International Journal of Nanomedicine

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ORIGINAL RESEARCH

Investigating Neuroplasticity Changes Reflected by BDNF Levels in Astrocyte-Derived Extracellular Vesicles in Patients with Depression

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Purpose: To investigate the neuroplasticity hypothesis of depression by measuring brain-derived neurotrophic factor (BDNF) levels in plasma astrocyte-derived extracellular vesicles (ADEVs) and to evaluate their potential as biomarkers for depression compared with plasma BDNF levels.

Patients and Methods: Thirty-five patients with major depressive disorder (MDD) and 35 matched healthy controls (HCs) were enrolled. Plasma ADEVs were isolated using a combination of ultracentrifugation and immunoaffinity capture. Isolated ADEVs were validated using transmission electron microscopy, nanoparticle tracking analysis, and Western blotting. BDNF levels were quantified in both ADEVs and plasma. ALG-2-interacting protein X (Alix) and cluster of differentiation 81 (CD81) levels, two established extracellular vesicle markers, were measured in ADEVs.

Results: After false discovery rate correction, patients with MDD exhibited higher CD81 levels (P_{FDR} = 0.040) and lower BDNF levels (P_{FDR} = 0.043) in ADEVs than HCs at baseline. BDNF levels in ADEVs normalized to CD81 (P_{FDR} = 0.002) and Alix (P_{FDR} = 0.040) remained consistent with this finding. Following four weeks of selective serotonin reuptake inhibitor treatment (n=10), CD81 levels in ADEVs decreased (P_{FDR} = 0.046), while BDNF levels normalized to CD81 increased (P_{FDR} = 0.022). BDNF levels in ADEVs were more stable than in plasma. Exploratory analysis revealed no correlation between BDNF levels in ADEVs and plasma (ρ=0.117, P = 0.334).

Conclusion: This study provides human in vivo evidence supporting the neuroplasticity hypothesis of depression by demonstrating altered BDNF levels in ADEVs. ADEVs may be more suitable for developing biomarkers of depression than plasma-derived biomarkers.

Keywords: major depressive disorder, ADEV, BDNF, biomarker, treatment

Introduction

Major depressive disorder (MDD) is a severe mental illness characterized by persistent low mood, loss of interest, and anhedonia.[1] MDD increases the risk of suicide and represents a significant global public health concern.[2] Approximately 5% of the global population experiences depression each year, with the highest prevalence observed in young individuals.[3] Despite this significant health burden, depression remains a largely neglected health crisis. The global prevalence of depression reached 279.6 million individuals in 2019, incurring a substantial impact on individuals, families, and society.[4]

The pathogenesis of depression is very complex, and various hypotheses have been proposed to explain its development. Of these, the neuroplasticity hypothesis is a widely studied and recognized theory.[5–7] Neuroplasticity refers to the nervous system's ability to reorganize its structure, function, and connections in response to intrinsic or extrinsic stimuli[8] and regulated by neurotrophic factors and their associated signaling pathways.[9,10] Neurotrophic factors not only support the metabolic needs of neurons but also play vital roles in their proliferation and survival. Functionally, they can act as signaling molecules that mediate learning and memory processes in the brain.[11] The main neurotrophic factors include brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), neurotrophin 3 (NT-3), and neurotrophin

Received: 12 May 2024
Accepted: 28 August 2024
Published: 2 September 2024

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4/5 (NT-4/5), with previous studies on the neuroplasticity hypothesis of MDD mainly focusing on BDNF. A meta-analysis of 1095 drug-naïve first-episode MDD patients and 1399 healthy controls (HCs) revealed a trend towards decreased BDNF levels in the peripheral blood of MDD patients.[12] Furthermore, the neuroplasticity hypothesis may explain the delayed onset of efficacy of antidepressant drugs.[13] Therefore, neurotrophic factors and their signaling pathways are considered as primary therapeutic targets in depression.[14]

Despite the data implicating neurotrophic factors in depression, the results are heterogeneous due to variations in clinical samples and cohorts, treatment modalities, detection techniques, and analytical methods. To minimize the potential influence of clinical factors, Chiou et al conducted a six-year longitudinal study of 71 first-episode drug-naïve patients with MDD rigorously matched with HCs.[15] They observed significantly lower baseline serum BDNF levels in patients with MDD compared with HCs. Conversely, Hung et al found no significant differences in serum BDNF levels in patients with MDD using age-adjusted covariance analysis.[16] Several longitudinal studies have shown no correlation between changes in BDNF levels during treatment and improvements in depressive symptoms.[17–19] Studies of other peripheral blood neurotrophic factors such as NGF, glial cell-derived neurotrophic factor (GDNF), and NT-4/5 in depression similarly show considerable heterogeneity.[20–23] A recent systematic review and meta-analysis further indicated that candidate biomarker levels in the peripheral blood do not correlate significantly with those in brain tissue nor cerebrospinal fluid (CSF).[24] Nevertheless, depression is a disorder characterized by structural and functional changes in the brain,[25,26] and it remains unclear whether detecting peripheral circulating neurotrophic factors accurately reflect their state within the brain. Therefore, it is essential to obtain direct evidence of neurotrophic factor alterations within the brains of patients with MDD.

Astrocytes, the most abundant glial cells in the brain, play vital roles in providing nutritional support to neurons, mediating synaptic formation and function, transmitting signals across brain regions to regulate neuronal activity, and maintaining central nervous system (CNS) homeostasis.[27] Astrocytes are recognized as key neuroglial cells implicated in the pathophysiology of depression,[28,29] serving as crucial regulators of brain function via mechanisms such as neuroplasticity and neuroinflammation.[30] In vitro, antidepressants can induce the expression and secretion of neurotrophic factors including BDNF, fibroblast growth factor 2 (FGF2), and GDNF in primary astrocytes.[31] A recent meta-analysis suggested that selective serotonin reuptake inhibitors (SSRIs) exert antidepressant effects by modulating the expression of specific molecules in astrocytes.[32] However, the exact mechanisms, transport, and distribution of astrocyte-related BDNF expression in patients with depression remain uncertain.

Studying astrocytes directly in the human brain is challenging due to methodological limitations; brain tissues and biofluids are not usually available from patients with MDD. Recent advances in extracellular vesicle (EV) extraction techniques offer a non-invasive approach to assessing brain status through the isolation of CNS-derived EVs.[33,34] Almost all types of cells can release EVs, small vesicles that carry various biological substances such as proteins, lipids, and nucleic acids that target recipient cells as a form of intercellular communication.[35,36] Since EVs can cross the blood-brain barrier (BBB) and can be isolated from various peripheral fluids,[37] CNS-derived EVs may provide insights into the pathophysiology of brain-related diseases. Our previous research showed that astrocyte-derived extracellular vesicles (ADEVs) from patients with MDD contain significantly higher levels of proinflammatory factors, with higher diagnostic values compared with serum.[38] This suggests that brain-derived EVs such as ADEVs can serve as a "window" into the health of the CNS[39] and provide more information about the brain than peripheral blood values.

Here we aimed to investigate: (1) whether BDNF levels in ADEVs differ in patients with MDD and HCs; (2) correlations between BDNF levels in ADEVs and plasma; (3) associations between BDNF levels in ADEVs and depression severity and cognitive function; and (4) whether BDNF measured in ADEVs offers diagnostic advantages over plasma BDNF levels in patients with depression.

Materials and Methods

This observational study was conducted jointly at the Renmin Hospital of Wuhan University and the Affiliated Hospital of West Anhui Health Vocational College. All experiments were performed at the Psychiatric Center Laboratory of Renmin Hospital of Wuhan University.

Study Design

To minimize potential interference from psychotropic medications, we recruited 35 patients with MDD between April 1, 2023 to March 31, 2024 who were not taking any antidepressant medications. Drug-naïve MDD patients were prescribed selective serotonin reuptake inhibitors (SSRIs) and were followed up for four weeks, ten of whom completed four-week follow-up. Inclusion criteria were: (1) meeting ICD-10 criteria for MDD by using the Mini-International Neuropsychiatry Interview;[40] (2) Hamilton Depression Rating Scale (HAMD-17)[41] total score ≥ 17; (3) drug-naïve or off antidepressant medications for more than three months; (4) Han Chinese ethnicity, aged between 18 and 65; and (5) provided written informed consent. Exclusion criteria were: (1) severe physical illness, especially neurological disorders; (2) infection, fever, or allergies within the previous two weeks; (3) pregnant or lactating; (4) presence of other psychiatric disorders; and (5) deemed unsuitable for participation upon assessment by the researchers. Additionally, we recruited 35 age- and sex-matched HCs through offline advertisements during the same period. HCs were required to be physically healthy with no personal nor first-degree family history of psychiatric illness. Other exclusion criteria for HCs were identical to those for MDD patients. All participants were assessed by two experienced and rigorously trained psychiatrists to ensure consistency. Additionally, HCs underwent a comprehensive health examination at enrollment to ensure the absence of any psychiatric or significant physical illnesses.

Sample Size Calculation

Due to the absence of studies investigating neurotrophic factors in ADEVs isolated from patients with MDD, we used findings from a clinical study examining BDNF and pro-BDNF in total exosomes isolated from patients with MDD as a reference for our sample size calculation.[42] In that study, the effect size of BDNF in serum total exosomes between MDD and control groups was 0.78, while pro-BDNF had an effect size of 1.16.[42] We therefore set the effect size for this study at 0.75, with a power of 0.85 (1-β) and a significance level of 0.05 (two-tailed). Sample size calculations were performed using G*Power software (v3.1.9.2),[43] which indicated that the study would need 66 subjects (33 per group). Finally, to account for unforeseen factors and dropout, we set the sample size to 70 (35 per group).

Clinical and Cognitive Measurements

The HAMD-17 and its subscales were used to assess depressive symptoms. The Montreal Cognitive Assessment (MoCA)[44] and the Digit Symbol Substitution Test (DSST)[45] were used to measure objective cognitive functioning. In our study, we conducted targeted symptom analysis of MDD patients using four subscales of the HAMD-17: Maier (items 1, 2, 7, 8, 9, and 10), anxiety/somatization (items 10, 11, 12, 13, 15, and 17), retardation (items 1, 7, 8, and 14), and sleep (items 4, 5, and 6).[46] The MoCA subscales contain five domains: visuospatial, frontal functioning, language, memory, and orientation.[47]

Plasma and ADEV Collection

All participants fasted for at least 8 h before blood collection. Five-milliliter venous blood samples were collected from patients and HCs between 7:00 and 10:00 am into Vacutainer tubes containing EDTA-K2 anticoagulant (BD Biosciences, Franklin Lakes, NJ, USA). Blood samples were centrifuged at 2000 g for 15 min at 4°C within 2 h of collection to separate plasma. After excluding hemolysis and lipemia, plasma samples were divided into 500 µL aliquots and stored at −80°C until use. Two days before testing, plasma samples stored at the Affiliated Hospital of West Anhui Health Vocational College were transported on dry ice to the Psychiatric Center Laboratory of Renmin Hospital of Wuhan University and immediately stored in a −80°C freezer.

ADEVs were separated using a two-step method combining ultracentrifugation with immunoaffinity capture.[48–50] First, total EVs (TEVs) were isolated from plasma samples using ultracentrifugation (Optima XPN-100, SW60Ti horizontal rotor; Beckman Coulter, Brea, CA, USA). Subsequently, biotinylated glutamate-aspartate transporter (GLAST) antibodies (Miltenyi Biotec, Bergisch Gladbach, Germany) targeting an astrocyte surface marker were used to isolate GLAST$^+$ EVs, which ultimately constituted the ADEV samples (Figure 1). All samples were assayed simultaneously on the testing day. A detailed protocol describing the isolation of ADEVs is provided in the Supplementary Material 1.

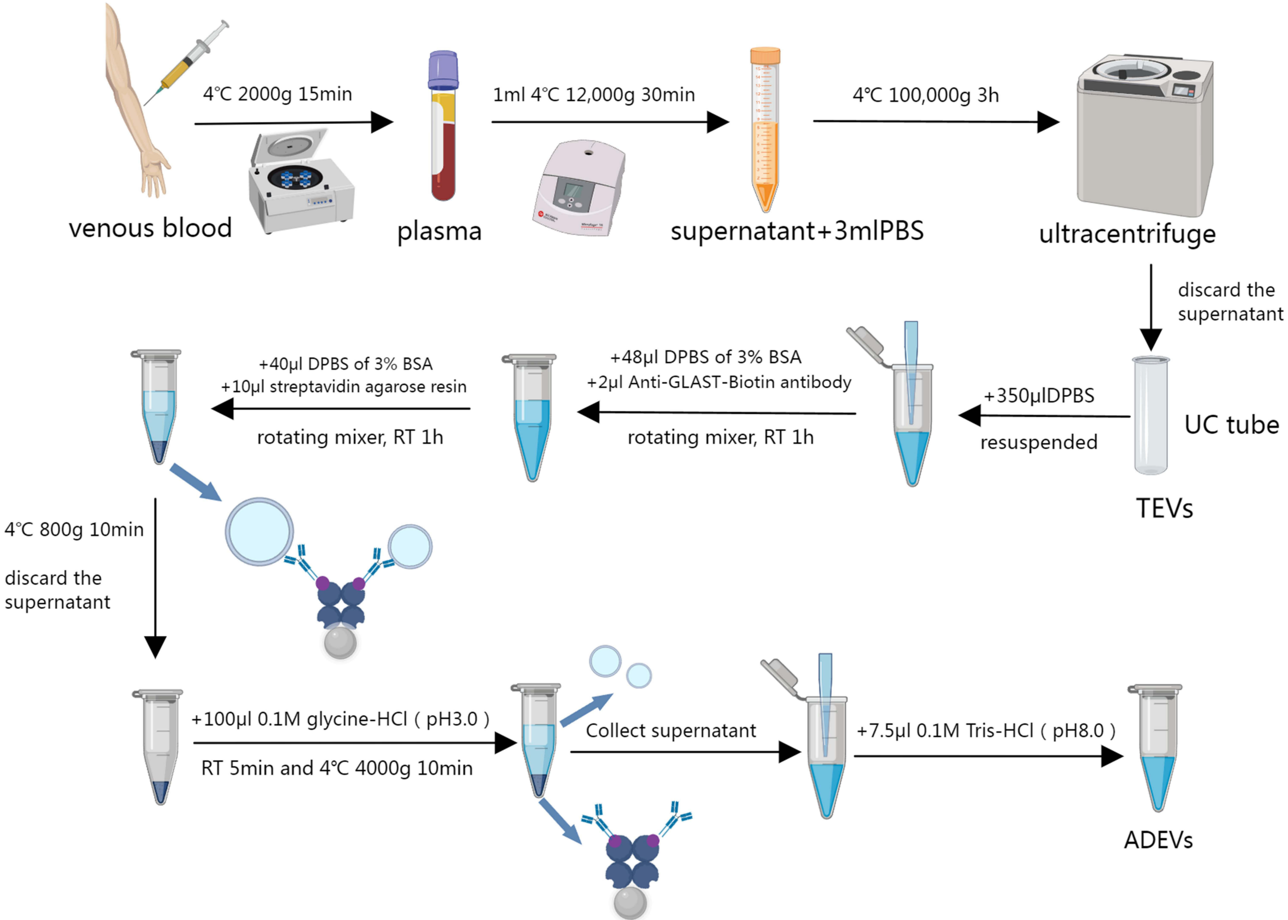

Figure 1 Protocol for the isolation of ADEVs by ultracentrifugation and immunoaffinity capture.

Confirmation of ADEVs

Transmission Electron Microscopy

Twenty microliters of ADEV samples were added dropwise to 200-mesh grids and incubated at room temperature for 10 min. Then, grids were negatively stained with 2% phosphotungstic acid for 3 min. The remaining liquid was removed with filter paper and ADEVs observed on a JEM1400 transmission electron microscope (JEOL Ltd., Tokyo, Japan).

Nanoparticle Tracking Analysis (NTA)

A Zetaview-PMX120-Z (Particle Metrix, Meerbusch, Germany) was used for NTA along with its corresponding software ZetaView (v8.05.14 SP7) to measure particle diameter and concentration.

Western Blotting

Three EV markers [ALG-2 interacting protein-X (Alix) (Proteintech, Rosemont, IL, USA), heat shock protein 70 (HSP70) (Abcam, Cambridge, UK), cluster of differentiation (CD) 9 (Abcam)] and one astrocyte marker [glial fibrillary acidic protein (GFAP) (Abcam)] were detected, and TEV supernatant was used as a negative control.

Protein Measurements

Enzyme-linked immunosorbent assay (ELISA) kits were used to measure BDNF (Elabscience, Wuhan, China) levels in ADEVs and plasma. Additionally, levels of CD81 (CUSABIO, Wuhan, China) and Alix (CUSABIO) in ADEVs were measured.

Statistical Analyses

In the baseline descriptive data analysis, continuous numerical variables were compared using Welch's two-sample *t*-tests. For categorical variable such as sex, we used Pearson's chi-squared test. General linear models (GLMs) were used to compare differences in target proteins between the two groups, setting the group as the independent variable and target proteins as the dependent variables, while sex, age, and BMI were added as covariates. The model-fitted estimated marginal means (EMMs) and their associated 95% confidence intervals (CIs) were used to describe differences between the two groups. Spearman coefficients were employed to examine correlations between BDNF levels in plasma and ADEVs as well as with clinical and cognitive characteristics. Finally, receiver operating characteristic (ROC) curves were used to further explore the diagnostic value of BDNF in ADEVs compared with plasma in patients with MDD. Pre- and post-treatment values were compared using paired sample *t*-tests.

In the main analysis, we used a false discovery rate (FDR) correction (Benjamini and Hochberg method)[51] to detect possible false positive results. A two-tailed *P*-value < 0.05 indicated a significant difference. All statistical analyses were performed in R v4.3.3, and the *ggplot2* package was used for visualizations.

Results

Confirmation of ADEVs

Isolated ADEVs were validated using three methods: transmission electron microscopy, NTA, and Western blotting (Figure 2). Small vesicles were clearly observed by transmission electron microscopy, and NTA results showed that the diameter distribution of these small EVs corresponded to previous studies.[52] Western blotting further confirmed the expression of three EV markers, Alix, HSP70, and CD9, as well as the astrocyte marker GFAP in ADEV samples. These results confirm successful collection of ADEVs.

Descriptive Clinical Data

The two groups of participants showed no significant differences in sex, age, BMI, and education (Table 1).

Detection of Plasma Lipoproteins

Pre-isolation plasma lipid levels were quantified to evaluate potential interference in ADEV isolation. There were no significant differences in total cholesterol (TC) (Roche, Basel, Switzerland), triglycerides (TG) (Roche), high-density

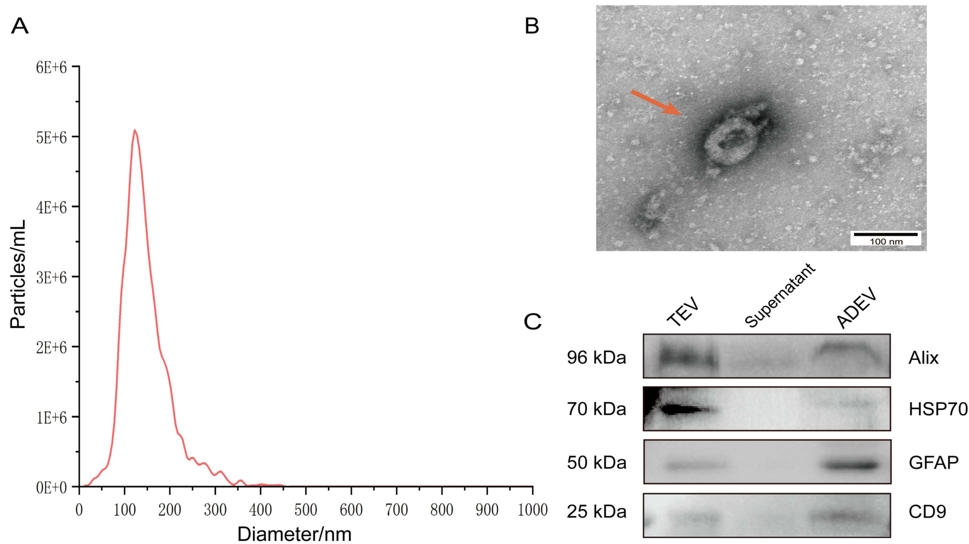

Figure 2 Confirmation of ADEVs. (**A**) The results of nanoparticle tracking analysis show the diameter distribution of isolated extracellular vesicles (EVs) (median diameter: 134.0 nm, concentration: 3.7×10^{10} particles/mL), consistent with prior knowledge about EVs. (**B**) Transmission electron microscope image of ADEVs isolated from a patient with MDD, clearly displaying the EV-like shape (red arrow). (**C**) Western blotting results of total EV (TEV), TEV supernatant, and ADEV from a healthy control. Three EV markers (Alix, HSP70, CD9) and the astrocyte marker GFAP were identified.

Table 1 Comparison of Baseline Demographic and Clinical Characteristics

Parameters	MDD (n=35)	HC (n=35)	P^a
Sex, Female/Male	23/12	26/9	0.434
Age, years, mean±SD	30.8±12.4	30.8±11.3	0.992
BMI, kg/m^2, mean±SD	21.6±2.2	21.5±2.6	0.947
Education, years, mean±SD	14.2±3.3	15.3±2.2	0.098
Onset age, years, mean±SD	27.4±12.2	–	–
Disease course, years, mean±SD	3.0±3.5	–	–
Drug-naïve, Yes/NO	25/10	–	–
HAMD-17 Total Score, mean±SD	27.0±5.2	0.5±0.8	<0.001
HAMD-17: Maier	11.5±2.9	0.2±0.4	<0.001
HAMD-17: anxiety/somatization	8.4±2.2	0.2±0.4	<0.001
HAMD-17: retardation	6.9±2.2	0.1±0.2	<0.001
HAMD-17: sleep	5.1±1.3	0.2±0.5	<0.001
MoCA Total Score, mean±SD	23.4±4.3	26.9±2.3	<0.001
MoCA: visuospatial	3.5±1.4	4.0±1.1	0.110
MoCA: frontal functioning	6.8±1.3	7.4±0.7	0.013
MoCA: language	4.4±1.2	5.2±0.8	0.003
MoCA: memory	3.1±1.3	4.3±1.1	<0.001
MoCA: orientation	5.6±0.8	6.0±0.0	0.003
DSST, mean±SD	46.2±14.8	67.0±12.9	<0.001

Notes: [a]For categorical data, Pearson's chi-square test was used, and for numerical data, Welch's *t*-test was used.
Abbreviations: MDD, major depressive disorder; HC, health control; BMI, body mass index; HAMD-17, Hamilton Depression Rating Scale; MoCA, Montreal Cognitive Assessment; DSST, Digit Symbol Substitution Test.

Table 2 Comparison of Plasma Lipid Levels Between MDD and HC Groups

Parameters	MDD (n=35)	HC (n=35)	*P*
TC (mmol/L)	4.53±1.03	4.76±0.69	0.282
TG (mmol/L)	1.21±0.55	1.12±0.65	0.537
HDL-C (mmol/L)	1.40±0.30	1.46±0.39	0.431
LDL-C (mmol/L)	2.75±0.84	2.77±0.60	0.900

Abbreviations: MDD, major depressive disorder; HC, health control; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

lipoprotein cholesterol (HDL-C) (YONGHE-SUN, Changsha, China), and low-density lipoprotein cholesterol (LDL-C) (YONGHE-SUN) levels between the two groups (Table 2).

Results of the Main Analyses

At baseline, BDNF levels in ADEVs from the MDD group were significantly lower, while CD81 levels in ADEVs were significantly higher, than in controls. There were no significant differences in BDNF levels in plasma and Alix levels in ADEVs between the two groups. Furthermore, BDNF levels normalized to CD81 and Alix in ADEVs were significantly lower in the MDD group compared with the HC group, and the result remained consistent when normalized to plasma volume (Figure 3A–F). Detailed results are presented in Table 3. In ROC analysis, the area under curve (AUC) values ranged from 0.647 to 0.744 (Figure 3G; see detailed results in the figure caption).

Ten drug-naïve patients with MDD completed four weeks of follow-up after SSRI treatment. Among them, seven patients were treated with escitalopram (10 mg/day) and the other three patients were treated with sertraline (50 mg/day).

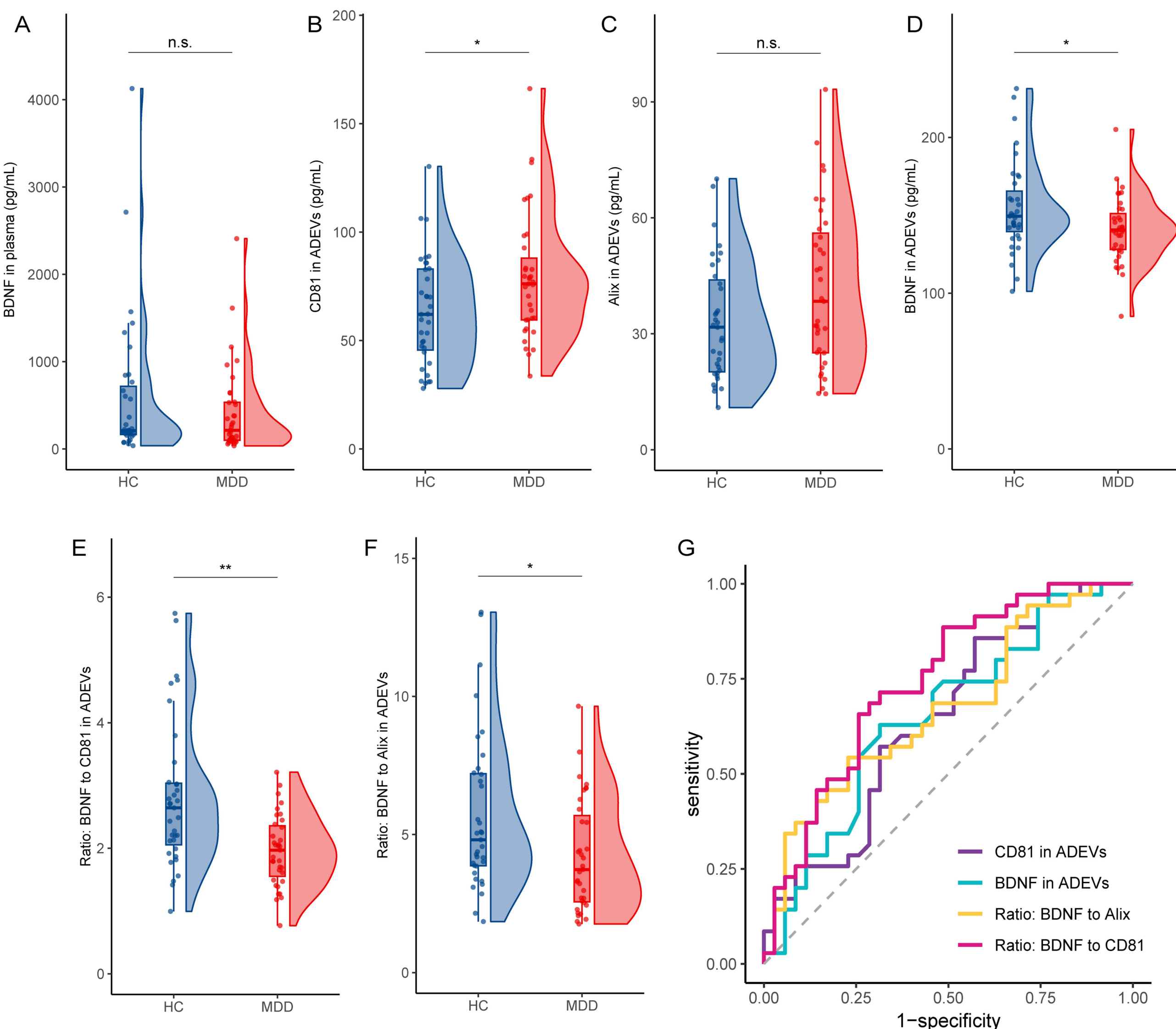

Figure 3 Half-rain half-boxplots of target protein concentrations in ADEVs and plasma between MDD and HC groups at baseline (**A–F**). (**G**) Receiver operating characteristic curves (ROCs) of target proteins in ADEVs. Area under the curve (AUC) values: CD81 in ADEVs: 0.647, 95% CI: 0.518 to 0.776; BDNF in ADEVs: 0.653, 95% CI: 0.524 to 0.782; ratio of BDNF to Alix in ADEVs: 0.672, 95% CI: 0.545 to 0.798; ratio of BDNF to CD81 in ADEVs: 0.744, 95% CI: 0.629 to 0.860.
Note: n.s.: not significant; $*P_{\text{false discovery rate (FDR)}} < 0.05$; $**P_{\text{FDR}} < 0.01$.

Following treatment, MDD patients exhibited significant improvements in depressive symptoms. Similarly, significant improvements were observed in all cognitive domains except orientation and language (Table 4).

In the longitudinal analysis, CD81 levels in ADEVs significant decreased, while BDNF levels in plasma and ADEVs, as well as Alix in ADEVs, did not significantly change after treatment. Furthermore, BDNF levels normalized to CD81 in ADEVs significantly increased after treatment, while there were no significant differences when BDNF levels were normalized to Alix in ADEVs (Figure 4A–F and Table 4).

Results of Exploratory Analyses

First, there was no significant correlation between BDNF levels in ADEVs and plasma (ρ=0.117, P=0.334) (Figure 5A). Second, there was a positive correlation between CD81 and Alix in ADEVs (ρ=0.699, P<0.001) (Figure 5B). Third, in patients with MDD, BDNF levels in ADEVs were positively correlated with CD81 (ρ=0.452, P=0.006) and Alix

Table 3 Comparison of BDNF, CD81, and Alix Between MDD and HC at Baseline Based on General Linear Model

Parameters	MDD (n=35)		HC (n=35)		*P*	P_{FDR}
	EMM	95% CI	EMM	95% CI		
In plasma						
BDNF (pg/mL)	431.5	194.4–668.5	578.8	341.8–815.9	0.384	0.384
In ADEVs (normalized to plasma volume)						
CD81 (pg/mL)	78.9	69.8–88.0	63.4	54.2–72.5	0.020	0.040
Alix (pg/mL)	41.8	35.5–48.1	33.2	26.8–39.5	0.058	0.069
BDNF (pg/mL)	140.8	132.0–149.6	154.8	146.0–163.7	0.029	0.043
Ratio: BDNF to CD81	1.97	1.66–2.28	2.82	2.50–3.13	<0.001	0.002
Ratio: BDNF to Alix	4.22	3.37–5.07	5.69	4.84–6.55	0.018	0.040

Abbreviations: BDNF, brain-derived neurotrophic factor; CD, cluster of differentiation; Alix, ALG-2 interacting protein-X; ADEVs, astrocyte-derived extracellular vesicles; EMM, estimated marginal mean; CI, confidence interval; FDR, false discovery rate; MDD, major depressive disorder; HC, health control.

Table 4 Clinical Features and Protein Changes in MDD Patients After 4 Weeks of SSRI Treatment (n = 10)

Parameters	Change from Baseline	95% CI	Cohen's d	95% CI	*P*	P_{FDR}
Clinical features						
HAMD-17 Total Score	−17.0	−23.7 to −10.3	−1.80	−2.81 to-0.76	<0.001	0.003
Maier	−7.0	−10.2 to −3.8	−1.56	−2.48 to −0.60	<0.001	0.003
Anxiety/somatization	−4.2	−6.2 to −2.2	−1.53	−2.45 to −0.58	<0.001	0.003
Retardation	−4.0	−5.9 to −2.1	2.71	−2.37 to −0.55	0.001	0.004
Sleep	−4.0	−5.2 to −2.8	1.63	−3.71 to −1.16	<0.001	<0.001
MoCA Total Score	4.3	2.4 to 6.2	1.59	0.62 to 2.52	<0.001	0.003
Visuospatial	0.8	0.4 to 1.3	1.27	0.40 to 2.09	0.003	0.008
Frontal functioning	0.9	0.2 to 1.6	0.91	0.14 to 1.63	0.019	0.031
Language	0.9	0.0 to 1.8	0.75	0.03 to 1.44	0.041	0.057
Memory	1.2	0.3 to 2.1	0.91	0.15 to 1.64	0.018	0.031
Orientation	0.5	−0.3 to 1.3	0.42	−0.24 to 1.06	0.213	0.273
DSST	11.9	4.9 to 18.9	1.22	0.37 to 2.03	0.004	0.009
In plasma						
BDNF (pg/mL)	129.8	−238.6 to 498.2	0.25	−0.39 to 0.88	0.446	0.472
In ADEVs (normalized to plasma volume)						
CD81 (pg/mL)	−10.8	−20.3 to −1.3	−0.81	−1.51 to −0.72	0.031	0.046
Alix (pg/mL)	9.5	−8.9 to 27.8	0.37	−0.28 to 1.00	0.273	0.328
BDNF (pg/mL)	4.3	−9.6 to 18.2	0.22	−0.41 to 0.84	0.503	0.503
Ratio: BDNF to CD81	0.51	0.15 to 0.87	1.01	0.22 to 1.76	0.011	0.022
Ratio: BDNF to Alix	−0.90	−2.94 to 1.15	−0.31	−0.94 to 0.33	0.347	0.390

Abbreviations: HAMD-17, Hamilton Depression Rating Scale; MoCA, Montreal Cognitive Assessment; DSST, Digit Symbol Substitution Test; BDNF, brain-derived neurotrophic factor; Alix, ALG-2 interacting protein-X; CD, cluster of differentiation; ADEVs, astrocyte-derived extracellular vesicles; CI, confidence interval; FDR, false discovery rate.

(ρ=0.454, P=0.006) (Figure 5C and D). Finally, there were no significant correlations between these target proteins and the severity of depression nor cognitive function (Supplementary Figure 1).

Discussion

Here, by comparing BDNF levels in ADEVs and plasma from patients with MDD and HCs, we demonstrate for the first time that detecting neurotrophic factors in ADEVs has greater value than measuring these factors in plasma. Patients with MDD had significantly higher CD81 levels and lower BDNF levels than HCs. Notably, these changes were not observed

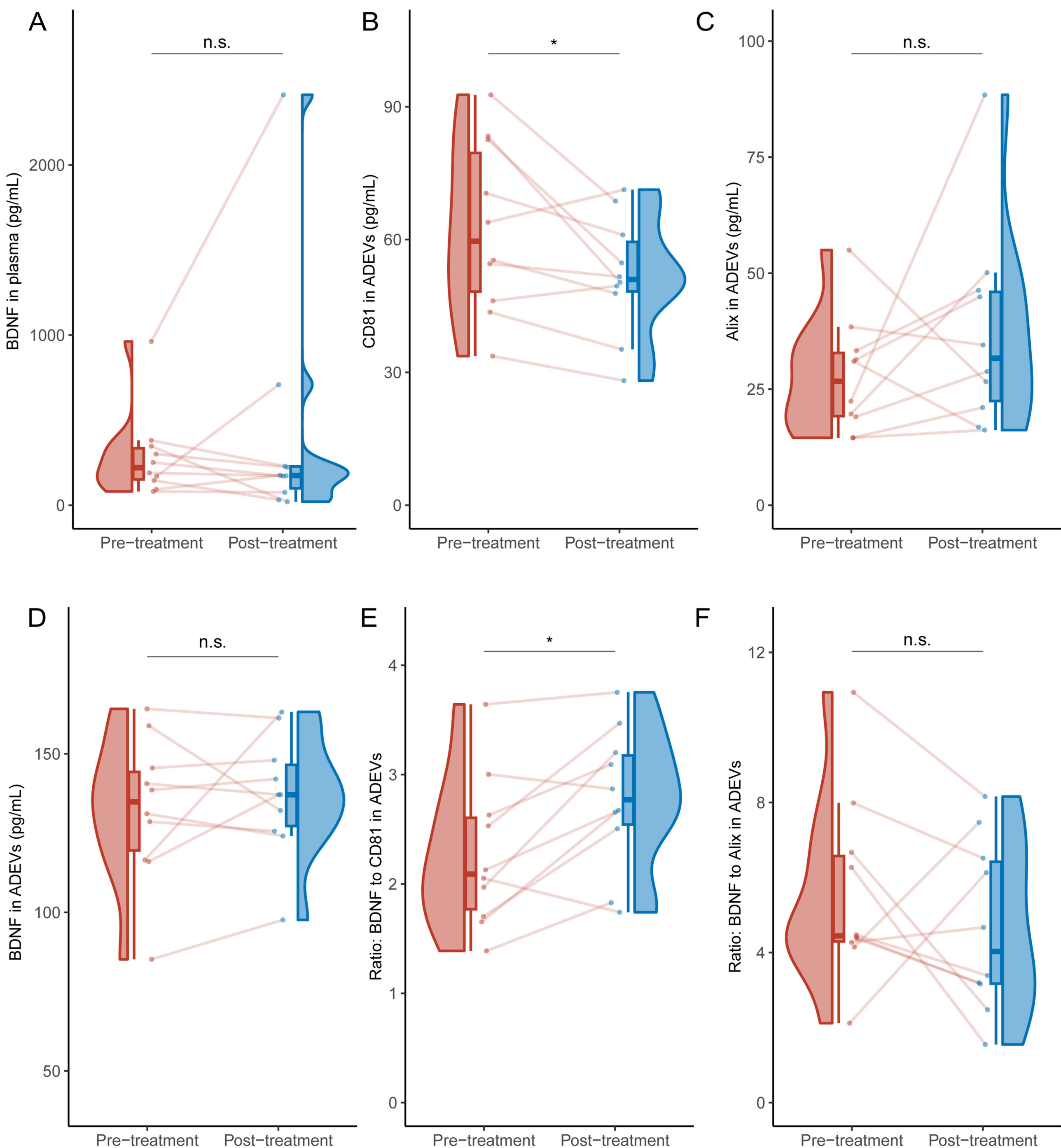

Figure 4 Half-rain half-boxplots of target protein concentrations in ADEVs and plasma between pre- and post-treatment patients with MDD (**A–F**).
Note: n.s.: not significant; $^{*}P_{\text{false discovery rate (FDR)}} < 0.05$.

in plasma. Our exploratory follow-up study revealed that after four weeks of SSRI treatment, patients with MDD had a significant decrease in ADEV CD81 levels and a significant increase in ADEV BDNF levels normalized to CD81.

Quality Control of ADEV Isolation

We used ultracentrifugation to separate TEVs in the first purification step, which achieves highest separation purity than other size-based or precipitation-based methods.[53] We also measured the concentration of plasma lipoproteins prior to ADEV extraction and detected no significant difference in plasma lipoprotein levels between the two groups. This quality

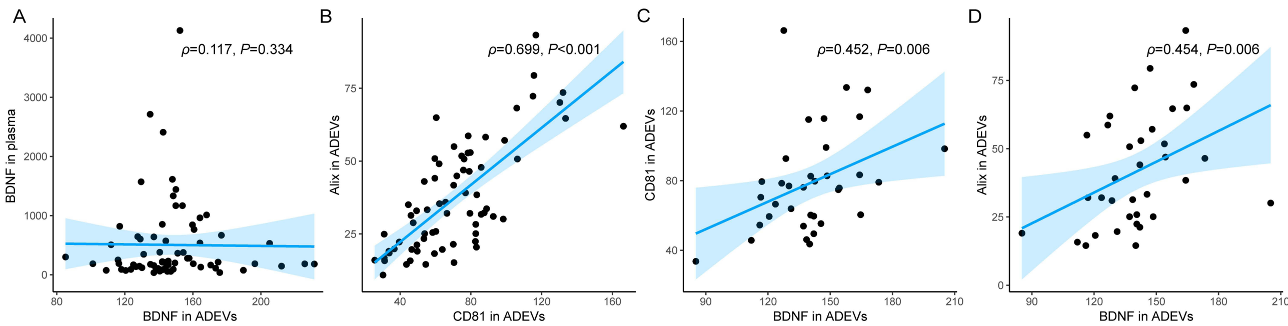

Figure 5 Correlation scatter plots (**A-D**). (**A**) BDNF in plasma and BDNF in ADEVs; (**B**) Alix and CD81 in ADEVs; (**C**) CD81 and BDNF in ADEVs of MDD at baseline; (**D**) Alix and BDNF in ADEVs of MDD at baseline.

control step was necessary, as lipoproteins and EVs share some physical properties, such as particle size and density,[54] especially with low-density lipoproteins,[55] which can lead to their co-separation during ultracentrifugation. Third, we strictly followed standardized operating procedures during ADEV isolation to minimize the introduction of human error. Together, these procedures enhanced the robustness of our results.

Astrocytes, Neuroplasticity, and Depression

Astrocytes are key participants in neuroplasticity, playing crucial roles in synaptic development and remodeling through dynamic and bidirectional interactions with neurons.[56] Astrocytes regulate neuroplasticity through various mechanisms, with neurotrophic factors a pivotal component. Astrocytes activate intracellular responses by sensing extracellular BDNF through TrkB receptors. They also facilitate synaptic plasticity by taking up and circulating BDNF/pro-BDNF at synapses.[57] Mesencephalic astrocyte-derived neurotrophic factor has been shown to prevent neuronal loss,[58] and astrocytes can release EVs containing neurotrophic factors such as GDNF and BDNF in vitro and necroptotic astrocytes can induce neuronal apoptosis through EV-derived pro-BDNF.[59] In animal experiments, conditional BDNF delivery from astrocytes contributes to the restoration of neural plasticity.[60] Notably, ADEVs are crucial mediators of cellular communication and signal transduction in astrocytes, and detecting changes in the ADEV cargo provides direct evidence of astrocyte activity or dysfunction.[61] Therefore, ADEV-derived BDNF may play an important role in the regulation of neural plasticity. We found that patients with MDD had lower levels of BDNF in ADEVs, consistent with the findings of a recent meta-analysis of CSF in patients with depression.[62] A reduction in BDNF in ADEVs may be an important feature of impaired neural plasticity in patients with depression.

Neurotrophic Factors, Depression, and ADEVs

Previous studies on neurotrophic factors in depression have mainly focused on their levels in peripheral blood, with little data on EVs. However, the results of peripheral blood studies have yielded inconsistent and even contradictory results with respect to state or trait changes in patients with depression and whether these changes serve as predictors of treatment response. We detected no significant difference in BDNF levels in plasma between patients with MDD and HCs but we did detect significantly lower BDNF levels in ADEVs from patients with MDD. This difference may be because neurotrophic factors are not solely expressed in the nervous system. For example, platelets can store and release BDNF, and their levels are much higher than in the brain.[63] A previous study reported lower serum and plasma BDNF levels in patients with depression compared with HCs, but this change was not observed in whole blood,[64] suggesting that the observed differences may not be due to platelet BDNF content but rather their ability to release BDNF. It remains unknown whether MDD affects the release of platelet BDNF. Many studies have assumed that peripheral blood BDNF is a biomarker reflecting brain diseases, but there is no direct evidence of an association between peripheral blood BDNF levels and BDNF expression in the brain. Moreover, the penetration of neurotrophic factors through the BBB is generally poor, and their plasma half-life is short.[9] Our study also showed no significant correlation between BDNF levels in ADEVs and plasma. The assessment of biomarkers such as neurotrophic factors in plasma/serum may be influenced by

other peripheral processes in individuals, and CNS-derived EVs may largely reduce the effects of such interference. Consistent with this, previous studies have shown large inter-individual differences in plasma/serum BDNF levels.[19,65] Similarly, our study revealed that the coefficient of variation (CV) of BDNF in plasma was 1.20 for MDD and 1.43 for HCs, while in ADEVs, the CVs were 0.15 and 0.19, respectively. Additionally, there is evidence that brain-derived exosomes in the peripheral blood have a similar diagnostic efficacy to CSF biomarkers, and the levels of various biomarkers in exosomes are highly correlated with those in CSF.[66] ROC curve analysis showed that the AUC for diagnosing MDD using BDNF normalized to CD81 concentration in ADEVs was 0.744. Therefore, we believe that ADEVs, as a "liquid biopsy" of the CNS,[67] have significant advantages over peripheral fluids for future biomarker development for psychiatric disorders like MDD.

We found that levels of BDNF normalized to CD81 in ADEVs within patients with MDD significantly increased after four weeks of SSRI treatment, while plasma BDNF levels did not significantly alter. This is consistent with previous research suggesting that peripheral measurements of neurotrophic factors may not predict treatment responses in patients with MDD.[22,68] However, it is interesting to note that, compared with untreated patients with MDD, those receiving antidepressant treatment showed increased BDNF expression in the post-mortem hippocampus.[69] Although we observed some changes in ADEVs from patients with MDD after antidepressant treatment, which may help to explain the delayed effects of SSRIs, the molecular characteristics of antidepressant responses are often highly heterogeneous. Considering that ADEVs originate from the CNS, it is more accurate to say that they may have an advantage in reflecting treatment responses in patients with MDD.

Exploratory Analyses

A common mechanism of astrocyte activation in many neurological disorders is an increase of reactive astrocytes, which can exert neuroprotective or neurotoxic effects.[70] Astrocyte activation may be accompanied by an increase in ADEV release.[71] CD81 and Alix are established biomarkers of EVs, with CD81 a transmembrane protein and Alix a cytoplasmic protein.[72–74] In this study, we observed an increase in CD81 levels within ADEVs, consistent with our previous findings.[50] However, Alix levels in ADEVs were not significantly different between the two groups. This might be due to variations in vesicle size. A clinical study revealed that both TEVs and CNS-EVs were smaller in patients with MDD than in HCs.[75] Assuming that ADEVs are spherical, CD81 levels within ADEVs could theoretically correlate with their surface area, while Alix levels might be associated with their volume. As the radius changes, the volume of a sphere changes more than its surface area. In our exploratory analysis, we found a significant positive correlation between CD81 and Alix in ADEVs. Furthermore, at baseline, BDNF levels in ADEVs were positively correlated with both CD81 and Alix in ADEVs in patients with MDD. These findings also confirm the high purity of our ADEVs, and the release of BDNF from ADEVs might exhibit particle-dependent characteristics. This could explain why the differences in BDNF levels within ADEVs were more pronounced when normalized to CD81 and Alix. In EV research, different normalization methods may amplify or mask differences, so we suggest employing appropriate and standardized normalization methods to ensure reliability and comparability.

Limitations

Our study has several limitations. First, due to the limited volume of extracted ADEVs, we only measured BDNF levels, a representative neurotrophic factor. A comprehensive assessment of known neurotrophins and growth factors would be valuable to obtain a full profile of such factors in the brain. Second, while ultracentrifugation is considered the "gold standard" for EV separation due to its high purity,[53] the recovery rate of EVs is low.[76] A single-step ultracentrifugation protocol (100,000 g, 2 h) for EV isolation has a reported recovery rate of 40%, and repeating the ultracentrifugation process under the same conditions results in a significantly lower recovery rate of only 16%.[53] To increase recovery of EVs, we modified the two-step ultracentrifugation to a one-step process and extended the centrifugation time.[77] This adjustment may have increased co-isolated protein aggregates and lipoproteins. However, in the second separation step, we utilized immunocapture with astrocyte-specific antibodies, which mitigated against the introduction of errors. Third, due to the uncontrollable nature of clinical follow-up, only ten drug-naïve patients with MDD completed the four-week follow-up on SSRI treatment. Consequently, the sample size for longitudinal observations in this study was small,

limiting our ability to conduct further analysis based on the specific SSRI used. Fourth, we attempted to quantify residual platelets in the pre-isolation plasma of ADEVs. However, the count fell below the lower detection limit of the clinical blood cell analyzer (BC-7500, Mindray, China). Fifth, lifestyle factors such as diet and exercise can also affect BDNF levels,[78,79] but this study did not strictly control for these variables. Sixth, while we observed changes in CD81 levels in ADEVs from patients with MDD, we did not perform NTA on all samples. Therefore, we cannot confirm whether the quantity of ADEVs in patients with MDD also changed. The relationship between EV biomarker levels and particle counts warrants further investigation. Finally, although we detected a significant decrease in BDNF levels in ADEVs from patients with MDD, suggesting potential changes in neuroplasticity, this observation cannot establish a causal relationship between neuroplasticity and depression.

Conclusions

In conclusion, our results indicate that BDNF levels are modified in MDD patient-derived plasma ADEVs compared with HCs and after SSRI treatment, potentially reflecting changes in neuroplasticity. This study provides human in vivo evidence supporting the neuroplasticity hypothesis of depression by demonstrating altered BDNF levels in ADEVs. Furthermore, ADEVs are emerging as a novel tool for investigating astrocyte status in the brain and assessing hypotheses related to depression.

Abbreviations

MDD, Major depressive disorder; HCs, healthy controls; BDNF, brain-derived neurotrophic factor; NGF, nerve growth factor; NT-3, neurotrophin 3; NT-4/5, neurotrophin 4/5; FGF2, fibroblast growth factor 2; GDNF, glial cell-derived neurotrophic factor; SSIR, selective serotonin reuptake inhibitor; EVs, extracellular vesicles; BBB, blood-brain barrier; CNS, central nervous system; ADEVs, astrocyte-derived extracellular vesicles; CSF, cerebrospinal fluid; HAMD-17, Hamilton Depression Rating Scale; MoCA, Montreal Cognitive Assessment; DSST, Digit Symbol Substitution Test; TEVs, total Evs; NTA, Nanoparticle tracking analysis; Alix, ALG-2 interacting protein-X; CD, cluster of differentiation; HSP70, heat shock protein 70; GFAP, glial fibrillary acidic protein; ELISA, Enzyme-linked immunosorbent assay; BMI, body mass index; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; GLMs, general linear models; EMMs, Estimated Marginal Means; CIs, confidence intervals; ROC, receiver operating characteristic; AUC, area under curve; FDR, false discovery rate.

Data Sharing Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics Approval

The study followed the Declaration of Helsinki (2013 revision) and obtained approval from the Ethics Committees of Renmin Hospital of Wuhan University (WDRY2020-K191) and Affiliated Hospital of West Anhui Health Vocational College (LAEY-2023-008). Informed consent was obtained from all participants or their legal guardians before they participated in the study.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This work was supported by the National Natural Science Foundation of China (U21A20364) and the National Key Research and Development Program of China (2018YFC1314600).

Disclosure

The authors report no conflicts of interest in this work.

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