control mice spent the most time in the interaction zone and little time in the corner zone when an unfamiliar target mouse was presented. However, defeated mice intensely displayed aversive responses to the target mouse, which spent less time in the interaction zone ($F = 12.086$, degree $= 55$, $P < 0.001$, **Figure 1C**) and preferred to staying in the corner zone ($F = 14.017$, degree $= 55$, $P < 0.001$, **Figure 1D**). This difference was observed exclusively in the presence of a social target and was not significant in an empty wire cage. No difference was observed in total movement throughout the arena ($F = 2.124$, degree $= 53$, $P = 0.109$, **Figure 1B**).

## Anxiety-Like Behavior Test

The open-field test was performed to assess whether defeated mice displayed altered anxiety-like behaviors. Compared with that in the control group, defeated mice spent less time in the center zone of the open field ($t = 2.445$, df $= 25$, $P = 0.022$, **Figure 2A**). Furthermore, defeated mice traveled within shorter distances in the central zone and even was found to scarcely enter into the center zone ($t = 5.425$, df $= 25$, $P < 0.001$, **Figure 2B**). To further confirm the anxiety-like behaviors inflicted by chronic social defeat stress, an EPM test was also conducted. The phenomenon suggested that, relative to the control group, defeated mice significantly traveled within shorter distances ($t = 2.444$, df $= 24$, $P = 0.022$, **Figure 2C**) and less entries into the open arms ($t = 2.239$, df $= 26$, $P = 0.034$, **Figure 2D**).

## Depressive-Like Behavior Test

To evaluate the depressive-like behavioral changes in mice suffered from chronic social defeat stress, forced swimming, and tail suspension tests were conducted in sequence. As expected, mice defeated by aggressors displayed increased immobility time during the forced swimming test ($t = -2.534$, df $= 26$, $P = 0.018$, **Figure 3A**). To further confirm our results, we conducted a tail suspension test, where the immobility time of mice subjected to chronic social defeat stress was also increased during tail suspension ($t = -2.979$, df $= 26$, $P = 0.006$, **Figure 3B**).

## Chronic Social Defeat Stress Represses HDAC7 Expression in the NAc

To analyze whether HDACs contributed to depression caused by chronic social defeat stress, western blot assay was adopted. The results showed that HDAC7 protein expression was significantly decreased in the NAc in the brain of mice subjected to chronic social failure stress compared to that in control mice ($t = 2.614$, df $= 14$, $P = 0.020$, **Figure 4A**). However, other HDACs such as HDAC1 ($t = 0.057$, df $= 20$, $P = 0.955$, **Figure 4B**), HDAC2 ($t = -1.595$, df $= 7$, $P = 0.155$, **Figure 4C**), and HDAC6 ($t = -1.781$, df $= 7$, $P = 0.118$, **Figure 4D**) were not changes in the NAc. Strikingly, it was observed that HDAC7 mRNA was

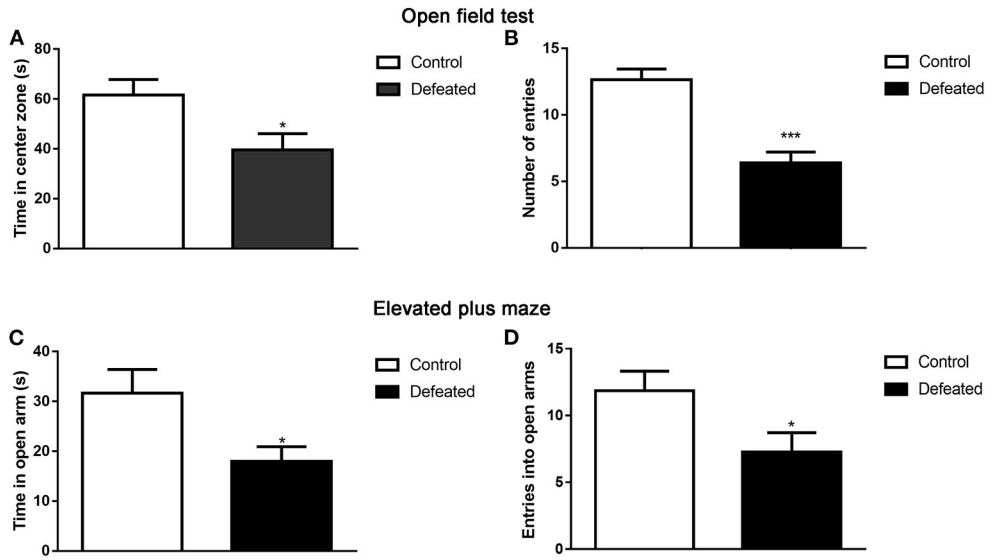


**FIGURE 2 |** Effect of chronic social failure on anxiety-like behavior. **(A)** Compared with control mice, mice subjected to chronic social failure stress spent less time in the middle area of the open field. **(B)** Mice subjected to chronic social failure stress entered the central region less often. **(C)** Compared with control mice, mice with chronic social failure stress spent less time on the elevated cross arm. **(D)** Mice subjected to chronic social failure stress spent significantly fewer times on the open arm. Data are expressed as means ± S.E.M. ($n = 14$ per group). Data comparisons between defeated and control groups were evaluated via two independent samples $t$-test. $^{*}P < 0.05$, and $^{***}P < 0.001$ compared to control.

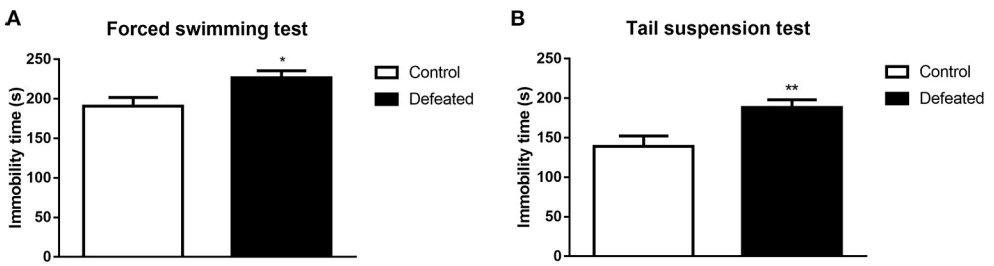


**FIGURE 3 |** Effects of chronic social failure on depression-like behavior. **(A)** Mice subjected to chronic social failure stress were significantly more sedentary during forced swimming than control mice. **(B)** Compared with control mice, mice subjected to chronic social failure stress spent significantly more time resting in the tail suspension test. Data are expressed as means ± S.E.M ($n = 14$ per group). Data comparisons between defeated and control groups were evaluated via two independent samples $t$-test. $^{*}P < 0.05$, and $^{**}P < 0.01$ compared to control.

also significantly decreased in the NAc ($t = -22.588$, df $= 4$, $P < 0.001$, **Figure 5A**) rather than in the cerebral cortex ($t = -1.229$, df $= 4$, $P = 0.286$, **Figure 5B**) and dorsal hippocampus ($t = 0.007$, df $= 4$, $P = 0.995$, **Figure 5C**) under chronic social defeat stress, indicating a brain region-specific role of HDAC7 in behavior changes inflicted by chronic social defeat stress. These data suggested that HDAC7 reduction in the NAc might be associated with behavior changes inflicted by chronic social defeat stress.

## DISCUSSION

Recently, accumulated evidence have indicated that alteration of histone acetylation caused by abnormal expression of HDACs in the NAc plays an important role in depression, and overexpression of specific HDACs in the NAc can exert therapeutic effects in depressive mice (18). Here, we implemented a chronic social defeat stress mouse model for depression, in which mice were continuously defeated for 10 min by a new CD-1 aggressor every day for 10 days. Social approach-avoidance test, open field test, EPM, and forced swimming test were used to evaluate the changes in behaviors induced by the depression model. The levels of social avoidance, depression, and anxiety in the defeated mice were significantly higher than those in normal mice. Studies based on models about chronic social defeat stress obtained comparable results in social avoidance behavior, anxiety-like behavior, and depressive-like behaviors, thus corroborating our findings (23). These results suggested that mice were suitable for exploring depression- and anti-depressive response-related cellular and molecular mechanisms.

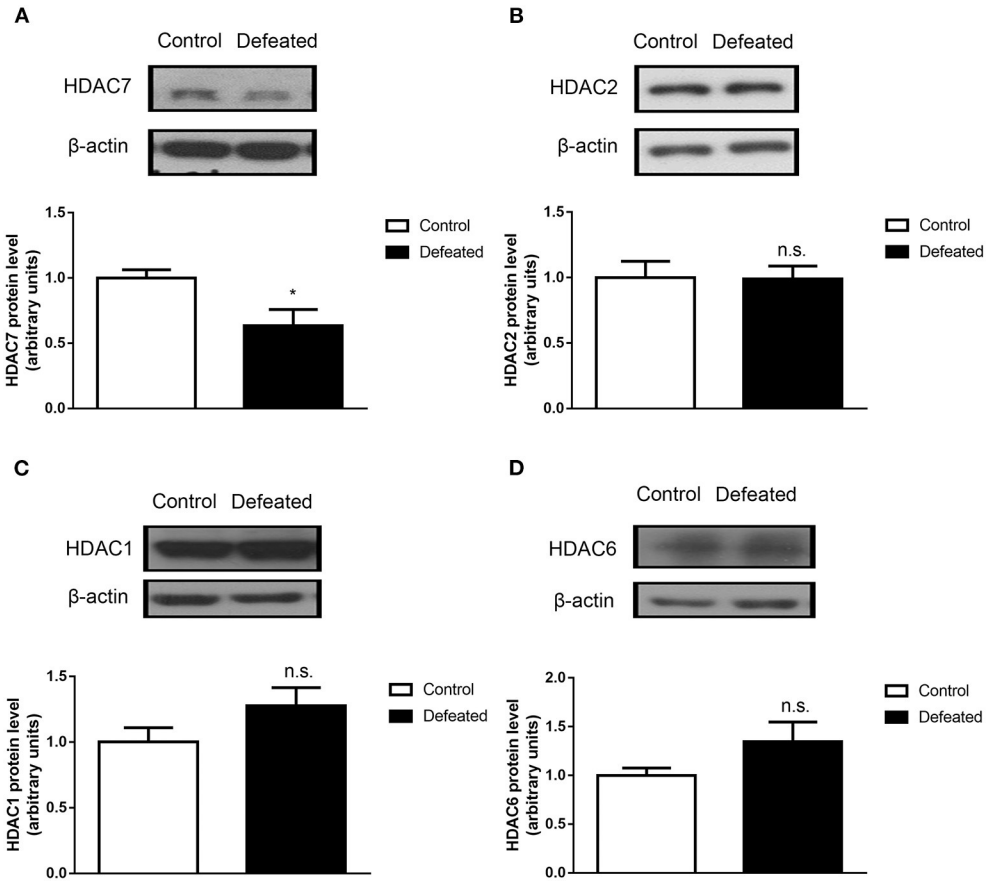


**FIGURE 4 |** Chronic social stress failure significantly reduced HDAC7 protein expression in the nucleus accumbens. **(A)** Compared with control mice, HDAC7 protein expression in the nucleus accumbens region in the brain of mice subjected to chronic social failure stress was significantly decreased. **(B–D)** There was no significant change in the expression of HDAC1, HDAC2, and HDAC6 in the nucleus accumbens region in the brains of mice subjected to chronic social failure stress compared to those in the control group. Data are expressed as means ± S.E.M. ($n \geq 4$ per group). Data comparisons between defeated and control groups were evaluated via two independent samples *t*-test. $^*P < 0.05$ compared to control.

The NAc is implicated in depression, and abnormal expression of HDACs such as HDAC5 and HDAC2 has been observed in the NAc (31, 32). However, as an essential member of class II HDACs, the function of HDAC7 in depression is unclear. Our previous work indicated that HDAC7 was ubiquitously expressed in the brain including the PFC, hippocampus, and amygdala (22). The results presented here showed that the level of HDAC7 was remarkably decreased in the NAc under chronic social defeat stress.

The strengths of our study were that a well-validated mouse model of depression and several complementary behavioral assays were used to confirm that social defeat stress generated a pattern of depressive-like and anxiety-like behaviors in mice. Moreover, we found that chronic social defeat stress in mice resulted in the reduction in HDAC7 levels in the NAc, which provided a new insight for the potential mechanism involved in depression. Despite the clear strength of our study, some limitations merit further consideration. Firstly, we only observed that chronic social defeat stress represses HDAC7 expression in the NAc, whether HDAC inhibition resulted in antidepressant-like activity and some mood-stabilizing drugs (such as valproate) had HDAC inhibition properties were largely unknown. Secondly, the genes regulated by HDAC7 during chronic social defeat stress were not investigated. Exploration of manipulating HDAC7 on patterns of gene expression will facilitate to provide new insight into the possible mechanisms underlying depression. Thirdly, it is revealed that detecting the role of manipulating HDAC5 in the behavioral adaptations to chronic emotional stimuli provides strong evidence to support the effects of HDAC5 in the pathogenesis of drug addiction, depression, and other stress-related syndromes (33). Future studies elucidating the role of knockdown or overexpression of HDAC7 in NAc in regulating the behavioral adaptations to chronic social defeat stress are required. Lastly, investigation of the molecular basis of susceptibility and resistance to social defeat stress in brain reward regions helps to better maintain emotional homeostasis (34). Therefore, it is worth investigating the role of susceptibility and resilience to 10 days of social defeat stress. Observation of any degree of variation in social interaction scores that may be correlated with HDAC7 expression in NAc will confirm the function of HDAC7 in the regulation of depression.

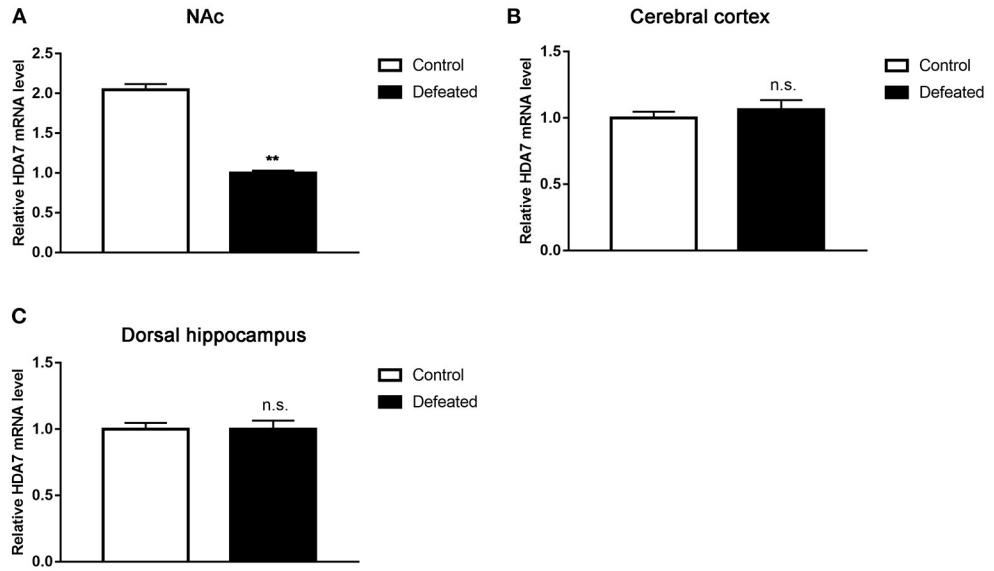


**FIGURE 5 |** Chronic social stress failure significantly reduced HDAC7 mRNA expression in the nucleus accumbens **(A)** rather than in the cerebral cortex **(B)** and dorsal hippocampus **(C)**. Data are expressed as means ± S.E.M. ($n \geq 4$ per group). Data comparisons between defeated and control groups were evaluated via two independent samples *t*-test. $^{**}P < 0.01$ compared to control.

Notably, only male mice were used in this study. The reason is to avoid the interference of the female estrus cycle. The monthly changes in estrogen and progesterone are significant in females, and these changes can affect the response state of the tested animals to certain experimental factors. Since female humans have higher rates of depression than male humans, it is unclear whether studies using only male mice have translational promise at all for understanding depression. More studies are still required to confirm our findings.

## CONCLUSION

In summary, our study demonstrated that HDAC7 was a critical histone deacetylase, whose reduction might be associated with the depressive-like behaviors induced by chronic social defeat stress. Therefore, HDAC7 may be a promising therapeutic target for curing depression. Our findings will provide a new insight for elucidating the mechanism underlying depression.

## 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 animal study was reviewed and approved by all handling procedures for animals were conducted following the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and were approved by the institutional animal care and use committee of Shandong University.

## AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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# Development and Evaluation of a Therapist Training Program for Psilocybin Therapy for Treatment-Resistant Depression in Clinical Research

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**Introduction:** Psychological support throughout psilocybin therapy is mandated by regulators as an essential part of ensuring participants' physical and psychological safety. There is an increased need for specially trained therapists who can provide high-quality care to participants in clinical studies. This paper describes the development and practical implementation of a therapist training program of psychological support within a current phase IIb international, multicenter, randomized controlled study of psilocybin therapy for people experiencing treatment-resistant depression.

**Description of Training Program:** This new and manualized approach, based on current evidence-based psychotherapeutic approaches, was developed in partnership with different mental health researchers, practitioners, and experts; and has been approved by the FDA. Training consists of four components: an online learning platform; in-person training; applied clinical training; and ongoing individual mentoring and participation in webinars.This paper provides a brief overview of the method of support, the rationale and methodology of the training program, and describes each stage of training. The design and implementation of fidelity procedures are also outlined.

**Lessons Learned:** As part of the phase IIb study of psilocybin therapy for treatment-resistant depression, 65 health care professionals have been fully trained as therapists and assisting therapists, across the US, Canada and Europe. Therapists provided informal feedback on the training program. Feedback indicates that the didactic and experiential interactive learning, delivered through a combination of online and in-person teaching, helped therapists build conceptual understanding and skill development in the therapeutic approach. Clinical training and engagement in participant care, under the guidance of experienced therapists, were considered the most beneficial and challenging aspects of the training.

**Conclusions:** Clinical training for therapists is essential for ensuring consistently high-quality psilocybin therapy. Development of a rigorous, effective and scalable training methodology has been possible through a process of early, active and ongoing collaborations between mental health experts. To maximize impact and meet phase III and post-approval need, enhanced online learning and establishing pathways for clinical training are identified as critical points for quality assurance. This will require close public, academic and industry collaboration.

**Keywords: psilocybin, therapist training, psychological support, treatment resistant, depression, psychedelics, psychdelic therapy, treatment-resistant depression**

## INTRODUCTION

Increasing interest in the therapeutic potential of psychedelic compounds for a range of mental health conditions highlights the need for training therapists in supporting participants in clinical studies. This paper describes the rationale, methodology and key learnings from a therapist training program for a phase IIb international, multicenter, randomized controlled study of psilocybin therapy in participants with treatment-resistant depression (TRD); clinical trial number NCT03775200.

Psilocybin is a molecule present in over 200 species of psychoactive mushrooms. It has a dose-dependent capacity to facilitate the experience of non-ordinary states of consciousness, and together with compounds like lysergic acid diethylamide (LSD), mescaline, and N,N-Dimethyltryptamine (DMT), is part of a group of drugs called psychedelics. Psilocybin is a partial-agonist of the 5HT group of receptors, including the $5HT_{2A}$ receptor subtype. The activation of $5HT_{2AR}$ results in subjective alterations of perception, mood and cognition during the acute effects of psilocybin. This has potential benefits for mood disorders, as perception and cognition appear to become more flexible, enabling opportunities for new perspectives and insights to be generated, potentially leading to new and novel solutions for ongoing psychological distress (1). Psilocybin effects generally peak around 90 min after ingestion, then gradually subside and resolve in 4–6 h.

Psilocybin and other psychedelic compounds have attracted attention from researchers and clinicians for their potential to catalyze therapeutic change, when taken within a therapeutic setting, in people diagnosed with depression (1), obsessive-compulsive disorder (2), alcohol dependence (3), nicotine dependence (4), and anxiety associated with cancer (5–7). The effects of psychedelics in general, and specifically psilocybin, for other problems are currently being investigated in several pilot studies. Early research with psilocybin has shown signals of immediate, significant and often enduring clinical improvements in depression and anxiety. Such effects are thought to result from a combination of the psychopharmacological effects of psilocybin and the participants' subjective experiences, including generation of insights and subsequent changes in cognition and behavior (8, 9).

Administration of psilocybin in a research setting requires approval from regulators, such as the FDA and United States Drug Enforcement Administration, in addition to approval from an Institutional Review Board or Research Ethics Committee. Just as regulators require consistent data about the synthesis, purity and stability of a specific formulation of psilocybin used in the clinical development program, they similarly require the shared consistency of another equally important component of the treatment approach - the psychological support. To address the need for participant safety and consistency in the delivery of the psychological support, we have created and implemented a therapist training program for the phase IIb clinical trial of psilocybin therapy for TRD.

The study aims to determine the optimal single dose of psilocybin, administered with psychological support, in a clinical setting. The 216 participants are randomized to low (1 mg), medium (10 mg) or high (25 mg) doses of COMP360, a high-purity polymorphic crystalline formulation of psilocybin produced under Good Manufacturing Practice, administered in conjunction with psychological support by specially trained therapists. The study design has been agreed with the European Medicines Agency, FDA, and approved by regulators in 10 countries in North America and Europe. Therapists' professional qualifications, as well as their training format and content, were approved by the FDA and included in the Investigational New Drug program. In 2018, the FDA granted a Breakthrough Therapy designation to COMP360 for TRD. The program of research is managed by COMPASS Pathways PLC., a mental health care company dedicated to accelerating patient access to evidence-based innovation in mental health.

## DESCRIPTION OF TRAINING PROGRAM

### Therapeutic Model

The principles of psychological support in the current trial, to be outlined in a separate publication, are informed by several factors:

- An empirical theoretical framework based on Perceptual Control Theory (10) that integrates validated evidence-based psychotherapeutic theories and approaches common to therapies such as Cognitive Behavioral Therapy (11),

**Abbreviations:** DMT, N,N-Dimethyltryptamine; FDA, United States Food and Drug Administration; GCP, Good Clinical Practice; LSD, Lysergic acid diethylamide; MDMA, 3,4 methylenedioxy-methamphetamine; TRD, Treatment-resistant depression.

Mindfulness-based Therapy (12), Method of Levels (13), Acceptance and Commitment Therapy (14), and Focusing (15);
- Extensive historic experience in the use of psilocybin in research and clinical settings (7, 16, 17);
- Current understanding of the psychopharmacology and mechanism of action of psilocybin (18–21);
- Regulatory and payor perspectives on the clinical development of psilocybin therapy.

Perceptual Control Theory (PCT) (10) informed the current approach as it offers a biopsychosocial framework for understanding and integrating key principles evident across the general literature on psychological functioning and the psilocybin literature. PCT proposes that human health is dependent on effective homeostatic control of important biological, social, and psychological variables, through feedback. This has particular relevance for TRD, where people's difficulties in controlling variables such as attention and negative thinking act as maintaining factors (11). From a PCT perspective, when we develop awareness of the internal variables we are controlling, we can develop solutions to balancing incompatible variables. As an example from psilocybin therapy, a client experiencing depression described holding two conflicting standards; wanting meaningful intimate relationships whilst also wanting to avoid vulnerability. As psilocybin therapy potentiated changes in usual patterns of perception, mood and cognition, it brought new opportunities for reorganizing and balancing such psychological variables (22). Therapists' training incorporates a basic overview of this theoretical framework to inform practice; describing how participants might develop awareness of personal goals through increased attentional mobility, introspection, and problem solving. This informs therapists' patient-centered and non-directive practice, utilizing a questioning style which supports participants in generating their own potential solutions for resolving incompatible goals, rather than teaching, offering advice, or making assumptions. These principles remain consistent with core therapist skills identified in other psychedelic interventions (23), meaning that there is considerable similarity between the current approach and support in other psilocybin therapies. These principles also informed item development in a fidelity scale, as described below in the section "Ensuring Consistency."

The primary purpose of psychological support throughout is to ensure participants' physical and psychological safety and minimize the number of any potential adverse events. The most common adverse effect in psilocybin sessions is mild-to-moderate transient anxiety that could potentially lead to more intense or longer-lasting anxiety and paranoia. Extended periods of overwhelming emotional arousal are not only distressing to participants, but could decrease positive treatment outcomes (24). Skillful psychological support helps participants engage with all aspects of their experiences, while keeping more challenging emotions within the participant's bandwidth of tolerance, whereby emotions are not so prolonged and overwhelming that they are unproductive, thus facilitating a sense of safety. To do this, two key principles - *self-directed enquiry* and *experiential processing* - are introduced to and practiced with participants during preparation.

### Self-Directed Enquiry

A key goal for therapists is to help participants direct attention toward internal experiences as they emerge in the present moment, which may involve noticing foreground and background thoughts, emotions, physical sensations, images and memories. The aim is to bring experiences into awareness and potentially consider them from different perspectives, a key process within most psychological interventions (25), to increase psychological flexibility, introspection and problem solving (11–15, 26). This is described as "self-directed enquiry." To facilitate this process, therapists use sensitive but searching questions to help participants mobilize their attention to experiences as they arise. Therapists demonstrate and practice self-directed enquiry with participants at all stages of the therapeutic process. If practiced sufficiently during preparation, self-directed enquiry could be particularly helpful should challenging experiences, such as anxiety-inducing material, emerge during a psilocybin session.

### Experiential Processing

Experiential processing refers to the sustaining of attention on an experience in the present moment so it can be processed in a meaningful way. Awareness of and re-evaluation of important biological, social, and psychological variables can potentiate new insights and facilitate change (10, 13). The importance of experiential "exposure" is well documented throughout the literature (26) Experiential processing thus requires a willingness to stay with thoughts, feelings, sensations or emotions until they have passed or evolved. This is expressed as moving "in and through" (27).

## Overview of the Therapeutic Process

Psilocybin therapy has three phases: *preparation*, *psilocybin session*, and *integration*. Safety is a foundation for all phases. The therapeutic focus is different at each phase.

Preparation focuses on establishing the therapeutic alliance between the therapist and participant, and demonstrating and practicing self-directed enquiry and experiential processing. Therapeutic alliance develops when the therapist is genuinely curious and cultivates trust through focused presence; considered one of the core elements in ensuring a safe and meaningful psilocybin session.

During preparation, the therapist aims to build the participant's capacity to navigate distressing experiences through self-directed enquiry and experiential processing, using topics chosen by the participant. Practicing this approach during preparation helps the participant engage with their experiences in the psilocybin session, including physical and psychological challenges.

During the psilocybin session, the focus is to ensure psychological distress is not overwhelming, in order to facilitate a meaningful experience. The participant is encouraged to maintain a courageous, trusting and open attitude in order to move "in and through" the full range of internal experiences.

Therapists are encouraged to maintain awareness of their own experiences during the session, including boredom, anxiety and fear, while modeling the same attitudes of openness and interest toward participants.

The therapeutic focus of integration is to reflect on the experience and generate insights following the psilocybin session. Participants are encouraged to describe and connect with the range of emotional, cognitive and physical experiences of the psilocybin session and relate them to their personal narrative. The integration process will generally unfold over time as the participant begins to implement changes in their life based on the experiences and insights of their psilocybin session.

## Therapist Qualifications, Background, Experience, and Selection Criteria

All therapists in the clinical development program of psilocybin therapy for TRD are required to be mental health care practitioners with a professional license in good standing. They must have demonstrated clinical experience in areas required for psychotherapy or mental health counseling. The FDA currently requires all United States therapists to have at least a Masters' level of education and that two trained therapists are present for all psilocybin sessions. The lead therapist on each treating team must be fully trained and the co-therapist is able to gain their necessary clinical training alongside the lead therapist during the study. Although it is not mandatory for one of the therapist dyad to be a psychiatrist, a psychiatrist must, however, be present on premises near to the psilocybin session to respond to emergencies. Psychiatrists in this role must complete specific training, separate from the therapists' training.

Therapists for the phase IIb study were selected by study sites according to country, state, and institution-specific requirements, and interviewed by members of the COMPASS training team. Emphasis was placed on exploring therapists' motivations for getting involved in the study; their essential qualities of presence, openness and patience; and their familiarity with principles of Good Clinical Practice (GCP)-compliant research. We sought therapists with clinical experience and a demonstrated ability to care for people experiencing severe psychological distress, as these skills were likely to translate well into maintaining calm and reassuring presence while caring for participants during a psilocybin session.

At the time of writing, more than 65 therapists and assisting therapists had been fully trained to work across several studies within the COMPASS clinical development program of psilocybin therapy for TRD in North America and Europe. The therapists currently working on the phase IIb study includes predominantly psychologists, as well as psychiatrists, masters level practitioners, nurses, diploma level CBT therapists and PhD mental health specialists. Years of experience since qualification averaged 25.9 years (SD 8.8) with a range of 2–32 years. Therapists reported clinical experience in a number of areas (some more than one), including adult mental health; addictions; dementia; physical health; child/developmental; family therapy, and eating disorders.

## Training Program Structure

Development and piloting of the training was overseen by a team with expertise and experience in therapeutic and research applications of psilocybin, research and training methodology in psychological treatments, and extensive clinical experience of people with TRD. The goal was to engage advisors and trainers with sufficient clinical experience in conducting psilocybin research sessions, which among our trainers, ranged between 25 and 300+ psychedelic research sessions. Having an "expert through experience" on the team, who had previously participated in a clinical study of psilocybin for TRD, firmly oriented the training processes around participant experience and needs. The expert group approach was applied to every stage of the training process, from therapist selection to mentoring and skill maintenance during the active stage of clinical trials.

The therapist training program for the phase IIb study entails four components: (1) an online learning platform, (2) in-person training, (3) clinical training, and (4) ongoing individual mentoring and webinars. All therapists are required to complete steps 1–3 of the training before they can lead sessions independently, and to engage in step 4 to continue their professional development.

### Step 1. Self-Paced Online Learning

An interactive online platform was developed, consisting of over 20 hours of video modules and training materials covering a general overview of psilocybin research and information about the therapeutic approach. Information contains essential, but not exhaustive, resources on the neuroscience and psychopharmacology of psilocybin; medical, psychological and ethical considerations of psilocybin research; and privacy, confidentiality and GCP. Training modules on the therapeutic approach focus on practicalities of each stage of the therapeutic process, including video re-enactments of clinical scenarios, a therapy manual with study-specific appendices, and **Supplementary Materials** such as slides, academic papers, adherence rating scales, and other resources.

As all subsequent training steps are interactive, requiring a thorough knowledge of the principles and methods, therapists cannot progress before completing the online training. The platform continues to play an important role in professional development, even after therapists begin leading psilocybin sessions independently. The platform was named "The Shared Knowledge Platform" to emphasize the importance of continuous learning and incorporating clinically-relevant insights from study therapists in a timely manner. Although the core principles of the therapeutic approach remain the same, the knowledge and feedback from study therapists are integral to the optimization of therapeutic care delivery and participant safety.

### Step 2. Interactive 5-Day In-person Training

The purpose of interactive training is for therapists to translate their learning from the online training modules into practice and demonstrate their understanding of core principles and methods of psychological support throughout all stages of psilocybin therapy. Training is conducted in groups of 10–15 people, led by experienced therapists and trainers. Most of the time is spent

in role-plays of short clinical scenarios, anonymized and adapted from previous or current studies. Role-plays based on examples from therapists' own lives, provide first-hand experience of self-directed enquiry and experiential processing. During in-person training, emphasis is placed on providing feedback from peers and trainers. Peer interactions are particularly important as they engage the entire group and enable the trainer to assess therapists' understanding of the approach, and their interpersonal and communication skills as well.

On rare occasions, trainers might have concerns about a therapist's conduct or there is evidence of poor understanding of the core principles of psychological support which could prevent a therapist from being effective. If the conduct or understanding cannot be addressed through feedback or additional training, it is recommended that the study site engage another therapist. In-person training provides opportunities to assess the skills and areas of focus for additional training, and also build a community of competent, motivated therapists who support each other in building a body of mutual knowledge.

### Step 3. Clinical Training

As agreed with the FDA, all therapists must gain clinical experience in at least four different psilocybin research sessions before leading psilocybin sessions independently. Most therapists in phase IIb gained their clinical experience by supporting participants in our phase I study: COMP002 – The Effects of Psilocybin on Cognitive and Emotional Function in Healthy Participants. This requirement is waived, in part or in full, for therapists with prior experience in supporting people in 3,4 methylenedioxy-methamphetamine (MDMA), LSD, or other psilocybin trials.

To build further capacity at active sites and mitigate potential turnover of therapists, therapists-in-training are now attending the psilocybin sessions in the current phase IIb study, as assisting therapists, alongside more experienced therapists, to gain clinical experience. Clinical training is always supervised by experienced therapists, and used as an opportunity to deepen therapists' understanding of core principles and to build their confidence.

After completing steps 1–3, therapists are eligible to lead psilocybin therapy sessions independently. All therapists are expected to engage in mentoring sessions at least once a month and to engage in continuous professional development.

### Step 4. Continuing Professional Development

#### *Mentoring*

The term "mentoring" is closely related to supervision practices common in psychotherapy training (28). The word "mentor" is used instead of "supervisor" as mentors in the phase IIb study do not have a formal managing, evaluating or enforcing role in relation to their mentees, nor do mentees work under their mentors' licenses. Otherwise, the content of mentoring is similar to that of clinical supervision.

The goal of mentoring is to develop and maintain strong professional skills and capabilities as a psilocybin therapist, and to ensure the fidelity of the treatment approach within the study context. Mentoring is not personal psychotherapy, nor is it a form of, or substitute for, fundamental clinical training or line management.

A common structure is followed in mentoring meetings, as recommended in evidence-based mentoring practices (28). Study-specific mentoring guidelines were developed by the mentoring team during a 3-day working group meeting. Guidelines are provided to all mentees at the outset, and the mentoring team meets monthly for "peer mentoring" to ensure consistent delivery of mentoring and resolve any emerging challenges. Mentoring sessions are structured around discussions of clinical scenarios and lessons to be learned from supporting participants in the study. Mentors share fidelity ratings and deliver feedback, along with discussing areas for improvement and potential protocol deviations, with the objective of ensuring consistency and therapist skill development. After each meeting, the mentor and mentee formulate key "take-home messages" and specific action points together, and provide a written summary.

#### *Webinars*

Therapists are required to participate in at least 50% of the professional development webinars usually conducted monthly. In these webinars, therapists present clinical case studies to their peers and trainers. Discussion content and key learning points are recorded and shared with therapists for future reference. Based on therapists' requests and learning needs that emerge during the study, some of the webinars also concentrate on specific themes, such as integration practices. Webinars aim to address emerging questions and issues in a timely manner and sustain and improve consistency across sites in the study. As different sites recruit at different rates, group webinars create an environment in which to share best clinical practices and maintain therapists' skills.

## Additional Considerations

Several additional considerations regarding the specific challenges of a multisite clinical trial with a psychedelic compound are important to the training program.

### Ensuring Consistency

Fidelity to the therapeutic approach ensures good internal validity, increases the replicability of an intervention, is likely to lead to better outcomes and participant care, and facilitates further therapy optimization research (29). Demonstrated fidelity to the approach is a regulatory requirement for research and will subsequently determine whether a course of treatment is reimbursed post-approval.

To assess consistency, fidelity scales specific to the intervention were developed to quantify adherence to the therapeutic approach in the context of research. In this study, the fidelity rating scale comprises items related to general safety and GCP, and items that assess principles and methods of the therapeutic approach, as outlined in the psilocybin therapy manual. These include key competencies and actions required of therapists during preparation (25 items), session (18 items) and integration (12 items). Example items for preparation include how well the therapist, "created and communicated a setting of safety and support"; for session, how they "encouraged and facilitated the participant attending to inner experiences

and bodily sensations (including challenging experiences and sensations) balanced with periods of communication and movement, where necessary"; and for integration, "facilitated the participant talking freely about the psilocybin session, using active listening and curious questioning" (integration). Therapists' fidelity is rated against criteria for core competencies, on a three-point scale of "*Yes*"; "*Yes, but below the desired level*"; or "*No.*" The current version of the fidelity scale is in the process of ongoing validation and refinement, and will be made available in a separate publication.

At the time of writing, fidelity raters in our phase IIb study are study trainers or therapists with clinical experience, or trained raters with no clinical experience. Video-recorded therapy sessions are used to assess fidelity. Non-therapist raters are required to complete online modules and attend at least three group training sessions, including the analysis and rating of standard clinical scenarios, followed by group discussion led by experienced trainers.

Across the study, all therapists are asked to use the fidelity scale to self-rate every session they conduct. For the purpose of training, such self-rating reports are collated with the fidelity ratings completed by raters and discussed at mentoring meetings.

### Safety Considerations

Ensuring consistent ethical behavior of therapists is vitally important in psychedelic therapy and research. Participants might be more suggestible, and therefore more vulnerable, during a psychedelic therapy session (30). We ask all study therapists to commit to a therapists' code of ethics, placing the participant's well-being above all, and setting aside personal, ideological, religious or spiritual convictions. Trainers observe therapists during in-person and clinical training, and ongoing mentoring, in order to identify and address any attitudes or behaviors that could place vulnerable participants at risk for psychological manipulation or other unethical behavior.

### Cultural Sensitivity

For the phase IIb trial, we have established 21 sites in 10 countries across North America and Europe. Given the prevalence of TRD across cultures and socio-economic structures, developing culturally informed approaches to psilocybin therapy and training is paramount. We have actively engaged with participants and experts, trial authorities, culturally diverse study teams and therapists to help identify and address potential challenges and create opportunities for ensuring cultural sensitivity.

We have encountered the challenge of therapists conducting therapy in their national language, which is not the same across all sites. To mitigate potential language discrepancies, a shared language document has been created to guide the selection of suitable translations for words or phrases that do not directly translate from English to the destination language, such as German, Dutch, Danish, Portuguese and Spanish. Therapists are asked to use suitable translations consistently. When possible, therapists are assigned a mentor who speaks the language in which they are conducting the trial, to ensure a thorough understanding of concepts and practices.

The implications of cultural variation on the efficacy of training, and the consistency and quality of support, are not yet clear. We recognize the need for understanding and training in cultural sensitivity, especially when interventions based on interpersonal trust are delivered. We also recognize that cultural sensitivity is not limited to nationalities or languages, but extends to socio-economic background and social determinants of health, including the ability to access quality care, and we plan to introduce a new module on cultural competency in the future.

## LESSONS LEARNED FROM THERAPIST FEEDBACK

To develop and improve the training program, all therapists were invited to complete a short anonymous survey after completing the training, and 37 responded.

Therapists rated the quality of the training overall, as well as each section of training (online manual, online videos, face-to-face training, and clinical experience). Ratings were on a Likert scale from 1 to 5 [poor (1), fair (2), average (3), very good (4), excellent (5)]. All therapists rated the training overall as very good ($N = 14$) or excellent ($N = 23$); in-person training was rated as very good ($N = 13$) or excellent ($N = 24$); the online therapy manual was rated as average ($N = 2$), very good ($N = 13$) and excellent ($N = 22$); and the online demonstration videos were regarded as average ($N = 1$), very good ($N = 12$) and excellent ($N = 24$).

Therapists also reported whether the feedback they received from trainers was sufficient, using a 3-point Likert scale (1 = Insufficient, 2 = Sufficient, 3 = More than enough); of which 18 rated "sufficient" and 19 "more than enough." For ratings of how useful the feedback was, on a 3-point scale (1 = not useful, 2 = somewhat useful, 3 = very useful), 2 reported feedback as "somewhat" and 34 reported it as "very" useful.

Therapists also provided qualitative feedback about what they had found most and least useful, challenging, and whether they felt ready to work with participants. After 35% of participants in the phase IIb trial had received psilocybin therapy, we conducted 90-min interviews with 11 therapists at high-recruiting sites to explore the same questions.

Qualitative data from the surveys and interviews were not subject to formal analysis, least of all because there was little variation in what was reported.

All therapists commented that the training was comprehensive and well-structured, and prepared them well for seeing participants in the study. They reported that the online Shared Knowledge Platform served as a good resource before and during the study, and even after the clinical training was completed. Based on this feedback, we plan to expand the clinical content of the online training to further support therapists who are actively working with participants in the trial. Specifically, additional modules on the implications and support for psychiatric and medical co-morbidities, suicidality, and medication washout were valued resources and might further improve participant safety. When the current approach is used in other studies, additional information on specific diagnostic

categories should be included in the platform. We also plan to expand the modules on cultural competencies, and self-care resources for therapists, as an essential part of training.

All therapists reported that the 5 days of initial in-person training was sufficient. They also commented that to fully benefit from in-person training, it was important first to have familiarized themselves with the principles of the approach and methods for the study, as outlined on the Shared Knowledge Platform. To ensure this is the case, we plan to introduce short quizzes and other forms of assessment to monitor engagement and understanding of the materials on the platform.

Therapists also commented that while didactic and experiential interactive training help with conceptual understanding and skill development in the therapeutic approach, clinical training and engagement in participant care during psilocybin therapy research sessions are particularly beneficial, although challenging.

Feedback from the survey and interviews has limitations. First, the study is in its early stages and most therapists have supported only a small number of participants. Second, given the limitations of the study design, we are not able to examine any potential relationship between therapists' training experience and the safety and efficacy outcomes of their participants.

## DISCUSSION

The training of clinicians for clinical work with psychedelics has not yet been a subject of formal inquiry or research. Related programs run by research centers or sponsors of psychedelic drugs progressing through the FDA drug development process are drug, indication, and trial specific[1]. Outside clinical trials, some postgraduate certificate programs offer training for licensed therapists wishing to add education and skills related to psychedelic medicines to their professional development, but do not directly or fully authorize graduates to participate as research therapists[2, 3]. Although other programs are related in their focus on the clinical applications of psychedelics, they are not specific to the COMPASS protocol, and are not a substitute for the training program described herein.

Another salient question in the field of psychedelic therapy training is the need for, or relevance, of a therapist's personal experience of the study drug. Some advocate for the inclusion of such experiences in training programs (23), and others have cautioned that the decision to have and discuss such experiences requires careful forethought by clinicians (31). No research has yet demonstrated the impact of therapists' training, or other kinds of personal experience, with psychedelics on clinical outcomes, and the inclusion of such experiences may be a barrier to the inclusion of a diverse group of therapists, place trainers and trainees in dual roles, and even stigmatize those who choose to pursue psychedelic-assisted therapy as a professional. Still, there is anecdotal evidence that some therapists find some personal experiences to be helpful in their professional development [e.g., Halberstadt (32)]. The current program does not include opportunities for personal experience of psychedelics yet respects and allows for discussion of therapists' experiences during training.

Clinical training is critical in ensuring high-quality psilocybin therapy in the context of clinical trials. While didactic training could be outsourced to academic institutions and experienced private therapists, clinical training can only be conducted at selected research sites that have the capacity for a consistently high enrolment and the availability of experienced therapists motivated to train and mentor new therapists. Focusing on selected academic and clinical partners as a base for clinical training will ensure a consistent process of certification of therapists-in-training, provide trainees with continuous educational and professional development credits, and establish training centers of excellence involved in clinical research and care delivery.

Ongoing mentoring and professional development activities, although straightforward, could be logistically challenging at present, given that therapists span time zones from California to Eastern Europe. With increasing numbers of trainees and psilocybin studies consolidating in selected academic centers, these challenges can potentially be resolved with more regional training centers and therapist communities. This consideration leads us to focus on a "train the trainer" program, in which selected experienced therapists undergo additional training to be able to lead future training events and programs.

The design and implementation of fidelity procedures are essential and require a dedicated team with a specific set of skills. We have learned from experience that introducing fidelity assessments early in the training process is essential to guide the direction of training and consolidate the learning for therapists. For a phase III study, we plan to provide raters with a specially designed fidelity rating manual that would be an essential part of training for fidelity raters. In addition, we are investigating the value of machine learning and artificial intelligence to improve fidelity measures as well as the quality of training and feedback.

## CONCLUSION

Training for psilocybin therapists is essential to ensure consistent and high-quality care for participants in clinical studies and potential post-approval applications. Early, active and ongoing collaborations between mental health experts is essential for the continuous development of a training methodology that is rigorous, effective and scalable. To maximize impact and to meet phase III and post-approval need, we consider the adoption of technology for enhanced online learning and the establishment of pathways for clinical training to be critical points for quality assurance. These goals can only be achieved through close public, academic and industry collaborations united by the common goal of transforming mental health care.

[1]https://mapspublicbenefit.com/training

[2]https://www.ciis.edu/research-centers/center-for-psychedelic-therapies-and-research/about-the-certificate-in-psychedelic-assisted-therapies-and-research

[3]https://www.fluence8.com/postgraduate-certificate-in-psychedelic-integration-therapy

## DATA AVAILABILITY STATEMENT

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

## ETHICS STATEMENT

Ethical approval was not provided for this study on human participants because the research involved a survey of training participants that was conducted as part of an internal educational program evaluation process and therefore not requiring IRB. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

## AUTHOR CONTRIBUTIONS

ST led the development of the psychological approach and therapeutic model, and the design and content development of the therapist training program, including mentoring and fidelity procedures and delivered the majority of the therapist in-person training, and contributed to the writing of this manuscript. EN provided some of the therapist in-person training, contributed to the development of the mentoring and fidelity procedures, and contributed to the writing of the manuscript. PG, WR, BR, R-LJ, and FR contributed to the development of the therapeutic model, therapist recruitment, training, supervision and mentoring, and manuscript development. ML-J co-led the development and the implementation of the training program, contributed to the selection, recruitment, training and supervision of therapists, and the development of the manuscript. RW contributed to therapist recruitment, training and further development of the training, adherence monitoring, supervision and mentoring program, evaluation of the training program, and the development of the manuscript. EM led the development of the therapeutic model, and the design and the implementation of the training program and also contributed to the evaluation of the survey results and development of the manuscript. All authors contributed to the article and approved the submitted version.

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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.2021.586682/full#supplementary-material

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# Botulinum Neurotoxin Therapy for Depression: Therapeutic Mechanisms and Future Perspective

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Depression is one of the most common mental disorders, which causes global burden. Antidepressants and psychotherapies are the mainstay of treatment for depression, which have limited efficacy. Thus, alternative approaches for preventing and treating depression are urgently required. Recent clinical trials and preclinical researches have clarified that peripheral facial injection of botulinum neurotoxin type A (BoNT/A) is a rapid, effective and relative safe therapy for improving some symptoms of depression. Despite its safety and efficacy, the underlying therapeutic mechanisms of BoNT/A for depression remains largely unclear. In the present review, we updated and summarized the clinical and preclinical evidence supporting BoNT/A therapy for the treatment of depression. We further discussed the potential mechanisms underlying therapeutic effects of BoNT/A on depression. Notably, we recently identified that the anti-depressant effects of BoNT/A associated with up-regulation of 5-HT levels and brain-derived neurotrophic factor (BDNF) expression in the hippocampus in a preclinical mouse model. In summary, these studies suggest that BoNT/A therapy is a potential effective and safe intervention for the management of depression. However, fundamental questions remain regarding the future prospects of BoNT/A therapy, including safety, efficacy, dose-response relationships, identification of potential predictors of response, and the precise mechanisms underlying BoNT/A therapy.

**Keywords: depression, botulinum neurotoxin, hippocampus, brain-derived neurotrophic factor, 5-HT**

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## INTRODUCTION

Major depressive disorder (MDD) is a complex mental disease, which is characterized by symptoms of emotional, motivational, cognitional, and physiological domains (1). MDD is a highly prevalent disease of mental disorders which ranging from 6 to 18% across different countries, and it has substantially increased since 1990, possibly driven by global population growth and aging (2). According to World Health Organization (WHO), it was estimated that more than 350 million people suffer from MDD all over the world (3). In addition, many studies indicate women have been shown to be at greater risk for MDD than men (4). MDD is one of the top ten causes of disability all over the world. And MDD has been predicted to be a leading cause of global disease burden by 2030, in view of the overall disability and sufferings caused by it (5). For instance, the economic costs due to depression worldwide is estimated to be 1.15 trillion US dollars per year (3),

and it is estimated that in the United States alone, this figure exceeds US$210 billion, resulting in 45% direct costs, 5% costs related to suicides and 50% costs related to work (3).

The symptoms of depression include low mood, decreased interest in the daily activities, decreased motivation, appetite and sleep disturbance, psychomotor agitation or retardation, cognitive impairment, and suicidal thought (6, 7). In addition, the patients with MDD have poorer physical health, including increased prevalence of cardiovascular disease, diabetes, and premature mortality compared with the general population (8). MDD which was untreated or partially treated has prodigious influence for the patients, their family, health-care system, and society (9, 10).

Clinically, the current treatments for depression are pharmacological and psychological interventions. Early clinical observations indicated that decreased monoamine function in the brain contributed to the pathogenesis of depression. Thus, antidepressants were developed in order to up-regulate monoamines levels in the brain either by inhibiting neuronal reuptake of them or by inhibiting their degradation. Although antidepressants are typically more efficacious than placebo in many clinical trials, some evidence suggested that ∼50% of patients with depression were not responsive to antidepressant treatments (11). In addition, antidepressant medication may cause significant side effects, such as weight gain, increased risk of diabetes, and sexual dysfunction. Notably, cognitive behavioral therapy is also shown to have only moderate therapeutic effect on depression (12). Given the enormous disease burden of MDD and limited efficacy of current antidepressants or cognitive behavioral therapy, there is an imminent need to develop an alternative effective therapy for depression. To this end, there is growing evidence supporting botulinum neurotoxin type A (BoNT/A) therapy as useful method to treat major depression (13–16). In the present review, we have provided clinical and preclinical evidence supporting BoNT/A therapy for treatment of depression and discussed the potential therapeutic mechanisms and future perspectives.

## Overview of Botulinum Neurotoxins

Botulinum neurotoxins (BoNTs) are produced by *Clostridium botulinum*, of which there are 7 identified and different serotypes (A-G) (17, 18). BoNTs has a molecular weight of 150 kDa, which consist a light chain (LC; 50 kDa) and a heavy chain (HC; 100 kDa) (19–21). The main action of BoNTs occurs in the neuromuscular junction. In botulism poisoning, flaccid paralysis occurs by inhibiting the release of neurotransmitters from the peripheral cholinergic nerve terminals of the skeletal and autonomic nervous system (20). BoNTs are a typical example of bacterial exotoxins which target intracellular substrates. BoNTs have developed a structural organization aiming at delivering the metalloprotease domain into the host cell cytosol and by exploiting several physiologic functions of nerve terminals can achieve it (21). When local injection of BoNTs, they have limited diffusion, and their action can be reversible with time. Based on this above-mentioned feature, BoNTs (especial BoNT/A) have become the safe and most efficacious treatment for various kinds human syndromes that are characterized by hyperactivity of nerve terminals (21).

The classical mechanisms underlying the actions of BoNT/A is the inhibition of acetylcholine (ACh) release from the presynaptic nerve terminals, then reducing the activity of muscle fibers (22, 23). There are five major steps of BoNT/A transport in the nerve terminal, including binding and specificity, internalization into nerve terminals, membrane translocation, inter-chain disulfide reduction and SNARE protein cleavage (20), especially endocytosis, intracellular trafficking and transcytosis are important. It has been reported that HC/A can bind to and activate fibroblast growth factor receptor 3 (FGFR3), which is a tyrosine kinase receptor, in neuroblastoma cells (24). Recently, it was reported that by using non-toxic full-length BoNT/A(0) mutant, a catalytically inactive, BoNT/A(0) enters cortical neurons via a pathway dependent on FGFR3 receptor (25). Further study showed that BoNT/A(0) enters neurons through both dynamin-dependent and dynamin-independent endocytosis (25).

BoNT/A may also act similarly to the action of tetanus neurotoxins on the nervous system, which is the well-known example of retro-axonal transport inside motor axons (26). It is noteworthy that peripheral injection of BoNT/A can have a transynaptic action on the central neuronal circuits, including the spinal cord and higher brain regions (27, 28). So far, convincing evidence supporting BoNT/A1 retrograde transport to the CNS was supplied by tracking down the cleavage of the SNARE within the CNS neurons after peripheral injection, using a specified antibody for the novel epitope generated from the cleavage of SNAP-25 by BoNT/A1 (17, 29, 30). By injecting BoNT/A1 into the rat whisker pad, it caused the appearance of truncated SNAP-25 in the somatodendritic area of primary efferent facial motoneurons (31). It was also found that after the catalytically active BoNT/A was injected into the nasolabial muscle tissue of rats and mice, it was transported to the facial nucleus (FN) in the brain (27). Retrograde transportation of BoNT/A1 can also occur through primary sensory afferents, as it was observed that arrival of truncated SNAP-25 is not only in the trigeminal nucleus (29) but also in the spinal cord dorsal horn after subcutaneous or intramuscular injection (30). The transportation of BoNT/A1 includes undergoing retrograde from periphery to the ganglia and anterograde from ganglia to the afferent innervations in the brain stem and/or the spinal cord (28). Therefore, these results suggested that the intracellular transportation of BoNT/A1 may be an active retro-axonal transport, rather than through their passive diffusion or diffusion of split productions (31).

## Clinical Application of BoNTs in Neurology

Currently, BoNTs therapy is widely administrated in clinical neurology, including dystonia, spasticity, autonomic disorders, and chronic pain (22, 23, 26) (**Figure 1A**). BoNTs can not only block the skeletal neuromuscular transmission, but also the autonomic innervation (24). Therefore, BoNTs therapy may also be beneficial for hyper-hidrotic disorders, urologic or gastrointestinal disorders (21). BoNT/A therapy had been applied for the management of many neurological disorders, such as dystonia and spasticity (25). Recently, a meta-analysis

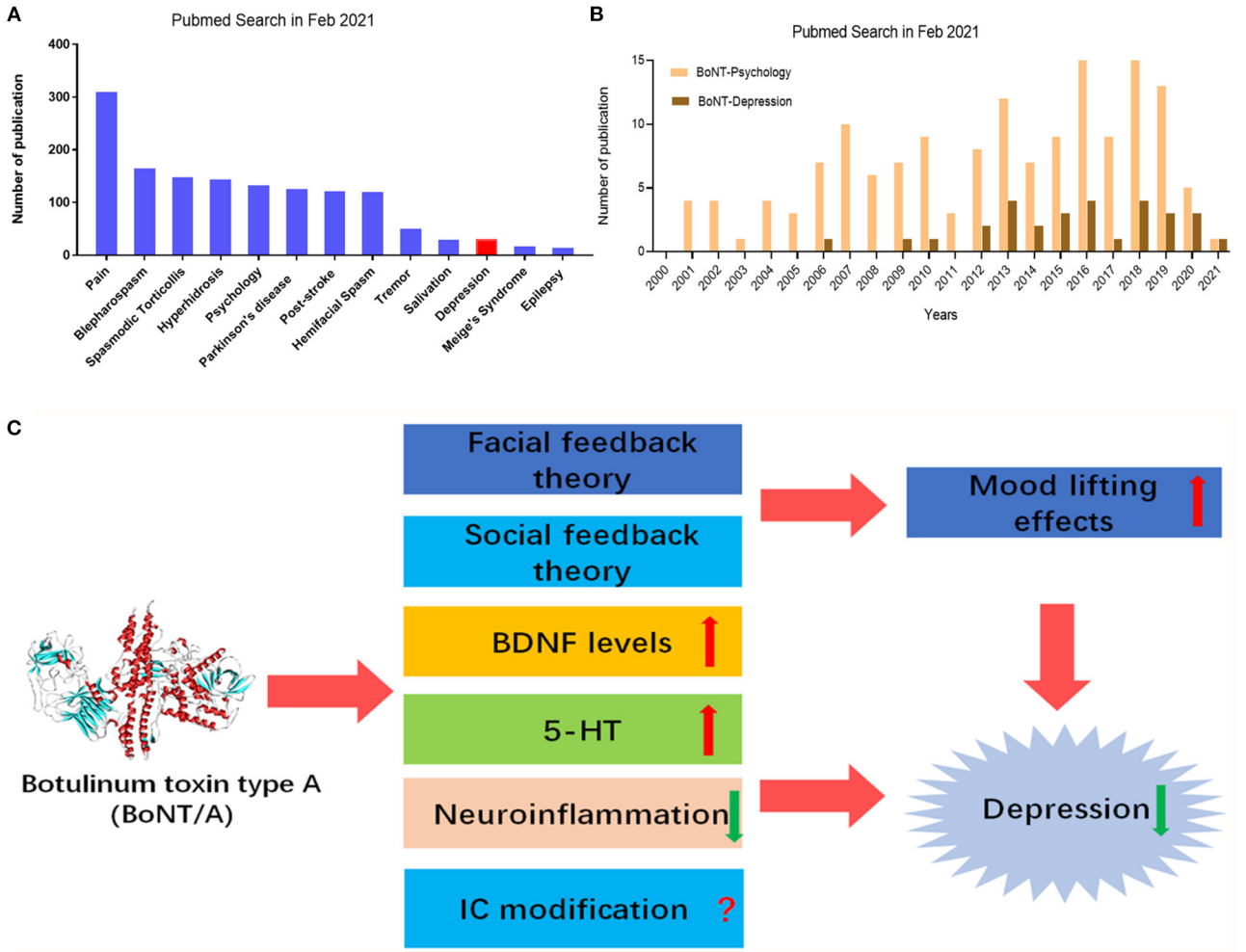


**FIGURE 1 |** PubMed search for BoNT/A and neurological diseases, including depression. **(A)** Number of publications applying BoNT/A in Parkinson's disease, pain, hyperhidrosis, post-stroke, tremor, salivation, epilepsy, spasmodic torticollis, hemifacial spasm, blepharospasm, Meige's Syndrome, depression, and psychology. **(B)** Number of publications for applying BoNT/A in depression and psychology in the last 20 years. **(C)** Therapeutic mechanisms of BoNT/A in depression. Peripheral injection of BoNT/A produces antidepressant effects possible though multiple mechanisms, such as facial feedback and/or social feedback to lift mood, IC modifications, up-regulation of BDNF levels, increasing 5-HT levels, and dampening neuroinflammation. IC, insular cortex; BDNF, brain-derived neurotrophic factor; 5-HT, 5-hydroxytryptamine.

study including a total of 42 clinical trials reassessed the efficacy of BoNTs for movement disorders treatment, such as blepharospasm, hemifacial spasm, and laryngeal dystonia (27, 28). BoNT/A therapy was though as a choice of the pharmacological treatment in focal spasticity to improve limb position, functional ability and to release pain (29, 30). The application of BoNTs within the broad category of autonomic indications includes the hypersecretory disorders, such as hyperhidrosis and sialorrhea (31). Preclinical and clinical evidence have showed that BoNTs therapy had analgesic effects on neuropathic pain, trigeminal neuralgia, and chronic migraine (32). Notably, BoNT/A has been given official approval for preventive therapy of chronic migraine by Food and Drug Administration (FDA) in pain medicine (33). BoNTs can alleviate trigeminal neuralgia and can last for 6 months or more (34). Recently, BoNTs is also applied for the management of motor and non-motor symptoms in Parkinson's disease (PD) patients (35). Intriguingly, recent meta-analysis and literatures support that clinical application of BoNT/A may have antidepressant properties (14, 36, 37) (**Figure 1B**). In the current review, we summarized the clinical and preclinical studies on BoNT/A-induced antidepressant effects, discussed the therapeutic mechanisms, and proposed the future directions of BoNT/A therapy for depression.

## BoNT/A Therapy for Depression: Clinical Evidence

Although pharmacological and psychological treatment for depression are available, a considerable proportion of depressed patients are resistant to current standard treatment (38, 39). In

esthetic medicine, the injection of BoNT/A in the glabellar region is a commonly used intervention (40). The contraction of the corrugator muscles is able to produce glabellar frown lines, which is also required for the facial expression of negative emotion, such as anger, fear, and/or sadness (41). Thus, it was concluded that BoNT/A therapy can actually make facial expression of emotion less negative, suggesting BoNT/A therapy may have mood-lifting effects in some depressed patients.

In 2006, Finzi et al. first reported open-label application of BoNT/A in 10 depressed patients led to significant improvement of self-rated depression score by using Beck Depression Inventory II (BDI-II) (15). Two months after the injection of BoNT/A, nine patients were not depressed and one patient reversed negative mood. Although there is no control experiment and the number of cases is too small, this study inspired people to further investigate the therapeutic effects of BoNT/A on depression. Subsequently, there are several randomized controlled trials (RCT) of BoNT/A therapy for the management of depression. In addition, injection of BoNT/A into frown muscles and glabellar region in humans was performed in most studies. Other injection sites were also increasingly used, such as bilateral lateral canthus (42) in humans. The injection sites of five reports were glabellar regions and the concentration of the injection were 29 U applying in females and 39 to 40 U for males. After that, Montgomery Asberg Depression Rating Scale (MADRS), BDI-II, and Hamilton Depression Scale (HAMD) were employed for the clinical evaluation of depression. They found that patients had a remission rate of more than 50% following BoNTs treatment, while females had a higher rate of remission than males (13). However, this study lasted only 6 weeks. In 2018, Finzi's another study showed that BoNTs injection was also effective for bipolar depression in men (43).

A randomized and placebo-controlled trial was conducted to evaluate the possible beneficial effect of injecting BoNT/A into the glabellar area as an adjuvant therapy for antidepression (44). This study lasted 16 weeks, making up for the shortcomings of abovementioned Finzi's research. Thirty participants randomly joined into the BoNT/A-treated ($n = 15$) or saline-treated ($n = 15$) group. The response rates of BoNT/A and placebo groups were 60.0 and 13.3%, respectively. This trial suggested that a single injection of BoNT/A in the glabellar region may rapidly reach an intense and sustained remission for some depressed patients, who did not response to former medication. This study is relatively perfect, except for a small number of samples. Lewis et al. assessed the effect of BoNT/A on mood by comparing the patients who received BoNT/A therapy in glabellar region and who had other cosmetic treatment (45). Total 25 female participants participated in this study. BoNT/A treatment group was lower than the control group in the scores of Irritability-Depression-Anxiety Scale (IDAS). For some participants, their first BoNT/A treatment was 2 weeks prior to the measurement, however one participant first BoNT/A treatment was 6 years ago. In order to avoid large skewness, the geometric mean is used to measure the central trend. The geometric mean was 195 days, which is the longest remission duration.

Brin et al. evaluated the antidepression effect of BoNT/A using two doses (30U and 50U) in females (36). At week 6, BoNT/A (50 U) treatment group did not separate from placebo group for any parameters. BoNT/A (30 U), administered in a standardized injection pattern in a single session, had a consistent efficacy signal across multiple depression symptom scales for 12 weeks or more. BoNT/A (30 U)/placebo MADRS differences of (observed ANCOVA) was $\geq$4.0 points (up to week 15) and $\geq$2.0 points (weeks 18–24), which is agree with the 2-point change threshold considered clinically relevant in MDD.

Magid et al. evaluated the effect of BoNT/A lasting 24 weeks (46). The response rates were 55% (6/11) in the BoNT/A-first group, 24% (4/17) in the BoNT/A-second group, and 0% (0/19) in the placebo group, respectively. The results suggested that the first injection of BoNT/A significantly decreased the BDI scores compared to that before treatment. An additional dose (no more than 20 U) were secondly injected to those patients who still had a severity score or greater for glabellar frown lines (47). Together, clinical studies (summarized in **Table 1**) have demonstrated that BoNT/A may be effective for the management of depression.

## BoNT/A Therapy for Depression: Preclinical Evidence

To date, preclinical animal studies of BoNTs treatment for depression are still limited, but there are some clues. A single facial injection of BoNT/A induced a rapid and continuous improvement about depression-like behaviors in naive and space-restriction-stressed (SRS) mice. The antidepressant-like effects of BoNT/A was reflected by a decreased duration of forced swimming test and tail suspension test immobility (14). Techniques currently used to assess antidepressants effects by using rodent models include olfactory bulbectomy, chronic mild stress, chronic forced swim test, novelty-suppressed feeding, novelty-induced hypophagia, social defeat stress, and learned helplessness (49). Given that different models have different sensitivity to behaviors tests, using different animal models to validate the anti-depressant effects of BoNT/A may be necessary in the future.

To date, the clinical trials and animal studies of BoNTs treatment of depression are still relatively lacking, and the number of cases needs to be increased to evaluate its safety and efficacy, especially the long-term efficacy. However, it was noticed that the publication about the application of BoNTs in depression is increasing (**Figure 1B**). Subsequently, we will discuss the therapeutic mechanisms underlying the effects of BoNTs on depression.

## Therapeutic Mechanisms of BoNT/A in Depression

To date, the potentially mechanisms underlying the therapeutic effects of BoNT/A in the treatment of depression is still elusive. There are some review article or meta-analysis that discussed its efficacy and possible mechanisms (37, 50). Based on current clinical and preclinical evidence, several hypotheses were proposed to explain the therapeutic mechanisms of BoNT/A on depression to date. The facial feedback hypothesis claims that the injection of BoNT/A between the eyebrows interfere with emotional feedback. Because botulinum toxin can

**TABLE 1 |** Clinical trials and preclinical studies on botulinum toxin in the treatment of depression.

| Year | Authors | Periodicals | study | Sex | Number | Groups | Brands | Doses | Dissolution | Injection | Tests | Results | Duration |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2006 | Finzi et al. (15) | Dermatol Surg | RCT | female | 10 | BoNT/A | — | 29 U | — | Frown muscles | — | Nine of ten experienced resolution of their depression symptoms. | 2 months |
| 2009 | Lewis et al. (45) | J Cosmet Dermatol | RCT | female | 25 | Placebo-BoNT/A | — | — | — | Frown muscles | IDAS | BoNT/A-treated group increased by 6 points. | 195 days |
| 2012 | Wollmer et al. (44) | J Psychiatr Res | RCT | female and male | 30 | Placebo-BoNT/A | Botox Cosmetic, Allergan | 29U/female, 39U/male | 0.9% NaCl solution, 100U/2.5 ml | Glabellar region | HAMD, BDI | Response rate 60.0 vs. 13.3% | 16 weeks |
| 2013 | Hexsel et al. (47) | Dermatol Surg | RCT | — | 25 | Depressed–non-depressed | Botox, Allergan Inc, Irvine, CA | 20U | 0.9% NaCl solution, 100U/1 ml | Glabellar region | BDI | BDI scores were significantly lower than before. | 12 weeks |
| 2013 | Finzi et al. (13) | J Psychiatr Res | RCT | female and male | 74 | Double-Blind,placebo-BoNT/A | Botox Cosmetic, Allergan | 29U/female, 39U/male | 0.9% NaCl solution, 100U/1 ml | Frown muscles | MADRS | Response rates at 6 weeks from the date of injection were 52% and 15% in the OBA and placebo groups. | 6 weeks |
| 2014 | Magid et al. (46) | J Clin Psychiatry | RCT | female and male | 30 | Double-Blind, placebo-BoNT/A | — | 29U/female, 39U/male | 0.9% NaCl solution, 40U/1 ml | Glabellar region | BDI, HDRS-21 | Response rates were 55% (6/11) in the BTA-first group, 24% (4/17) in the BTA-second group, and 0% (0/19) in the placebo group. | 24 weeks |
| 2018 | Chugh et al. (43) | J Psychiatr Pract | RCT | female and male | 37 | Chronic and treatment resistant depression | — | 29U/female, 39U/male | — | Glabellar region | BDI, HDRS-21, MADRS | Almost all of the patients (41/42) showed clinically meaningful improvement in the symptoms of depression. | 3 weeks |
| 2018 | Finzi et al. (48) | J Psychiatr Res | RCT | female and male | 6 | Bipolar depression | — | 29–46 U | — | Glabellar region | BDI | This is the first report of successful BT therapy of bipolar depression in six patients. | 15 weeks |

*(Continued)*

**TABLE 1** | Continued

| Year | Authors | Periodicals | study | Sex | Number | Groups | Brands | Doses | Dissolution | Injection | Tests | Results | Duration |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2019 | Li et al. (14) | Neurosci Bull | Animal testing male | | 5–10 per groups | Placebo-BoNT/A-fluoxetine | BoNT/A, Lanzhou, China | 0.18 U | 0.9% NaCl solution, 0.18U/100 μl | mouse cheeks | TST, FST | BoNT/A improves depressive-like behaviors in mice undergoing spatial restraint stress. | 2 weeks |
| 2020 | Brin et al. (36) | Int Clin Psychopharmacol | RCT | female | 255 | double-blind placebo-controlled | — | 30 U<br>50U | 0.9% NaCl solution | — | MADRSH AMD-17 | Neither BoNT/A 30 U nor 50 U demonstrated statistically significant superiority over placebo at the primary endpoint, but 30 U showed consistent. | 24 weeks |

*BDI, Back depression inventory; BoNT/A, botulinum neurotoxin type A; FST, forced swim test; HAMD, hamilton depression scale; IDAS, irritability-depression-anxiety scale; MADRS, Montgomery Asberg depression rating scale; RCT, randomized controlled trial; TST, tail suspension test.*

paralyze muscles, it is impossible to modify facial expression according to mood states, such as happy, sadness and anger, which often appear in depressed patients. Social feedback hypothesis indicated that happy facial expressions will get positive social feedback and improve mood. Finally, facial injection of BoNT/A causes structure or function changes in the brain to alleviate depression, for example, upregulation of brain-derived neurotrophic factor (BDNF) expression in the brain (51). We will further discuss these abovementioned hypotheses as follow (**Figure 1C**).

## Facial Feedback Hypothesis

The possible relationship between facial expression and depression can be traced back to the Darwin period (52). Strack et al. tested the hypothesis that people's facial activity affects their emotional responses (53). These results suggest that inhibition and promotion mechanisms may contribute to the observed emotional response (53). Other researchers have presented supportive results, indicating that signaling between the emotional center of the brain and the facial muscles is bidirectional, which further supports the facial feedback hypothesis (54).

It was confirmed that after the injection of BoNTs between the eyebrow and the orbicularis muscle, the depressed emotional state was reduced compared with depressed patients who had a saline injection. The improvement of depressed emotional state produced by BoNTs therapy was more obvious, when dealing with mild emotional stimuli (55). Wollmer et al. analyzed existing studies in an attempt to find better predictors to reflect the effects of BoNTs on patients with MDD. After data analysis, they found that high tension was the main predictor of BoNTs response, which sensitivity, specificity and overall accuracy of 100, 56, and 87%, respectively (56). The better effect of BoNTs for mood may be by the intervention of a proprioceptive feedback loop from the facial musculature to the emotional brain (57). Tension may be associated with more dynamic activities, resulting in more facial expression changes (58). In addition, BoNTs therapy reduced anxiety caused by frowning muscles, supporting the facial feedback hypothesis (59).

## Social Feedback Hypothesis

Through its action of reversible paralysis of mimic muscles, peripheral injection BoNT/A is considered to be able to prevent the emotional facial expression, including anger, sadness, and fear from being perceived by seeing of the face. Thus, BoNT/A therapy may improve the social interaction with people around, improve social contact, and then have benign social feedback. Namely, people would enter a virtuous circle of positive mood and social feedback, resulting in the persistent improvement of self-esteem. Furthermore, it was demonstrated that brain limbic system and mirror neuron system is involved in the recognition of emotional facial expression (60, 61).

## BDNF

The down-regulation of the expression of several neurotrophic factors has been involved in the pathogenesis of MDD. The most prominent and widespread representative is a polypeptide BDNF,

which promotes cyclic adenosine monophosphate (cAMP) response element binding protein (CREB) phosphorylation through the activation of extracellular signal-regulated kinase (ERK). In the brain, especially in the hippocampus, BDNF plays a functional role in neuronal differentiation and survival, neurogenesis, synaptic plasticity, connectivity, maintenance of morphology, learning and memory (62). Intriguingly, BDNF levels in several brain regions are remarkably reduced in depression-like animals and depressed patients. Several commonly-used antidepressants, such as serotonin-selective reuptake inhibitors (SSRIs) (63) can up-regulate the level of BDNF expression in the hippocampus and the prefrontal cortex in order to exert antidepressant effects (64) (**Figure 1**). Our recent work also showed that facial injection of BoNT/A was also able to up-regulate the protein expression level of BDNF in the hippocampus not only at the mRNA but also at protein in mice. Furthermore, BoNT/A injection also activated the downstream ERK-CREB signaling pathways of BDNF in the hippocampus in stressed-mice (14). Thus, the up-regulation of BDNF levels may be a new mechanism underlying the therapeutic effects of BoNT/A in the management of depression.

## Monoamine Theory

The monoamine theory claims that there is an association between emotional disorders and reduced availability of the absence of 5-hydroxytryptamine (5-HT) or norepinephrine (NE) in the brain (65). Antidepressants can increase their brain content by inhibiting the reuptake of two important neurotransmitters (5-HT and NE) into nerve terminals (65). Some of the antidepressants based on monoamine theory include: tricyclic antidepressants (TCAs), monoamine oxidase inhibitors (MAOI), and SSRIs. Unilateral administration with BoNT/A into rat whisker significantly increased the NE levels in the striatum and 5-HT levels in the hypothalamus (14, 66). Interestingly, facial injection of BoNT/A significantly increased the 5-HT level in the hippocampus, hypothalamus, and prefrontal cortex in chronic stressed mice (14, 66). Thus, the results showed that BoNTs can up-regulate the levels of monoamines (e.g., 5-HT and NE) in the brain. Further clinical and basic studies need to identify the precise alteration of the neurotransmitters in the brain during BoNT/A therapy for depression.

## Insula cortex modification

Series of evidence support the importance of several brain areas associated with MDD, by using magnetic resonance imaging (MRI) (67–69) or meta-analysis the MRI data (70, 71). The most extensive research has been done on the insular cortex (IC), prefrontal cortex (PFC), anterior cingulate cortex (ACC), amygdala and hippocampus (62), which play a key role in sensation and emotion. IC is a brain region responsible for the coding of emotional and social aspects (51). Previous studies demonstrated abnormal insular connectivity may mediate mood disorders in the symptom of depression (72). A new hypothesis for the therapeutic effects of BoNTs on depression, refers to "insula cortex (IC) modification," was recently proposed from transgenders' study (51). In patients suffering from mental illness, depression or bipolar disorder, it is possible to find both mental and physical dysphoria. The morphological changes of IC may be related to the difference between physical image and mental perception of one's body (51). Although the authors did not provide any evidence supporting BoNT/A therapy causes morphological alterations of IC, they proposed that the structural and functional modification of IC caused by BoNT/A therapy warrants further investigation.

## Neuroinflammation

An important part of the physiological stress-sensing system is the immune system, which interacts with main integrative systems of body, including the hypothalamic–pituitary–adrenal (HPA) axis, the autonomic nervous system and the central nervous system (CNS) (73). Immune disorders affecting CNS function would cause neuroinflammatory diseases, such as multiple sclerosis and autoimmune encephalitis. Low-level chronic systemic inflammation may play an important role in mediating the interface among psychological stress, depressive symptomatology, and association with depressive symptoms (74). However, there is no direct evidence to support the therapeutic mechanisms underlying the effects of BoNTs for depression involved in neuroinflammation. But there are some clues indicated the potential modulation effects of BoNTs on the development of neuroinflammation. The mechanisms underlying therapeutic effects of BoNTs on neuropathic pain are involved in restraining the release of inflammatory mediators and peripheral neurotransmitters from sensory nerves (75). Increased expression of inflammatory factors has been shown to be inhibited by monoclonal anti-Toll-like receptor 2 (TLR2) and inhibitors specific to intracellular proteins such as c-Jun N-terminal kinase (JNK), extracellular signal-regulated kinase (ERK), and p38 mitogen-activated protein kinase (MAPK) (76). Furthermore, BoNT/A treatment did not decrease LPS-induced release of pro-inflammatory factors in the astroglia, suggesting BoNT/A may have little effect on astrocytes (77). In contrast, BoNT/A treatment decreased the upregulation of expression of microglia-derived pro-inflammatory factors (77, 78), suggesting that BoNT/A may modulate the development of neuroinflammation by the inhibition of microglia activation in the CNS. The direct evidence supporting that therapeutic effects of BoNT/A on depression may owe to inhibiting neuroinflammation is still lacking.

# FUTURE PERSPECTIVE

Clinical and preclinical experiments have showed that BoNTs may be beneficial for the management of depression. However, the therapeutic application of BoNTs for depression has not USA or EU or Evidence-based therapeutic approved indication. The therapeutic mechanisms of BoNTs in the treatment for depression are needed to be further clarified. In view of the heterogeneity of the clinical findings and various influencing factors (16), more clinical trials should be performed to evaluate the dose equivalence, especially considering the different BoNT/A1 formulations. Notably, the potency of the preparations of BoNTs can be expressed as Units (U), where 1 U corresponds to 1 LD50 in the mouse bioassay (79). It is noteworthy that the

clinical effect of 1 unit is not interchangeable between different preparations, because the bioassay methods used by different brands are different, (80).

Functional magnetic resonance imaging (fMRI) was used to assess neural responses to BoNTs treatment by radionuclide imaging in human (81). However, it remains a major challenge in objectively reflecting emotional state especially in animal models. Interestingly, a recent study showed that mice can display stereotyped facial expressions responding to emotionally salient events, and upon targeted manipulations in emotion-relevant neuronal circuits (82). By using brain imaging methods and monitoring facial expression of emotion in rodents, the therapeutic mechanisms of BoNT/A on depression may be better understood.

Recently, recombinant techniques (including site-directed mutations) have been used to create engineering botulinum neurotoxins as potential novel drugs for improving their therapeutic efficacy (83, 84). For instance, Yin et al. generated a "gain-of-function" mutation by replacing two non-aromatic residues at an extended loop in the C-terminal receptor-binding domain of BoNT/B (85). This engineering BoNT/B showed enhanced binding to neuronal membrane, enhanced efficacy in paralyzing muscles, and lowered systemic diffusion (85). In addition, BoNTs were designed as novel analgesic by utilizing the ability of BoNT to cleave SNARE complexes (83). Another engineering BoNT designed by mixing BoNT/E LC to the N-terminus of BoNT/A produced locally-applied and long-acting analgesic effects in a rat neuropathic pain model (86). Scheps et al. designed a mutant with three mutations (T420E; F423M; Y426F) in the C-terminus of BoNT/A1 light chain, which exert a faster onset and a shorter duration than wild-type BoNT/A1 (87). There are several lines of evidence supporting that BoNT/A1 therapy is generally safe and may be used as a new, alternative option for the treatment of depression (37). However, safety issue must be considered for all recombinant engineering BoNTs. Together, these novel engineering botulinum neurotoxins warrant further investigation for depression treatment.

## CONCLUSIONS

Although both clinical and preclinical studies have demonstrated that BoNT/A therapy may be an effective alternative intervention for depression, future investigations are needed to improve our understanding of the therapeutic mechanisms of BoNT/A for depression. Thus, studies on BoNT/A therapy may provide novel targets for the development of effective antidepressant drugs.

## AUTHOR CONTRIBUTIONS

YL performed literature search and prepared the draft. TL and WL wrote the manuscript. All authors contributed to the article and approved the submitted version.

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# Detecting Depression Through Gait Data: Examining the Contribution of Gait Features in Recognizing Depression

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While depression is one of the most common mental disorders affecting more than 300 million people across the world, it is often left undiagnosed. This paper investigated the association between depression and gait characteristics with the aim to assist in diagnosing depression. Our dataset consisted of 121 healthy people and 126 patients with depression who diagnosed by psychiatrists according to the Diagnostic and Statistical Manual of Mental Disorders. Spatiotemporal, temporal-domain, and frequency-domain features were extracted based on the walking data of 247 participants recorded by Microsoft Kinect (Version 2). Multiple logistic regression was used to analyze the variance of spatiotemporal (12.55%), time-domain (58.36%), and frequency-domain features (60.71%) on recognizing depression based on Nagelkerke's $R^2$ measure, respectively. The contributions of the different types of features were further explored by building machine learning models by using support vector machine algorithm. All the combinations of the three types of gait features were used as training data of machine learning models, respectively. The results showed that the model trained using only time- and frequency-domain features demonstrated the same best performance compared to the model trained using all the features (sensitivity = 0.94, specificity = 0.91, and AUC = 0.93). These results indicated that depression could be effectively recognized through gait analysis. This approach is a step forward toward developing low-cost, non-intrusive solutions for real-time depression recognition.

**Keywords: depression, gait analysis, machine learning, diagnosis, skeletal joints**

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## INTRODUCTION

Depression is one of the most common mental disorders affecting more than 300 million people across the world (1). It is associated with decreased life satisfaction, impaired psychosocial functioning, and high disability and suicide rates (2–4). Early treatment can reduce healthcare costs, as well as morbidity and mortality rates associated with depression (5, 6). However, more than half of depressed patients actually have not received treatment for various reasons such as the difficulty in diagnosing (7, 8). One reason mentioned a lot is that primary care physicians generally initiate guideline-concordant care for depressed patients requesting help (9, 10), but they often fail to recognize patients with depressive symptoms (11, 12). Another long-standing reason is traditional questionnaire-based approaches for depression which may increase the risk of misdiagnosing and

thus mistreating depression in primary care settings (13–15). Therefore, more efficient methods for detecting depression are required to improve the delivery of services to those in need.

Motor symptoms (e.g., gait) have been shown to be an essential manifestation of depression (16–18). In particular, gait and postural control modulated by a complex neural network (19), which is also implicated with the pathophysiology of major depression (20). Till now, many studies investigated the association between depression and gait characteristics by using instrumental assessments. For example, Sloman et al. (21) analyzed photograms of a single stride during a natural walk and found that depressed patients' walks were more slowly with a lifting motion of the leg. In contrast, healthy participants propel themselves forward with increased foot push-off. Another study used a combination of electronic walkways and photogrammetry to show that depressed patients have shorter strides and slower gait velocity than healthy controls (22). Michalak et al. (23) demonstrated that depressed individuals exhibit reduced vertical head movements, more slumped posture, and lower gait velocity than controls by using three-dimensional (3D) motion capture.

While many studies have demonstrated significant differences in gait patterns between depressed and healthy individuals, gait is relatively neglected in clinical practice as a tool for diagnosing depression (24). For instance, basic clinical gait assessments are mainly observational or based on gait speed to functional assessment (25–27). Nevertheless, judgments formulated by clinicians on the basis of observed behavior are subjective. Methodologies of gait speed tests vary widely from study to study, making it difficult to obtain a general description of patients' gait patterns with depression (28). Furthermore, until recently, automated gait analysis has been requiring expensive equipment and auxiliary operations often unavailable in clinical settings (29). However, modern cost-effective intelligent devices provide new perspectives for gait-based depression recognition. For example, Microsoft Kinect, designed for Xbox, has been used to monitor body movement patterns continuously (30), and its effectiveness in estimating body posture and movement has been proven (29, 31, 32). Using fast Fourier transforms, Zhao et al. (33) extracted frequency domain features from gait data captured by Kinect from 179 graduate students. They then trained machine learning models to predict depression levels of the participants estimated through a questionnaire; the correlation coefficient between the prediction score of models and questionnaire scores reached 0.51. A random forest classifier built based on 12 spatiotemporal features (e.g., walking speed, stride length, arm swing, and body sway) for detecting depression among postgraduate students achieved an accuracy rate 91.58% (34). Wang et al. (35) first extracted time- and frequency-domain features through power spectral density analysis, and spatial geometric features through covariance matrices and the symmetric Stein divergence from gait data captured by Kinect. A framework for detecting depression based on fused features was proposed with a classification accuracy of 93.75%. Therefore, features extracted from Kinect-captured gait data using mathematical methods are effective in model-based depression recognition.

Previous studies showed that machine learning models trained with gait-related features can predict depression accurately, and Kinect provided objective and easily accessible data. However, few of these studies quantified the contribution of each type of gait feature (e.g., spatiotemporal, time-domain, and frequency-domain features) on depression recognition. More importantly, depression severity in these studies was assessed based on depression symptoms scales. While the effectiveness of questionnaire-based scales in accessing depression severity has been well-validated (36), questionnaire scores themselves cannot be used as a diagnosis. Furthermore, research results obtained based on one scale may not replicate to other scales since symptoms on different depression scales do not overlap completely (24).

Instead of considering depressive symptoms in the general population, this study considers the relationship between gait characteristics and depressive symptoms of clinical cases. The aims of the study include (1) evaluating the effect of different types of gait features in recognizing depression and (2) building machine learning models consisting of gait features for detecting depression. Once the contribution and effectiveness of different types of gait features in recognizing depression have been quantified, future research can confidently explore deeper insights into gait patterns of depressed patients and more robust classification models for diagnosing depression in the clinical setting.

# MATERIALS AND METHODS

## Participants

In this study, depressive patients were recruited from Beijing Anding Hospitals of Capital Medical University. Psychiatrists diagnosed these patients according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria. Inclusion criteria for the case group included: (a) diagnosed as major depressive disorder, (b) no psychotropic medicines are taken within past 2 weeks, (c) without a current or historical DSM-IV diagnosis of any other mental diseases, (d) without current or historical DSM-IV diagnosis of alcohol or drug abuse, and (e) without disability or injury that affected their walking ability.

For the control group, healthy people were recruited via local advertisements. Inclusion criteria for the control group included: (a) both mentally and physically healthy, (b) without long-term use of analgesics, sedatives, sleep drugs, cortisol drugs, anti-epileptic drugs, and treatment of high blood pressure, (c) without positive family history of mental disorders in three generations, and (d) without disability or injury that affected their walking ability.

Finally, 126 depressive patients and 121 healthy people completed the study. **Table 1** shows the demographic characteristics of depressed people and healthy people.

## Experimental Settings

We used a Kinect (Version 2) to record the gait data of participants. All the participants were asked to walk naturally back and forth for 2 min on a 6 × 1 m footpath (**Figure 1**). With Kinect continuously shooting, the 3-dimensional position changes of participants' 25 main body joints during walking were

recorded by 30 Hz sampling rate (**Figure 2**). The study was a part of a clinical research project about the potential biological and behavioral indicators of major depressive disorder, approved by the Institutional Review Board of the Institute of Psychology, Chinese Academy of Sciences (approved number: H15010).

## Data Preprocessing

### Coordinate System Transformation

By default, Kinect takes its position as the origin of the 3D Cartesian coordinate system, in which the X-axis grows to the Kinect's left, Y-axis grows up, and Z-axis grows out in the direction Kinect is facing (**Figure 1**). Given that different participants may have different positions relative to the Kinect during their walk, which means that the default coordinate system may introduce significant errors in gait pattern analysis. Therefore, we need the coordinate system transformation, in which we take the position of the spine joint (joint 9) as the origin of the coordinate system. Taking a segment of one participant's left-foot (joint 24) data on the Y-axis when walking toward Kinect as an example, we demonstrate the effect of the coordinate system transformation (**Figure 3**).

### Data Segmentation

Since gait is a cyclic physical activity, large amounts of repetitive data may lead to data redundancy and low computational efficiency. Since each participant's gait data contained back and forth walking, we need to do data segmentation. To do so, we first divided these data into face-toward and back-toward segments based on whether the participant was facing the Kinect or not. We only retained the face-toward segments due to the better accuracy of Kinect in estimating body posture and movement. In order to reduce the influence of participants' unnatural movements at the beginning/end of this experiment, we then selected the middle segment from all the face-toward segments for further analysis. Finally, we identified the beginning of a gait cycle as the lift (toe-off) of left-foot, and chose gait records of two cycles in the middle face-toward segment as the final data segment according to the change of the left-foot (joint 24) on the Y-axis. The length of these segments ranged from 1.73 to 3.43 s (mean = 2.37, SD = 0.26).

**TABLE 1 |** Demographic characteristics of participants.

| Characteristic | Depressed group | Control group |
|---|---|---|
| **Gender, *n* (%)** | | |
| Male | 57 (45.2) | 61 (50.4) |
| Female | 69 (54.8) | 60 (49.6) |
| **Height, M ± SD** | 167.4 ± 7.9 | 166.4 ± 8.2 |
| **Weight, M ± SD** | 63.3 ± 11.5 | 66.4 ± 13.3 |
| **Age, M ± SD** | 31.0 ± 9.8 | 34.7 ± 11.5 |

### Low-Pass Filtering

As unexpected body wobble or the systematic errors of Kinect may introduce high-frequency components and noise into the collected data, the original gait record needs to be filtered before data analysis. Gaussian filter is a non-uniform low-pass filter with a kernel whose coefficients decrease with the increase of distance from the kernel's center. It can be used to attenuate noises and high-frequency components in signal data (37), and its effectiveness in filtering Kinect-captured gait data has been proved in several studies (33, 38). Specifically, we set the Gaussian filter's kernel coefficient to $g = \frac{1}{16}[1, 4, 6, 4, 1]$, and then calculated the convolution of each joint' records in 3 dimensions and the Gaussian filter. The procedure of filtering is defined as:

$$\mathrm{y}\,(\mathrm{n}) = \sum_{t=-\infty}^{\infty} x\,(t)\,g(n-t) = x\,(n)\ * g(n) \qquad (1)$$

$x$ is the records of each joint in 3 dimensions, $n$ refers to the frame number, $g$ refers to the Gaussian filter, and * refers to convolution operation. We take a segment of one participant's left-foot (joint 24) data on Y-axis when walking toward Kinect as an example. After low-pass filtering, many little burrs and fluctuations in the original records are removed compared to the filtered data (**Figure 4**).

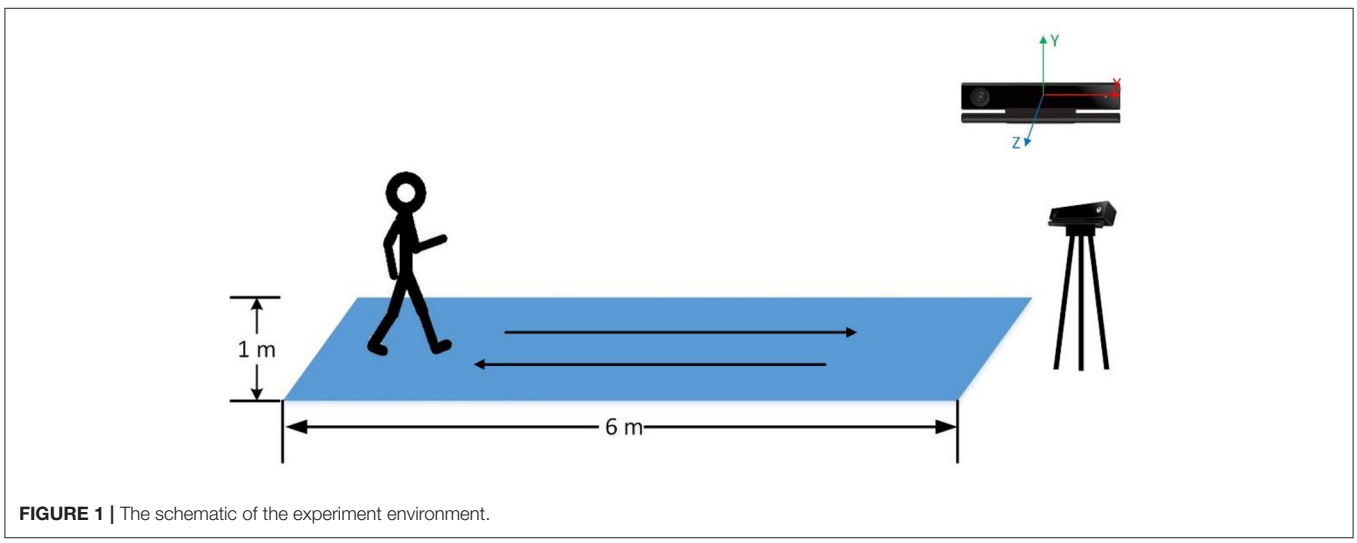
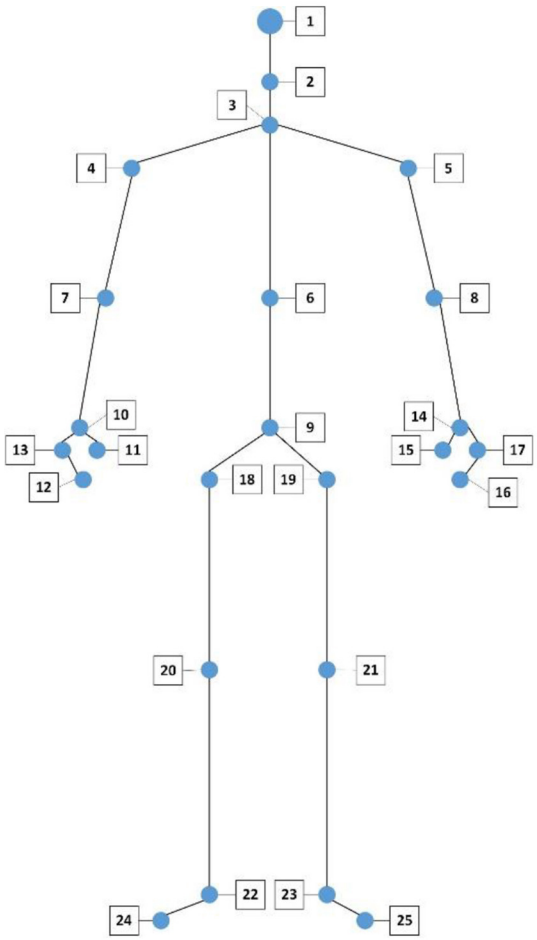



**FIGURE 1 |** The schematic of the experiment environment.




Joint 1: Head
Joint 2: Neck
Joint 3: SpineShoulder
Joint 4: ShoulderLeft
Joint 5: ShoulderRight
Joint 6: SpineMid
Joint 7: ElbowLeft
Joint 8: ElbowRight
Joint 9: SpineBase
Joint 10: WristLeft
Joint 11: HandLeft
Joint 12: HandTipLeft
Joint 13: ThumbLeft
Joint 14: WristRight
Joint 15: HandRight
Joint 16: HandTipRight
Joint 17: ThumbRight
Joint 18: HipLeft
Joint 19: HipRight
Joint 20: KneeLeft
Joint 21: KneeRight
Joint 22: AnkleLeft
Joint 23: AnkleRight
Joint 24: FootLeft
Joint 25: FootRight

**FIGURE 2 |** The 25 joints captured by a Kinect.

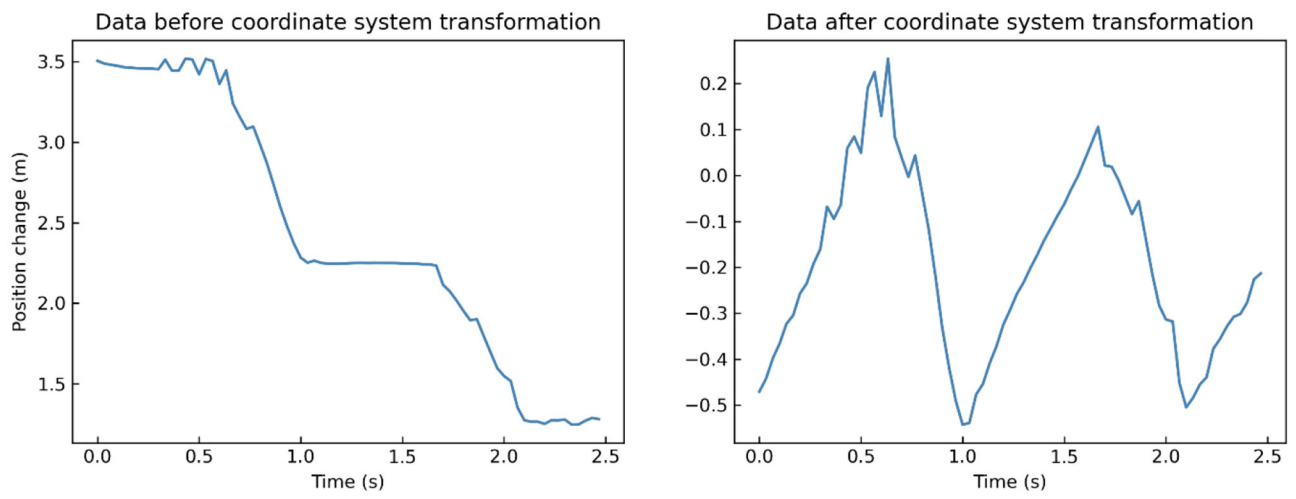



**FIGURE 3 |** A segment of one participant's left-foot (joint 24) data on Y-axis when walking toward Kinect before (**left**) and after (**right**) coordinate system transformation.

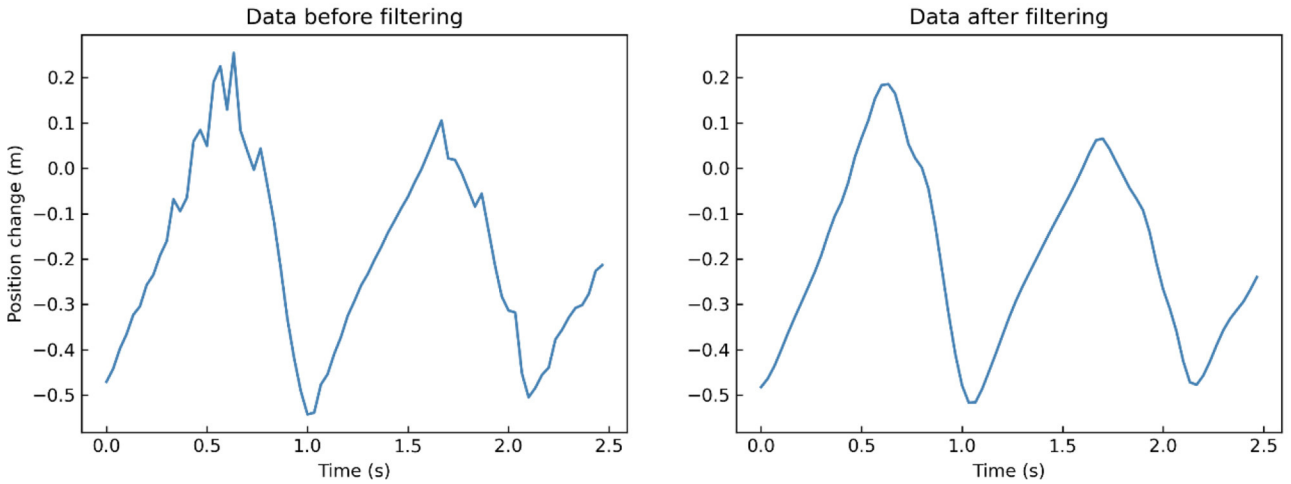



**FIGURE 4 |** A segment of one participant's left-foot (joint 24) data on Y-axis when walking toward Kinect before (**left**) and after (**right**) low-pass filtering.

# Feature Extraction

## Spatiotemporal Features Extraction

Previous research found that gait patterns associated with depression were characterized by increased body swaying, shortened strides, reduced walking speed and arm swing, etc. (23, 39, 40). Therefore, we extracted spatiotemporal features from Kinect-captured gait data that might potentially differentiate gait patterns between the case and control groups. Specifically, we extracted the following spatiotemporal features:

1) **Body swaying**: Body swaying is measured by the maximum difference in position of the left-shoulder (joint 4) and the right-shoulder (joint 5) on the X-axis during a gait cycle.
2) **Left-arm/Right-arm swing**: It was defined as the maximum difference of the left/right wrist (joint 10/14) moving along the Z-axis during a gait cycle.
3) **Vertical head movement**: We measured the vertical head movement as the maximum vertical amplitude of head (joint 1) along the Y-axis during a gait cycle.
4) **Head posture**: We quantified the head posture during a gait cycle by averaging the angle between the vertical direction and the connection line between the neck (joint 2) and the clavicle (joint 3) in the plane which consisted of the Y-axis and the Z-axis.
5) **Left/Right stride length**: It measured the maximum change in the horizontal direction of the left/right foot (joint 22/23) during a gait cycle.
6) **Left/Right toe clearance**: It measured the maximum change in vertical height of the left/right foot (joint 22/23) during a gait cycle.
7) **Walking speed**: We measured participants' walking speed according to their spine (joint 9) movement along Z-axis.

At last, the mean values of these features in each participant's two gait cycles were calculated as the final spatiotemporal features. Finally, we obtained a total of 10 spatiotemporal features.

## Time-Domain Features Extraction

Previous studies have confirmed the effectiveness of time-domain features in gait analysis not only in clinical settings but also in laboratory settings (41, 42). Time-domain information related to the statistical value of data on the 25 joints was used to characterize individuals' movement patterns. In this study, we calculated the mean, standard deviation, skewness, and kurtosis of the original data. Specifically, mean is a measure of the central tendency of the random variable characterized by that distribution. Standard deviation measures the amount of dispersion of a dataset. To examine the asymmetry that deviates from the symmetrical bell curve of a dataset, we estimated skewness. Kurtosis measures outliers present in the probability distribution. For the data on the three axes of the 25 joints, we calculated the above four statistical features respectively. Finally, we obtained a total of $3 \times 25 \times 4 = 300$ time-domain features.

## Frequency-Domain Features Extraction

In addition to using statistical methods to extract time-domain features, we conducted discrete Fourier transform to convert time-domain signals to frequency-domain features (43). The formula is defined as

$$F_k = \sum_{j=0}^{n-1} x_j^t e^{-i2\pi k \frac{j}{n}},\ k = 0, 1, \ldots, n-1 \qquad (2)$$

in which, $i$ denotes the imaginary number, $x_j^t$ stands for data on the $t$ axis ($t \in \{X, Y, Z\}$), $n$ refers to the width of a segment. For the data on the three axes of the 25 joints, we first obtain the amplitudes and phases of the data through the discrete Fourier transform. Then we calculated the direct current component, zero frequency component, which is the average value of the signal, as well as the mean, variance, standard deviation, skewness, and kurtosis of amplitudes and phases, respectively. Finally, we obtained a total of $3 \times 25 \times (1 + 5 \times 2) = 825$ frequency-domain features.

## Data Analysis

### Binary Logistic Regression

The multiple logistic regression analysis was used to investigate the contributing effect of different types of gait characteristics in recognizing depression. In this analysis, the variable selection was performed using stepwise forward selection, subsequently including one by one the variables that were not statistically significant ($\alpha = 0.05$). Specifically, the dependent variable was composed of dichotomous depressive state (case group vs. control group), and different types of gait features were analyzed as independent variables via multiple logistic regression analysis separately.

Compared with spatiotemporal features, the number of time- and frequency-domain features may be too much, which brings much redundancy and should be filtered out. To avoid the multicollinearity problem and reduce data dimension, principal component analysis (PCA) was initially conducted on the time- and frequency-domain features separately. The principal components (PCs) are the linear combinations of the original features that account for the variance of the data. Then spatiotemporal features, PCs containing 95% cumulative contribution rate of time-domain features, and PCs containing 95% cumulative contribution rate of frequency-domain features entered the logistic regression model.

To measure the contribution effect of variables to depression, we calculated both the odds ratio (OR) (44) and Nagelkerke's $R^2$ (45). OR is a measure of association between an outcome and exposures. It represents the odds that an outcome will occur given a particular exposure, compared to the odds of the outcome occurring in the absence of that exposure. Nagelkerke's $R^2$, the adjusted $R^2$ in linear regression, can provide the amount of variance of the dependent variable explained by the explanatory variables.

### Classification Modeling

In this stage, we tested the actual classification efficacy of different types of gait features on depression using supervised learning methods, and tried to find the optimal combination of features that makes the depression recognition models have the best performance. Specifically, the output of models was composed of dichotomous depressive state (case group vs. control group). All the combinations of the three types of gait features were used as training data to build machine learning models, respectively. To obtain the optimal performance of machine learning models, we conducted Sequential backward selection (SBS) to remove useless features from training data before building recognition models. SBS is a greedy search algorithm to find the best subset of features, which can minimize the performance loss of machine learning models while reducing the feature dimension (46). It starts from the whole feature set and sequentially discards the feature so as to improve (or minimally worsen) the evaluation measure. The algorithm stops when all remaining features are useful for the model, and removing one of them could lead to a decline in accuracy.

In this study, we trained classification models to detect depression using the support vector machine (SVM) (47) with linear kernel function. SVM is one of the most state-of-the-art classification algorithms, which first maps feature vectors to a higher-dimensional feature space using kernel tricks and then makes predictions based only on support vectors. To evaluate the predictive performance of the models, we considered sensitivity, specificity, and the area under the ROC Curve (AUC) (48). We applied 10-fold cross validation and averaged performance measures across all folds within a single prediction model.

**TABLE 2 |** Differences of spatiotemporal features between depressed group and control group.

| Spatiotemporal features | Depressed group | | Control group | | *t* statistic | *P*-value |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | | |
| Body swaying (m) | 0.36 | 0.04 | 0.36 | 0.04 | −0.58 | 0.562 |
| Left-arm swing (m) | 0.27 | 0.11 | 0.31 | 0.12 | −2.45 | 0.015* |
| Right-arm swing (m) | 0.23 | 0.09 | 0.27 | 0.10 | −2.97 | 0.003** |
| Vertical head movement (m) | 0.06 | 0.05 | 0.06 | 0.04 | 0.42 | 0.672 |
| Head posture (degree) | 1.23 | 0.10 | 1.27 | 0.06 | −3.97 | <0.001*** |
| Left stride length (m) | 0.62 | 0.07 | 0.62 | 0.07 | −0.27 | 0.789 |
| Right stride length (m) | 0.59 | 0.08 | 0.61 | 0.07 | −1.46 | 0.146 |
| Left toe clearance (m) | −0.69 | 0.06 | −0.71 | 0.06 | 1.92 | 0.056 |
| Right toe clearance (m) | −0.70 | 0.06 | −0.71 | 0.07 | 1.54 | 0.126 |
| Walking speed (m) | 0.99 | 0.18 | 1.01 | 0.17 | −0.97 | 0.332 |

*These spatiotemporal features were calculated in the coordinate system with the spine joint (joint 9) as the origin. *P < 0.05, **P < 0.01, ***P < 0.001.*

## RESULTS

### Binary Logistic Regression

Results showed that depressed patients walk more slowly (walking speed) and with fewer movements (e.g., left-arm swing, right-arm swing, head posture, right stride length, left toe clearance, right toe clearance) than participants of the control group (**Table 2**). And the independent sample *t*-test indicated that depressed group had significantly less left-arm swing, right-arm swing, and head posture [$t_{(245)} = -2.45$, $P = 0.015$; $t_{(245)} = -2.97$, $P = 0.003$; $t_{(245)} = -3.97$, $P < 0.001$] than the control group.

We examined how many different types of gait features contributed to depression recognition, using Nagelkerke's $R^2$ statistic, and estimated the effect of each variable using ORs. The results showed that with all the spatiotemporal features in the logistic model, it accounted for 12.55% (Nagelkerke's $R^2$) of the variance in the dependent variable depression. ORs for each spatiotemporal feature retained in this model after stepwise forward selection and their significance are shown in **Table 3**; left-arm swing (OR = 0.017, $P = 0.010$), and head posture (OR = 0.001, $P < 0.001$) significantly predicted depression.

The results indicated that when all the time-domain PCs entered the logistic model, it accounted for 58.36% (Nagelkerke's $R^2$) of the variance in the dependent variable depression. ORs for each PC retained in this model after stepwise forward selection and their significance are shown in **Table 4**; PC2

**TABLE 3 |** Binary logistic regression model of spatiotemporal features.

| | β | SE | Wald | Odds ratio | Corrected *P*-value |
|---|---|---|---|---|---|
| Left-arm swing | −4.047 | 1.458 | 7.708 | 0.017 | 0.010* |
| Head posture | −7.031 | 1.981 | 12.593 | 0.001 | <0.001*** |

*The P-value is corrected by Bonferroni correction. *P < 0.05, ***P < 0.001.*

**TABLE 4 |** Binary logistic regression model of time-domain principal components.

| | β | SE | Wald | Odds ratio | Corrected *P*-value |
|---|---|---|---|---|---|
| PC2 | −0.301 | 0.058 | 27.001 | 0.740 | <0.001*** |
| PC5 | 0.304 | 0.064 | 22.833 | 1.355 | <0.001*** |
| PC8 | 0.410 | 0.077 | 28.234 | 1.507 | <0.001*** |
| PC9 | −0.187 | 0.74 | 6.362 | 0.829 | 0.198 |
| PC11 | 0.225 | 0.073 | 9.404 | 1.253 | 0.037* |
| PC19 | 0.231 | 0.103 | 5.049 | 1.260 | 0.159 |
| PC24 | −0.280 | 0.108 | 6.752 | 0.756 | 0.128 |
| PC26 | 0.299 | 0.108 | 7.631 | 1.349 | 0.098 |
| PC28 | 0.326 | 0.118 | 7.580 | 1.385 | 0.100 |
| PC29 | 0.255 | 0.118 | 4.658 | 1.290 | 0.525 |
| PC31 | 0.413 | 0.129 | 10.257 | 1.512 | 0.023* |
| PC35 | 0.655 | 0.156 | 17.715 | 1.926 | <0.001*** |
| PC39 | 0.310 | 0.146 | 4.502 | 1.364 | 0.575 |
| PC41 | 0.341 | 0.148 | 5.291 | 1.406 | 0.364 |
| PC46 | 0.471 | 0.165 | 8.200 | 1.602 | 0.071 |
| PC70 | −0.685 | 0.248 | 7.590 | 0.504 | 0.100 |
| PC77 | −0.709 | 0.264 | 7.196 | 0.492 | 0.124 |

*The P-value is corrected by Bonferroni correction. *P < 0.05, ***P < 0.001.*

(OR = 0.740, $P < 0.001$), PC5 (OR = 1.355, $P < 0.001$), PC8 (OR = 1.507, $P < 0.001$), PC11 (OR = 1.253, $P = 0.037$), PC31 (OR = 1.512, $P = 0.023$), and PC35 (OR = 1.926, $P < 0.001$) significantly predicted depression. The time-domain PCs are the linear combinations of the original time-domain features, and **Supplementary Table 1** shows the Pearson correlation coefficients between these PCs and the original time-domain features.

The results showed that when all the frequency-domain features PCs entered logistic model, it accounted for 60.71% (Nagelkerke's $R^2$) of the variance in the dependent variable depression. ORs for each significant PC retained in this model after stepwise forward selection and their significance are shown in **Table 5**; PC2 (OR = 0.924, $P = 0.022$), PC4 (OR = 1.245, $P < 0.001$), PC5 (OR = 1.278, $P < 0.001$), PC6 (OR = 0.878, $P = 0.022$), PC7 (OR = 0.788, $P < 0.001$), PC10 (OR = 0.855, $P = 0.024$), PC24 (OR = 0.755, $P = 0.004$), PC27 (OR = 0.761, $P = 0.014$), and PC30 (OR = 0.757, $P = 0.016$) significantly predicted depression. The frequency-domain PCs are the linear combinations of the original frequency-domain features, and **Supplementary Table 2** shows the Pearson correlation coefficients between these PCs and the original frequency-domain features.

**TABLE 5 |** Binary logistic regression model of frequency-domain principal components.

| | β | SE | Wald | Odds ratio | Corrected *P*-value |
|---|---|---|---|---|---|
| PC2 | −0.079 | 0.025 | 10.349 | 0.924 | 0.022* |
| PC4 | 0.219 | 0.043 | 26.246 | 1.245 | <0.001*** |
| PC5 | 0.246 | 0.047 | 27.014 | 1.278 | <0.001*** |
| PC6 | −0.130 | 0.040 | 10.370 | 0.878 | 0.022* |
| PC7 | −0.238 | 0.049 | 23.821 | 0.788 | <0.001*** |
| PC10 | −0.157 | 0.049 | 10.216 | 0.855 | 0.024* |
| PC11 | −0.142 | 0.052 | 7.498 | 0.868 | 0.105 |
| PC12 | 0.120 | 0.055 | 4.780 | 1.127 | 0.490 |
| PC22 | 0.149 | 0.070 | 4.526 | 1.160 | 0.568 |
| PC24 | −0.281 | 0.077 | 13.419 | 0.755 | 0.004** |
| PC27 | −0.273 | 0.081 | 11.234 | 0.761 | 0.014* |
| PC30 | −0.279 | 0.084 | 10.900 | 0.757 | 0.016* |
| PC40 | 0.281 | 0.098 | 8.266 | 1.325 | 0.069 |
| PC50 | 0.291 | 0.115 | 6.424 | 1.337 | 0.191 |
| PC60 | 0.305 | 0.125 | 5.936 | 1.357 | 0.252 |
| PC70 | −0.355 | 0.140 | 6.447 | 0.701 | 0.189 |
| PC87 | −0.480 | 0.174 | 7.604 | 0.619 | 0.099 |

*The P-value is corrected by Bonferroni correction. *P < 0.05, **P < 0.01, ***P < 0.001.*

**TABLE 6 |** Depression recognition performance measures from 10-fold cross validation.

| | Sensitivity | Specificity | AUC |
|---|---|---|---|
| Spatiotemporal features | 0.59 | 0.58 | 0.58 |
| Time-domain features | 0.89 | 0.78 | 0.83 |
| Frequency-domain features | 0.86 | 0.88 | 0.87 |
| Spatiotemporal features + time-domain features | 0.89 | 0.78 | 0.83 |
| Spatiotemporal features + frequency-domain features | 0.82 | 0.83 | 0.83 |
| Time-domain features + frequency-domain features | 0.94 | 0.91 | 0.93 |
| All features | 0.94 | 0.91 | 0.93 |

## The Recognition of Depression

In **Table 6**, sensitivity, specificity and AUC of the classification models are presented. **Figure 5** displays the receiver operating characteristics (ROC) curves of different classification models, showing the balance between sensitivity and specificity throughout the decision space. When only spatiotemporal features were used to classify depression, the classification accuracy (AUC) was 0.58. When only time-domain features were used to classify depression, the classification accuracy (AUC) was 0.83. When only frequency-domain features were used to classify depression, the classification accuracy (AUC) was 0.87. The classification accuracy (AUC) achieved 0.83, when both spatiotemporal features and time-domain features were used to classify depression. The classification accuracy (AUC) achieved 0.83, when both spatiotemporal features and frequency-domain features were used to classify depression. The best accuracy

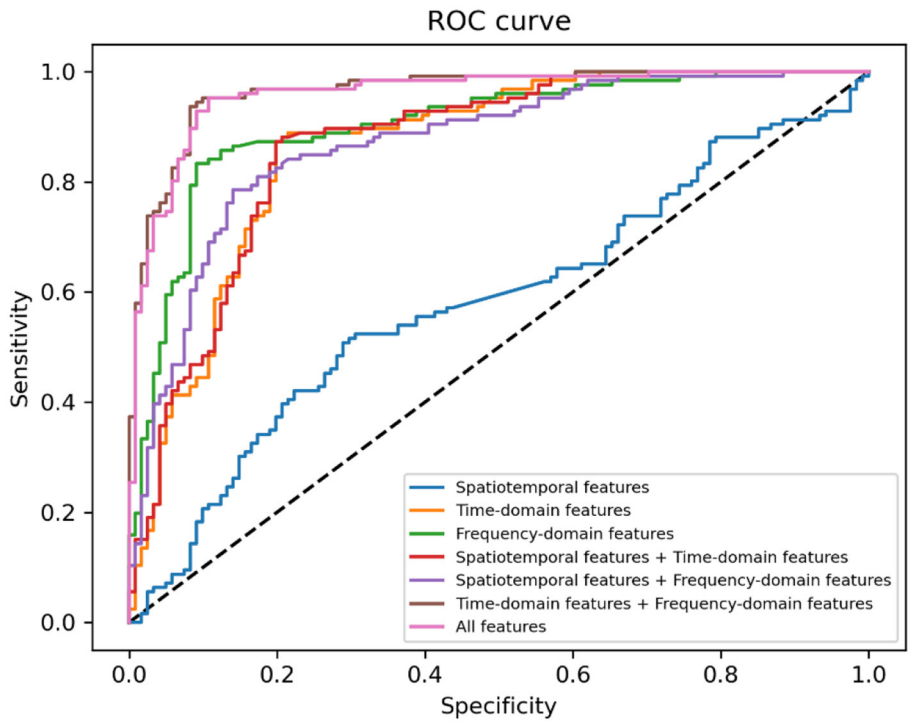



**FIGURE 5 |** Receiver operating characteristics (ROC) curve for different machine learning methods.

(AUC) is 0.93, when both time- and frequency-domain features or all features were used to classify depression.

## DISCUSSION

In this study, several machine learning models for detecting depression were trained using gait data captured via Kinect. Ten spatiotemporal features were extracted by following the approaches of previous studies. Besides, 300 time- and 825 frequency-domain features were extracted using time-frequency analysis methods. The results of multiple logistic regression analysis showed that the impacts of spatiotemporal, time-domain, and frequency-domain features on the dependent variable (depression diagnosis) were 12.55%, 58.36%, and 60.71% respectively. The classification models consisting of the above features were found effective in detecting depression. The performance of the optimal model was very outstanding (sensitivity = 0.94, specificity = 0.91, and AUC = 0.93). These findings address the primary goals of the study, which suggest that (1) depression can be reflected in gait, with different types of gait features contributing differently to depression detection and (2) machine learning is an effective approach to recognize depression.

One critical finding in this study is that gait patterns associated with depression are characterized by reduced arm swing and head posture, especially left-arm swing and head posture are predictors of depression in the logistic regression model, which is consistent with previous studies that found that patients with depression tended to walk with reduced movements (e.g., arm swing, head movements) (23, 39, 40). Furthermore, this study validated the effectiveness of both time- and frequency-domain features in recognizing depression, which is also consistent with previous studies (41, 42). However, significant time- and frequency-domain PCs can only provide us with mathematical relationships between gait patterns and time- and frequency- domain features. Although further insights can be gained by calculating the correlation coefficients between each significant PC and original features (as shown in **Supplementary Tables 1, 2**), they are also not intuitive. It is worth noting that while high-level spatiotemporal features may provide an intuitive understanding of individual gait patterns, they contribute less to depression recognition than low-level time- and frequency- domain features. The limited information contained in spatiotemporal features restricts the possibility of understanding the disorganization of gait control and early detection of gait impairments (49, 50), which is why the clinical gait assessment based on spatiotemporal features (e.g., gait speed) is mainly applicable to monitoring the overall health status of a large population (25).

It is worth noting that the models built of time- domain features and spatiotemporal and time- domain features have the same performances, which suggests that spatiotemporal features had very few contributions to recognize depression. It is also reflected in the fact that the model comprised of time- and frequency-domain features has the same performance as the model consisted of all features. These results are consistent with those reported in previous studies that demonstrated the superiority of signal features such as time- and frequency-domain features over spatiotemporal parameters (42). In

summary, time- and frequency-domain features are more efficient in constructing computational models to identify depression than spatiotemporal features.

This study is the first attempt to systematically investigate the impact of different types of gait features on depression recognition. The proposed method allows recognizing depression in real-time and remotely, which can be useful when immediate clinical assessment may not be available. This method is able to overcome many disadvantages of psychological questionnaires (24) include time-consuming (51), recall bias (52), and desirability bias (53). Furthermore, the proposed use of Kinect can be practical in daily-life settings since the devices are low-cost, widely available, and do not require any markers or sensors to be attached to the body. Therefore, the method proposed in this paper holds a promise for detecting depression on a fine-grained scale with ecological validity and low economic burden.

This study has several limitations. First, although Kinect is widely used for estimating body posture and movement (29, 31, 32), it may not record spatiotemporal body data as accurately as more expensive 3D motion capture systems. Second, we could not extract many indicators (e.g., skewness, kurtosis) because the footpath in our experiment is short (6 meters) that resulted in the length of valid gait data we could analyze was short (two cycles) as well. These indicators were computed by high-level spatiotemporal features that could have more contribution to depression recognition if they could be extracted from a longer time gait data. Third, the aim of this study is to examine the contribution of gait features in recognizing depression, thus there is a lot we can optimize to get better model performance (e.g., experimenting with more complex filters, tuning hyper-parameters of machine learning models) from the perspective of engineering.

## CONCLUSIONS

This study demonstrated that gait characteristics could be effectively utilized to identify depression, while gait-related features were used for building machine learning models. According to experimental results, spatiotemporal features are appropriate for interpreting gait patterns, while time- and frequency- domain features are effective in depression recognition. In conclusion, the study is a step forward toward developing low-cost, non-intrusive solutions for real-time depression recognition. In the future, this proposed method might be applied in both hospitals to aid diagnosis and scenarios that require a simple and rapid large-scale investigation.

## DATA AVAILABILITY STATEMENT

The datasets generated for this article are not readily available because the raw data cannot be made public, if necessary, feature data can be provided. Requests to access the datasets should be directed to liuxiaoqian@psych.ac.cn.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institutional Review Board of the Institute of Psychology, Chinese Academy of Sciences (approved number: H15010). The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

YW, JW, XL, and TZ contributed to conception and design of the study. YW organized the database and wrote the first draft of the manuscript. YW and JW performed the formal analysis. XL, JW, and TZ contributed to the writing-review and editing of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2021.661213/full#supplementary-material

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# Mapping the Presence of Anxiety Symptoms in Adults With Major Depressive Disorder

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**Background:** Patients with major depressive disorder (MDD) often present with co-occurring anxiety symptoms. The network method provides a novel view on understanding the co-occurrence of depressive and anxiety symptoms. Thus, the purpose of our study was to explore it by applying network analysis methods.

**Methods:** We used electronic medical records from West China Hospital in China. In total, 3,424 patients who met the criteria for MDD were included. R-studio 3.6 was used to estimate the network structure. First, we estimated the network structure of depression and anxiety symptoms using the graphic LASSO algorithm. Then, we estimated the centrality indices of nodes to determine which symptoms are more central in the network. We then estimated the bridge centrality indices using the *bridge* function via the R package *networktools.*

**Results:** Some strong connections were found like "easy to wake up," "wake up early," and "difficulty falling asleep," "suicidal thoughts," and "hopelessness." "Depressed mood," "somatic anxiety," "hopelessness," "anxiety mood," and "tension" have the higher centrality indices. Results revealed eight bridge symptoms (e.g., concentration/memory difficulty, gastrointestinal symptoms) in the co-occurrence network structure.

**Conclusions:** This research suggests that the described approach in mapping the presence of anxiety symptoms in individuals with major depression might potentially increase diagnostic precision and help choose more targeted interventions and potentially reduce the occurrence of treatment resistance.

**Keywords: major depressive disorder, anxiety, co-occurrence, network analysis, psychopathology**

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## INTRODUCTION

*Major depressive disorder* (MDD) is a debilitating psychiatric condition characterized by depressed mood, decreased energy level, and lack of interest in pleasurable activities (1). It has a significant burden on both individuals and society and is related to psychological impairments and health dysfunction. Among Chinese adults, MDD has a 12-month and lifetime prevalence rate of 3.6 and 6.9%, respectively (2). Gaspersz's study showed that 40–60% of patients with MDD also have anxiety symptoms (3). Another survey suggested that 50% of individuals with MDD meet the diagnostic criteria for anxiety disorders (4, 5).

The co-occurrence anxiety symptoms among patients with MDD have important clinical implications. Firstly, the co-occurrence anxiety symptoms predict a more chronic course and more severe disease progression (6). Secondly, compared with individuals who have MDD without anxiety symptoms, patients influenced by anxiety and depressive symptoms have shown greater functional disability, poorer quality of life, and higher risk of suicide behavior (7, 8). Thirdly, MDD with prominent coexisting anxiety symptoms is more difficult to treat than MDD without anxiety symptoms (9). Fourth, anxiety symptoms are associated with occupation of more health care resources among patients with MDD (10). Previous studies either concentrated on the count symptoms to clarify a diagnosis and calculate the prevalence rate of comorbidity or regarded the symptoms as indicators of latent dimensions, however, the between-symptom links are considered a byproduct of dimensional community (11).

Network theory provides us a novel perspective of mental disorders. Network analysis conceptualizes symptoms as constituents of mental disorders, as compared to traditional methods that assume an underlying disease in advance as the common cause of symptoms. Recently, network analysis models are rapidly growing, not only concerning methodological issues but also in offering an appealing interpretation of psychopathology (12, 13).

From the perceptive of topology, network structure consists of nodes(symptoms) and edges(association between symptoms) (14). The importance of nodes was evaluated via centrality indices. Edges represent the links between pairs of symptoms and thicker edges denote larger correlations (15). Specifically, centrality indices include strength, closeness, and betweenness that allow clinicians to discern the symptoms with the greatest importance in the network structure. Those with high centrality indices convey more clinical information (16, 17). Strength is one of the most commonly used centrality indices as it is easy to interpret and is the most stable centrality index (18). In the opinion of network theory, comorbidity is regarded as a constellation of symptom-level relationships (19). Symptoms that link two mental disorders are regarded as "bridge symptom." Bridge symptoms indicated by bridge centrality indices mainly included bridge strength, bridge closeness, and bridge betweenness (20). Bridge symptoms may have an important role in the development and maintenance of co-occurring mental disorder (21). Thus, when one mental disorder presents, intervention on potential bridge symptoms may contribute to preventing co-occurrence (20). For example, if we suppose that sleep disturbance is a bridge symptom between depression and anxiety, then patients who suffer poor sleep quality as one of their MDD symptoms would be at greater risk for anxiety compared to those without sleep disturbance. Thus, it would be wise for psychiatrists to treat these bridge symptoms therapeutically to reduce the co-occurrence.

At present, 10 studies utilized network analysis to explore comorbidity and co-occurrence in depression (22–31). Previous studies have explored the comorbidity between anxiety and depression (23), posttraumatic stress disorder and co-morbidity depressive symptoms (31), and comorbidity between obsessive-compulsive disorder and depression (26). While previous network researches in MDD have broadened our knowledge of the field, several limitations remain. Firstly, most studies use self-reported questionnaires (e.g., Patient Health Questionnaire-9 and General Anxiety Disorder-7) to assess symptoms among the general population (29, 30), while self-report questionnaires have a risk for response biases (32). Few studies used a sample that comprised treatment-seeking patients with MDD (23). Secondly, while sample size plays an important role in establishing a reliable network structure, most studies used a small sample size that ranges from 296 (25) to 1,029 (23, 33). Thirdly, no studies to date have explored the co-occurrence between MDD and anxiety symptoms based on real-world evidence. Real-word evidence is important for establishing the network structure of co-occurrence between MDD and anxiety symptoms. In this regard, electronic medical records (EMRs) bring new chances in clinical research, providing the potential chance for low-cost and high-volume data on clinical research (34).

The main aim of this study, which was based on the retrospective of EMRs, was to establish the co-occurring network structure between MDD and anxiety symptoms to identify the bridge symptoms.

# METHODS

## Study Population

This was a retrospective study based on pre-existing data derived from EMRs undertaken at West China Hospital. Eligible patients were those with a diagnosis of MDD, aged between 18 and 65. In this study, we identified MDD patients through recorded primary diagnosis at discharge and based on the International Classification of Disease, Tenth Revision (Clinical Modification Codes F32, single episode major depressive disorder and F33, recurrent major depressive disorder), which has been described in another study (35). We extracted anonymous clinical-related information. Exclusion criteria are the following 3 items. ① Patients' follow-up discharge diagnosis code is F30 or F31. ② Patients with MDD did not accomplish Hamilton Depression Scale-24 (HAMD-24) and Hamilton Anxiety Scale-14 (HAMA-14) at admission. ③ Age $\leq$ 18 or age $>$ 65. The details are shown in **Figure 1**. The data used to support the findings of this manuscript are restricted by the West China Hospital in order to protect patient privacy and avoid legal and ethical risks. Data are available from the West China Hospital for researchers who meet the criteria for access to confidential data (data.cd120.com).

We obtained approval from the Ethics Committee of the West China Hospital, Sichuan University (2017 N0.185). As this is a retrospective study, Institute Review Board (IRB) waived the requirement for obtaining informed consent from the individual patients. We did not use information on the identity of patients and all related information was kept confidential. All procedures were in accordance with the ethical standards of the Ethics Committee and the revised Helsinki Declaration of 2008 (36, 37).

## Measures

HAMD-24 is one of the most widely used scales in clinical practical assessment of depression. It is assessed by psychiatrists and takes about 15 to 20 min to finish (38). HAMA-14 is one of

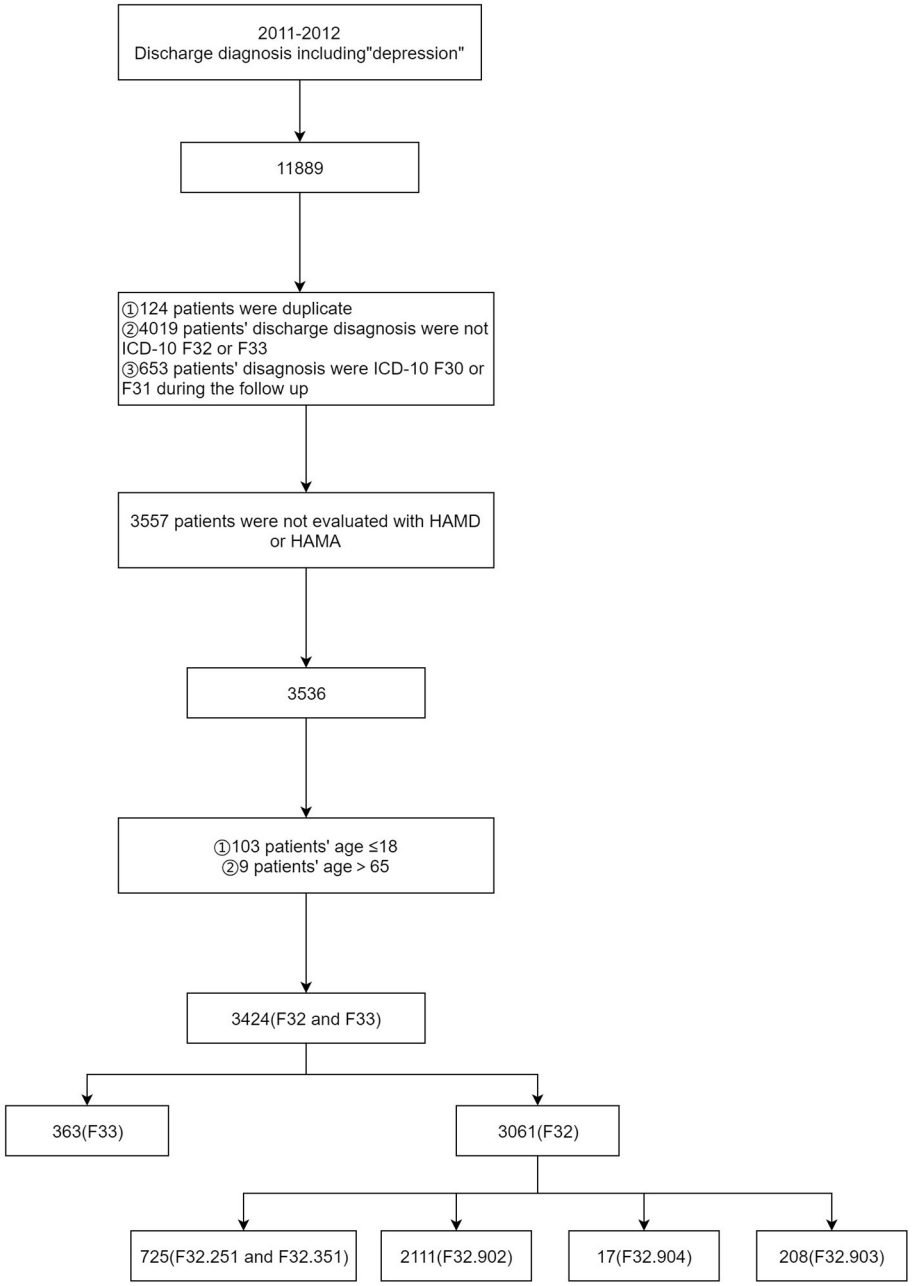


**FIGURE 1 |** The process of data extraction.

the most widely used scales in the clinical practical assessment of anxiety. HAMA-14 includes 14 items with each item divided into 5 levels from 0 to 4 (39). The Chinese versions of HAMD-24 and HAMA-14 have good reliability and validity (40).

## Data Analysis

All analysis was accomplished using the R-3.6 studio. In this research, the missing data belong to the category of missing at random (MAR). So, we used the method of unconditional mean imputation to handle the missing data (41). The network structure consists of two elements: nodes and edges. Every node represents a symptom, and each edge demonstrates a relationship between the two symptoms. In our study, the nodes (symptoms) were represented the scale items of HAMD-24 and HAMA-14. We used a graphical Gaussian model to estimate the networks. In addition, we used the least absolute shrinkage and selection operator (LASSO) to regularize our model and used the *qgraph* package to visualize the network. Next, we computed the centrality indices (i.e., betweenness, strength, and closeness) of nodes to find which symptoms are more central in

the network structure. Betweenness and closeness are often not reliably estimated (17). Thus, we only reported the strengths in this article, while other node centrality indices are provided in the **Supplementary Material**. We assessed the accuracy stability of the centrality using the bootstrap approach in the *bootnet* package. To gain a stable and interpretable centrality, the CS coefficient should be >0.25 (42). We estimated the stability of edge-weights by bootstrapping the 95% confidence intervals (CIs), where fewer overlaps in the CIs indicate higher stability. Jones uses the term community to demonstrate a theoretically based group of nodes that correspond to a mental disorder according to clinical criteria, instead of according to any network analytic procedure (20). This method contributes to identifying bridge nodes, especially when networks are large, complex, or difficult to account for visually. The *networktools* package (43) were used to calculate the values and create plots. You can get the code from the Git hub (https://github.com/fenfenge/Network-structure).

# RESULTS

## Descriptive Statistics

We included 3,424 patients with MDD comprising 2,349 females and 1,075 males, with ages ranging from 18 to 65 ($M = 42.5$, SD $= 13.25$). **Table 1** shows the items, item content, sample means, standard deviation, and missing items/percentage.

## Network Estimation

The graphical LASSO network is shown in **Figure 2**. A thicker edge indicates a stronger association between the symptoms. Green edges represent positive regularized partial correlations, and red edges represent negative regularized partial correlations. Network analysis demonstrated that five strong connections edges were among the HAMD-24 items. The top edge was between the items "easy to wake up," "wake up early," and "difficulty falling asleep" (D4: D5: D6). Additionally, HAMD-24 items "suicidal thoughts" and "hopelessness" (D3:D23) and "hopelessness" and "inferiority" (D23:D24) were among the strongest. There were two strongest edges between the HAMA-14 items. The strongest edge was between items "tension" and "afraid" (A2:A3), followed by "cardiovascular symptoms" and "respiratory symptoms" (A9:A10).

## Network Inference and Stability

Strength centrality is shown in **Figure 3**. Firstly, nodes demonstrating "depressed or sad mood" (D1), "somatic anxiety" (D11), and "hopelessness" (D23) were among the MDD symptoms exhibiting higher levels of strength. Secondly, "anxiety mood" (A1) and "tension" (A2) were among the anxiety symptoms exhibiting higher levels of strength. In terms of stability of network analysis, bootstrap 95% CI demonstrated moderate stability for the strength index (**Supplementary Materials**).

Bridge strength is shown in **Figure 4**. Nodes demonstrating "anxiety mood" (A1), "insomnia" (A4), "concentration/memory difficulty" (A5), "pessimism" (A6), and "gastrointestinal symptoms" (A11) were anxiety symptoms displaying higher levels of bridge strength on all MDD symptoms. The MDD symptoms "nervousness or anxiety" (D10), "somatic anxiety" (D11), and "gastrointestinal symptoms" (D12) exert a strong bridging effect on anxiety symptoms.

**TABLE 1 |** Items, item content, missing items, means, and standard deviations for HAMD-24 and HAMD-14.

| Item | Item content | Missing items/Percentage | M | SD |
|---|---|---|---|---|
| D1 | Depressed or sad mood | 0/0.00% | 2.55 | 0.95 |
| D2 | Guilty feelings | 0/0.00% | 0.88 | 0.95 |
| D3 | Suicidal thoughts | 0/0.00% | 1.07 | 1.15 |
| D4 | Difficulty falling asleep | 0/0.00% | 1.55 | 0.97 |
| D5 | Wake up early | 0/0.00% | 1.37 | 0.91 |
| D6 | Early awakening | 0/0.00% | 1.28 | 0.95 |
| D7 | Loss of interest/pleasure | 0/0.00% | 1.98 | 1.02 |
| D8 | Psychomotor retardation | 0/0.00% | 0.95 | 0.93 |
| D9 | Agitation | 0/0.00% | 0.61 | 0.88 |
| D10 | Nervousness or anxiety | 0/0.00% | 1.80 | 1.03 |
| D11 | Somatic anxiety | 0/0.00% | 1.55 | 1.07 |
| D12 | Gastrointestinal symptoms | 2/0.06% | 0.78 | 0.88 |
| D13 | Somatic symptoms | 2/0.06% | 0.93 | 0.89 |
| D14 | Loss of interest in sex | 5/0.15% | 0.32 | 0.69 |
| D15 | Hypochondria | 2/0.06% | 0.63 | 0.90 |
| D16 | Loss of weight | 2/0.06% | 0.61 | 0.82 |
| D17 | Insight | 2/0.06% | 0.46 | 0.68 |
| D18 | Changes in mood patterns | 2/0.06% | 0.45 | 0.68 |
| D19 | Depersonalization/Derealization | 7/0.20% | 0.13 | 0.43 |
| D20 | Paranoid symptoms | 8/0.23% | 0.28 | 0.65 |
| D21 | Obsessive-compulsive symptoms | 8/0.23% | 2.00 | 0.55 |
| D22 | Feel less capable | 8/0.23% | 1.48 | 1.02 |
| D23 | Hopelessness | 8/0.23% | 1.38 | 1.07 |
| D24 | Inferiority hopelessness | 8/0.23% | 1.14 | 1.00 |
| A1 | Anxiety mood | 52/1.52% | 2.01 | 0.97 |
| A2 | Tension | 51/1.49% | 1.51 | 0.99 |
| A3 | Afraid | 51/1.49% | 1.22 | 1.01 |
| A4 | Insomnia | 51/1.49% | 1.85 | 1.30 |
| A5 | Concentration/memory difficulty | 52/1.52% | 1.18 | 0.92 |
| A6 | Pessimism | 56/1.64% | 2.26 | 0.97 |
| A7 | Muscular symptoms | 60/1.75% | 0.71 | 0.94 |
| A8 | Sensory symptoms | 60/1.75% | 0.87 | 0.98 |
| A9 | Cardiovascular symptoms | 60/1.75% | 0.87 | 0.97 |
| A10 | Respiratory symptoms | 60/1.75% | 0.57 | 0.82 |
| A11 | Gastrointestinal symptoms | 61/1.78% | 0.84 | 1.00 |
| A12 | Genitourinary symptoms | 60/1.75% | 0.36 | 0.69 |
| A13 | Automatic symptoms | 60/1.75% | 1.05 | 0.98 |
| A14 | Behavior during the talk | 60/1.75% | 1.23 | 0.99 |

# DISCUSSION

Many patients with MDD also experience anxiety symptoms. As far as we know, this is the first research to explore network connectivity among treat-seeking patients with MDD

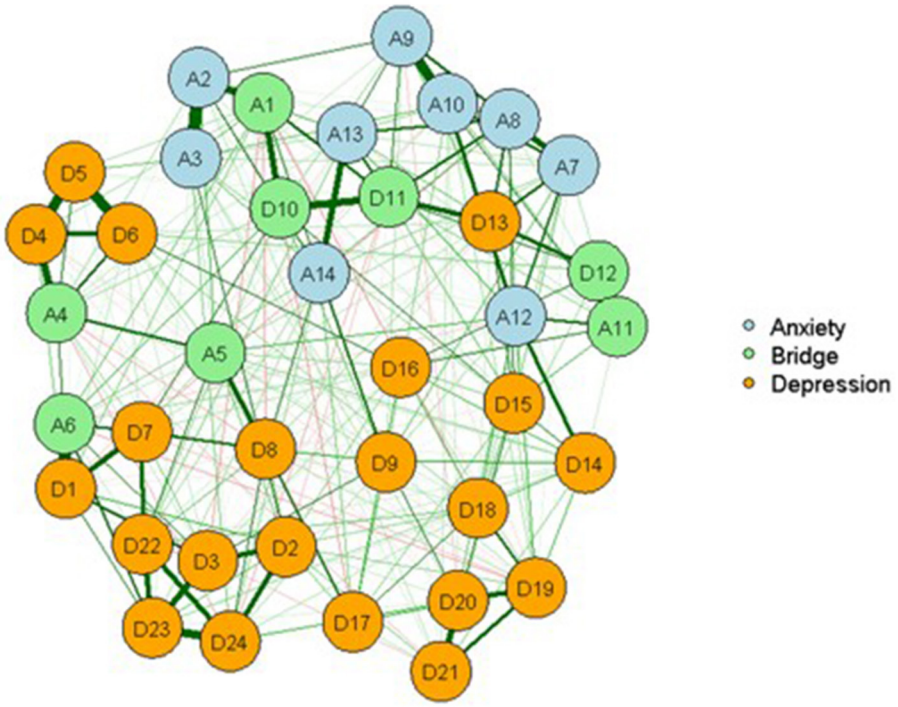


**FIGURE 2 |** Network of MDD and anxiety symptoms showing bridge symptoms among clinical samples. D1: Depressed or sad mood; D2: Guilty feelings; D3: Suicidal thoughts; D4: Difficulty falling asleep; D5: Easy to wake up; D6: Early awakening; D7: Loss of interest/pleasure; D8: Psychomotor retardation; D9: Agitation; D10: Nervousness or anxiety; D11: Somatic anxiety; D12: Gastrointestinal symptoms; D13: Somatic symptoms; D14: Loss of interest in sex; D15: Hypochondria; D16: Loss of weight; D17: Insight; D18: Changes in mood patterns; D19: Depersonalization/Derealization; D20: Paranoid symptom; D21: Obsessive-compulsive symptom; D22: Feel less capable; D23: Hopelessness; D24: Inferiority hopelessness; A1: Anxiety mood; A2: Tension; A3: Afraid; A4: Insomnia; A5: Concentration/memory difficulty; A6: Pessimism; A7: Muscular symptoms; A8: Sensory symptoms; A9: Cardiovascular symptoms; A10: Respiratory symptoms; A11: Gastrointestinal symptoms; A12: Genitourinary symptoms; A13: Automatic symptoms; A14: Behavior during the talk.


**FIGURE 3 |** Plot of standardized centrality indices for network. D1: Depressed or sad mood; D2: Guilty feelings; D3: Suicidal thoughts; D4: Difficulty falling asleep; D5: Easy to wake up; D6: Early awakening; D7: Loss of interest/pleasure; D8: Psychomotor retardation; D9: Agitation; D10: Nervousness or anxiety; D11: Somatic anxiety; D12: Gastrointestinal symptoms; D13: Somatic symptoms; D14: Loss of interest in sex; D15: Hypochondria; D16: Loss of weight; D17: Insight; D18: Changes in mood patterns; D19: Depersonalization/Derealization; D20: Paranoid symptom; D21: Obsessive-compulsive symptom; D22: Feel less capable; D23: Hopelessness; D24: Inferiority hopelessness; A1: Anxiety mood; A2: Tension; A3: Afraid; A4: Insomnia; A5: Concentration/memory difficulty; A6: Pessimism; A7: Muscular symptoms; A8: Sensory symptoms; A9: Cardiovascular symptoms; A10: Respiratory symptoms; A11: Gastrointestinal symptoms; A12: Genitourinary symptoms; A13: Automatic symptoms; A14: Behavior during the talk.

and co-occurrence anxiety symptoms based on real-world evidence. Perhaps the most prominent result was that not all nodes(symptoms) were equally important in the network with co-occurrence anxiety symptoms among patients with MDD (44, 45). Besides, we also found some strong linkage between symptoms, such as "easy to wake up" (D4), "wake up early" (D5),

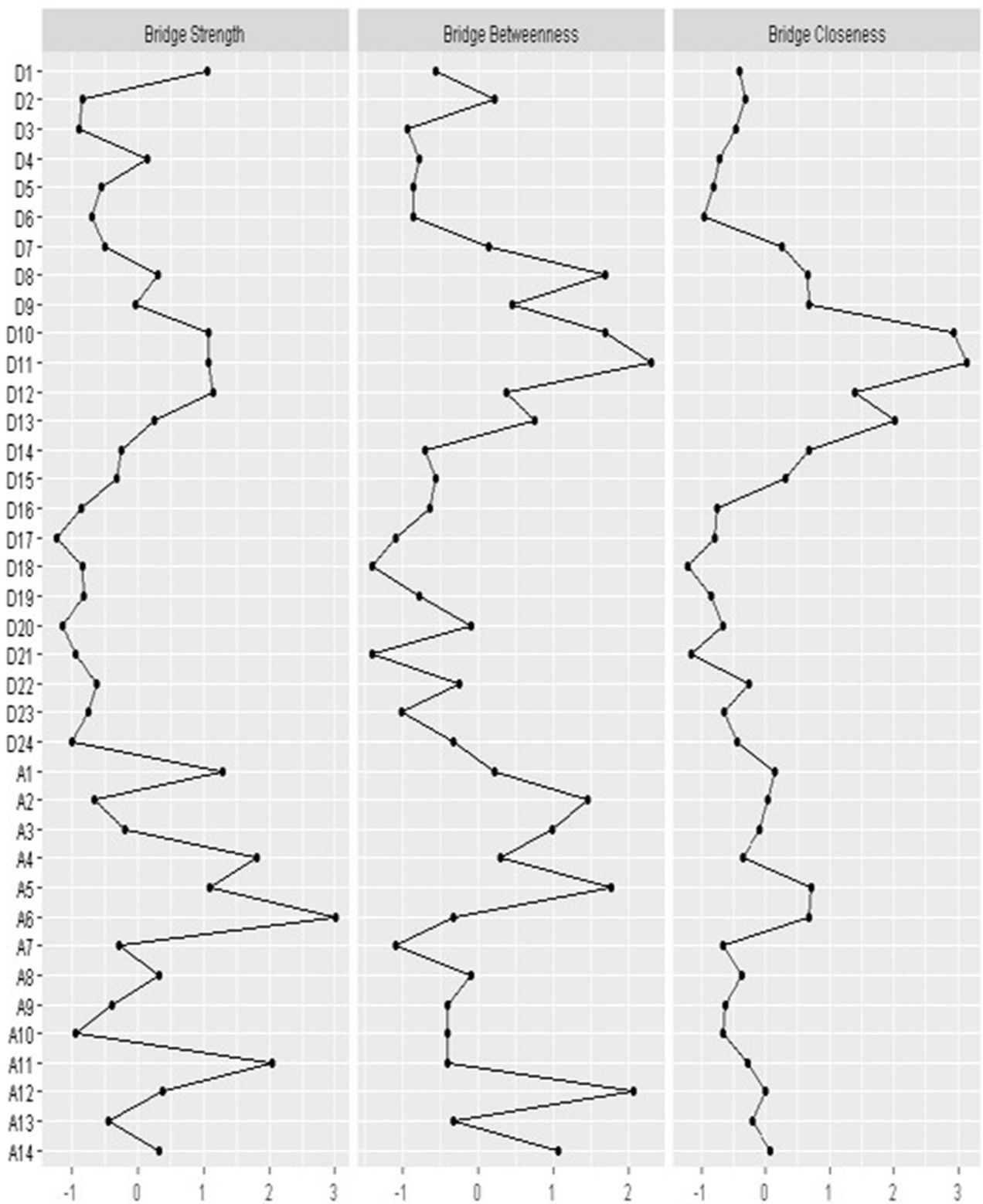


**FIGURE 4 |** Bridge centrality indices of network structure of MDD and anxiety symptoms.

and "difficulty falling asleep" (D6), which were closely associated with each other.

Moreover, "depressed or sad mood" (D1) and "hopelessness" (D23) have the highest centrality in the network structure. Such results do not come as a surprise; previous studies that used self-report questionnaires and composite international diagnostic interviews also suggested that sad mood and hopelessness carry more weight than other symptoms of MDD (14, 46–48). "Somatic anxiety" (D11) had one of the highest centrality indices, making it a hallmark symptom of MDD among Chinese. "Somatic anxiety" is involved via overt signs of excessive autonomic activity and/or skeletal/muscle-motor tension. Due to the following three reasons that including (1) emotional symptoms are stigmatized, (2) thinking deviated far from internal experiences, (3)a special perspective of the self is emphasized, (49) somatization has been a general symptom in the Chinese population (50, 51). It is worthy that higher somatization was related to poorer performance in the cognitive and poorer antidepressant treatment clinical outcome (52). Anxiety symptoms (e.g., anxiety mood and tension) also had higher centrality. Goghari's 2-year longitudinal study found that patients with MDD reported higher levels of anxiety than those with other mental disorders. To some extent, easing the patient's anxiety level helps improve the outcome and daily function of depression (53).

Network theory helps to understand the co-occurrence of psychopathology. We could reduce co-occurrence by effectively "burning the bridge symptoms" between disorders (45). In this study, the MDD nodes that displayed the strongest association with anxiety symptoms were "nervousness" (D10), "somatic anxiety" (D11), and "gastrointestinal symptoms" (D12). Conversely, the anxiety nodes that displayed the strongest connection with MDD symptoms were "anxiety mood" (A1), "insomnia" (A4), "concentration/memory difficulty" (A5), "pessimism" (A6), and "gastrointestinal symptoms" (A11). Gastrointestinal symptoms were bridge symptoms that link MDD to anxiety symptoms and similarly linked anxiety symptoms to MDD. Gastrointestinal symptoms are common features for both MDD and anxiety disorders. They are also prominent features in posttraumatic stress disorder, schizophrenia, and autism spectrum disorder (54–56), demonstrating that gastrointestinal symptoms may be a common feature associated with a multitude of mental disorders. Simpson's review indicated that the role of gut microbiota in mood regulation and emotional processing may be of particular relevance to depression and anxiety etiology (57). In this study, concentration/memory difficulty was a bridge symptom that linked anxiety to MDD. Pettit's research found that computer-based attention training can reduce anxiety symptoms among the youth (58). These results suggested that attention training could be used to ease anxiety symptoms among MDD patients.

We found a strong connection between "easy to wake up," "wake up early," and "hard to fall asleep" (D4: D5: D6). Interestingly, the three symptoms above belong to the diagnostic criteria for insomnia (59) and MDD patients with sleep complaints are prone to more severe symptoms (60). Thus, insomnia is a valuable therapeutic target in MDD patients. The theoretical promise of network analysis is that psychiatric symptoms are not isolated and may accentuate each other (61). Symptoms may reinforce one another via creating a feedback loop, such as, "inferior helplessness" (D24) could lead to "hopelessness" (D23), and at the same time prompt "suicidal thoughts" (D3). These links may contribute to the build of a self-sustained symptom constellation. Thus, it is necessary to find potential self-sustaining mechanisms and timely interventions for feedback loops (13). Somatic symptoms (e.g., cardiovascular symptoms and respiratory symptoms) were closely connected. This can be explained by the mind-body interaction model, which states that information flows not only from the body to the brain but also from the brain to the body. In the brain, "prediction" is derived from metacognition (conceptual knowledge), namely higher-order thoughts and cognition, which helps estimate the generation of behavioral commands (62).

## Clinical Implication

The network model provides a novel view to investigate the potential mechanisms underlying the etiology and maintenance of mental disorders. We can understand how the symptoms are interrelated via networks and how to intervene on comorbidities. Firstly, when depressive patients are admitted to the hospital, psychiatrists should prioritize the evaluation of identified bridging MDD symptoms to screen patients with a higher risk of suffering anxiety symptoms. Secondly, from the viewpoint of network structure, we can intervene mental disorders from the following aspects: (1) symptoms (nodes) interventions: direct intervention of one or more symptoms; (2) network interventions: intervention symptom-symptom connections (61); (3) bridge symptoms: intervention of bridge symptoms to prevent co-occurrence. For example, we can intervene on closely connected sleep symptoms to avoid using certain antidepressants that may cause or even worsen sleep problems (63), since improving sleep contributes to improving the outcomes of MDD (64, 65). Our findings may be used to demonstrate which symptoms (e.g., gastrointestinal symptoms, insomnia) drive the association, and which should be handled first to reduce MDD and anxiety co-occurrence. These findings emphasize the importance of assessing anxiety symptoms among patients with MDD. Owing to the limitations of earlier versions of the Diagnostic and Statistical Manual of Mental Disorder (DSM) on grading targeting rules, anxiety in mental disorders has been underestimated, underdiagnosed, and undertreated. CBT is very effective for the treatment of anxiety symptoms and sleep disturbance (66). Thus, patients with MDD can be treated with suitable medicines and CBT.

## Strengths and Limitations

This research has several key strengths. First, we employed a sample comprised of treatment-seeking patients diagnosed with MDD according to the ICD-10. Moreover, HAMD and HAMA are evaluated by psychiatrists; thus, ambiguous/vague answers to the self-assessment scale were avoided. Second, we used a relatively large sample that contributed to establishing a reliable network structure with robust edge weights and centrality (42). To date, only one study has used large psychiatric samples of over 1,000 participants (23).

Despite the strengths, some limitations should be considered in this study. Firstly, this is a cross-sectional study, and we cannot explore changes in the co-occurrence network structure over time. Specifically, we are not sure whether the connections between symptoms appear temporarily or continuously, and what will happen to those strong connections under the intervention of strategy. Considering that network modes require the estimation of many parameters and these models need power to reliably detect small coefficients, exploration of larger data sets is necessary (67, 68). Thus, in this study, we do not compare the network structure in different severity of subgroup. Secondly, in our study, the target population was patients with MDD diagnosis so that the results could be generalized to the whole population. Specifically, the results do not suit individuals who suffer from certain depression symptoms, while failing to meet the diagnostic criteria for MDD. Third, Gureje's study showed that the presentation of mental disorders is influenced by culture and social milieu (69), while we only focused on Chinese patients. Future studies should consider the cross-cultural variation and explore how culture influences the presentation of mental disorders. Finally, we included patients with ICD-10 F32 and F33 diagnoses only. We did not check whether the patients had a comorbidity diagnosis with an anxiety disorder or any other co-morbidity. However, Wise's study (70) may support the notion that comorbidities might not be so important, at least concerning functional connectivity. Specifically, Wise's research supports the notion that biological abnormalities in functional connectivity in major depression across independent samples might overlap irrespective of the presence of anxiety comorbidities.

## CONCLUSION

This research is the first to explore the association between depressive and anxiety symptoms among MDD patients based on EMRs, thus offering an essential basis on how the two disorders co-vary. We found that some high central symptoms (e.g., hopelessness, somatic anxiety, and tension) and some bridge symptoms (e.g., concentration/memory difficulty, pessimism, and gastrointestinal symptoms). We summarize the evidence from the current research that treatment for co-occurring anxiety symptoms among MDD patients at symptom level may be efficacious. Multiple interventions, such as improving sleep, CBT, or attention training, could be applied to address these co-occurring symptoms.

## DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: The dataset belong to the West China Hospital, Sichuan University. Requests to access these datasets should be directed to Jingwen Jiang, jiangjingwen@wchscu.cn.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of the West China Hospital, Sichuan University. The ethics committee waived the requirement of written informed consent for participation.

## AUTHOR CONTRIBUTIONS

FG participated in the study design, data analysis, interpretation of findings, literature search, writing, implementation, and approval of the final manuscript. WZ conceived and designed the study. JJ, YW, and MW participated in the study data analysis. All authors have approved the final manuscript.

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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.2021.595418/full#supplementary-material

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# Current Advances in Wearable Devices and Their Sensors in Patients With Depression

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In this study, a literature survey was conducted of research into the development and use of wearable devices and sensors in patients with depression. We collected 18 studies that had investigated wearable devices for assessment, monitoring, or prediction of depression. In this report, we examine the sensors of the various types of wearable devices (e.g., actigraphy units, wristbands, fitness trackers, and smartwatches) and parameters measured through sensors in people with depression. In addition, we discuss future trends, referring to research in other areas employing wearable devices, and suggest the challenges of using wearable devices in the field of depression. Real-time objective monitoring of symptoms and novel approaches for diagnosis and treatment using wearable devices will lead to changes in management of patients with depression. During the process, it is necessary to overcome several issues, including limited types of collected data, reliability, user adherence, and privacy concerns.

**Keywords: wearable devices, sensors, major depression, biomarkers in psychiatry, mood monitoring**

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## INTRODUCTION

The rapid development of wearable devices has led to their active use in research on depression. Sensors in wearable devices can collect physiological data related to mental health. These devices enable monitoring and assessment of patients in real time and in an unobtrusive way. In addition, the data collected through wearable devices can be monitored by the patient, and health care providers can receive and use the patient-generated health data to personalize healthcare. It is expected that the big data obtained by wearable technologies will facilitate the delivery of personalized, interactive, noncontact healthcare in a cost-effective manner.

Studies have shown that some types of data obtained from various wearable devices concern depression. Vallance et al. (1) reported that greater physical activity as measured by an accelerometer was correlated with lower rates of depression. Other research found that skin conductance as measured by wearable patches can be a sensitive biomarker for depression (2–4). A meta-analysis reported that heart rate variability (HRV), which is defined as spontaneous fluctuations in heart rate mainly reflecting the activity of the autonomic system, is reduced in patients with depression, even without concomitant cardiovascular disease (5, 6). HRV often is

measured by photoplethysmography (PPG) to determine changes in microvascular perfusion by illuminating the skin with light and measuring the transmitted or reflected amount (7).

Although the market of wearable devices is growing rapidly, the use of wearable technology for diagnosis and treatment of depression remains limited. Therefore, the purpose of this study was to provide a current overview of the developments and utility of wearable devices in research on depression. Here, we collected original studies examining wearable devices for diagnosis and treatment of depression and highlight the developments and trends in this field.

## METHODS AND RESULTS

To identify studies on the use of wearable devices in depression research, a literature search was performed in PubMed and the Web of Science databases, focusing on articles published prior to December 31, 2020. The specific search string was as follows: (wearable* OR actigraph* OR actigraphy OR actiwatch OR actimetry OR smartwatch OR wrist-worn OR "fitness tracker" OR "inertial sensor" OR "digital outcome measure") AND (depress* OR MDD OR "major depressive disorder" OR bipolar OR unipolar OR "affective disorder" OR "mood disorder"). This search string was applied only to article titles.

For this study, we excluded papers not written in English and the following types of manuscripts: conference papers, meeting abstracts, research notes, brief/short reports, letters, corrections, protocols, reviews, systematic reviews, meta-analyses, and editorial materials. We also excluded papers with an irrelevant theme (e.g., wearable bipolar batteries) or that were only distantly related to the aim of this study. In addition, we excluded papers that were not focused on depressive symptoms or focused on the general population or statistical and technical analysis. The search process is presented in **Figure 1**. We collected a total of 18 studies that made use of wearable devices to assess or monitor depressive symptoms or to predict major depressive disorder (MDD) (**Table 1**).

## DISCUSSION

### Overview

Among the collected studies on wearable devices for patients with depression, two-thirds used actigraph units, while the rest used other devices such as a novel wearable device with three accelerometers (18); medically used wearable devices, such as a Parkinson's KinetiGraph (PKG) (23) and the E4 wristband (Empatica, Boston, MA, USA) (25); and commercial wearable devices not originally intended for medical use, such as the Fitbit Flex™ (Fitbit, Inc., San Francisco, CA, USA) (19), Apple watch (Apple Inc., Cupertino, CA, USA) (20), and Silmee™ W20 wristband (TDK Corporation, Tokyo, Japan) (22). Among the reviewed studies, no wearable devices were used for treatment. In all studies, specific physiological, activity/sleep, or subjective parameters of individuals were collected through wearable devices, and the relationship between these parameters and depression was investigated. Except for one study that employed a waist actigraph unit (17), all included studies used wrist-worn devices to assess their study populations. Because wearing accessories on the wrist is nondistracting and familiar to most people, wrist-worn wearable devices have been used actively for research.

The use of actigraphy for monitoring depressive symptoms has been investigated since the 1990s and involves using wristwatch-like devices to collect activity or sleep data generated by movements (26). An actigraph unit is typically equipped with linear or a three-axis accelerometer to detect movements. Actigraph units are produced by various companies, with most studies (9, 11, 14, 15) using actigraph units manufactured by Cambridge Neurotechnology Ltd (Cambridgeshire, England). However, new devices and algorithms are being developed constantly, and there is no consensus regarding which device or algorithm is most appropriate for assessing patients with depression (26).

Some relevant medical devices include PKG and the Empatica E4 wristband. The PKG is a wristwatch-like device with a three-axis accelerometer originally designed to assess motor symptoms such as bradykinesia and tremor in patients with Parkinson's disease (27). However, Powell et al. (23) recently used it to assess motor symptoms in patients with depression. On the other hand, the Empatica E4 is a wrist-worn device that contains a PPG sensor capable of detecting heart rate, an electrodermal activity (EDA) sensor, an optical thermometer for measuring peripheral skin temperature, and the three-axis accelerometer for estimating motion and sleep characteristics. This device was used by Pedrelli et al. (25) to monitor changes in depressive symptom severity of patients.

Some consumer devices not originally indicated for medical use are the Fitbit Flex™, Apple watch, and Silmee™ W20 wristband. The Fitbit Flex™ is a commercially available fitness tracker equipped with a three-axis accelerometer that measures motion patterns of users. The utility of the Fitbit Flex™ in evaluating sleep patterns in depressive patients was compared against polysomnography (PSG) and actigraphy (19). On the other hand, the Apple watch is a so-called "smartwatch" featuring various apps as well as capabilities for phone calls and text messaging. It contains a small touchscreen, which was used by Cormack et al. (20) to offer tasks like cognitive and mood assessments to study participants. Finally, the Silmee™ W20 wristband was used previously (22) for unintended medical use and is equipped with a three-axis acceleration sensor, pulse sensor, ultraviolet sensor, and temperature sensor.

### Measured Parameters

Various parameters related to depression have been measured by each wearable device and can be classified as activity/sleep, physiological, and subjective parameters.

Activity characteristics, sleep patterns, and circadian rhythm can be estimated by accelerometers in wearable devices. Previous studies have consistently revealed the association between physical activity and MDD. People who do not regularly engage in physical activity are more likely to show depressive symptoms (28), and depression can be a significant risk factor for a sedentary lifestyle (29). Most studies employing

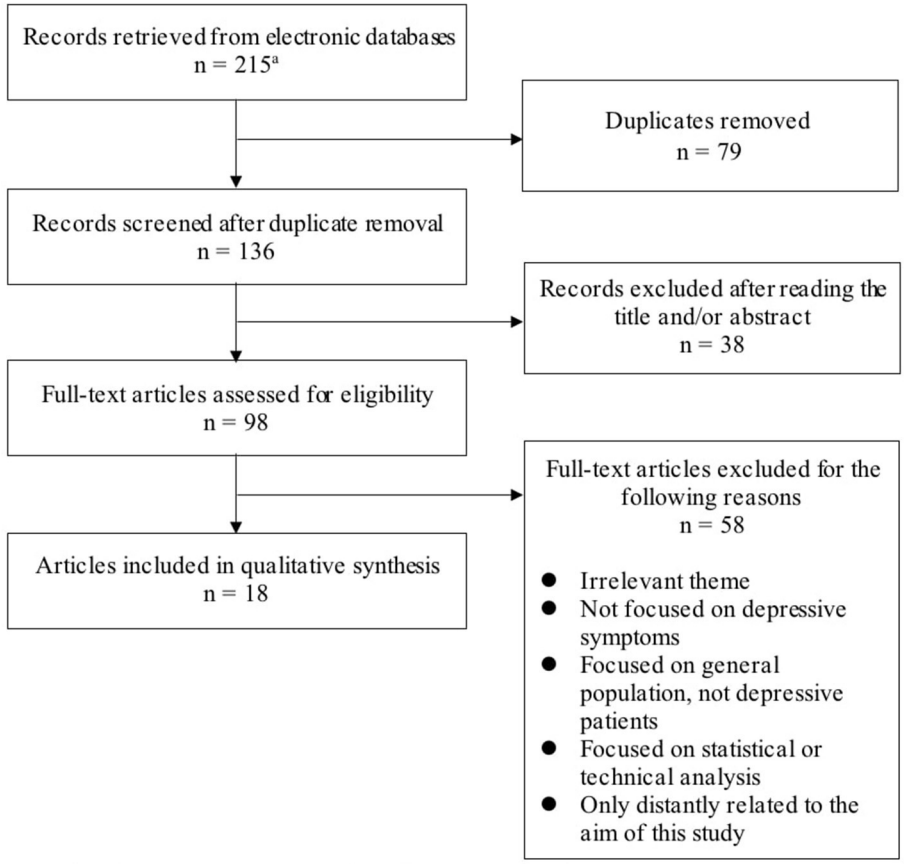


**FIGURE 1 |** Flow diagram of the literature search. [a]PubMed/Medline: *n* = 89; Web of Science: *n* = 126.

wearable devices have reported results consistent with these statements. When inpatients with MDD were treated with antidepressants, their activity level as measured by actigraphy was significantly increased upon discharge (8). Elsewhere, Winkler et al. (9) observed that activity was increased even by means of electroconvulsive therapy (ECT). Finally, Peis et al. (24) reported that increased motor activity and early patterns of actigraphic measures allowed for accurate prediction of hospital discharge date using a Hierarchical Generalized Linear Regression Model.

In a previous study, MDD patients with motor retardation had decreased level of activity compared with those without motor retardation (16). Meanwhile, Powell et al. (23) showed the potential for PKG to be used to evaluate movement patterns such as bradykinesia in patients with MDD.

Insomnia is a risk factor for depression, and sleep-related parameters can be used for prediction of depression (30). In addition, depressive patients tend to report more unsatisfactory sleep quality than healthy controls (31). However, this finding was not supported by most studies using actigraphy for sleep assessment of depressive patients, even though McCall and McCall (12) showed that actigraphic measurement of sleep is closely approximated those of PSG. Specifically, with mirtazapine medication for MDD patients, no significant improvement was found for actigraphic sleep measures (13). Also, both Winkler et al. (14) and Hoogerhoud et al. (15) reported that ECT did not affect actigraphy-assessed sleep. Rojo-Wissar et al. (21) suggested that maternal bonding characteristics were associated with sleep characteristics in adulthood but were not dependent on depression status. However, unlike other studies, Chung and Tso (10) showed that insomnia symptoms measured by actigraphy can be used to predict pain symptoms in MDD patients by Pearson correlation analysis, so further research is needed.

Several studies have shown that circadian rhythm–related parameters are correlated with depressive symptoms. Winkler et al. (9) reported that circadian rhythms in patients with seasonal affective disorder can be restored with bright light therapy (BLT). In another study, remitters had increases in circadian amplitude (the gap between the peak and the mean of a wave), but no significant changes were observed in circadian acrophase (the time at which the peak of a rhythm occurs) (14).

Skin temperature (ST), skin conductance (SC), and HRV as measured by an EDA sensor, optical thermometer, and PPG sensor, respectively, are physiological parameters collected by

**TABLE 1 |** Clinical trials with wearable devices in research on depression.

| References | Subjects | Devices | Methods | Main points |
|---|---|---|---|---|
| Raoux et al. (8) | Inpatients with MDD ($n = 26$). | Wrist actigraph | 24-h motor activity pattern monitoring at Days 0 and 28. | Activity level was increased after pharmaceutical treatments. |
| Winkler et al. (9) | Outpatients with seasonal affective disorder ($n = 17$). | Wrist actigraph | 4 weeks of activity monitoring with BLT in the morning. | BLT normalized disturbed activity patterns and restored circadian rhythms in seasonal affective disorder patients. |
| Chung and Tso (10) | Patients during an acute episode of MDD ($n = 91$). | Wrist actigraph | Actigraphic data collected twice over a 3-month period. | Sleep data measured by actigraphy may predict pain symptoms in MDD. |
| Razavi et al. (11) | Medicated inpatients with MDD ($n = 76$). | Wrist actigraph | 24-h actigraphic monitoring. | Motor-related single item "activities" of HAMD were associated with motor activity parameters, while the total score was not. |
| McCall and McCall (12) | Patients with a current major depressive episode and chronic insomnia ($n = 54$). | Wrist actigraph | Overnight study with concurrent actigraphic and PSG monitoring. | There were moderate positive correlations between actigraphy and PSG for all variables. |
| Rothschild-Fuentes et al. (13) | MDD outpatients aged 60 years or more ($n = 10$). | Wrist actigraph | Actigraphic parameters recorded before mirtazapine treatment and at day 60 of the treatment. | Sleep fragmentation index was significantly decreased after mirtazapine treatment, while other sleep parameters were not significantly changed. |
| Winkler et al. (14) | Inpatients with treatment-resistant depression. | Wrist actigraph | Activity level measured before and after ECT. | Remitters had increases of light activity, total activity, and circadian amplitude. |
| Hoogerhoud et al. (15) | Severely depressed patients ($n = 12$). | Wrist actigraph | 5-day actigraph monitoring during ECT course. | Actigraphy-assessed sleep in the short-term was not affected by ECT. |
| Krane-Gartiser et al. (16) | MDD inpatients with and without motor retardation ($n = 25$ and 27). | Wrist actigraph | 24-h actigraphy recordings. | Reduced mean activity level, higher intraindividual variability, and lower complexity were shown in patients with motor retardation compared with patients without motor retardation. |
| Nishida et al. (17) | Patients with medication-resistant MDD. | Waist actigraph | Monitoring over the course of rTMS treatments. | Sleep variables did not show significant changes, but *post-hoc* tests indicated a significant increase in mean steps per day. |
| O'Brien et al. (18) | Adults with late-life depression and aged 60 years or more ($n = 29$). | A novel wrist-worn device with three accelerometers | Monitoring over 7 days. | Subjects with late-life depression showed significantly reduced physical activity and slower fine motor movements. |
| Cook et al. (19) | Patients with unipolar MDD ($n = 21$). | Fitbit Flex™ | An overnight study with concurrent actigraphic and PSG monitoring. | The Fitbit Flex™ is not adequate to be substituted for PSG when evaluating sleep in MDD. |
| Cormack et al. (20) | Patients with mild-to-moderate MDD ($n = 30$). | Apple watch | Cognition and depressed mood assessment by new Cognition Kit app every day over 6 weeks. | Daily mood and cognitive assessments correlated moderately with validated tests. |
| Rojo-Wissar et al. (21) | Adults with MDD ($n = 34$). | Wrist actigraph | Self-reported parental bonding instrument and wrist actigraphy (for 1 week) were evaluated. | Sleep characteristics in adulthood were associated with maternal bonding but were independent of depression status. |
| Tazawa et al. (22) | Depressed patients ($n = 45$). | Silmee W20 wristband | Machine learning models developed using data collected by the device over seven days. | Skin temperature and sleep parameters were the most significant features for prediction. |
| Powell et al. (23) | Patients with severe unipolar or bipolar depression ($n = 12$) | PKG | PKG used to assess motor symptoms in depression. | PKG measures were significantly correlated with clinically assessed melancholia. |
| Peis et al. (24) | Depressed patients ($n = 23$). | Wrist actigraph | Regression model was developed to predict clinical course and hospital discharge of depressed patients. | Increased motor activity and early patterns of actigraphic measures allowed for accurate prediction of hospital discharge date. |
| Pedrelli et al. (25) | Patients with MDD ($n = 31$). | Empatica E4 wristband | Assessment by smartphone, wristband sensors, in-person clinical interviews, HDRS for 8 weeks. | The predicted score of the developed model and clinician-rated HDRS showed moderate-to-high correlation; skin conductance, HRV, and activity were important features of the model. |

wearable devices. Tazawa et al. (22) reported that ST can be an indicator of depression using a machine learning model with parameters detected from the Silmee™ W20 wristband. This corresponds with the report that depressive patients have a higher body temperature than that of healthy controls (32).

The study by Pedrelli et al. (25) corresponds with previous research suggesting that SC is significantly correlated with depression (33), and that HRV reflects autonomic dysregulation affected by mental health status (34). They have proposed a machine learning model that predicts clinical scores of MDD from various data collected through a smartphone and wristband. According to them, the 10 most predictive features for MDD include parameters related to SC and HRV. However, further research is necessary since machine learning models usually remain black boxes and do not clearly conclude to what degree and why the underlying parameters are related to the prediction (35).

## Future Trends

The advantage of incorporating wearable devices into research in patients with depression is that wearable devices enable continuous and objective monitoring of patients. With this technology, real-time changes between patient hospital visits can be tracked objectively and treatment effects can be monitored more accurately. Assessments of MDD often are conducted by mood questionnaires such as the Hamilton Depressing Rate Scale (HDRS). However, these types of mood assessments are easily disturbed by various biases, such as recall bias (36). This can lead to inaccurate results depending upon the degree of inconsistency among patients or clinicians (37). In addition, errors that can occur during manual data entry by physicians or researchers can be prevented when using wearable devices.

Wearable devices also enable patients to monitor their symptoms. For example, a patient can check their current level of stress or depression using wearable devices or a smartphone connected via Bluetooth. In a study using Psymate (PsyMate BV, Maastricht, the Netherlands), a "personal digital assistant"–based system for mood assessment, personalized feedback interventions appeared to help patients improve their depressive symptoms and prevent maladaptive behaviors that can worsen their moods, suggesting that the provision of such feedback through wearable devices will have similar effects (38). It has shown that, if patients are notified about their mood frequently, it may help them to manage their own depression.

Wearable devices can potentially be used to develop novel diagnostic methods or treatments. Sensors can measure various parameters other than those introduced above; for example, speech pattern and voice analyses through sensors can be used to assess depression severity and treatment response (39). In addition, using sensors like inertial sensors, bending sensors, and electromyography signals, it is possible to capture human motion through wearable devices (40). Considering studies showing that depressive patients can exhibit gait variability (41) and motor abnormalities (42), future research is expected to use novel wearable devices to investigate how movement changes with depression.

Recently, wearable devices that can treat depression at home have been developed. One study (43) showed that daily morning light therapy for 60 min using home light-therapy glasses can trigger an improvement in depressive symptoms. If this technology can be applied to normal glasses with light sensors tracking the daily light exposure of patients, daily use can help to monitor and treat depression.

Wearable devices can be used as a part of personalized exercise-treatment programs for MDD (44). In addition, wearable devices can increase patient physical activity and medication adherence according to an ongoing trial enrolling patients with heart failure and diabetes mellitus (45); based on step count data from wearable devices in this study, individualized feedback is provided to patients. We can expect a study in the future that attempts this type of intervention for patients with depression.

An aripiprazole tablet with an ingestible sensor (Abilify MyCite; Otsuka America Pharmaceutical, Inc., Rockville, MD, USA) was approved recently by the United States Food and Drug Administration. The sensor on the pill sends a signal to a cutaneous wearable so that clinicians can track the medication adherence of patients. This model is expected to be used in depression studies considering that aripiprazole can be administrated as an adjunctive treatment for depression.

## Challenges

There are challenges in the application of wearable devices in the field of depression. First, it is difficult to detect various symptoms of depression using wearable devices. In particular, evaluation of subjective mood symptoms is difficult; for example, wearable devices mostly detect physiological data and have limitations in evaluating subjective symptoms. However, a recent study using the Apple watch showed the possibility of high-frequency cognitive and mood assessments using wearable devices (20). Second, physiological data that can be measured by wearable devices are lacking. For example, although a change in appetite is a common symptom in patients with depression, it is difficult to measure it with wearable devices. Vu et al. (46) previously tried to estimate eating behaviors by detecting wrist movements or the sound of eating, but the accuracy was low.

There are various issues with adherence and compliance of patients. Wearable devices have variations in the degree of convenience of use, and there is a difference in user comfort when wearing the devices. The effectiveness of mobile health interventions varies greatly according to design of the intervention (47), and acceptance of wearable devices can vary depending on user age (48). Therefore, further research is required about how to increase adherence and compliance rates so that users can continuously wear wearable devices for 24 h.

Unreliability and inaccuracy are problems in use of wearable devices. Various wearable devices for fitness and wellness are available on the market. However, since these devices are heterogeneous, they are not simple to use for those wishing to monitor clinical symptoms. The reliability of the sensor system and data-processing algorithm of wearable devices also makes it difficult to introduce these devices into the medical field.

Furthermore, data can be disrupted by various kinds of noises generated by the surrounding environment and the physical condition of the person wearing the device. Although some studies have been carried out to validate the accuracy of wrist actigraphy in comparison with PSG (49, 50), accelerometers on the wrist are not effective in detecting sleep patterns not involving limb motion, so other instruments like a pressure sensor sheet or chest-worn sensor are needed to obtain a higher degree of accuracy comparable to that of PSG (51, 52).

Another issue involves feature extraction with regard to unreliability issues. According to a layered hierarchical framework presented by Mohr et al. (53), raw sensor data (e.g., location, movement) are transformed into low-level features (e.g., activity, total sleep time). The low-level features are combined and constitute high-level behavioral markers (e.g., psychomotor activity, sleep disruption). The clinical state (e.g., depression) is inferred through a combination of high-level behavioral markers. Although it is a logically valid framework, whether the data, such as the movements measured with an accelerometer, can accurately represent psychomotor agitation or retardation is unclear. For example, sleep is estimated based on patient motion and pulse not by measuring their brainwaves (54).

There also is a problem of privacy and ethics. Since data obtained through wearable devices are stored on an external server, there is a possibility that this data can be leaked. Because of this problem, legal regulations for use in the medical field will be essential.

## CONCLUSION

There are rapid ongoing developments of wearable devices for clinical use. Depressive symptoms can be estimated by many parameters collected objectively in real-time by wearable devices. The possibility of diagnosis and prediction remotely using these devices has been supported in this review. Future trends are expected with the emergence of new wearable devices that will bring novel diagnostic and therapeutic approaches like motion capture, speech analysis, and portable light therapy. This suggests the potential for fundamental changes in diagnosis and treatment of depression in the future by developments of wearable devices. These developments can lead to early and accurate diagnosis of depression, the capability to provide more personalized treatment to patients with depression, and to develop preventive measures for groups at risk of depression. Wearable devices will have a critical role in medicine with the advent of personalized telemedicine.

## AUTHOR CONTRIBUTIONS

HK, MP, and HJ contributed to conception and design of the study. HK and MP performed the bibliographical search. SL wrote the first draft of the manuscript. SL, HK, MP, and HJ wrote sections of the manuscript. All authors contributed to the article and approved the submitted version.

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# The Heterogeneity of Longitudinal Cognitive Decline in Euthymic Bipolar I Disorder With Clinical Characteristics and Functional Outcomes

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We characterized the heterogeneity and risk factors of cognitive decline in euthymic bipolar disorder (BD), and their magnitude of associations with subjective daily functions. In this retrospective cohort, BD type I patients ($N = 128$) were followed for an average of 6.5 years. Intelligence quotient (IQ) at index date was recorded, and premorbid IQ was estimated. We used Brief Assessment of Cognition in Affective Disorders (BAC-A) to assess cognition at follow-up. We evaluated current functions with World Health Organization Disability Assessment Schedule 2.0. Clinical and sociodemographic factors were examined for their independent effects on longitudinal cognitive decline. In addition, we employed multivariate adaptive regression spline to detect inflection points for the nature of slope changes in cognitive decline among BD patients. During follow-up years, 21 BD patients (16.4%) showed longitudinal cognitive decline. In cognitive decline group, all cognitive domains of BAC-A were significantly worsened. We found that density of episodes with psychotic features was an independent risk factor for cognitive decline after adjusted for age, gender and dose of mood stabilizer. After the age of 42 years, a steeper cognitive change was observed in the cognitive decline group. The correlation pattern between cognitive domains and functional outcomes differed between patients with and without cognitive decline. The present study characterized cognitive heterogeneity longitudinally in BD patients. As density of episodes play roles for cognitive decline, our results emphasize the importance of relapse prevention. Our findings provide hints for future personalized interventions and facilitating genetic and biological studies for dissecting the heterogeneity of bipolar illness.

**Keywords: BAC-A, bipolar disorder, function assessment, heterogeneity, longitudinal**

## INTRODUCTION

Bipolar disorder (BD), a recurrent chronic disorder, is characterized by episodes of mania and depression interspaced by euthymia, and it affects different aspects of daily living (1). BD is also associated with cognitive deficits in a number of domains, which may persist in patients in remission (2). For example, Robinson and Ferrier reported that euthymic patients with BD showed cognitive impairments compared with healthy controls, particularly in executive function and verbal learning (3). A meta-analysis using individual patient data further confirmed significant impairments in a wide-range of cognitive domains in BD. In particular, verbal learning test, executive function and processing speed showed robust impairments even controlling for age, educational years, gender, residual mood symptoms, and medications (4). Cognitive deficits have substantial negative impacts on social functioning and are responsible for poor inter-episode recovery or poor quality of life in a high proportion of patients (5–7), suggesting the importance of studying cognitive functions in BD to enhance future clinical care and prognosis outcomes.

The presence of cognitive impairment can regard as a marker of neuroprogression in BD patients (8). The underlying neurobiological mechanisms may include high pro-inflammatory activity, reduced neurotrophic support, and high oxidative stress burden in BD (9). Other factors such as higher rate of medical comorbidity, unhealthy related behaviors, and substance abuse (10, 11) also contribute to lower brain and cognitive reserve, thereby increasing risk of cognitive declines. In addition, recent studies reported substantial genetic overlap between cognitive function and BD risk (12).

On the other hand, previous cross-sectional studies have examined clinical factors with cognitive deficits in BD. Although findings were inconsistent, some studies have reported the correlations of cognitive impairments with illness severity (4). However, it is difficult to infer the causal link between cognitive deficits and clinical features due to the cross-sectional nature of these studies. One early study adopted a first-episode design to compare first-episode BD, schizophrenia, and healthy controls, and found that cognitive deficits are evident right from the first mood episode (13). During the disease course, cognitive impairment in BD patients then varies, with some studies suggesting that cognitive deficits stabilize over time (14–16), whereas others have shown a pattern of progressive deterioration (17, 18) and even increased risk of dementia in later life (19). It is believed that heterogeneity is widely observed among BD patients in this regard, in terms of the impaired cognitive domains, longitudinal cognition stability, and the speed of deteriorative progression. Longitudinal study design is preferable to explore cognitive declines over the course of bipolar illness, though it was relatively scarce in the literature.

So far, the heterogeneity of longitudinal cognitive decline in BD patients is not well-understood. First, insufficient data were available to investigate all cognitive domains in few longitudinal studies, and findings on deficits among different cognitive domains were inconsistent. Whether these are discrete areas of impairment or reflect an underlying single, more basic cognitive abnormality is as yet unclear. Second, previous longitudinal studies often consisted of small sample sizes and different follow-up periods, resulted in various findings. For example, the synthesis of longitudinal evidence suggests that the cognitive path of individuals with BD may be set early on and may not deteriorate over time (20, 21). However, another study with a much longer follow-up period showed significant test–retest differences in executive measures revealing decline (22), which implied the existence of cognitive instability over longer disease course. Third, the differences in medication variables or mood scales between assessment times could not be well-controlled in every study and may have influenced the results. In addition, we should consider the effect of repeated testing, wherein the true decline of cognitive functioning would be partially masked by learning effects during short period of follow-up.

We hypothesized that both the clinical course and cognitive decline are heterogeneous among BD patients, and thus may partially account for the inconsistency of findings in previous reviews. The majority of cross-sectional studies have suggested that 38–40% of patients with BD have no neurocognitive deficits, and 20–30% had obvious cognitive deficits (23–25), while less data on longitudinal cognitive decline. The complexity of cognitive impairment in BD may include neurodevelopmental, neuroprogressive, or combination of the two. The lack of longitudinal assessment of cognitive performance in BD hinders to explore the heterogeneity in this topic.

We aim to establish a retrospective cohort of bipolar disorder patients and to evaluate their cognitive changes over time by looking at age hinge points of cognitive decline, to characterize heterogeneous cognitive profiles, and to examine the risk factors for longitudinal cognitive decline. Lastly, we further evaluate whether different cognitive changes impact daily functions in patients with and without cognitive decline.

## MATERIALS AND METHODS

### Participants

In this retrospective cohort, individuals who were diagnosed with BD subtype I according to Diagnostic and Statistical Manual of Mental Disorders, fourth Edition, Text Revision (DSM-IV-TR) by board-certified psychiatrists from outpatient clinics were invited to join this study between July 1, 2018 to the end of 2019 (study entry). They were more than 20 years of age, and we excluded (a) known substance use disorder (except nicotine use disorder); (b) any disorder with known neurological symptoms or complications such as brain injury or stroke; (c) a previous diagnosis of intellectual disabilities, schizophrenia or schizoaffective disorders; or (d) inability to complete the standard clinical assessment or providing informed consent. The information for psychiatry comorbidities and exclusion criteria is obtained through medical diagnosis record and by the Chinese version of the modified Schedule of Affective Disorder and Schizophrenia-Lifetime (SADS-L), which has been report to have high interrater reliability values of mood disorders (major depression: 0.79; bipolar disorder: 0.71) (26). Individuals were also required to be euthymic at the time of study entry and under stable medications (no change of medications in previous

1 month) for current cognitive assessment. To evaluate cognitive deficits, eligible participants who had a prior index intelligence quotient (IQ) testing during euthymic state in medical record were retained in the following analysis. Mood symptoms were obtained through clinician-administered measures of the 17-item Hamilton Depression Rating Scale (HDS) and Young Mania Rating Scale (YMRS). For euthymic state, we defined HDS and YMRS scores $\leq$8 within 7 days before assessment (27, 28).

## Measurements

### Demographic Data, Clinical Course, and Index IQ

Patients' demographic and clinical course data were collected when study entry, from medical records and interviews by psychiatrists, if required. The clinical characteristics of participants included the number of affective episodes (total, manic, and major depressive), number of episodes with psychotic features, number of hospitalizations, age at illness onset, maximum length of free intervals, and number of suicide attempts. Adverse childhood events were assessed using Chinese version of Childhood Trauma Questionnaire-Short Form (CTQ-SF) (29). Considering the duration of illness varied across individuals, we also calculated episode density by dividing number of episodes by duration of illness as the total episode density, manic episode density, major depressive episode density and episode with psychotic features density separately. Psychopharmacological treatments used at the time of study entry were recorded and then transformed to defined daily dose (DDD). DDD is a unit of measurement assumed average maintenance dose per day for a drug used for its main indication in adults, which can be derived for comparisons of drug consumption (30, 31). Through reviewing medical records, we retrospectively recorded physical comorbidities, anthropometry, and index IQ at the euthymic state from the first clinical visit or admission of BD diagnosis. The index IQ was measured by a licensed psychologist using the Wechsler Adult Intelligence Scale (WAIS-III or WAIS-IV) in BD euthymic state and was regarded as a stable trait in adults. It was routinely assessed for the clinical practice in the recruitment hospital for assisting attending psychiatrists to establish proper rehabilitation plans for patients. In addition, we collected patients' premorbid educational years and occupational status with age and gender to estimate premorbid IQ (32, 33). Therefore, the cognitive information using IQ from medical records was defined at two time-points: T1, the premorbid estimated IQ; T2, the index IQ after BD onset (Details were illustrated in **Supplementary Figure 1**).

### Current Cognitive Measurements

Enrolled patients were assessed using the Brief Assessment of Cognition in Affective Disorders (BAC-A) during their euthymic state for at least 1-year interval apart from the date of index IQ measure to ensure evaluating longitudinal change and minimize possible learning effects. The time point of BAC-A assessment was defined as T3 as study entry (**Supplementary Figure 1**). BAC-A has extensively been used as a rapid and reliable measure of cognitive assessment in a range of clinically affective patients (34). It takes ∼35 min to administer. It provides measure of affective memory and emotional inhibition (Affective Processing Tests [APTs]) from Affective Auditory Verbal Learning Test and Emotional Stroop Task, and also measure for six traditional neurocognitive domains, namely, working memory (Digit Sequencing Task), motor speed (Token Motor Task), verbal fluency (Category Instances and Controlled Oral Word Association Test), attention and processing speed (Symbol Coding), verbal memory (List Learning), and executive function (Tower of London) with comparable norm references (35, 36). APTs were further applied with the indexes of Affective Interference Test (AIT), Emotion Inhibition Test (EIT), Delayed Recognition (DR), and Emotion Inhibition Index (EII) (37). The criterion and construct validity of each test for cognitive impairment as well as the sensitivity of these tests to changes in cognition have been demonstrated in the scientific literature, and each test has also been shown to be valid for use in different cultures and language groups (38).

### Functional Assessment

Trained interviewer administered the World Health Organization Disability Assessment Schedule 2.0 (WHODAS 2.0) for multidimensional assessment of subjective function in the participants also in the time of study entry (T3). WHODAS 2.0 is a practical, generic assessment instrument that can measure health and disability at a population level or in clinical practice. It evaluates subjective function in six domains: Domain 1: cognition– understanding and communicating; Domain 2: Mobility– moving and getting around; Domain 3: Self-care– attending to one's hygiene, dressing, eating, and staying alone; Domain 4: Getting along– interacting with other people; Domain 5: Life activities– domestic responsibilities, leisure, work, and school; and Domain 6: Participation– joining in community activities and participating in society (39). The raw score is transformed into item response theory (IRT) scoring (0–100), with higher scores indicating increasing severity of subjective function impairment (39).

## Statistical Analysis

We explored cognitive heterogeneity in BD longitudinally and defined subjects dichotomously into groups with or without cognitive decline. We transformed cognitive performance into Z1, Z2b and Z3 scores for the estimated premorbid IQ at T1, index IQ at T2 and BAC-A composite score at T3, respectively. This transformation was done based on the same outside reference from standardization samples in Taiwan (33, 36, 40), to make different assessments comparable at each time point. First, we compared the cognitive performance between T1 and T2. Among 128 subjects, there were 23 who showed cognitive deficits comparing premorbid Z1 and Z2 at disease index date, while 105 retained their cognitive function at disease index date (having $<$2 standard deviations [SD] of cognitive changes from T1 to T2). Second, there were 21 revealed longitudinally cognitive decline (16 from 105 and 5 from 23, having $>$ 2 SD cognition downward changes from T2 to T3), and 89 were classified into the cognitive non-declined group who had $<$ 2 SD cognition changes from T2 to T3. Finally, there remained 18 patients (stable cognitive deficits) who showed cognitive deficits at T2 with cognitive functions stable over the follow-up period at T3.

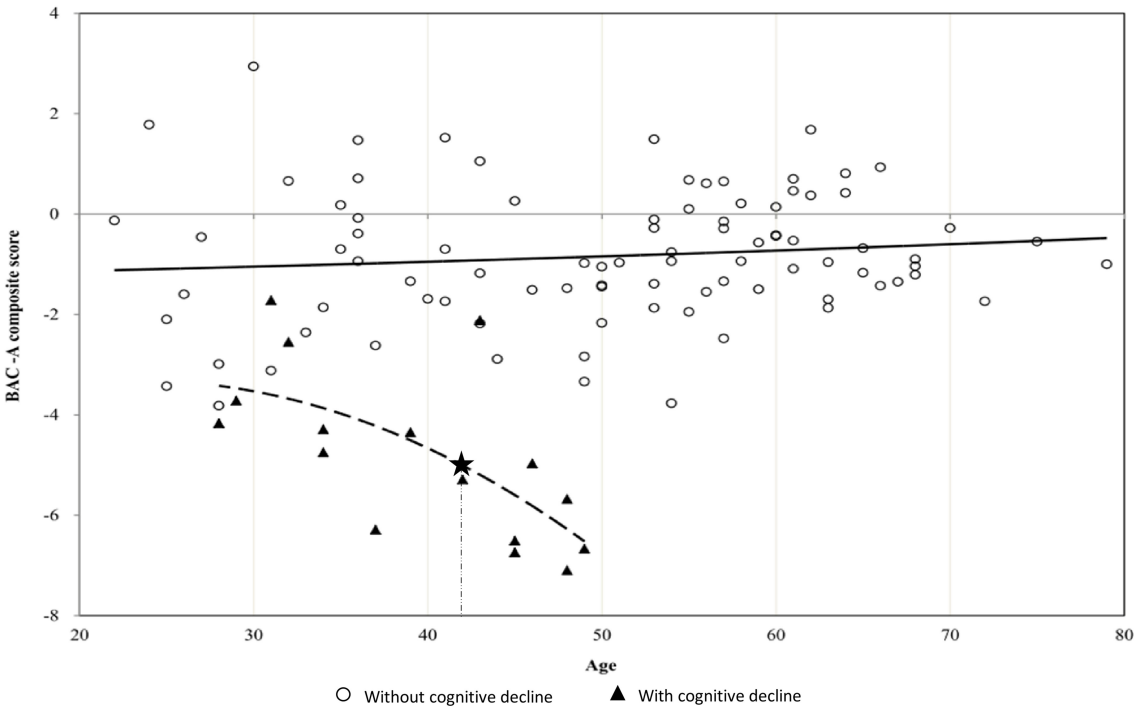


**FIGURE 1 |** Scatterplot between age and BAC-A composite score in BD patients with or without cognitive decline. The figure depicts steeper decrease in BAC-A composite score with hinge points (asterisk mark) at the age of 42 years in BD patients with cognitive decline. The slope of dotted curve showed −0.276 SD per year after age 42.

In the following analyses, we compared BD groups with ($N = 21$) and without longitudinal cognitive decline ($N = 89$) only.

We compared demographic and clinical characteristics between BD patients with and without cognitive decline using the chi-square and Student's *t*-test for categorical and continuous variables, respectively. The differences in BAC-A and functional outcomes were also examined. Normality of data was assessed using the Kolmogorov-Smirnov test. For non-normally distributed data, we used non-parametric Wilcoxon rank sum test for analysis. The variables that showed significant differences between the two groups were further assessed by univariate logistic regression to reveal their influences on cognitive outcome. The significant factors from univariate analysis were then included in multivariable logistic regression with stepwise selection to evaluate their independent effects, while adjusted for age and gender. In the multivariable regression analyses, we excluded variables with high correlation with others to avoid multicollinearity in the model (e.g., correlation coefficient between manic episode density and density of episodes with psychotic features was high, equals to 0.59). Pearson correlations between specific cognitive and functional domains were demonstrated in each group. Moreover, multivariate adaptive regression spline (MARS) (41) and scatterplot with Loess curve were used to detect hinges or inflection points to characterize the timing and nature of slope changes in the different cognitive groups. MARS is a non-parametric regression technique that can model non-linearities, which is much suitable for detecting the hinge points (42). MARS analysis was conducted using the earth package in R (43); all other analyses were conducted using SAS (version 9.4; SAS Institute Inc.; Cary, NC, USA). There were very few missing numbers in each different variable, and were treated as missing data in all analysis. Significance level was set at $p < 0.05$.

# RESULTS

## Patient Characteristics

In our retrospective cohort study, BD-I patients demonstrated longitudinal heterogeneous course in terms of cognitive functions, who presented with stable cognitive deficits (14.1%), with longitudinal cognitive decline (16.4%) and without cognitive decline (69.5%), respectively.

The characteristics between BD patients with ($N = 21$) and without ($N = 89$) longitudinal cognitive decline are presented in **Table 1**. Only two participants had psychiatry comorbidity with cluster B personality disorders. None of the participants was comorbid with schizophreniform disorder, delusional disorder, anxiety disorders, alcohol or illegal substance use disorders, organic mental disorders, obsessive-compulsive disorder, attention deficit/hyperactivity disorder or autistic spectrum disorders. The mean follow-up period from index IQ to current BAC-A assessment (T2 to T3) was 6.5 years, which showed no difference between the two groups. Compared with patients without cognitive decline, those with cognitive decline

**TABLE 1 |** Sociodemographic and clinical characteristics of BD patients with and without cognitive decline.

| | Without cognitive decline (*N* = 89) | With cognitive decline (*N* = 21) | *P*-value |
|---|---|---|---|
| Gender Male, n (%) | 34 (38.20) | 10 (47.62) | 0.428 |
| Marriage: married or lived together, n (%) | 34 (38.20) | 4 (19.05) | 0.097 |
| Job: regular job, n (%) | 23 (25.84) | 4 (19.05) | 0.515 |
| With family psychiatry history, n (%) | 48 (53.93) | 13 (61.90) | 0.509 |
| Smoking, n (%) | 15 (17.24) | 3 (14.29) | 0.999 |
| Alcohol use habit, n (%) | 1 (1.14) | 1 (4.76) | 0.443 |
| Current physical comorbidity, n (%) | 18 (20.69) | 4 (19.05) | 0.999 |
| Seasonality, n (%) | 30 (33.71) | 6 (28.57) | 0.652 |
| Age (yr)[a] | 50.35 ± 13.49 | 43.81 ± 10.49 | 0.040* |
| Onset of age (yr)[a] | 27.86 ± 12.05 | 22.81 ± 8.48 | 0.072 |
| Education years[a] | 12.99 ± 3.16 | 12.95 ± 2.73 | 0.962 |
| Index full IQ | 93.39 ± 16.08 | 88.75 ± 19.87 | 0.460 |
| Performance IQ | 90.00 ± 18.06 | 81.83 ± 20.63 | 0.235 |
| Verbal IQ | 96.91 ± 15.45 | 97.67 ± 19.9 | 0.902 |
| Follow up duration (month)[a] | 57.39 ± 49.34 | 110.00 ± 88.45 | 0.076 |
| Duration of illness (year) | 22.44 ± 11.08 | 21.71 ± 10.03 | 0.784 |
| Total episode density[a] | 0.40 ± 0.27 | 0.51 ± 0.24 | 0.070 |
| Manic episode density[a] | 0.26 ± 0.21 | 0.40 ± 0.24 | 0.013* |
| Major depressive episode density[a] | 0.13 ± 0.15 | 0.10 ± 0.13 | 0.413 |
| Episode with psychotic features density[a] | 0.15 ± 0.16 | 0.29 ± 0.25 | 0.023* |
| Maximum of free interval (month)[a] | 91.38 ± 70.27 | 67.24 ± 49.04 | 0.140 |
| Number of suicide attempt[a] | 0.77 ± 1.60 | 0.71 ± 0.85 | 0.817 |
| Days of hospitalization[a] | 191.48 ± 195.97 | 354.38 ± 201.68 | 0.001* |
| Number of admission[a] | 5.13 ± 5.31 | 8.00 ± 5.29 | 0.028* |
| Index body mass index, BMI[a] | 22.53 ± 5.29 | 24.01 ± 5.21 | 0.258 |
| **Current psychoactive agents (DDD)** | | | |
| First-generation antipsychotics[a] | 0.06 ± 0.17 | 0.16 ± 0.48 | 0.357 |
| Second-generation antipsychotics[a] | 3.29 ± 4.48 | 6.52 ± 7.52 | 0.071 |
| Mood stabilizers[a] | 0.51 ± 0.35 | 0.73 ± 0.46 | 0.018* |
| Antidepressants[a] | 0.07 ± 0.28 | 0.00 ± 0.00 | 0.016* |
| Benzodiazepines[a] | 0.50 ± 0.75 | 0.47 ± 0.62 | 0.829 |

*$p < 0.05$; [a]*Assessed using Wilcoxon rank sum test.*
*IQ, intelligence quotient; DDD, defined daily dose.*

were younger, experienced more manic episodes, especially episodes with psychotic features, more number of admissions, and higher hospitalization duration. In addition, the cognitive decline group revealed using higher dose of mood stabilizer and less dose of antidepressant at time point T3 than those without cognitive decline. No significant differences were observed in variables such as physical comorbidities, duration of illness, age of onset, or index IQ measurement between the two groups.

## Functional Outcomes and BAC-A in BD Patients With Cognitive Declines

No significant differences were observed in current functional outcomes assessed using WHODAS 2.0 between BD patients with or without cognitive decline. **Table 2** shows the IRT scoring of the groups; a higher score indicated more severity of subjective impairment, that is, greater disability. We noted that common functional impairments in the chronic phase of BD were observed in both groups, regardless of presence of cognitive decline. Even for the most preserved functional domain (self-care with IRT score 14.0), patients showed impairment that was the last 30% in the population percentile when compared with norm data (39). In addition, there were no difference from HDS, YMRS and childhood trauma experience between two groups. For cognitive profiles, each patient's performance in individual tests was compared with an age- and gender-matched Taiwan norm to calculate the z-score for six traditional neurocognitive domains (36). As observed in BAC-A assessment, the cognitive decline group showed significant worsening of all six neurocognitive domains, not limited to specific domains. Moreover, APT indexes indicated the different emotional inhibitions between the groups (**Table 2**).

## Risk Factors for Cognitive Decline and Decline Curve in Patients With BD

We used univariate and multivariable logistic regression to analyze the independent risk factors for cognitive decline in

**TABLE 2 |** Mood status, functional assessment and cognitive profiles at follow-up in BD patients with and without cognitive decline.

| | Without cognitive decline ($N = 89$) | With cognitive decline ($N = 21$) | *P*-value |
|---|---|---|---|
| WHODAS2.0 Total score | 31.15 ± 18.57 | 23.90 ± 11.41 | 0.258 |
| D1 cognition | 26.54 ± 18.43 | 26.00 ± 13.7 | 0.934 |
| D2 walk[a] | 19.44 ± 27.87 | 17.60 ± 6.65 | 0.284 |
| D3 self-care[a] | 13.11 ± 17.54 | 14.00 ± 6.99 | 0.730 |
| D4 along with others | 35.19 ± 22.17 | 28.50 ± 15.81 | 0.390 |
| D5-1 housekeeping[a] | 35.26 ± 32.76 | 27.00 ± 20.58 | 0.110 |
| D5-2 job & learn[a] | 23.00 ± 29.56 | 25.25 ± 10.50 | 0.285 |
| D6 social[a] | 32.93 ± 23.41 | 25.60 ± 14.61 | 0.364 |
| HDS score[a] | 2.40 ± 1.95 | 2.81 ± 1.94 | 0.393 |
| YMRS score[a] | 2.72 ± 2.27 | 2.86 ± 2.33 | 0.803 |
| CTQ total[a] | 61.47 ± 7.80 | 64.5 ± 19.39 | 0.675 |
| BAC-A composite score[a] | −0.83 ± 1.33 | −4.51 ± 1.63 | <0.0001* |
| Verbal memory[a] | −0.18 ± 1.37 | −2.69 ± 2.52 | 0.0002* |
| Motor speed[a] | −1.32 ± 1.19 | −3.34 ± 1.56 | <0.0001* |
| Working memory[a] | −0.37 ± 0.98 | −1.99 ± 1.51 | <0.0001* |
| Verbal fluency | −0.20 ± 1.10 | −1.44 ± 0.71 | <0.0001* |
| Attention and processing speed | −0.92 ± 1.31 | −2.62 ± 0.92 | <0.0001* |
| Executive function[a] | −0.08 ± 1.15 | −2.39 ± 2.11 | <0.0001* |
| **Affective processing tests** | | | |
| AIT: total non-affective words[a] | 13.36 ± 4.45 | 10.95 ± 4.18 | 0.026* |
| AIT: total affective words | 10.7 ± 4.47 | 8.48 ± 4.49 | 0.043* |
| AIT: cued non-affective words[a] | 3.69 ± 2.01 | 3.10 ± 2.19 | 0.238 |
| AIT: cued affective words[a] | 5.38 ± 2.00 | 4.19 ± 1.47 | 0.012* |
| DR: correct non-affective words[a] | 17.85 ± 2.08 | 16.29 ± 2.90 | 0.028* |
| DR: correct affective words[a] | 16.94 ± 2.39 | 15.95 ± 3.41 | 0.219 |
| DR: non-affective false alarms[a] | 2.15 ± 2.08 | 3.71 ± 2.90 | 0.028* |
| DR: affective false alarms[a] | 3.06 ± 2.39 | 4.05 ± 3.41 | 0.219 |
| EII (emotion inhibition index) | −79.38 ± 23.28 | −66.62 ± 23.51 | 0.026* |

*$p < 0.05$; [a]*Assessed using Wilcoxon rank sum test.*
*HDS, Hamilton depression scale; YMRS, Young mania rating scale; CTQ, Childhood Trauma Questionnaire; AIT, affective interference test; DR, delayed recognition; EIT, emotion inhibition test.*

BD, and results are displayed in **Table 3**. Because the extreme low dose of antidepressants used among BD patients (mean DDD was 0.00 in cognitive decline group and 0.07 in without cognitive decline group), we were not able to assess the effects of antidepressants DDD on cognitive decline in regression models. In multivariable logistic regression models controlling for age and gender, we found that density of episodes with psychotic features during the disease course (odds ratio [OR] 25.21, 95% CI 2.15–259.61, $p = 0.010$) and DDD of mood stabilizers (OR 3.86, 95% CI 1.03–14.89, $p = 0.049$) were independent risk factors for cognitive decline.

**Figure 1** depicts a steeper decline in BAC-A composite score in patients with cognitive decline compared with those without cognitive decline. In addition, MARS yielded a hinge point at the age of 42 years for the cognitive decline group, and their BAC-A composite score steeply worsened by 0.276 SD per year after this age.

## Correlation Between Cognitive and Functional Domains

**Figure 2** demonstrated correlations between cognitive and subjective functional domains in patients with (**Figure 2A**) and without cognitive decline (**Figure 2B**). We evaluated whether there was the same trend of correlations for both groups of BD, where better cognitive scores were correlated to lower subjective dysfunction. We found that in the group without cognitive decline, no significant correlation was found between functional and cognitive domains after adjusted for age. In contrast, the cognitive decline group showed strong links between various cognitive domains and daily functions ($r$ ranging from 0.64 to 0.76). For example, the range of impaired functions, such as self-care and getting along with other people were negatively correlated with verbal fluency. Working memory and composite score of cognition also have negative correlation with subjective social and housekeeping function, respectively. We tested differences for the aforementioned four pairs of correlation coefficients between the two groups using Z test, and the correlation differences reached significance ($P < 0.05$). In this part, the different correlation patterns of the two groups suggested the heterogeneous effects on daily function by their longitudinal cognitive profiles.

# DISCUSSION

To the best of our knowledge, this retrospective cohort study is the first to provide a comprehensive picture of BD patients with cognitive decline through long-term follow-up, and the results suggested the existence of subgroups with different cognitive trajectories. We classified our patients into two groups according to presence or absence of longitudinal cognitive decline and correlated cognitive profiles to their daily dysfunctions. We revealed that more number of episodes with psychotic features and current high DDD of mood stabilizers were risk factors to be associated with cognitive decline. Furthermore, we demonstrated a hinge point at the age of 42 years with steeper decline of cognition in the cognitive decline group.

In our study, the picture of BD patients with longitudinal cognitive decline shows wide-range of domains of impairment, rather than on only some specific cognitive domains, which is consistent with most previous cross-sectional studies (44). Moreover, the magnitude and proportion of cognitive dysfunction reported in previous cross-sectional studies, which used BAC-A to assess cognitive function in BD, is similar to that obtained by combining the three subgroups in the present study (35, 45). Therefore, our findings, showing longitudinal changes of BD are robust as suggested by those of previous cross-sectional literature. More specifically, our findings of longitudinal cognitive heterogeneity in a small subset of patients with BD are important and can partially explain the diverse and inconsistent findings for clinical characteristics and risk factor identification in the literature. In our study, the longitudinal cognitive decline group comprised around 16.4% of our sample, and this group may be established as a specific subtype of BD. Such longitudinal cognitive profiles can potentially facilitate

**TABLE 3 |** Risk factors for cognitive decline in BD using univariate and multiple logistic regression analyses.

| | Univariate | | | Multivariable | | |
|---|---|---|---|---|---|---|
| | **OR** | **(95% CI)** | ***p*-value** | **OR** | **(95% CI)** | ***p*-value** |
| Age | | | | | | |
| ≧40 | 1.00 | | | 1.00 | | |
| >40 | 0.55 | (0.20–1.46) | 0.231 | 0.68 | (0.23–1.98) | 0.457 |
| Gender | | | | | | |
| Female | 1.00 | | | 1.00 | | |
| Male | 1.47 | (0.57–3.83) | 0.430 | 0.99 | (0.34–2.97) | 0.996 |
| Manic episode density | 10.02 | (1.35–74.57) | 0.024* | | | |
| Episode with psychotic features density | 26.90 | (2.44–297.13) | 0.007* | 25.21 | (2.15–259.61) | 0.010* |
| DDD of Mood stabilizers | 4.24 | (1.23–14.67) | 0.022* | 3.864 | (1.03–14.89) | 0.049* |

*$p < 0.05$.

*Multivariable regression using stepwise selection for age, gender, manic episode density, psychotic episode density and DDD (defined daily dose) of mood stabilizers. By the selection, final model including age, gender, episode with psychotic features density and DDD of mood stabilizers.*

**A** BD patients with cognitive decline

| | WHO DAS total | D1 Cognition | D2 Walk | D3 Self-care | D4 Along with | D5-1 Housekeeping | D5-2 Job & Learn | D6 Social |
|---|---|---|---|---|---|---|---|---|
| **BAC-A composite score** | 0.15 | -0.17 | 0.12 | 0.14 | -0.12 | -0.71* | 0.09 | 0.41 |
| **Verbal memory** | 0.20 | -0.23 | 0.07 | 0.24 | 0.26 | -0.49 | 0.52 | 0.29 |
| **Motor speed** | -0.06 | -0.06 | 0.34 | -0.41 | -0.52 | -0.15 | -0.65 | 0.19 |
| **Working memory** | 0.51 | 0.20 | 0.29 | 0.30 | 0.38 | -0.29 | 0.71 | -0.76* |
| **Verbal fluency** | 0.45 | -0.01 | -0.30 | -0.64* | -0.64* | -0.06 | 0.91 | 0.42 |
| **Attention and processing speed** | -0.28 | -0.13 | 0.21 | 0.16 | -0.14 | -0.27 | -0.16 | -0.06 |
| **Executive function** | -0.49 | -0.73* | 0.13 | -0.54 | -0.62 | -0.38 | -0.48 | -0.03 |

**B** BD patients without cognitive decline

| | WHO DAS total | D1 Cognition | D2 Walk | D3 Self-care | D4 Along with | D5-1 Housekeeping | D5-2 Job & Learn | D6 Social |
|---|---|---|---|---|---|---|---|---|
| **BAC-A composite score** | -0.02 | -0.09 | -0.06 | -0.06 | -0.07 | -0.01 | -0.37 | 0.17 |
| **Verbal memory** | 0.01 | -0.01 | -0.14 | -0.06 | 0.02 | 0.01 | -0.44 | 0.24 |
| **Motor speed** | 0.27 | 0.22 | 0.36 | 0.34 | 0.01 | 0.32 | 0.23 | 0.29 |
| **Working memory** | -0.17 | -0.43* | -0.04 | -0.14 | -0.27 | -0.09 | -0.50 | 0.05 |
| **Verbal fluency** | -0.06 | -0.05 | -0.18 | -0.13 | 0.03 | -0.08 | 0.22 | 0.21 |
| **Attention and processing speed** | 0.00 | -0.03 | -0.02 | 0.01 | -0.05 | 0.01 | -0.26 | 0.11 |
| **Executive function** | -0.01 | -0.17 | -0.19 | -0.27 | 0.02 | -0.04 | 0.18 | 0.17 |

**FIGURE 2 |** Pearson correlations between cognitive and functional domains in BD. *$p < 0.05$; Gray base indicating the significance after adjusting the effect of age.

further genetic and biological studies and help clinicians develop more effective and personalized intervention strategies (46, 47). As we had suggested, the heterogeneity of the cognitive trajectory conformed to the progress of illness in clinical manifestation staging models including cognitive deterioration (48). Our findings revealed that cognitive decline varies from one patient to another, with decline in certain patients while others remain stable. Conversely, we may say that some patients maintained relatively stable cognitive function might be cognitive reserve, which reflects the partial capacity of the brain to endure neuropathology and minimize clinical cognitive deficits (49). Our findings also suggested the paradigms of cognitive impairment in BD as persistently stable or progressive are not exclusive or this may partially overlap (50). The verification of heterogeneity of longitudinal cognitive change in small part of BD samples further comprehend the different faces of cognition in this disorder.

The risk factors identified for cognitive decline in BD suggest the converging evidence that patients with BD show cognitive impairment related to the clinical course. However, most previous studies have not considered potential longitudinal heterogeneity and have thus compared all BD patients together, leading to masking risk factors findings in subgroups of cognitive change over time in BD (51–54). Recently, a cohort study that

assessed longitudinal cognitive changes in BD showed that a higher number of manic episodes is associated with a decrease in global cognition as well as working memory and visual memory (51). Our findings were consistent with this study and further implied the association of cognitive impairment with episodes with psychotic features (55, 56). Another risk factor noted in this study is the dose of mood stabilizer used at the BAC-A assessment. It is clear that higher dose of lithium or anticonvulsants has varying effects on cognitive function (57, 58); however, we cannot conclude the causal effect of dose of mood stabilizer to the risk of cognitive decline from this findings, as the dose recorded at T3 of the study and there may exist some confounding by indications with the patients presence of more manic episodes and cognitive deficits at the same time.

Although our findings implied the associations between episodes with psychotic features and cognitive decline in patients with BD, very little is known about the underlying mechanism why some patients with BD develop significant cognitive decline while others remain cognitively intact. There may exist unmeasurable confounding factors in this cohort. The association of psychotic features with cognitive decline in BD was corroborated by studies from psychosis populations who have been found to exhibit smaller total brain volume (59) and reduced functional connectivity in frontoparietal control network (60). In addition, meta-analyses pooling inconsistent results from each individual study revealed findings of reduced white matter integrity and volume in BD (61, 62). We need large sample size in future studies to tackle the heterogeneity issue for cognitive impairment. In addition, we recommend that further genetic risk or biomarker studies should be conducted in this subgroup of patients. Therefore, the precise intervention can apply earlier in this subgroup, such as active relapse prevention or cognitive remediation. Moreover, the subgroup of BD patients with cognitive decline showed steeper changes after the age of 42 years compared with those without cognitive decline. The hinge point of age 42 in cognitive decline group is on average 20 years after the disease onset, implying that long-term follow-up is needed for detecting substantial cognitive change. On the other hand, there is time to design and implement intervention strategies to alleviate potential cognitive decline. Our results are consistent with the findings that there was accelerated aging in executive functions in BD (63), therefore, we suggested that clinicians should pay attention to patients' cognitive function in their early middle age.

Psychosocial function is a person's ability to perform activities of daily living and to be involved in meaningful interpersonal relationships. As per our results, patients with BD have difficulties in several areas of function during their remitted status. These results are consistent with those of other studies demonstrating that most patients with BD have functional difficulties assessed by Functioning Assessment Short Test (FAST) (64–66) and WHODAS 2.0 (67). However, our findings suggested no difference of functional outcomes in patients with and without cognitive decline assessed by WHODAS 2.0. There may be possible floor effect of this subjective assessment for this population, as both groups exhibit poor functional scores. Thus, we are not able to detect meaningful differences between the two subgroups with relatively small samples. We used the WHODAS 2.0 for evaluating our patients, rather than disease-specific questionnaires, such as FAST, because the former disability assessment is more informative by comparing with general norm or other diseases. Further results from the partial correlation analysis between cognitive and functional domains, controlling for age, indicated that the cognitive profile was still correlated with some functional domains mainly in the group of BD patients with cognitive decline. These results are in line with those of cross-sectional studies showing that the cognition-function relationship may be weaker among patients without cognitive deficits than among those with cognitive impairment (68). The distinct correlation results among patients with BD also responded to those of a previous study conducted by Sole et al. They suggested the more robust correlation between the poor function group of BD to their cognitive function and smaller correlations in less functional impaired BD (69).

Several limitations should be considered of this study. First, our study sample was recruited from a tertiary psychiatry hospital where patients have more severe degree of illnesses. This potentially biased sample may limit the generalizability of our findings to the whole BD population, especially the proportion with cognitive decline. Moreover, we cannot exclude the possibilities of recall bias in reporting clinical related information, such as lifetime psychiatric comorbidities. Nevertheless, we had checked medical charts to verify records in comorbidities and medication to minimize such bias. Second, without a healthy control group, cognitive decline in patients should be viewed as evidence of relative cognitive decline. Third, as mention before, we used different measurements for three time-points of cognitive assessment. Despite of this limitation, all of the raw scores have been transformed into standardized Z scores according to Taiwan's norm data. Forth, despite the correlation patterns between functional and cognitive domains showed significant differences between the two subgroups (**Figure 2**), such differences required further validation, as well as to minimize potential confounding effects from other variables. Furthermore, the restricted sample size limited the power to examine interaction effects among potential variables, such as interaction of disease course and medications. Fifth, WHODAS 2.0 is an interviewer-administered tool assessing subjective disability; a combination of WHODAS 2.0 administered from caregivers or other objective measures may provide a more comprehensive and accurate outcome picture for BD patients with and without cognitive decline. Sixth, we have no data about inter-rater reliability in relation for measuring IQ and function. Finally, the dichotomous categorized definition about cognitive decline (difference more than 2SD) in our study is arbitrary. However, the potential of misclassification is non-differential and bias the odds ratio toward the null, which suggested the robustness of our findings. In the current study, we adjusted the medication effect only at time point of T3, the full picture of medication usage during the disease course cannot be easily captured. In addition, due to the extreme low dose of antidepressants used in our samples, we're not able to evaluate the effect of antidepressants use on cognition decline. Further detailed data

collection in larger samples and advanced methodology might be helpful to overcome these issues. On the other hand, with a retrospective cohort design, we have the strength to confirm the diagnosis as bipolar disorder through the disease course. Future prospective studies should be cautious about including patients that may change diagnosis from BD to schizoaffective disorder or schizophrenia during follow-up period. Further investigations in the field are necessary for uncovering the underlying mechanisms linking risk factors, cognitive decline, and functional outcomes.

In conclusion, our results specify and characterize cognitive heterogeneity in BD longitudinally, which may facilitate further genetic and biological studies to define more valid BD subtypes to reveal the underlying mechanisms. We identified risk factors for cognitive decline and therefore suggested aggressive relapse prevention. The heterogeneity of cognitive decline in BD should be considered, thus individualized intervention for patients with BD could be applied in the future.

## 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/s.

## ETHICS STATEMENT

The Research Ethics Committee of Taipei City Hospital approved our study (TCHIRB-1-703103). The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

W-YC performed conceptualization, funding acquisition, project administration, and writing the original draft. M-CH, S-KL, H-CL, P-YC, and CC performed investigation and resources. Y-CL, S-HW, and C-EC done software analysis and data curation. C-JK performed the suggestion for investigation, resources, and data curation. P-HK was major for supervision, methodology, and reviewing the manuscript. All authors read and approved the final manuscript.

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## SUPPLEMENTARY MATERIAL

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# Gut Microbiome: A Potential Indicator for Differential Diagnosis of Major Depressive Disorder and General Anxiety Disorder

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**Background:** Major depressive disorder (MDD) and general anxiety disorder (GAD) share many common features, leading to numerous challenges in their differential diagnosis. Given the importance of the microbiota–gut–brain axis, we investigated the differences in gut microbiota between representative cases of these two diseases and sought to develop a microbiome-based approach for their differential diagnosis.

**Methods:** We enrolled 23 patients with MDD, 21 with GAD, and 10 healthy subjects (healthy crowd, HC) in the present study. We used 16S rRNA gene-sequencing analysis to determine the microbial compositions of the gut microbiome based on Illumina Miseq and according to the standard protocol.

**Results:** GAD showed a significant difference in microbiota richness and diversity as compared with HC. Additionally, Otu24167, Otu19140, and Otu19751 were significantly decreased in MDD relative to HC, and Otu2581 and Otu10585 were significantly increased in GAD relative to MDD. At the genus level, the abundances of *Sutterella* and *Fusicatenibacter* were significantly lower in MDD relative to HC, and the abundances of *Fusicatenibacter* and *Christensenellaceae*_R7_group were significantly lower in GAD than in HC. The abundance of *Sutterella* was significantly higher whereas that of *Faecalibacterium* was significantly lower in GAD relative to MDD. Moreover, we observed that *Christensenellaceae*_R7_group negatively correlated with the factor score (Limited to Hopelessness) and total score of HAMD-24 ($p < 0.05$), whereas *Fusicatenibacter* negatively correlated with FT4 ($p < 0.05$). Furthermore, the GAD group showed significant differences at the genus level for *Faecalibacterium*, which negatively correlated with PTC ($p < 0.05$).

**Conclusions:** This study elucidated a unique gut-microbiome signature associated with MDD and GAD that could facilitate differential diagnosis and targeted therapy.

**Keywords: gut microbiome, anxiety, depression, 16S ribosomal RNA, differential diagnosis**

## INTRODUCTION

Major depressive disorder (MDD) is characterized by deep sadness, reduced energy, vegetative nervous system dysregulation, cognitive dysfunction, and even a high suicidal tendency (1). Generalized anxiety disorder (GAD) is characterized by extreme anxiety about issues, such as security, money, and health, and accompanied by restlessness and autonomic dysfunction (2). Anxiety and depression are two common disorders that show high comorbidity (3–5). Although they share several causal and descriptive features, there are some associated differences in their clinical features and etiological factors (6). The separation of anxiety and depression disorders is extremely important for the elucidation of the underlying disease mechanisms and development of specific pharmacological and psychological treatments. Although many studies have distinguished anxiety and depression from the perspective of symptomatology and psychological, social, and physiological etiology (7–10), there remains no convincing evidence of their distinction. To further elucidate the substantial but incomplete overlap between these disorders, this study sought to determine whether patients with clinical diagnoses of MDD and GAD can be differentiated based on gut-microbiota features.

The studies conducted in recent decades indicate that gut microbiota play a crucial role in modulating brain function and human behavior (11). Furthermore, differences in gut microbiota have been identified in various psychiatric diseases, including depression, bipolar disorder, and schizophrenia (12, 13), as well as several animal models of psychiatric diseases (14–16). There is evidence for altered microbiota composition in depressed individuals (17–19), with levels of *Faecalibacterium* negatively correlating with symptom severity (20) and suggesting that the clinical phenotype of mental illness might be affected by gut microbiota. Additionally, studies show that probiotic administration of *Bifidobacterium longum* and *Lactobacillus helveticus* can decrease anxiety (21–23). Moreover, Chen et al. and Jiang et al. found several consistent taxonomic differences, including higher abundances of Enterobacterales, *Bacteroidaceae*, *Escherichia/Shigella*, *Bacteroides*, and *Tyzerella* and lower abundances of Firmicutes, Mollicutes, *Prevotellaceae*, *Ruminococcaceae*, Subdoligranulum, *Coprococcus*, and *Dialister* between participants with GAD and controls (24, 25).

In general, recent studies independently investigated the characteristics of gut flora in depression and anxiety patients but did not conduct a comparative analysis. In fact, few studies have explored the use of gut flora as a marker for disease diagnosis. For example, recent studies suggest that changes in intestinal microflora might be used as a biomarker for depression diagnosis and monitoring (26, 27). Zheng et al. recently identified distinct gut-microbial compositions in MDD as compared with bipolar disorder and provided a novel marker panel to distinguish MDD from bipolar disorder based on gut-microbiome signatures (28). To the best of our knowledge, there is currently no information concerning differences in intestinal flora as a biological marker identifying anxiety and depression.

Emerging evidence points to a bidirectional communication between the neuroendocrine system and gut microbiota (29). Gut microbiota can modulate central processes via endocrine pathways within the microbiota–gut–brain axis (30, 31). Sudo et al. found that elevations in plasma adrenocorticotropic hormone (ACTH) and corticosterone levels in response to stress were substantially higher in germ-free mice than in specific pathogen-free mice (32). Additionally, recent studies report that germ-free mice, which are devoid of any bacterial contamination, show reduced depression-like behaviors along with changes in the hypothalamic–pituitary–adrenal (HPA) axis as compared with specific pathogen-free mice (33, 34). On the other hand, numerous studies have verified the relationship between the hypothalamic–pituitary–thyroid (HPT) or HPA systems and the onset of depression and anxiety (32, 35). For example, Brownlie et al. proposed that a dynamic decrease in thyroid hormone levels, particularly FT3 and FT4 (36), could be related to depression. Moreover, increased activation of the HPA axis has been repeatedly observed in depressed patients, especially in the melancholic subtype (37). In most studies, these two relationships [between depression/anxiety and gut microbiota and between neuroendocrine (HPA/HPT) and gut microbiota] have been studied independently. However, whether the gut microbiota can affect the neuroendocrine system and lead to mental illnesses, including anxiety and depression, remains unclear.

In this study, we compared the differences in the intestinal flora of patients with anxiety diagnosis to those with depression diagnosis in order to determine whether intestinal flora can help distinguish between the two groups. To achieve this, we examined MDD and GAD patients without obvious anxiety and/or depressive symptoms, respectively, and used 16S rRNA gene-sequence analysis to help distinguish differences in their intestinal flora. Additionally, we analyzed the effects of different bacteria on clinical symptoms and the neuroendocrine system in order to further explore their function in these conditions.

## MATERIALS AND METHODS

### Subjects

Patients with MDD and GAD and normal control subjects participated in this study. Both MDD and GAD patients included a series of outpatients who received treatment at the West China Hospital from January to June 2019. All samples were from Chengdu, Sichuan, China, a relatively geographically closed area harboring residents with similar eating habits. The patients were diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) (38) at the first clinical examination, with diagnoses were confirmed by two psychiatrists (39). Patients <18 or >45 years of age and with organic etiology for their psychiatric symptoms, psychotic features, or intellectual disability were excluded. Patients included in the study were either newly diagnosed with depression or had not used psychotropic drugs for at least 6 months. The normal control subjects included 10 worker volunteers (aged 18–45 years) without current or past major psychiatric disorders. Subjects with the following conditions were also excluded: a lifetime history of bipolar disorder, schizophrenia, schizoaffective, or other psychiatric disorders; hypertension; cardiovascular disease; diabetes mellitus; obesity; liver cirrhosis; fatty liver disease;

irritable bowel syndrome; inflammatory bowel disease; drug or alcohol abuse in the previous year; use of antibiotics, probiotics, prebiotics, or synbiotics in the 6 months before fecal sample collection; known active bacterial, fungal, or viral infections; and obvious dietary preferences (e.g., vegetarians). All patients completed the Hamilton Depression Rating Scale (HAMD-24) and the Hamilton Anxiety Scale (HAMA) to obtain a clinical rating of the severity of depression and anxiety (40, 41). Both scales were independently administered by two psychiatrists that were blinded to the clinical status of the participants and had attended a training session on how to administer the tests before the start of the study. To minimize the impact of accompanying symptoms, we also excluded GAD patients with HAMD-24 $\geq$20 and MDD patients with 14-item HAMA $\geq$14.

All procedures contributing to this work comply with the ethical standards of national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. All procedures involving human subjects and patients were approved by the Ethics Committee of West China Hospital of Sichuan University (approval number: 2019-268). Written informed consent was obtained from all study subjects.

## Neuroendocrine Hormone Analysis

The HPT axis test indicators include thyroid-stimulating hormone (TSH; normal value: 0.27–4.2 mU/L), triiodothyronine (T3; normal value: 1.3–3.1 mmol/L), thyroxine (T4; normal values: 62.0–164.0 mmol/L), free triiodothyronine (FT3; normal value: 3.6–7.5 pmol/L), and free thyroxine (FT4; normal value: 12.0–22.0 pmol/L). The HPA axis test indicators include ACTH (normal value: 5.0–78.0 ng/L) and 8:00 A.M. cortisol (PTC; normal value: 147.3–609.3 mmol/L). Fasting venous blood was taken by drawing 4 mL of cubital venous blood at 8 A.M. after overnight fasting. All analyses were performed using a Roche Cobas e601 module (Roche, Basel, Switzerland) *via* electrochemiluminescence. All reagents and calibrations were performed according to manufacturer instructions.

## Sample Collection and DNA Extraction

Fecal samples were immediately frozen upon collection in a sterile plastic cup and stored at −80 °C before analysis. Microbial genomic DNA was extracted using the QIAamp DNA stool mini kit (Qiagen, Hilden, Germany) according to manufacturer instructions. The 16S rRNA V3-V4 amplicons were generated using the National Institutes of Health (NIH) Human Microbiome Project protocols (16S 454 Sequencing Protocol HMP Consortium; https://www.hmpdacc.org).

## 16S rRNA Gene-Sequencing Analysis

Libraries were prepared and paired-end sequenced with Illumina Miseq according to manufacturer instructions (42). These QIIME2 16S rRNA sequencing protocols were used to select and analyze operational taxonomic units (OTUs) (43). Sequences from this project were deposited in the NCBI Short Read Archive under BioProject ID PRJNA647236.

**TABLE 1 |** Clinical and demographic characteristics of the MDD, GAD, and HC groups.

| GROUP | MDD | GAD | HC | *p* |
|---|---|---|---|---|
| Age (*y*) | 30.04 ± 5.90 | 30.43 ± 7.95 | 30.22 ± 6.50 | 0.982 |
| BMI | 21.87 ± 3.00 | 21.19 ± 2.89 | 21.45 ± 2.80 | 0.743 |
| HAMD-24 | 29.26 ± 7.51 | 12.10 ± 5.25 | *NA* | <0.001 |
| HAMA | 8.00 ± 3.55 | 23.71 ± 7.30 | *NA* | <0.001 |
| Sex, *n* (%) | | | | 0.929[a] |
| Male | 7 (30.43) | 7 (33.33) | 4 (40.00) | |
| Female | 16 (69.57) | 14 (66.67) | 6 (60.00) | |
| Marital status, *n* (%) | | | | 0.935[a] |
| Never married | 9 (39.13) | 8 (38.10) | 3 (30.00) | |
| Married | 14 (60.87) | 13 (61.90) | 7 (70.00) | |
| Family history, *n* (%) | | | *NA* | 0.481[b] |
| Yes | 4 (17.39) | 6 (28.57) | | |
| No | 19 (82.61) | 15 (71.43) | | |
| TSH | 2.29 ± 1.32 | 2.55 ± 1.56 | *NA* | 0.543 |
| TT3 | 1.50 ± 0.23 | 1.55 ± 0.30 | *NA* | 0.531 |
| TT4 | 96.83 ± 14.95 | 88.22 ± 19.08 | *NA* | 0.102 |
| FT3 | 4.54 ± 0.75 | 4.41 ± 0.77 | *NA* | 0.587 |
| FT4 | 16.34 ± 2.71 | 15.04 ± 3.41 | *NA* | 0.168 |
| ACTH | 33.00 ± 17.77 | 29.83 ± 15.62 | *NA* | 0.535 |
| PTC | 389.58 ± 257.00 | 373.57 ± 267.43 | *NA* | 0.841 |

[a] *Fisher's exact probability method.*
[b] *Chi-squared test.*

## Bioinformatic and Statistical Analyses

The sequence index file generated from the sequencing experiment was used to identify and extract the sample data saved in FASTQ format. Barcodes and the primers in the beginning and the end were used to identify and select sequence reads. The sequence number of each sample was normalized, and OTUs with 97% identity thresholds were used in the UPARSE (v.7.1; http://drive5.com/uparse/) software program. Chimeric sequences were identified and removed using UCHIME (v.4.1; http://drive5.com/uchime/). The taxonomy of each 16S rRNA gene sequence was analyzed with RDP Classifier (http://rdp.cme.msu.edu/) using the SILVA (SSU 138) 16S rRNA database at a confidence threshold of 70% (44).

Gut-microbiota-specific microbial characteristics were subjected to analysis of variance (ANOVA), emphasizing both statistical significance and biological relevance. ANOVA was used to compare the relative abundance of microbes identified with 16S rRNA sequencing.

Statistical analyses were performed using SPSS (v.21.0; IBM Corp., Armonk, NY, USA). One-way ANOVA was used to compare the continuous variables, including age, BMI, and clinical scales. Fisher's exact test was used to analyze contingency tables, and the chi-squared method was used to compare the variables of all three groups. A $p < 0.05$ was considered significant. The false recovery rate representing the threshold correction was generated using the Benjamini–Hochberg method (45).

The $\alpha$-diversity was calculated by the ACE, Chao, Simpson, and Shannon indices. Mann–Whitney $U$ tests were used to identify differences between the two groups. The $\beta$-diversity was calculated using the Bray–Curtis index as the distance method and reported according to principal component analysis. A hierarchical clustering tree was used to describe similarities among different data-point categories using the Bray–Curtis distance method and visualized using iTOL (https://itol.embl.de/).

PICRUSt, a bioinformatics software package that predicts metagenome functional content from marker gene (e.g., 16S rRNA) surveys and full genomes (46), was used to determine species function.

Circos software (http://circos.ca/) visualizes data in a circular layout (47) and was employed to visualize the relationship between samples and species.

Cytoscape is an open-source software platform for visualizing molecular-interaction networks and biological pathways and integrating these networks with annotations, gene-expression profiles, and other state data (48). We used this to determine the correlation between significantly different KEGG orthologs (KOs) and significantly different OTUs or species.

# RESULTS

## Demographic Features and Levels of Neuroendocrine Hormone

We collected 54 fecal samples from the study participants, including 10, 23 (18 newly diagnosed and 5 relapsed), and 21 (19 newly diagnosed and two relapsed) subjects in the healthy crowd (HC), MDD, and GAD groups, respectively. The mean age at assessment was 30.04 ± 5.90, 30.43 ± 7.95, and 30.22 ± 6.50 years for the MDD, GAD and HC groups, respectively, with no significant difference found between groups according to ANOVA. Moreover, the BMI, sex ratio, marital status, family history, and levels of neuroendocrine hormones did not differ significantly among the three groups (**Table 1**).

## Diversity Analysis ($\alpha$ and $\beta$)

Accounting for 70% of the valid sequences, we obtained 1,620,000 high-quality sequences from the 54 fecal samples of all participants: the HC, MDD, and GAD groups contained 630,000, 690,000, and 300,000 sequences, respectively. The richness of gut bacterial communities in all three groups was estimated by ACE and Chao indices, and the diversity was estimated using the Shannon and Simpson diversity indices. ACE and Chao analysis showed that most of the gut microbial diversity in each sample had been captured with the current sequencing depth. After rarefying the sequencing depth among all samples using a bootstrap method (30,000 reads per sample), the Shannon and Simpson diversity index estimates were calculated, revealing no significant difference in richness and diversity between HC and MDD. However, GAD showed a significant difference in microbiota richness and diversity as compared with HC (**Figures 1A–D**). To explore the differences in the comprehensive microbial phenotypes of MDD, GAD, and HC, we performed $\beta$-diversity analysis. Among the three groups, statistical analysis of $\beta$-diversity at the genus level indicated that the distance was similar according to principal component analysis (**Figure 1E**). The hierarchical clustering tree used to describe the similarities among different data point categories using the Bray–Curtis distance method showed sample similarities between the three groups (**Figure 1F**).

## Analysis of Fecal Species Community

We obtained 10,996, 14,406, and 15,010 species-level OTUs from the HC, MDD, and GAD groups, respectively, using the OTU cluster method (**Table 2**). Results of the Venn diagram showed that 5,069 OTUs were common to all three groups (**Figure 2A**). The relationship between samples and OTUs is presented as the Circos diagram (**Figure 2B**). The general overview of gut bacterial composition at the phylum and genus levels is shown in **Figures 2C,E**. At the phylum level, the groups were rich in Bacteroidetes, Proteobacteria, and Firmicutes, but there were differences in terms of abundance. Compared with that in the HC group, we found that the relative abundance of Proteobacteria and Firmicutes increased considerably, and that of Bacteroidetes decreased considerably in the MDD group. Additionally, the abundance of Firmicutes decreased, whereas that of Bacteroidetes and Proteobacteria increased in GAD relative to HC vs. MDD (**Figure 2C**). The relationship between samples and phylum-level species is presented as the Circos diagram (**Figure 2D**). At the genus level, we found that *Bacteroides* and *Prevotella* were abundant in the three groups. The abundance of *Faecalibacterium* decreased and *Sutterella*, *Fusicatenibacter*, and *Christensenellaceae*_R7_group increased

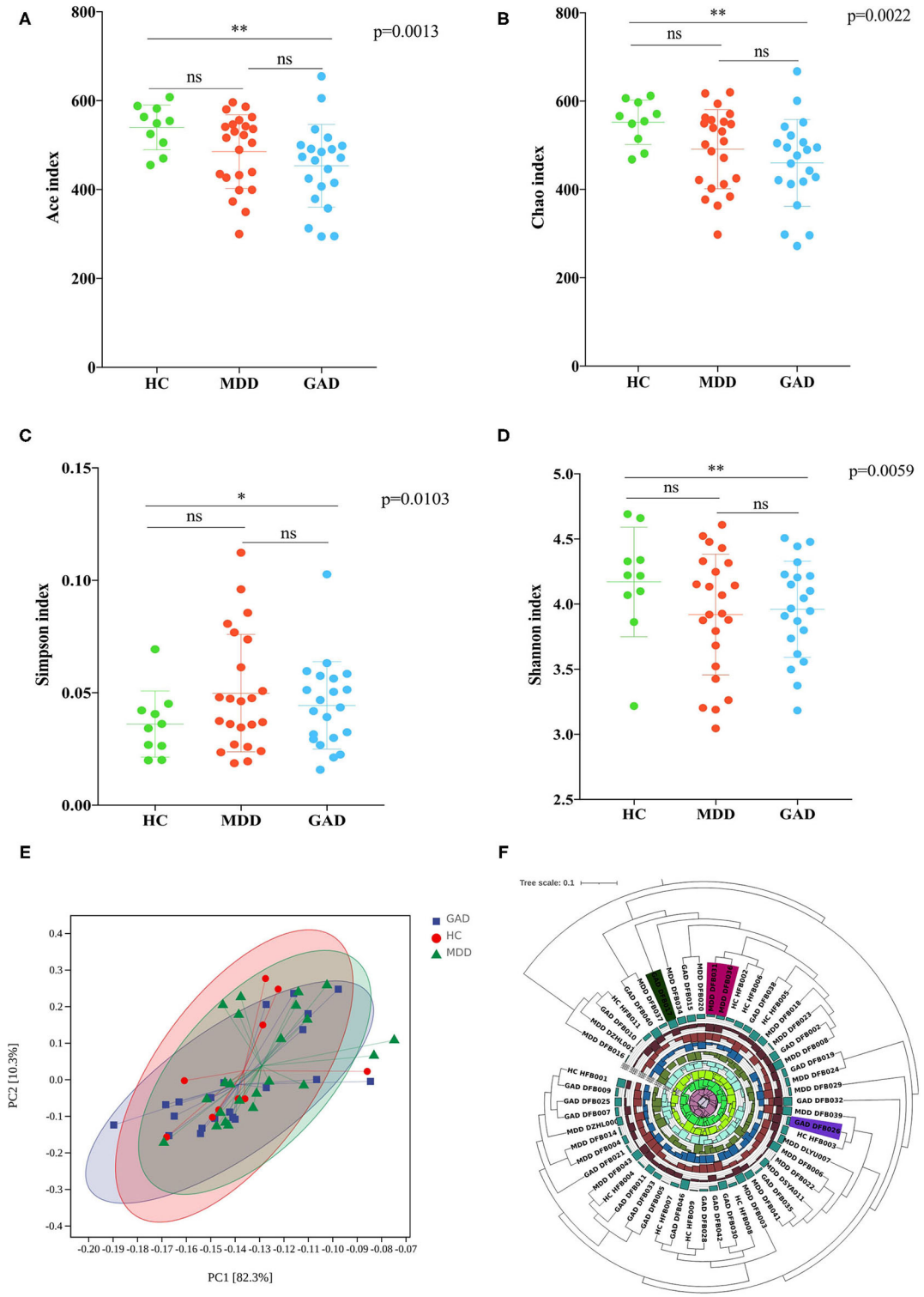


**FIGURE 1 |** Diversity analysis ($\alpha$ and $\beta$). **(A–D)** Analysis of variations in richness (Chao and ACE indices) and diversity (Simpson and Shannon indices). MDD compared with HC revealed no significant difference in richness and diversity. GAD compared with HC revealed a significant difference in the richness and diversity. *$p < 0.05$ (Bonferroni $< 0.017$), **$p < 0.01$ (Bonferroni $< 0.0033$). MDD compared with GAD revealed no significant difference in richness and diversity. **(E)** Results of β-diversity visualized using principal component analysis (PCA; Bray–Curtis distance method). **(F)** Hierarchical clustering tree showing the similarities among different categories of data points by the Bray–Curtis distance method. Diversity analysis showed similar species diversity among the three groups.

**TABLE 2 |** Comparison of phylotype coverage and diversity estimation of the 16S rRNA gene libraries at 97% similarity from the sequencing analysis.

| Group | No. of reads | No. of OTUs | Coverage (%) | Richness estimator | | | | Diversity index | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | ACE | 95% CI | Chao | 95% CI | Shannon | Simpson | Evenness |
| HC | 630,000 | 10,996 | 97.19 | 4257.84 | 4031.74–4506.03 | 3090.89 | 2840.24–3395.07 | 4.733331714 | 0.032098 | 0.354556683 |
| MDD | 690,000 | 14,406 | 97.47 | 3762.74 | 3557.59–3989.13 | 2790.49 | 2562.74–3069.66 | 4.647938217 | 0.036133 | 0.34454694 |
| GAD | 300,000 | 15,010 | 96.15 | 5978.93 | 5689.91–6291.70 | 4170.2 | 3857.99–4538.97 | 5.017976 | 0.025108 | 0.397936241 |

*CI, confidence interval.*

in MDD relative to HC. The abundance of *Fusicatenibacter* and *Christensenellaceae*_R7_group decreased in GAD relative to HC. Compared with that in the MDD group, we found an increase in the relative abundance of Fusobacteria, Tenericutes, Verrucomicrobia, and Bacteroidetes but a decrease in that of Proteobacteria, Actinobacteria, and Firmicutes in the GAD group (**Figure 2E**). The relationship between samples and species is illustrated by the Circos diagram (**Figure 2F**). In summary, we found that patients with MDD or GAD showed considerable changes in gut microbiota, and that there were differences in the relative abundance of gut microbiota in patients with both disorders.

## Analysis of the Signatures of Gut Microbiota

We the compared the relative abundance of microbial composition among the three groups in the discovery set at both the OTU and genus levels. We found taxonomic differences in fecal microbiota among the HC, MDD, and GAD groups and identified six significantly different OTUs that were altered among the three groups (**Table 3** and **Figure 3A**). The levels of Otu24167, Otu19140, and Otu19751 were significantly decreased in MDD relative to HC, whereas Otu2563 levels were significantly increased in MDD relative to GAD or HC. Otu2581 and Otu10585 levels were significantly increased in GAD relative to MDD. Furthermore, we found no significant difference in the abundance of OTUs between GAD and HC. Similarly, at the genus level, the abundances of *Sutterella* and *Fusicatenibacter* were significantly lower in MDD relative to HC, and both *Fusicatenibacter* and *Christensenellaceae*_R7_group abundances were significantly lower in GAD relative to HC. Additionally, the abundance of *Sutterella* was significantly higher, whereas that of *Faecalibacterium* was significantly lower in GAD relative to MDD (**Table 4** and **Figure 3B**).

## Functional Prediction of Gut Microbiota

We obtained 6910 KOs and mapped them to the KEGG database using PICRUSt. A total of 69 significantly different KOs were obtained by ANOVA (**Supplementary Material**). The correlation between significantly different KOs and significantly different OTUs is depicted by a heatmap (**Figure 4A**). We found that the levels of two KOs (K01205 and K09011), which significantly correlated with Otu10585_Bacteroides, were significantly lower in MDD and higher in GAD relative to HC. The levels of one KO (K01201), which significantly correlated with Otu19751_Prevotella-9 and Otu19140_Prevotella-9, were lower in MDD and higher in GAD relative to HC. Another KO (K00163), which significantly correlated with Otu24167_Megamonas, showed higher levels in MDD relative to GAD and HC. Two KOs (K08281 and K03782), which significantly correlated with Otu2563_Enterobacteriaceae, showed higher levels in MDD relative to GAD and HC. Another KO (K07713), which significantly correlated with Otu2581_Bacteroides, showed lower levels in MDD relative to GAD.

The correlation between the significantly different KOs and species is depicted by a heatmap (**Figure 4B**). We found that two KOs (K00657 and K04516) significantly correlated with Christensenellaceae_R7_group, and that their levels were higher in GAD and lower in MDD relative to HC. Two KOs (K08281 and K02067) significantly correlated with *Faecalibacterium*, and their levels were higher in MDD and lower in GAD relative to HC. One KO (K02549) significantly correlated with *Fusicatenibacter,* and its levels were higher in MDD and lower in GAD relative to HC. We used a network diagram to explain the relationship between the significantly different KOs and OTUs/species (**Figures 4C,D**).

## Relationship Between Gut Microbiota and Clinical Parameters

As noted, we found four bacterial genera (*Christensenellaceae*_R7_group, *Faecalibacterium, Fusicatenibacter,* and *Sutterella*) with differences in MDD and GAD relative to HC or between them. These four bacterial genera can be considered as important genera that affect the disease phenotype. To further explore the functions of these different bacterial genera, we evaluated correlations among the relative abundance of bacteria, hormones (including PTC, ACTH, FT3, FT4, TT3, TT4, and TSH), and the total and factor scores of HAMD (Hopelessness, Sleep disturbance, Block, Diurnal/variation, Cognitive impairment, Weight, and Anxiety/somatic) in the MDD group and those of HAMA (Psychic anxiety and Somatic anxiety) in the GAD group. We found significant differences in correlations at the genus level for *Fusicatenibacter* and *Christensenellaceae*_R7_group in MDD patients (**Figure 5A**). We observed that *Christensenellaceae*_R7_group negatively correlated with the HAMD factor score (Limited to Hopelessness) and total score ($p < 0.05$), *Fusicatenibacter* negatively correlated with FT4 ($p < 0.05$), and other key phylotypes showed no strong correlation. Further, the GAD

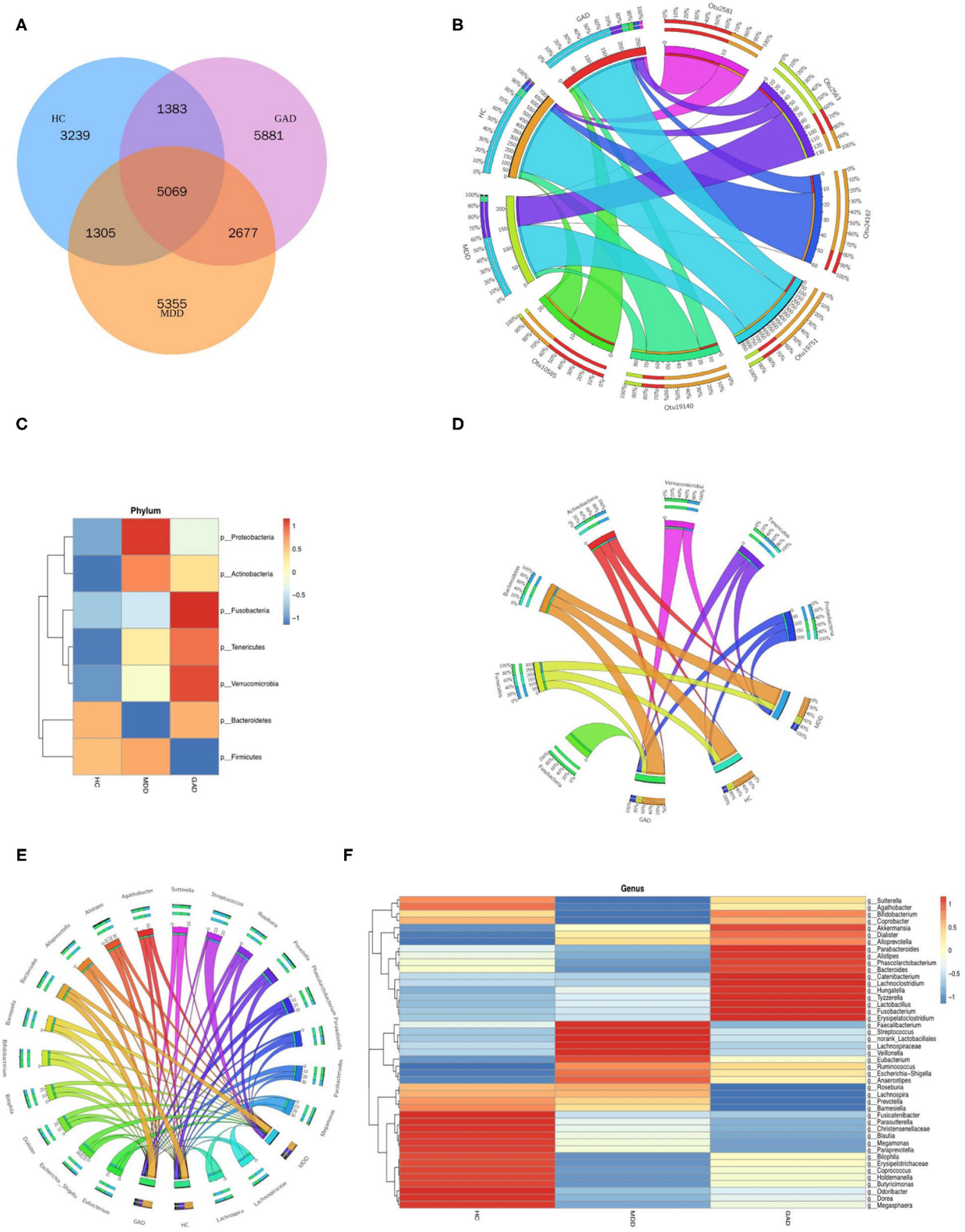


**FIGURE 2 |** Species composition analysis. **(A)** Venn diagram of samples with common and unique OTUs. **(B)** Distribution of OTUs in the three groups. Data were visualized using Circos software (http://circos.ca/). The length of the bars for each sample on the outer ring represents the percentage of species in each sample. **(C,E)** Stacked bar plots at the phylum and genus levels show species composition according to the relative abundance of species. **(D,F)** Distribution of species in the three groups at the phylum and genus levels.

**TABLE 3 |** Statistical analysis of the OTUs by ANOVA.

| Name | Mean (GAD) | Stderr (GAD) | Mean (HC) | Stderrn (HC) | Mean (MDD) | Stderr (MDD) | *p* | FDR | *P* (HC-GAD) | *P* (MDD-GAD) | *P* (MDD-HC) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Otu2581 | 10.0476191 | 3.1475434 | 4.5 | 2.42326685 | 0.91304348 | 0.63155822 | 0.01229678 | 0.8772808 | 0.3115826 | 0.00892584 | 0.60088641 |
| Otu24167 | 12.2380952 | 10.253897 | 47.5 | 24.3284242 | 0.39130435 | 0.39130435 | 0.02301388 | 0.8772808 | 0.10009871 | 0.64423469 | 0.01727706 |
| Otu19751 | 184.428571 | 73.8974377 | 572.4 | 260.248223 | 136.826087 | 49.1125453 | 0.03020169 | 0.8772808 | 0.06111859 | 0.92991901 | 0.02841122 |
| Otu19140 | 18 | 7.43799768 | 54.5 | 25.2666887 | 13.0434783 | 4.26377251 | 0.03421628 | 0.8772808 | 0.07042385 | 0.91925868 | 0.03153303 |
| Otu10585 | 13.3333333 | 5.16781861 | 7.6 | 3.10268701 | 1 | 0.36658881 | 0.03721526 | 0.8772808 | 0.60101603 | 0.0285381 | 0.50097406 |
| Otu2563 | 28.5238095 | 10.8974919 | 23.9 | 15.2683041 | 79.4347826 | 20.067458 | 0.0418317 | 0.8772808 | 0.98528601 | 0.0650306 | 0.1230169 |

*FDR, false discovery rate; Stderr, standard error.*

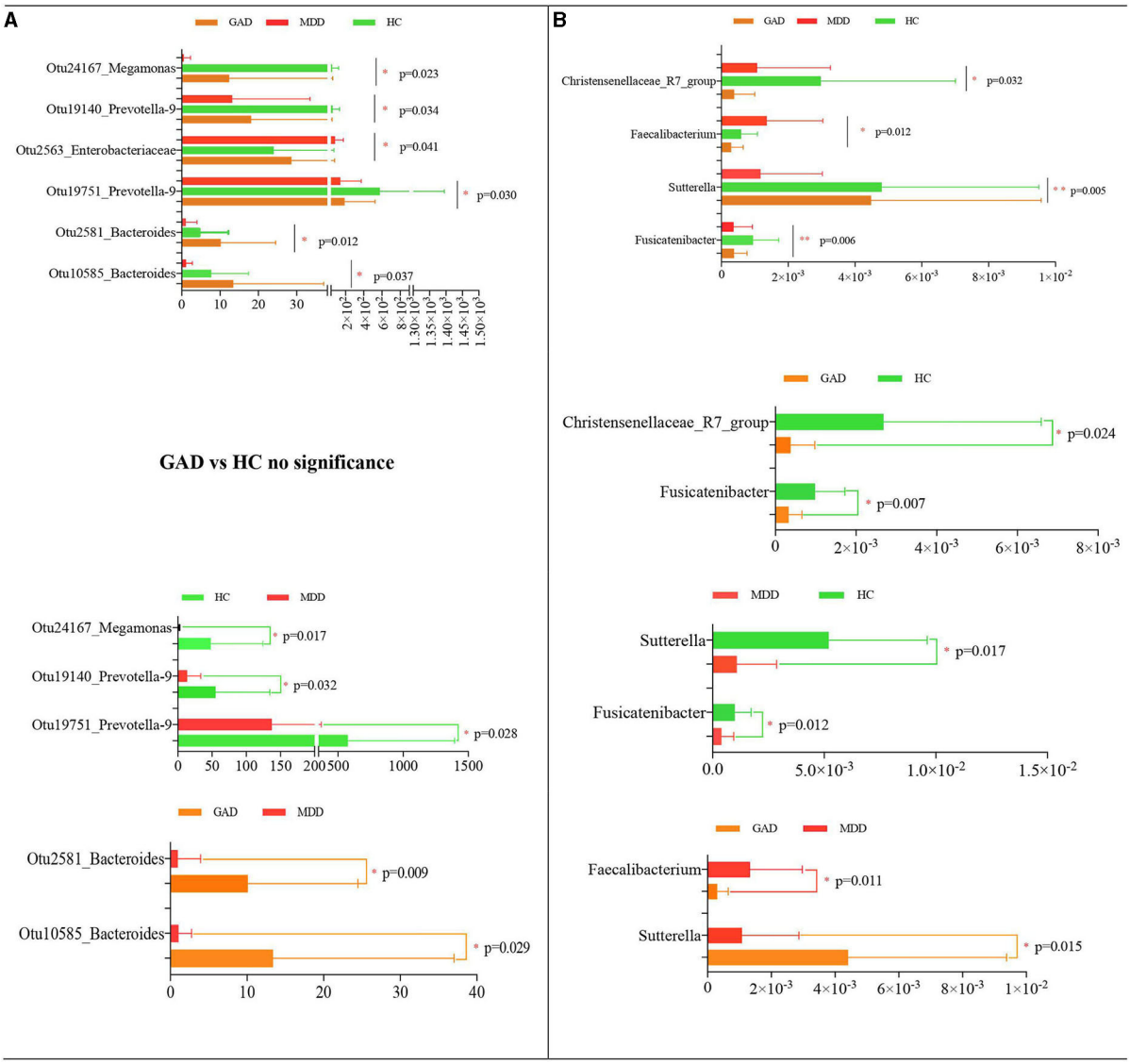


**FIGURE 3 |** Statistical analysis of species differences. Taxonomic differences of fecal microbiota between HC, MDD, and GAD groups. Altered levels of specific bacterial OTUs and genera in MDD, GAD, and HC. **(A)** Six dominant bacterial OTUs were altered among the three groups. Otu24167, Otu19140, and Otu19751 were significantly decreased in MDD relative to HC. By contrast, Otu2563 was significantly upregulated in MDD relative to GAD or HC. Otu2581 and Otu0585 were significantly upregulated in GAD relative to MDD or HC. **(B)** Four significantly different species at the genus level across the three groups are shown. The abundances of *Sutterella* and *Fusicatenibacter* were significantly lower in MDD relative to HC. *Fusicatenibacter* and *Christensenellaceae*_R7_group abundances were significantly lower in GAD relative to HC. The abundance of *Sutterella* was higher, whereas that of *Faecalibacterium* was lower in GAD relative to MDD (all multiple comparisons; ANOVA tests).

**TABLE 4** | Statistical analysis of the species by ANOVA.

| Name | Mean (GAD) | Stderr (GAD) | Mean (HC) | Stderr (HC) | Mean (MDD) | Stderr (MDD) | *p* | FDR | *P* (HC-GAD) | *P* (MDD-GAD) | *P* (MDD-HC) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Sutterella* | 0.00439143 | 0.00108852 | 0.005187 | 0.00139934 | 0.00106652 | 0.00037587 | 0.00479284 | 0.66214192 | 0.85118087 | 0.01566996 | 0.01722476 |
| *Fusicatenibacter* | 0.00031762 | 7.44E-05 | 0.000974 | 0.00023634 | 0.00037304 | 0.00011939 | 0.00595727 | 0.66214192 | 0.00663858 | 0.93707457 | 0.01242988 |
| *Faecalibacterium* | 0.00029 | 7.86E-05 | 0.000552 | 0.00015174 | 0.00131739 | 0.00034548 | 0.01248295 | 0.70261169 | 0.81872373 | 0.01100806 | 0.1834709 |
| *Christensenellaceae*_R7_group | 0.00036429 | 0.0001336 | 0.002671 | 0.00124013 | 0.00101304 | 0.00045173 | 0.03155333 | 0.71208711 | 0.02401158 | 0.59744199 | 0.12743151 |

*FDR, false discovery rate; Stderr, standard error.*

group showed significant differences at the genus level for *Faecalibacterium*, which negatively correlated with PTC ($p < 0.05$), whereas other key phylotypes showed no strong correlation (**Figure 5B**). The columnar stack diagram shows the correlation between clinical parameters and the significantly different species (**Figures 5C,D**).

## DISCUSSION

This study is the first to characterize and compare the gut-microbial compositions of patients with MDD and GAD. Our findings provide a better understanding of the differences between these two diseases in terms of their underlying mechanisms and will help in identifying novel therapeutic targets for better treatments.

We identified unique microbial signatures of patients with MDD and GAD relative to the HC. There was no significant difference in richness and diversity between the HC and patients with MDD. Consistent with this finding, numerous studies have reported no differences between MDD and control groups across all examined indices (49–51). However, some studies found decreased $\alpha$-diversity in depressive disorders using the Shannon index (52, 53), as well as significant differences in $\beta$-diversity between participants with a depressive disorder and those in the control group (19, 52, 54–58). Based on the taxonomic findings in this study, Otu24167, Otu19140, and Otu19751 were significantly decreased in MDD as compared with HC, and the abundance of *Sutterella* and *Fusicatenibacter* at the genus level was significantly lower in patients with MDD than in the HC. Previous studies reported a lower *Sutterella* abundance in patients with depressive disorders than in the HC (19, 53–55); however, the association of *Fusicatenibacter* with depression has not previously been reported.

We found that the GAD group showed significantly higher microbiota richness and diversity than the HC, but there was no significant difference in OTU abundance between the two groups. This result differs significantly from previous reports. Chen et al. and Jiang et al. reported no difference in $\alpha$-diversity between participants with an anxiety disorder and those in the control group, and that participants with GAD showed lower microbiota richness than control group subjects (24, 25). This heterogeneity of results might be attributed to numerous factors, including sample size, dietary intake, demographic characteristics of the participants, clinical status, sequencing methods, statistical methods, and/or the statistical significance threshold chosen to determine the disease-associated gut microbiota (59, 60). Based on the taxonomic findings (**Figure 3B**), *Fusicatenibacter* and *Christensenellaceae*_R7_group abundances were lower in the GAD group than in the HC. Mancabelli et al. reported *Christensenellaceae* as one of five taxa considered as a signature of a healthy gut (61). It is possible that its family might be related to affective disorders and neurological diseases (62). For example, patients with Parkinson's disease, multiple sclerosis, and autism have a remarkably lower relative abundance of *Christensenellaceae* (63–65).

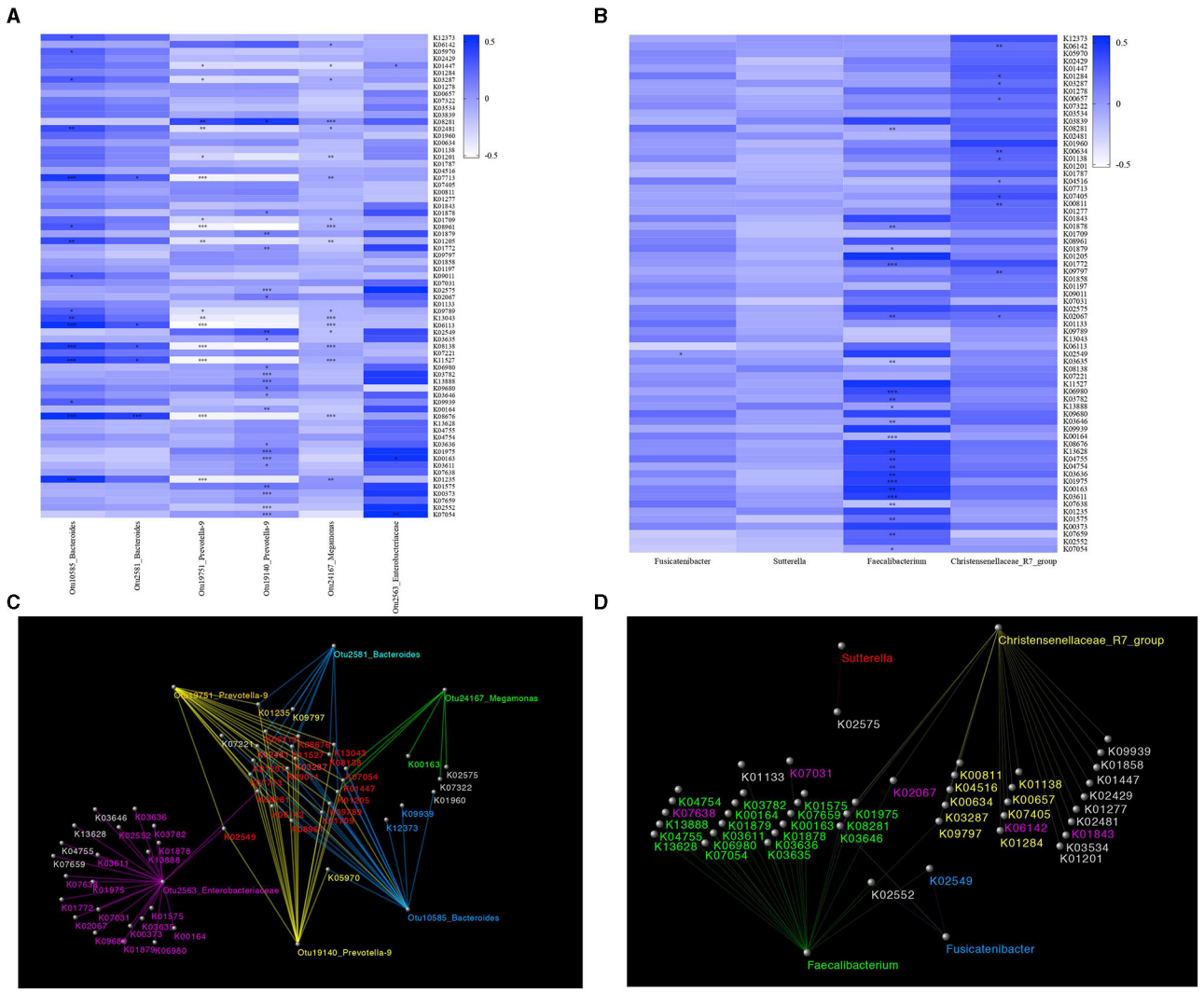


**FIGURE 4 |** Functional prediction. Results of gut microbial functional pathway analyses. KOs were determined using PICRUSt. The significance of functional pathway prediction was determined by ANOVA (**Supplementary Material**). We obtained 69 significantly different KOs. The correlations between OTUs or species and KOs according to a heatmap are shown. Color variation indicates correlation, "+" indicates positive correlation, and "–" indicates negative correlation. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.005$. The network was used to show the correlation between significantly different KOs and OTUs or species using Cytoscape software. **(A)** The correlation between significantly different KOs and OTUs is shown in a heatmap. Sixteen KOs were significantly correlated with Otu10585_ Bacteroides, five KOs were significantly correlated with Otu2581_Bacteroides, 16 KOs were significantly correlated with Otu19751_Prevotella-9, 18 KOs were significantly correlated with Otu19140_Prevotella-9, 22 KOs were significantly correlated with Otu2563_Enterobacteriaceae, and three KOs were significantly correlated with Otu24167_Megamonas. **(B)** The correlation between significantly different KOs and species is shown in a heatmap. Eleven KOs were significantly correlated with *Christensenellaceae*_R7_group, 22 KOs were significantly correlated with *Faecalibacterium*, and one KO was significantly correlated with *Fusicatenibacter*. **(C)** Network diagram showing the relationship between significantly different KOs and OTUs. Different colors represent different OTUs. The same color between Kos and OTUs indicates a significant correlation. The red KOs represent significant correlations between KOs and multiple OTUs. **(D)** Network diagram showing the relationship between significantly different KOs and species. Different colors represent different species, and the same color indicates a significant correlation.

The most important focus of this study was on distinguishing between MDD and GAD, and several significant differences were observed. Compared with patients with GAD, Otu2581 and Otu10585 levels were significantly reduced, whereas the abundance of *Sutterella* was decreased and that of *Faecalibacterium* was increased at the genus level in patients with MDD. However, no significant differences were observed in the α-diversity and richness of the intestinal floras between patients with GAD and patients with MDD, indicating that their intestinal floras were similar. Additionally, we found that Otu10585_Bacteroides significantly correlated with K09011 (map00290, valine, leucine, and isoleucine biosynthesis). Various studies also reported that MDD is associated with aberrant branched-chain amino acid and energy metabolism (66), suggesting that these amino acids (valine, leucine, and isoleucine) might serve as appropriate biomarkers for

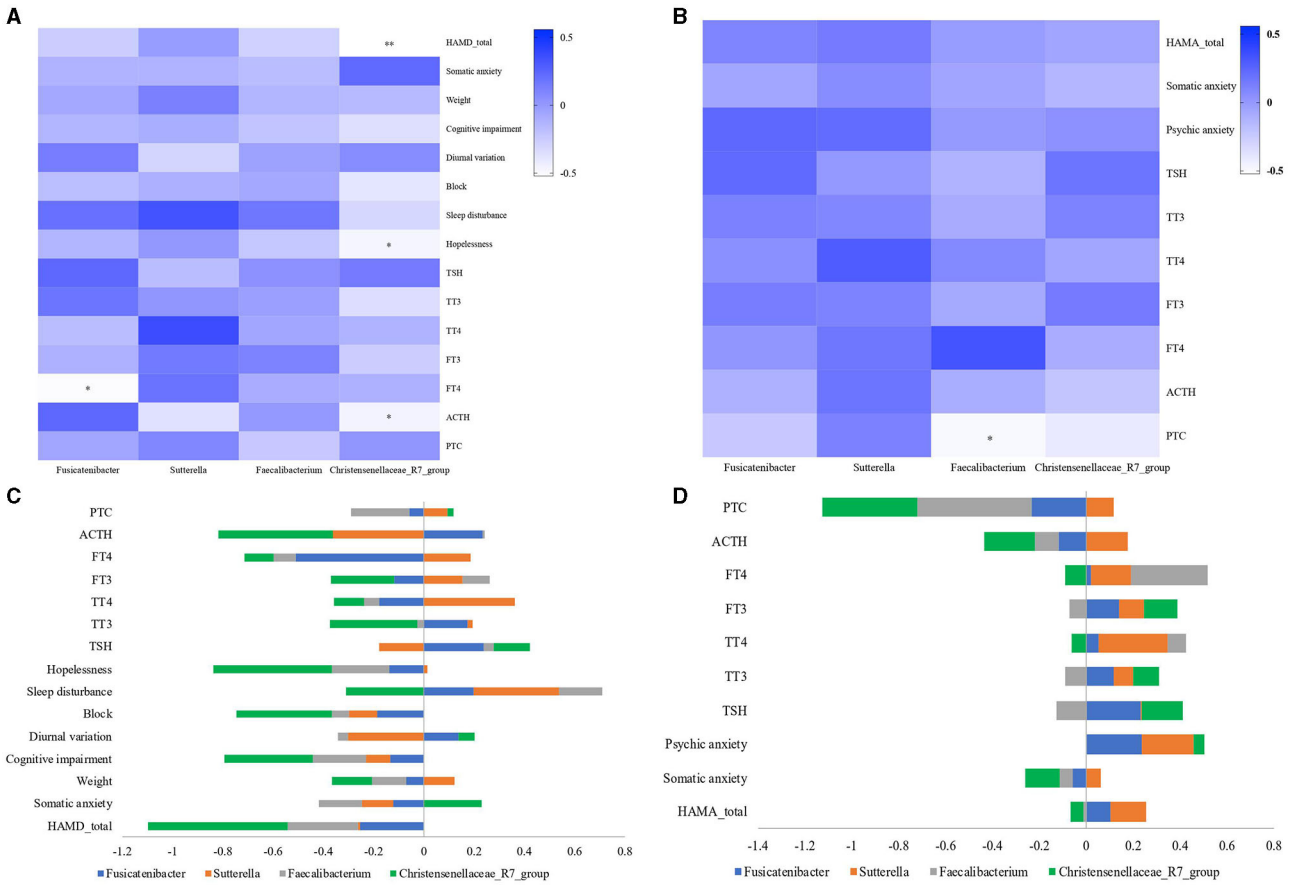


**FIGURE 5 |** Relationship between gut microbiota and clinical parameters. Heatmaps were used to show relationships between significant differences in microbiota and clinical parameters, including hormones (PTC, ACTH, FT3, FT4, TT3, TT4, and TSH), the total and factor scores of HAMD (Hopelessness, Sleep disturbance, Block, Diurnal/variation, Cognitive impairment, Weight and Anxiety/somatic) in the MDD group, and the total and factor scores of HAMA (Psychic anxiety and Somatic anxiety) in the GAD group. **(A)** In the MDD group, *Fusicatenibacter* was significantly negatively correlated with FT4 ($p < 0.05$), and *Christensenellaceae*_R7_group was significantly negatively correlated with HAMD ($p < 0.01$), Hopelessness, and ACTH ($p < 0.05$). **(B)** In the GAD group, *Faecalibacterium* was significantly negatively correlated with PTC ($p < 0.05$). **(C,D)** The stacked bar plot was used to show the relationship between gut microbiota and clinical parameters ("–" shows a negative correlation, and "+" shows a positive correlation).

depression (67). We speculate that depression might be caused by the influence of Otu10585 on branched-chain amino acids metabolism. Moreover, the present results showed that *Faecalibacterium* was significantly correlated with K08281 (map00760, nicotinate, and nicotinamide metabolism) and K02067 (map02010, ABC transporters). Niacin deficiency is reportedly a contributing factor in mental-illness development and symptom alleviation (66). We speculate that the decreased abundance of *Faecalibacterium* might affect nicotinate and nicotinamide metabolism, leading to variations in correlative metabolism that result in MDD or GAD. ABC transporters exert notable effects on pathogen–host interactions and bacterial physiology (68), which might indicate another pathway of *Faecalibacterium* that results in GAD or MDD; however, the specific mechanism requires further study. Furthermore, the roles of *Sutterella* and Otu2581 remain unclear, although previous studies report that *Sutterella* is an intestinal flora associated with inflammatory responses and is found in abundance in autistic patients (69–71).

To determine why intestinal flora affect the clinical phenotype, we analyzed the correlation of some representative floras in patients with MDD or GAD with respect to the HPA or HPT axis. Our findings (**Figure 5**) implied that changes in intestinal flora might first induce changes in the HPA and/or HPT axis, which ultimately lead to the different clinical phenotypes of MDD and GAD. Previous reports indicated that gut-microbiota deficiency exacerbates the neuroendocrine and behavioral responses to acute stress (72–74). Other studies have also found a close relationship between HPT/HPA-axis dysfunction and depression/anxiety (32, 35, 36, 75, 76). For example, a dynamic decrease in thyroid hormone levels (particularly FT3 and FT4) is reportedly closely related to depression (36). These observations are consistent with the present findings and implications.

Additionally, we observed that *Christensenellaceae*_R7_group negatively correlated with factor (Limited to Hopelessness) and total scores of HAMD, suggesting that although *Christensenellaceae_R7_group* has not been observed as enriched in patients with MDD, it might affect the clinical manifestations and severity of MDD. Similar conclusions have been confirmed in other studies (20, 54).

This study has some limitations. First, the sample size was relatively small with no power calculation, which might have resulted in sampling bias. Second, the 16S rRNA gene sequencing used in this study resulted in limited functional information; therefore, whole-genome and whole-macrotranscriptome sequencing need to be performed in future studies. Third, we did not use standardized diagnostic tools to diagnose patients, assess the mental state of HCs and exclude comorbidities, which may weaken the reliability of the results. Last, other influential factors, such as food intake and physical activity, were not considered, which might also cause bias.

In summary, this study characterized and identified different gut-microbial compositions in subjects with MDD, subjects with GAD, and the HC. We identified a correlation between the bacteria and clinical symptoms, including a significant negative correlation between *Christensenellaceae_R7_group* and HAMD score. Moreover, we conducted a preliminary analysis of possible mechanisms underlying intestinal flora-affecting diseases. Our findings suggest that intestinal microflora might serve as molecular markers for distinguishing MDD from GAD.

## DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/**Supplementary Material**.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of West China Hospital of Sichuan University. The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

ZD and WK: conception of the work, final approval of the version to be published, and agreement to be accountable for all aspects of the work. ZD, XS, YH, JL, HL, HX, and LY: acquisition of data. ZD and LY: analysis and interpretation of data for the work. ZD: writing. WK: revising the work. All authors contributed to the article and approved the submitted version.

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# Potential of Antithrombin III as a Biomarker of Antidepressive Effect in Major Depressive Disorder

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**Background:** The evaluation of treatment response to antidepressant therapy commonly depends on neuropsychologic assessments, as there are currently no suitable biomarkers. Previous research has identified a panel of increased proteins in patients with major depressive disorder (MDD), including antithrombin III (ATIII), as potential biomarkers of depression.

**Methods:** A total of 90 MDD patients were recruited. Of these, 74 patients received occipital repetitive transcranial magnetic stimulation (rTMS) as individualized, standard, or sham treatment for 5 days, and underwent the complete procedure, including clinical assessments, blood collection, and protein measurement.

**Results:** After treatment, ATIII was significantly decreased in both the individualized and standard groups (both $p < 0.001$) relative to the sham group. In the individualized group, reduction in ATIII was associated with improvements in several neuropsychological assessments. Furthermore, ATIII at baseline in the standard group and after individualized rTMS showed good performance for evaluating or predicting the response to five-day treatment (AUC = 0.771, 95% CI, 0.571–0.971; AUC = 0.875, 95% CI, 0.714–1.000, respectively) and remission at follow-up (AUC = 0.736, 95% CI, 0.529–0.943; AUC = 0.828, 95% CI, 0.656–1.000, respectively). Lastly, both baseline ATIII and change in ATIII showed good predictive value for the 24-item Hamilton Depression Rating Scale at follow-up ($p = 0.024$ and 0.023, respectively).

**Conclusion:** Our study revealed a reduction in ATIII after occipital rTMS in MDD patients and a relationship between change in ATIII and therapeutic response. Taken together, these findings provide evidence for the potential of ATIII as a biomarker for the evaluation and prediction of antidepressive effects.

**Keywords: major depressive disorder, antithrombin III, occipital repetitive transcranial magnetic stimulation, antidepressive effect, biomarker**

## INTRODUCTION

Major depressive disorder (MDD) is a mental illness characterized by low mood and anhedonia that affects 322 million people worldwide, leading to excess disability and mortality (1–3). For the period of 2007 to 2017, MDD was the third leading cause of global years lived with disability (YLDs) (1). According to the World Health Organization, this common mental disorder will be responsible for the greatest burden of disease worldwide by 2030 (4). Both biological factors and environmental milieu are related to the occurrence of depression (5, 6); however, little is known about the physiopathological mechanisms underlying MDD. Several hypotheses have been proposed (3, 7). In one of the mainstream theories, neuroinflammation is proposed to influence MDD through abnormalities in immune cells, like microglia, astrocytes and oligodendroglia, and increases in inflammatory factors (8–12). Accumulating evidence suggests that neuroinflammation might be a mediator of MDD in connection with other possible mechanisms, such as stress, neuroendocrine system dysfunction, neurotransmitters depletion, neurogenesis defects, and intestinal flora disorder (13–17). As reported in previous studies, various anti-inflammatory treatments can improve depression symptoms, supporting that inflammatory molecules or pathways may be potential targets for novel antidepressant therapy (18, 19).

At present, MDD and antidepressive therapy response are assessed by clinical features, rather than objective indicators, duo to the lack of confirmed clinical biomarkers (3, 20, 21). As a result, it is imperative to identify and evaluate potential biomarkers of MDD. A variety of techniques have been utilized in the aim to identify such biomarkers, including genomics, transcriptomics, proteomics, metabonomics, neuroimaging, electroencephalography, etc. (22–27). In comparison to other biological parameters, proteins play direct roles in the occurrence and development of MDD and the result of antidepressive treatment (24), and thus have great application as candidate biomarkers.

In our previous proteomic study, four proteins were noted: C-reactive protein (CRP), antithrombin III (ATIII), inter-alpha-trypsin inhibitor heavy chain H4 (ITIH4), and vitamin D binding protein (VDB) (28). As an acute-phase protein, CRP is synthesized by hepatocytes and increases rapidly in response to systemic inflammation with activated innate immunity (29, 30). A multitude of studies have demonstrated that high levels of CRP are associated with MDD (31). Notably, CRP values have been used to predict the outcome of depression and resistance to standard antidepressants (32–34). ATIII is a glycoprotein mainly produced in the liver that exerts anticoagulant and anti-inflammatory effects by targeting activated thrombin and other blood coagulation factors (35, 36). In addition, ATIII can promote prostacyclin release and deactivate leukocytes to inhibit inflammation independent from coagulation (36). As reported by Stelzhammer et al., elevated levels of ATIII were detected in MDD patients after the first session of electroconvulsive therapy (ECT) (37). ITIH4 is an acute-phase protein related to inflammatory response involved in cell proliferation, migration, anti-apoptosis, and matrix stabilizing (38, 39). A genome-wide analysis reported that single nucleotide polymorphisms (SNPs) in the *ITIH4* gene were associated with bipolar disorder (40). Furthermore, according to Finseth et al., there are associations between suicide attempt and *ITIH4* (41). The main biological function of VDB, as its name suggests, is to bind and transport the specific sterol and its metabolites. Additional physiological processes regulated by this glycoprotein include actin scavenging, macrophage and osteoclast activation, and phagocytic cells chemotaxis (42–44). In prior studies, increased VDB was observed in patients with major mood disorders, comprised of both MDD and bipolar disorder (45–47). These four proteins were observed to be elevated in the depressed sample compared with controls in our previous study (28), supporting their potential to differentiate MDD. However, whether they are associated with antidepressive treatment and curative effects is unknown.

Occipital repetitive transcranial magnetic stimulation (rTMS) therapy is a novel non-invasive intervention for MDD with established safety and efficacy (48). The occipital cortex has been demonstrated to be associated with the pathophysiologic changes of MDD, which was supported by altered visual evoked potential, diminished perception of ambient light, impaired synaptic plasticity and low density of calbindin-immunoreactive GABAergic neurons in the occipital cortex in MDD patients (49–51). Moreover, a previous study suggests that selectively neural response to emotional stimuli in the occipital cortex might be a useful biomarker in identifying responders to scopolamine (52), which partly supports the role of the occipital cortex in antidepressive treatment.

This present study aimed at exploring changes in four proteins included CRP, ATIII, ITIH4, and VDB after occipital rTMS, and to assess their potential as biomarkers of the outcomes and effects of antidepressive therapy.

## MATERIALS AND METHODS

### Subjects

The Ethics Committee of the Second Affiliated Hospital of Xinxiang Medical University approved this study, which was carried out at Henan Provincial Mental Hospital. All work involving human subjects followed the latest version of the Declaration of Helsinki. After the benefits and risks of the study were fully explained, a total of 90 drug naïve or drug free depressed individuals were recruited. Written informed consent was obtained from the participants or their legal guardians.

The inclusion criteria for MDD patients were as follows: (a) meeting the diagnostic criteria of MDD according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV), assessed by two well-trained psychiatrists utilizing the Structured Clinical Interview for DSM-IV; (b) a score of >17 on the 24-item Hamilton Depression Rating Scale (HAMD-24); (c) aged 18 to 55 and drug naïve or drug free for at least 2 weeks; and (d) able to cooperate with the trial and understand the items properly.

Patients suffering from the following disorders or diseases were excluded: (a) other DSM-IV axis I or axis II disorders; (b) organic brain diseases; (c) psychotic depression; (d) endocrine

diseases with abnormal biochemical indexes or organ functions; (e) treatment-resistant depression treated by electroconvulsive shock or rTMS with adequate dosage and duration; and f. diseases that result in any contraindications to magnetic resonance imaging (MRI) or rTMS. In addition, given potential effects due to alterations in hormones, female patients who were pregnant or in the puerperium or climacteric periods were excluded from this study.

## Neuropsychological Assessments

The scales for neuropsychological assessments in three dimensions (affective assessments, social-psychology assessments, and cognitive function assessments) were described in detail in our previous study (53). Briefly, to estimate the affective states of the MDD patients, the HAMD-24, Hamilton Anxiety Scale (HAMA), Beck Scale for Suicide Ideation-Chinese version-Current (BSI-CV-C), Self-Rating Depression Scale (SDS), Self-Rating Anxiety Scale (SAS), and Beck Hopelessness Scale (BHS) were conducted under unified instructions at baseline and day 1, 3, and 5 of treatment. In addition, during the 4 week follow-up study, the HAMD-24 was used once per week to evaluate the outcomes. To assess social psychology traits, patients were assessed once using the Life Event Scale (LES), Childhood Trauma Questionnaire-Short Form (CTQ-SF), family Adaptation, Partnership, Growth, Affection, Resolve (APGAR), and Self-Consciousness Scale Revised (SCSR). Cognitive functions were estimated in terms of information processing speed, executive function, and visuospatial memory and learning function (detailed evaluation tools are listed in **Supplementary Table 2**), and were tested at baseline and at the end of treatment.

## Occipital rTMS Treatment Regimens

The strategy and parameters of rTMS treatment were reported in the previous study (48). Briefly, MDD patients were randomly assigned to the individualized group, standard group, or sham group to receive the corresponding therapy for 5 consecutive days. For the individualized group, the coordinates of the stimulation points were personalized based on affective visual tasks completed during functional MRI. For the standard group, fixed stimulation sites were set in the left cortical visual area V1. Moreover, the following parameters were applied in rTMS treatment in this study: stimulation intensity = 90% resting motor threshold, frequency = 10 Hz, train duration = 4 s, inter-train interval = 26 s, pulse number = 1,600 in each session, total duration = 20 min, number of time = 2 times per day, total sessions = 10.

## Blood Samples

Once participants were enrolled, 5 mL of fasting venous blood was collected in a plasma separator tube (Xinle, China) between 6:00 and 10:00 in the morning. An additional 5 mL whole blood sample was drawn after the 5 day treatment. Two runs of centrifugation were completed within 2 h of blood collection. The first processing was for plasma separation with a rotational speed of 2,000 × g for 10 min at 4°C. Afterwards, the supernatant was centrifuged for the second time for purification with a rotational speed of 12,000×g for 10 min under the same conditions. Plasma samples were stored at −80°C until quantitative determination of proteins.

## Protein Quantification

Protein quantification was carried out using the enzyme-linked immunosorbent assay (ELISA) technique using commercial kits (R&D Systems, USA) according to the manufacturer's instructions. The concentration of diluted proteins was computed based on the standard curves, which was subsequently multiplied by the dilution factors to signify the actual protein level. Each sample was run in triplicate. Both the inter- and intra-assay coefficients of variation were <5%.

## Statistical Analysis

All data were analyzed using SPSS 20.0 (IBM, USA), GraphPad Prism 7.0 (GraphPad Software, USA), or SAS 9.4 (SAS Institute Inc., USA). Kolmogorov-Smirnov tests were used to assess the distributions of numerical variables. For variables with a non-normal distribution, Kruskal-Wallis tests were used to calculate the statistical significance of differences among groups. For variables with a Gaussian distribution, data were tested by one-way analyses of variance (ANOVA) with Bonferroni's *post hoc* tests. The protein levels and clinical assessments at the two time points were compared using paired *t*-tests or Wilcoxon signed-rank tests as required. In addition, Chi-squared tests were utilized to analyze categorical data. Partial correlation analyses and multivariate linear regressions were computed to determine the correlations and interactive effects, respectively. Receiver operating characteristic (ROC) curves were used to estimate and predict the antidepressive efficacy. Furthermore, a linear mixed model was established to explore predictive factors of therapeutic effects. Quantitative variables were expressed as mean [standard deviation (SD)] or median [interquartile range (IQR)], and categorical variables were expressed as absolute numbers. $P < 0.05$ was set as the level of statistical significance (two-tailed tests).

# RESULTS

## Demographic and Clinical Characteristics

A total of 16 patients were excluded from analysis for various reasons, which are detailed in **Supplementary Figure 1**. Of the 74 remaining patients, 23 were assigned to the sham group, 24 were assigned to the individualized rTMS treatment group, and 27 were assigned to the standard rTMS treatment group. Of all demographic and clinical characteristics considered, the three groups only differed significantly in their drinking histories ($p = 0.044$, **Supplementary Table 1**).

## Neuropsychological Assessments at Baseline and End of Treatment

As shown in **Supplementary Table 2**, the only significant differences in neuropsychological assessment scores for the three groups at baseline were for the CTQ-SF and Stroop A (CTQ-SF: $p = 0.010$; Stroop A: $p = 0.046$). Pairwise comparisons showed that CTQ-SF scores in the standard group were significantly lower than those in the other groups ($p = 0.027$, standard group vs.

individualized group; $p = 0.028$, standard group vs. sham group), while the differences in information processing speed evaluated by the Stroop A test mainly came from the contrast between the individualized group and the sham group ($p = 0.040$). Comparisons of affective scale and cognitive test scores from baseline to the end of therapy showed notable improvements in the individualized group (all $p < 0.05$). Similarly, patients treated by standard rTMS showed significant amelioration of symptoms, as shown by changes from baseline to after treatment for most assessments, except the Neuropsychological Assessment Battery: Mazes (NAB Mazes) and Brief Visuospatial Memory Test-Revised (BVMT-R) (**Supplementary Table 2**).

## Protein Levels at Baseline and End of Treatment

Protein levels at baseline were comparable among the three groups, as shown in **Figure 1** (CRP: F = 2.576, $p = 0.083$; ATIII: F = 0.802, $p = 0.452$; ITIH4: F = 0.341, $p = 0.712$; VDB: F = 0.219, $p = 0.804$). After individualized rTMS therapy, the levels

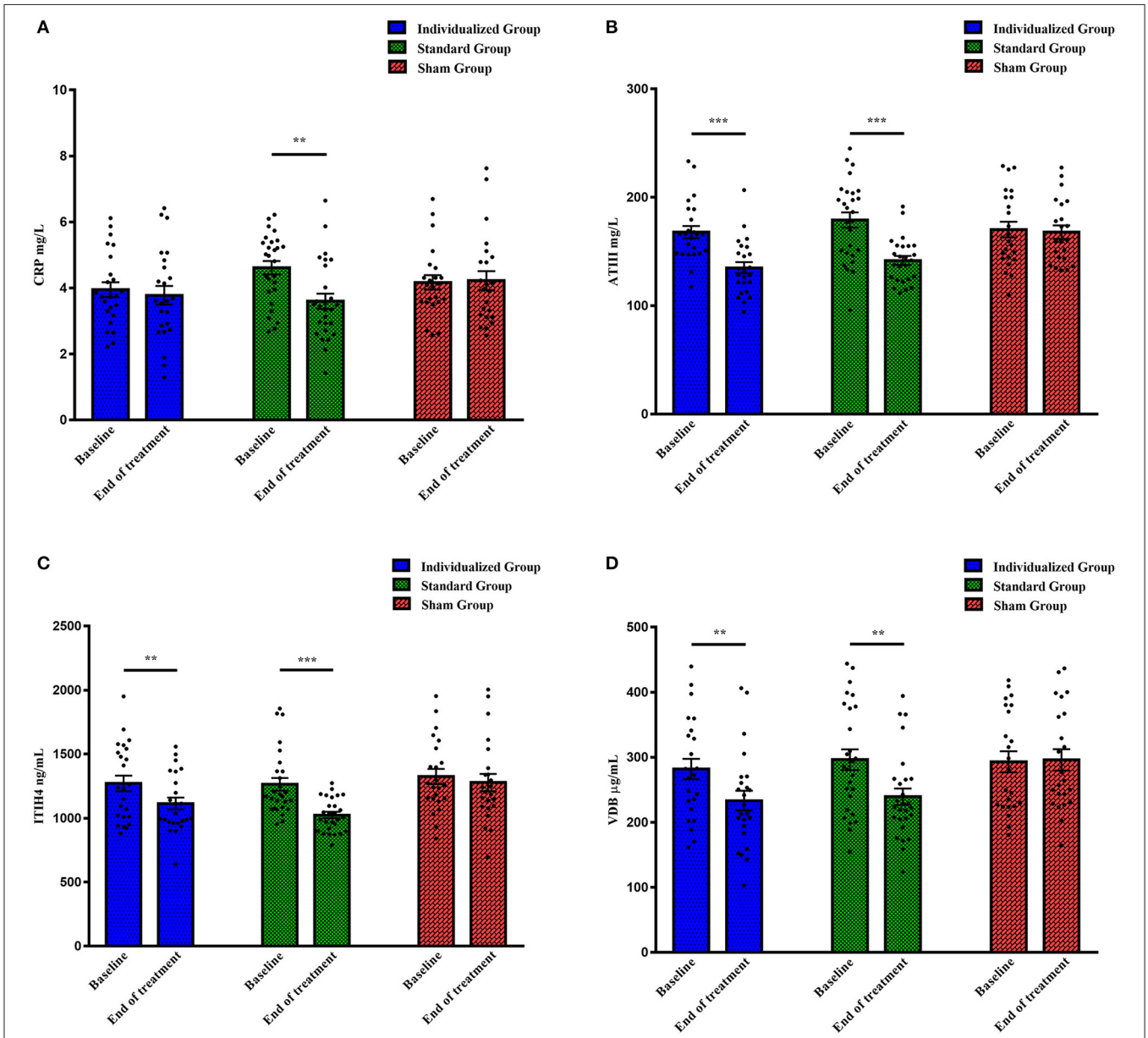


**FIGURE 1 |** The levels of four proteins in different treatment groups at baseline and the end of treatment. **(A)** CRP levels (mg/L); **(B)** ATIII levels (mg/L); **(C)** ITIH4 levels (ng/mL); **(D)** VDB levels (μg/mL). CRP, C-reactive protein; ATIII, antithrombin III; ITIH4, inter-alpha-trypsin inhibitor heavy chain H4; VDB, vitamin D binding protein. $^{**}p < 0.01$, $^{***}p < 0.001$.

of ATIII, ITIH4, and VDB significantly declined ($t = 5.586$, $p < 0.001$; $t = 2.893$, $p = 0.008$; $t = 2.955$, $p = 0.007$, respectively), while a downward trend with no statistical significance was observed for CRP ($t = 0.503$, $p = 0.620$) (**Figure 1**). Similarly, reductions in the expression of all proteins were demonstrated in the standard group when the whole treatment course was completed (CRP: $t = 3.298$, $p = 0.003$; ATIII: $t = 4.523$, $p < 0.001$; ITIH4: $t = 4.589$, $p < 0.001$; VDB: $t = 3.029$, $p = 0.005$) (**Figure 1**). In contrast to the rTMS treatment groups, as shown in **Figure 1**, the levels of proteins after five-day sham treatment were approximately equal to those at baseline (CRP: $t = -0.148$, $p = 0.884$; ATIII: $t = -0.040$, $p = 0.968$; ITIH4: $t = 0.971$, $p = 0.342$; VDB: $t = -0.209$, $p = 0.836$).

## Correlations of Changes in Protein Levels and Changes in Neuropsychological Assessments

There were significant correlations between changes in ATIII level, but not levels of the other three proteins, and antidepressive efficacy, as evaluated by changes in affective and cognitive assessments, in the individualized group. Controlling for ATIII level at baseline and the initial score, the alteration in ATIII was positively correlated with changes in several scales, including HAMD-24 ($r = 0.509$, $p = 0.016$), SDS ($r = 0.536$, $p = 0.010$), SAS ($r = 0.442$, $p = 0.039$), and BHS ($r = 0.479$, $p = 0.024$), while a negative correlation was observed for change in Stroop C ($r = -0.599$, $p = 0.003$) (**Figures 2A–E**). Further multiple linear regression was utilized to verify these correlations, and supported that change in ATIII could affect changes in HAMD-24 and Stroop C after individualized rTMS (standardized $\beta_{HAMD-24} = 0.423$, $p = 0.039$; standardized $\beta_{Stroop\,C} = -0.471$, $p = 0.020$).

Further analyses revealed an interactive effect of family APGAR and alteration in ATIII on change in SAS, indicating that family APGAR and change in ATIII affected change in SAS together in the individualized group (standardized $\beta = 0.418$, $p = 0.042$). As shown in **Figure 3**, patients with greater reduction in SAS showed more notable changes in ATIII and higher family APGAR scores.

## ROC Curve Analyses

Given the above-described findings, the ROC curve analysis used only ATIII rather than all four proteins. ATIII levels at two time points were analyzed for their ability to estimate or predict the response to 5 day treatment and remission at 4 week follow-up. In line with general practice, responders in this study were defined as patients with a change in HAMD-24 $\geq$50% and remission was evaluated by a HAMD-24 score $\leq$7.

For the individualized group, larger areas under the curve (AUCs) were observed based on the ROC curves of ATIII after rTMS therapy. There was higher specificity for identifying responders to 5 day treatment (AUC = 0.875, 95% CI, 0.714–1.000, sensitivity = 0.750, specificity = 0.938) and higher

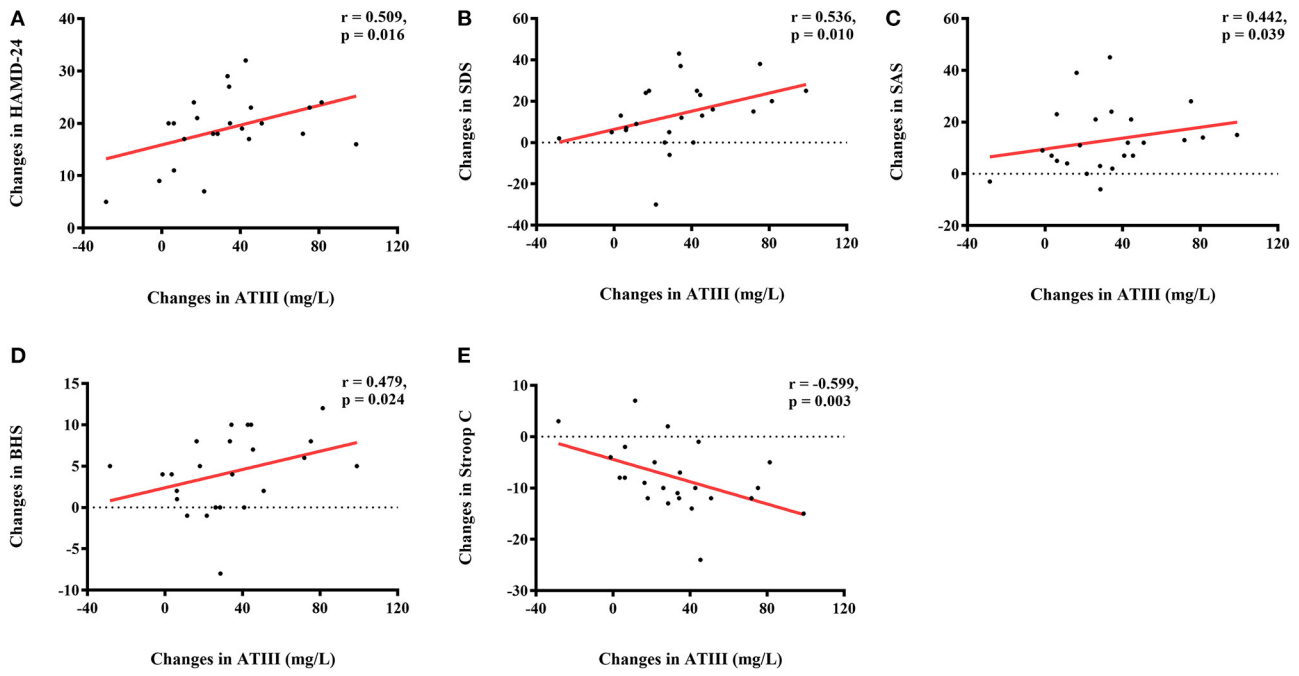


**FIGURE 2 |** Correlations of altered ATIII (mg/L) and changes in neuropsychological assessments in the individualized group. **(A)** Positive correlation between change in ATIII level (mg/L) and change in HAMD-24; **(B)** Positive correlation between change in ATIII level (mg/L) and change in SDS; **(C)** Positive correlation between change in ATIII level (mg/L) and change in SAS; **(D)** Positive correlation between change in ATIII level (mg/L) and change in BHS; **(E)** Negative correlation between change in ATIII level (mg/L) and change in Stroop C. ATIII, antithrombin III; HAMD-24, 24-item Hamilton Depression Rating Scale; SDS, Self-Rating Depression Scale; SAS, Self-Rating Anxiety Scale; BHS, Beck Hopelessness Scale.

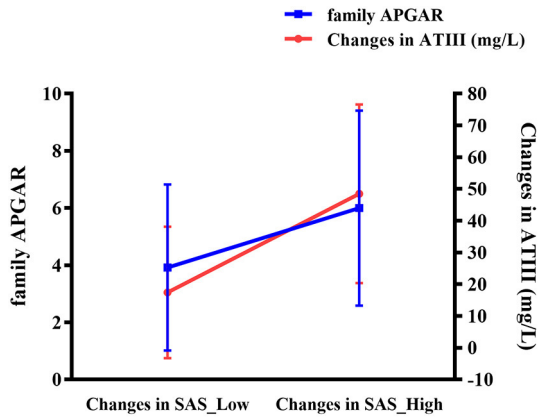


**FIGURE 3 |** Interaction of altered ATIII (mg/L) and family APGAR on change in SAS in the individualized group. ATIII, antithrombin III; APGAR, Adaptation, Partnership, Growth, Affection, Resolve; SAS, Self-Rating Anxiety Scale.

sensitivity for predicting remission 4 weeks later (AUC = 0.828, 95% CI, 0.656–1.000, sensitivity = 0.875, specificity = 0.625). Nevertheless, ROC curve analyses of ATIII at baseline showed a low predictive performance for response and remission with small AUCs (AUC = 0.547, 95% CI, 0.250–0.844, sensitivity = 0.375, specificity = 1.000; AUC = 0.688, 95% CI, 0.456–0.919, sensitivity = 0.750, specificity = 0.625, respectively) (**Figure 4**).

In contrast, according to the ROC curves presented in **Figure 5**, baseline ATIII in the standard treatment group showed better efficiency for predicting rTMS antidepressive effects, represented by both response and long-term remission (AUC = 0.771, 95% CI, 0.571–0.971, sensitivity = 0.588, specificity = 0.900; AUC = 0.736, 95% CI, 0.529–0.943, sensitivity = 0.643, specificity = 0.846, respectively), whereas ATIII at the end of treatment was ineffective for the same predictions (AUC = 0.507, 95% CI, 0.266–0.748, sensitivity = 0.444, specificity = 0.750; AUC = 0.538, 95% CI, 0.304–0.773, sensitivity = 0.769, specificity = 0.417, respectively).

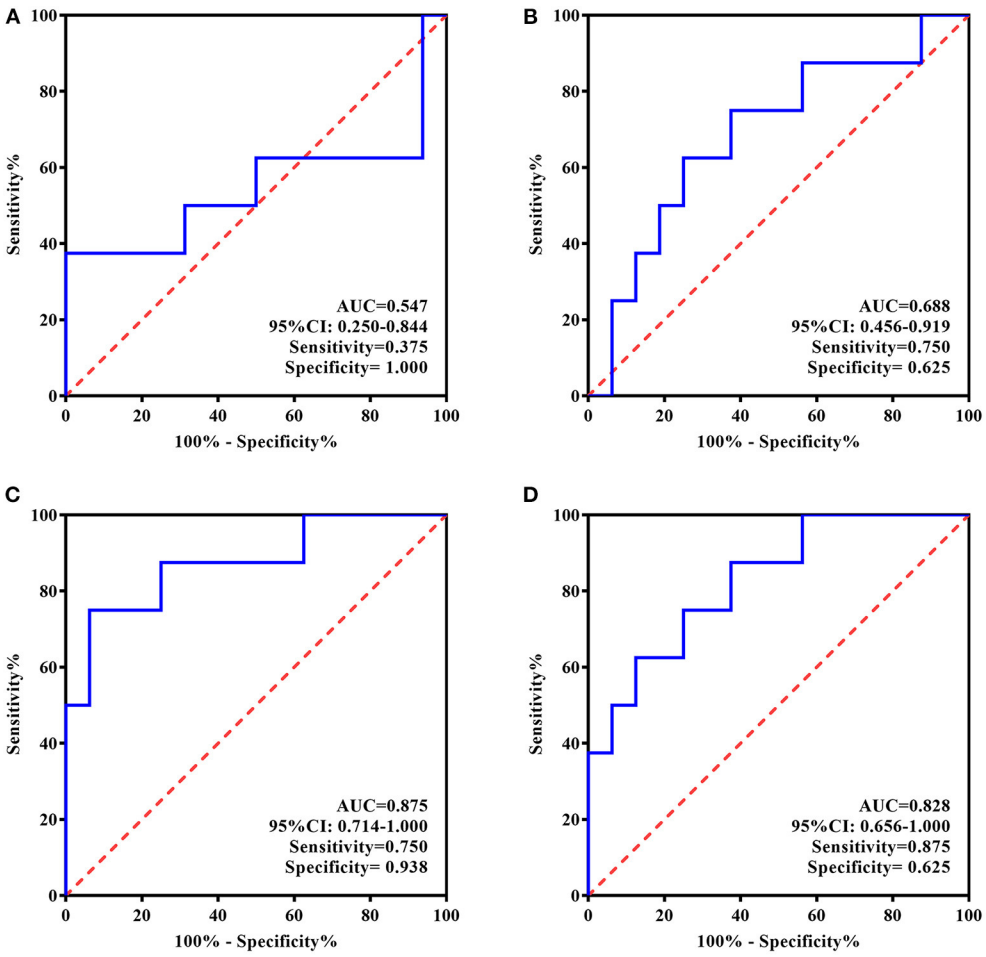


**FIGURE 4 |** ROC Curve for the evaluating and predicting performance of ATIII in the individualized group. **(A)** ROC curve of ATIII at baseline for predicting the response to 5-day treatment; **(B)** ROC curve of ATIII at baseline for predicting the remission in 4 weeks following the treatment termination; **(C)** ROC curve of ATIII at day 5 for estimating the response to 5-day treatment; **(D)** ROC curve of ATIII at day 5 for predicting the remission in 4 weeks following the treatment termination. ROC curve, receiver operating characteristic curve; AUC, area under the curve; CI, confidence interval.

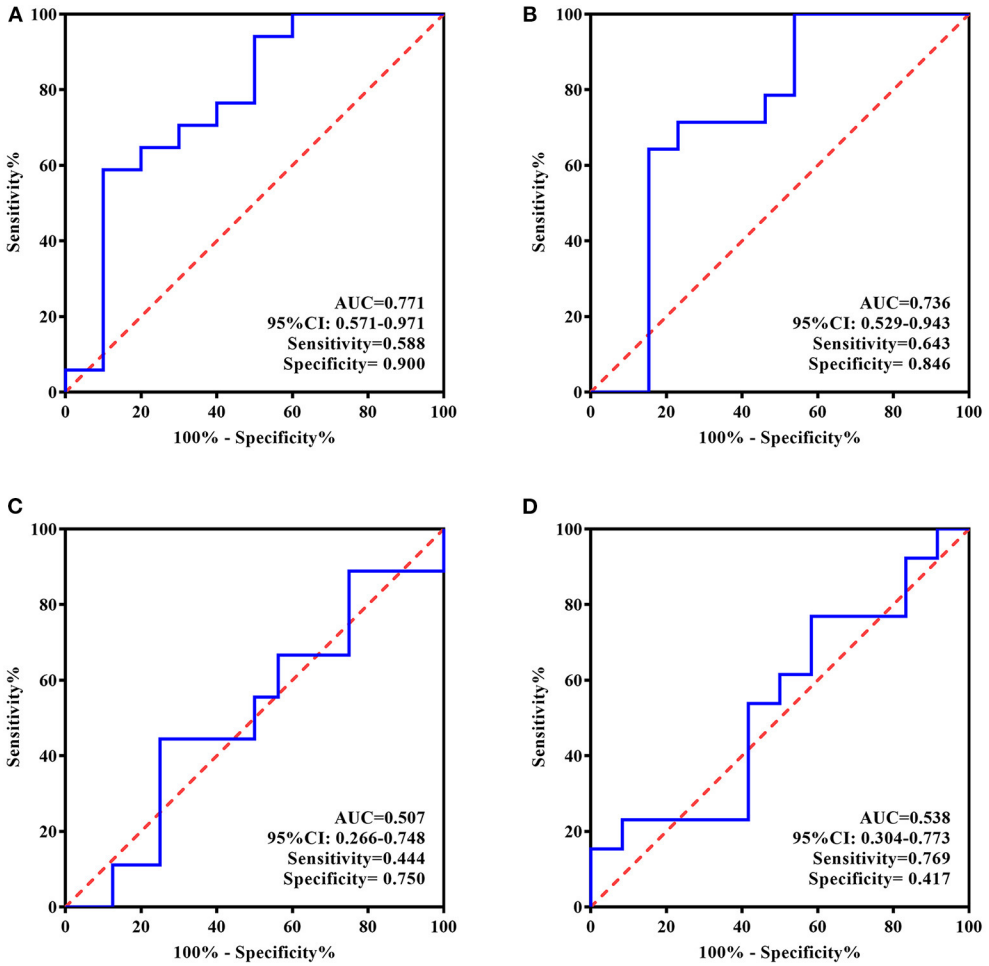


**FIGURE 5 |** ROC Curve for the evaluating and predicting performance of ATIII in the standard group. **(A)** ROC curve of ATIII at baseline for predicting the response to 5-day treatment; **(B)** ROC curve of ATIII at baseline for predicting the remission in 4 weeks following the treatment termination; **(C)** ROC curve of ATIII at day 5 for estimating the response to 5-day treatment; **(D)** ROC curve of ATIII at day 5 for predicting the remission in 4 weeks following the treatment termination. ROC curve, receiver operating characteristic curve; AUC, area under the curve; CI, confidence interval.

## Predictive Factors of Therapeutic Effects

Based on the assessments of affection at day 1, 3, and 5 (as well as additional assessments of HAMD-24 at week 1, 2, 3, 4 in the follow-up study) or cognitive tests after treatment, which were set as the curative effect indexes, multidimensional data were brought into the statistical model to estimate the potential predictive factors and their predictive values, including demographic and clinical information, days of treatment, treatment group, levels of the four proteins at baseline, and initial scores on all assessments and psychosocial scales. The value of baseline ATIII for predicting antidepressive efficacy is shown in **Table 1**. The antidepressive efficacy is supported by several assessments, such as HAMD-24 ($p = 0.020$), HAMA ($p < 0.001$), BSI-CV-C ($p = 0.044$), SDS ($p < 0.001$), SAS ($p = 0.002$), BHS ($p = 0.014$), Brief Assessment of Cognition in Schizophrenia: Symbol Coding (BACS SC, $p = 0.002$), and Stroop B ($p = 0.048$), while ITIH4 was only demonstrated to predict therapeutic effects represented by SAS ($p = 0.044$) (**Supplementary Table 3**). All potential predictive factors of each assessment are listed in **Supplementary Table 3**.

In addition, given the impacts of altered protein levels on long-term curative efficiency evaluated by HAMD-24 at week 1, 2, 3, and 4, additional data of changes in protein expression were included in the model. As shown in **Table 2**, both ATIII at baseline and change in ATIII were related to HAMD-24 assessments at follow-up ($p = 0.024$ and 0.023, respectively), supporting their power to predict later effect.

## DISCUSSION

The present study revealed downtrends in a panel of plasma proteins, namely CRP, ATIII, ITIH4, and VDB, after 5 day occipital rTMS treatment. Moreover, significant

**TABLE 1 |** ATIII at baseline involved in prediction of therapeutic effects.

| | *t* score | *p*-value |
|---|---|---|
| HAMD-24 | 2.41 | 0.020* |
| HAMA | 3.71 | <0.001*** |
| BSI-CV-C | 2.07 | 0.044* |
| SDS | 4.19 | <0.001*** |
| SAS | 3.24 | 0.002** |
| BHS | 2.57 | 0.014* |
| TMT-A | 1.04 | 0.304 |
| BACS SC | −3.29 | 0.002** |
| Stroop A | −1.61 | 0.114 |
| Stroop B | −2.03 | 0.048* |
| NAB Mazes | −0.43 | 0.672 |
| Stroop C | −1.41 | 0.167 |
| BVMT-R | −1.01 | 0.316 |

*$p < 0.05$; ** $p < 0.01$; ***$p < 0.001$.
*ATIII, antithrombin III; HAMD-24, 24-item Hamilton Depression Rating Scale; HAMA, Hamilton Anxiety Scale; BSI-CV-C, Beck Scale for Suicide Ideation-Chinese version-Current; SDS, Self-Rating Depression Scale; SAS, Self-Rating Anxiety Scale; BHS, Beck Hopelessness Scale; TMT-A, Trail Making Test A; BACS SC, Brief Assessment of Cognition in Schizophrenia: Symbol Coding; NAB Mazes, Neuropsychological Assessment Battery: Mazes; BVMT-R, Brief Visuospatial Memory Test-Revised.*

**TABLE 2 |** Potential predictive factors of HAMD-24 in the follow-up study.

| Factor | *t* score | *p*-value |
|---|---|---|
| ATIII at baseline | 2.34 | 0.024* |
| Change in ATIII | 2.35 | 0.023* |
| HAMD-24 at baseline | 4.70 | <0.001*** |
| Days of treatment | −9.68 | <0.001*** |
| History of smoking | 2.84 | 0.007** |
| CTQ-SF | 2.24 | 0.030* |

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.
*ATIII, antithrombin III; HAMD-24, 24-item Hamilton Depression Rating Scale; CTQ-SF, Childhood Trauma Questionnaire-Short Form.*

correlations between decrease in ATIII and improvements in neuropsychological assessments comprised of HAMD-24, SDS, SAS, BHS, and Stroop C were demonstrated in patients treated by individualized rTMS. This study also illustrated the predictive performance of ATIII, whether for rTMS treatment groups or all participants, suggesting the potential of this protein for estimating and predicting antidepressive efficacy.

There was no grouping bias in the affective assessments or levels of proteins, indicating that patients treated by different methods were in depressive states of similar clinical severity and had similar protein expression profiles at the group level at baseline. At the end of treatment, levels of three proteins, namely ATIII, ITIH4, and VDB, were decreased in both rTMS groups; a statistically significant reduction in CRP was only shown in the standard rTMS treatment group. As reported by Jha et al., reduced CRP was observed after 8 week sertraline treatment in MDD patients, in line with the findings of our study (54). However, alterations in the levels of the other three proteins after antidepressive treatment have not been investigated in previous studies. Of the four proteins, no notable differences were found in the sham group between baseline and day 5, suggesting the possible relevance of these proteins to the therapeutic process of occipital rTMS. Based on mounting evidence, inflammatory response is considered the common pathway of these four candidate biomarkers (55–58), and is speculated to be a promising target of multiple non-convulsive neurostimulation interventions, including rTMS (59, 60), which might partly account for the differences in protein alterations between rTMS and sham treatment.

As a member of the family of serine protease inhibitors (serpins), ATIII originates from *Serpin clade C, member 1 (SerpinC1)* with a reaction center for serine protease in the conservative spatial structure (61). Apart from its major suppressive roles in coagulation and hemostasis, ATIII exerts anti-inflammation action with or without mediation of anticoagulation (36, 56). A previous study detected higher ATIII mRNA levels in both gray and white matter in patients with Alzheimer's disease compared with controls (62); specifically, increased ATIII in astrocytes was speculated to be commensurate with astrogliosis, which has also been observed in MDD (63). Berk et al. reported a supersensitive platelet response to thrombin stimulation in depressed patients (64). A recent study of remitted MDD patients found a higher procoagulant index and fibrinogen level (65), suggesting an enhanced procoagulant state and a feasible mechanism for the increased risk of cardiovascular disease in MDD patients (66). ATIII can repress thrombin-induced activation of platelets and endotheliocytes and reduce their secretions, such as interleukin-1 and P-selectin, which are demonstrated to promote interactions between endothelial cells and neutrophils to further aggravate inflammation (67, 68). As reported by Stelzhammer et al., compared to baseline levels, ATIII in MDD patients increased significantly after first ECT treatment; however, this was identified by liquid-chromatography mass spectrometry and not in combination with multiplex immunoassay (37). Our inconsistent results might be ascribed to several reasons. Firstly, only 10 patients with treatment-resistant depression were analyzed in the study by Stelzhammer et al. without expanded and independent validation. Such a low sample size might result in a false positive, as the authors speculated, and differences in race and antidepressant resistance could exacerbate inconsistencies. Moreover, increased ATIII was observed in patients treated by ECT after just once session, indicating its involvement in acute ECT effect. By contrast, in the present study, reduced ATIII levels were detected after 5 day rTMS treatment. The diverse therapeutic courses and related pathways could lead to the different protein expression profiles.

As determined in the present study, the decline in ATIII was further associated with improvement of clinical symptoms comprising depression, anxiety, hopelessness, and cognitive deficiency in the individualized group. Moreover, for the same patients, an interaction with change in SAS was demonstrated

between altered ATIII and family APGAR, suggesting that family support may impact the relationship between protein levels and curative effect. On a deeper level, biological and environmental factors could interact to affect the antidepressive efficiency of occipital rTMS.

A biomarker should be useful for identifying and/or predicting response to treatment. In the present study, baseline ATIII in the standard group and decreased ATIII after individualized rTMS showed a high performance for estimating or predicting antidepressive effect, suggesting the prognostic value of ATIII at both baseline and the end of rTMS treatment. As a key factor, baseline ATIII was involved in the prediction of all emotional assessments, cognitive tests of information processing speed, and long-term efficacy evaluated by HAMD-24 at follow-up study together with changes in ATIII. Taken together, these findings demonstrate that ATIII could be a potential biomarker for curative effects in MDD treatment, regardless of the therapeutic method.

Previous proteomic research identified four proteins connected to MDD (28). In the present study, following rigorous scientific research, one of the four candidates, ATIII, was identified as a potential biomarker of both MDD diagnosis and antidepressive effect evaluation and forecasting by multi-verification. As ATIII level can be detected by a simple test, it is suitable for clinical screening. Another indicator identified in the proteomic study, VDB, was validated both *in vitro* and *in vivo* and an application has been filed for a patent. For this reason, we believe it is worthwhile to verify ATIII in a larger cohort and with further animal and cell experiments, followed by developing a convenient and efficient kit and filing a patent.

There are several limitations of this work. Firstly, this study explored biomarkers of curative efficacy based on the rapid antidepressive effects of occipital rTMS by analyzing changes in ATIII before and after rTMS treatment. As most MDD patients took medication or completed other therapies as prescribed at follow-up, homogeneity could not be maintained. In the light of this, blood samples were only collected at baseline and after 5 day rTMS stimulation. Due to the lack of blood collection at 4 week follow-up, it was not possible to assess changes in ATIII during this period or to evaluate its ability to estimate the long-term efficacy of antidepressive treatment. However, we were still able to demonstrate the predictive value of ATIII using a statistical model. Secondly, considering the importance of patient safety and need for patient cooperation, MDD patients who were suicidal or in a stuporous state were excluded. Therefore, our findings are limited to patients with moderate to severe depression without extreme negative symptoms. Thirdly, in order to eliminate interference from antidepressants and increase homogeneity, this study required patients to be drug naïve or drug free for at least 2 weeks before recruitment and reconfirmed MDD diagnosis at follow-up before statistics. In a future study, we will add rTMS stimulation along with standard medication to expand the scope of significance of ATIII. We will also carry out a non-inferiority study to compare the antidepressive effects of occipital and prefrontal rTMS in which there is no restriction related to the use of antidepressant drugs. Lastly, the small sample size could increase the risk of false-positive results; to validate the findings of this study, it is necessary to repeat this analysis in an independent cohort with a larger sample.

## CONCLUSION

In conclusion, this study revealed decreases in CRP, ATIII, ITIH4, and VDB after occipital rTMS therapy. Furthermore, we revealed a relationship between greater reductions in ATIII and greater improvements in neuropsychological assessments in patients who received individualized stimulation. Ultimately, we demonstrated the potential value of ATIII as a biomarker of MDD and antidepressive treatment outcomes.

## DATA AVAILABILITY STATEMENT

The raw data analyzed in this article are not publicly available. Requests to access the data should be directed to janemengzhang@vip.163.com.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of the Second Affiliated Hospital of Xinxiang Medical University. The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

ZZ conceived and designed the study. ZZ and HoZ supervised to carry out the protocols and enrolled subjects. ZZ, HoZ, and RS characterized subjects. RS collected specimens, analyzed data, prepared the tables and figures, wrote the manuscript, and followed ZZ's guidance and they were responsible for the data interpretation. HaZ, KL, and BW were responsible for multi-mode MRI scan. XL, JZ, and YY carried out rTMS treatment. YS measured levels of CRP, ATIII, ITIH4, and VDBP. YZ established the linear mixed model. LG and YS assisted with the structure design of the manuscript. All authors contributed to the article and approved the submitted version.

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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.2021.678384/full#supplementary-material

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