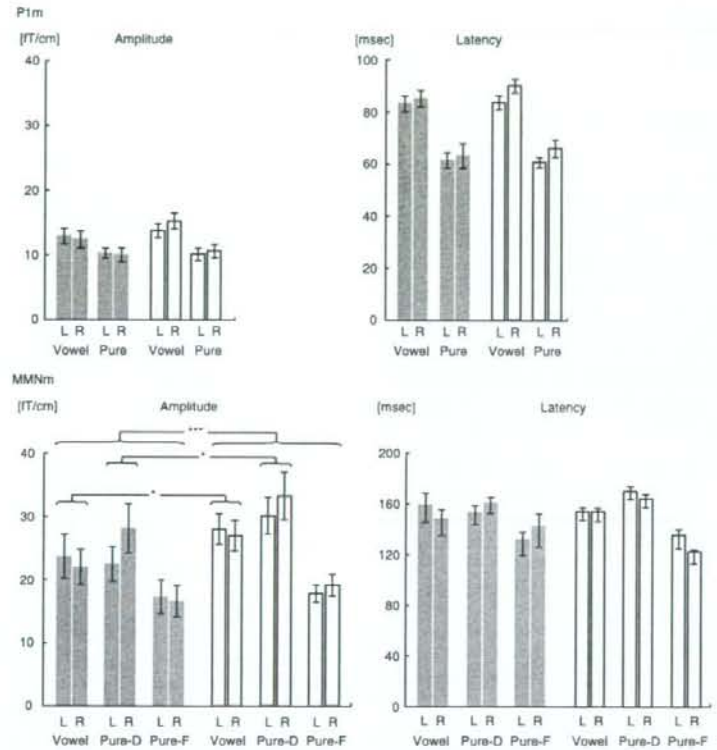


**Figure 5.** mGFP powers and latencies of P1m (upper panels) and MMNm (lower panels) averaged for each group (major depressive disorder patients [gray], healthy volunteers [white]), for each condition (vowel across-category change condition [Vowel], pure-tone duration change condition [Pure-D], and pure-tone frequency change condition [Pure-F]) and for each hemisphere (right [R], left [L]). Each error bar indicates standard error: $^{*}p<.1$; $^{**}p<.05$; $^{***}p<.01$.

task conditions described above. The differences in the severity of the depressive symptoms between the patients in the two studies may be one of the possible reasons for the difference in the results. The HDRS scores in this study were smaller those in the study by Kähkönen et al.

In the child subjects, Lepistö et al. (2004) found unchanged MMN amplitude and smaller MMN latency in major depressive disorder patients. In addition, Ogura et al. (1995) examined the N200 component in adult major depressive disorder and bipolar disorder and found small amplitudes of the early N200 component that is assumed to correspond to MMN. The results of this study are in agreement with those of Ogura et al. (1995) but not with those of Lepistö et al. Differences in task designs and in the age, severity of depressive symptoms, and medication status of the subjects may partly explain these discrepancies.

***Relationship between MMN and Clinical Variables***

The absence of significant correlations between MMNm power and doses of antidepressants, anxiolytics, and hypnotics in this study suggests that a smaller MMNm power in major depressive disorder patients is not due to the effects of psychotropic medication. In addition, although the MMN amplitude has been reported to be reduced in Alzheimer's disease and dementia (Pekkonen, 2000; Schroeder, Ritter, & Vaughan, 1995), MMNm power reduction in this study is not assumed to be due to intellectual decline in the subjects, because the subjects suspected of having dementia were excluded from our study on the basis of their MMSE scores.

The clinical significance of the MMNm power reduction in major depressive disorder patients can be speculated considering the lack of significant correlations of MMNm power with clinical variables. The lack of a significant correlation between MMNm power and clinical symptoms may suggest that the MMNm power reduction is not a state-dependent finding. The lack of a significant correlation between MMNm power and illness duration or age of onset may suggest its nonprogressive nature. Taken together, MMNm power reduction in major depressive disorder patients may be assumed to reflect the trait for developing major depressive disorder or the morbid process of developing major depressive disorder.

*Shuchaku-Seikaku* (*Shuchaku* the tendency for obsessive preoccupation with certain thoughts and affairs; *Seikaku* character) is a type of personality often observed as a premorbid personality of depression, particularly in Japan. MMN (N2a component) amplitude reduction in *Shuchaku-Seikaku* patients supports this interpretation (Ogura et al., 1991). This interpretation should be examined in future studies with more subjects and detailed personality assessments.

On the other hand, MMN amplitude for a pure-tone frequency change condition in schizophrenia was demonstrated to correlate with illness duration in a meta-analysis (Umbricht & Krljes, 2005), and MMN amplitude in schizophrenia was demonstrated to be reduced in recent-onset and chronic patients but not in first-episode patients (Umbricht, Bates, Lieberman, Kane, & Javitt, 2006). These findings support the deteriorating nature of MMN through the illness course. On the other hand, MMN amplitude has been reported to be reduced in adolescent-onset schizophrenia (Oades et al., 2006), which may suggest the trait-dependent or morbid-process-related nature of MMN reduction. Moreover, MMN amplitude reduction in schizophrenia may be interpreted to indicate state-dependent neurodegeneration based on the finding that MMN amplitude in a nonaffected member of twin pairs discordant for schizophrenia is unchanged when compared with that in healthy subjects (Ahveninen et al., 2006).

The results of the MMNm dipole location suggest an additional significance of MMNm abnormalities in major depressive disorder patients. The MMNm dipole has been estimated to be located more laterally in schizophrenia patients than in healthy volunteers in some studies (Kasai et al., 2003; Oades et al., 2006; Pekkonen et al., 2002), and the location shift is interpreted to reflect functional and structural abnormalities in the temporal lobe. The unchanged dipole location of MMNm in major depressive disorder patients in this study suggests that MMNm abnormalities in major depressive disorder patients are more functional than structural. However, this finding should be regarded as preliminary because correction for brain size was not considered. Preserved P1m power in this study also suggests additional significance of MMNm power reduction in major depressive disorder patients. Major depressive disorder patients may be relatively spared in sensory function (P1m) but more impaired in higher cognitive function, including preattentive level (MMNm), as compared with schizophrenia in which both P1m and MMNm powers are reduced (Ahveninen et al., 2006).

### *P1m Results*

Some of the results obtained in this study are different among the three task conditions. As for P1m, both its mGFP and latency were significantly smaller in the pure-tone condition than in the vowel across-category change condition. As for MMNm, its mGFP and latency were significantly smaller in the pure-tone frequency change condition than in the pure-tone duration change condition and the vowel across-category change condition. These results suggest that P1m may be affected mainly by the physical property of the sound, whereas in the case of MMNm, it may be affected by the task conditions in a more complex manner.

This assumption is also supported by the estimated locations of the dipoles. Although the locations of the P1m dipole were not estimated differently across the task conditions, the locations of MMNm were estimated more anteriorly in the vowel across-category change condition than in the pure-tone duration change condition but not in the pure-tone frequency change condition. Comparison between P1m and MMNm resulted in the differences in the location: The MMNm dipole is located more superiorly than the P1m dipole. These results suggest again that the location of MMNm is affected not only by the physical property of the sound but also by the task condition.

### *Limitations of This Study*

The limitations of this study are as follows: (1) The number of subjects is small; (2) the results were drawn from patients with mild to moderate symptoms; and (3) although we found no significant correlation of MMNm or P1m finding with psychotropic medication except mood stabilizers, the effect of psychotropic medication cannot be completely excluded because almost all the patients took psychotropic drugs. In the future, studies with more subjects in various mood states in a drug-free condition and longitudinal follow-up cohort studies including premorbid patients will be performed.

### *Conclusions*

We investigated preattentive information processing in major depressive disorder patients by MEG using MMNm and P1m. The MMNm power was smaller in major depressive disorder patients than in healthy volunteers. This result suggests the functional dysfunction of preattentive information processing irrespective of clinical symptoms and psychotropic medication in major depressive disorder patients; this dysfunction is not due to the dysfunction at the lower level of information processing.

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# Novel Augmentation Therapy with Cilostazol for the Geriatric Major Depressive Disorder Patient with Deep White Matter Hyperintensities on $T_2$-Weighted Brain MRI: A Case Report

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In our search of a new augmentation therapy for geriatric patients with intractable depression, we administered cilostazol, an antiplatelet agent, in addition to conventional antidepressants to a patient with persistent major depressive disorder showing deep white matter hyperintensities on a T2-weighted magnetic resonance image and evaluated cerebral blood flow before and after the administration of cilostazol by $^{99m}$Tc-ethyl-cysteinate dimer single photon emission computed tomography. This patient showed improvements of depressive symptoms as well as an increase in cerebral blood flow. These findings suggest a potential efficacy of cilostazol as a new drug for use in augmentation therapy for depressed patients with deep white matter hyperintensities.

## Introduction

It has been reported that patients with geriatric depression exhibit a higher percentage of cerebrovascular disorder including asymptomatic cerebral infarction than the non-depressed population [5]. Cerebrovascular disorder has been considered to be deeply related to the condition of geriatric depression, and Alexopoulus et al. [1] and Krishnan et al. [6] proposed the concept of vascular depression in 1997. Thus, the importance of cerebrovascular lesions has been emphasized for understanding the pathophysiology of mood disorder in the elderly. Treatment of depressed patients with cerebrovascular lesions including asymptomatic ones is often difficult because many patients with this condition respond poorly to antidepressants, frequently develop side effects, and are more susceptible to chronicity or recurrence. Therefore, a new therapeutic approach that takes into account cerebrovascular factors is required. Cilostazol is an antiplatelet agent with a vasodilating effect that results in an enhancement of cerebral blood flow [7]. The drug has mainly been used for chronic arterial occlusion but also for preventing recurrent cerebral infarction because its effectiveness to prevent recurrence of cerebral infarction has been demonstrated in a large-scale study [2]. To the best of our knowledge, however, there has been no report yet of its efficacy in depressed patients with asymptomatic cerebrovascular lesions. In this study, we examined the possibility of a new augmentation therapy by concomitantly administering cilostazol and conventional antidepressants to a patient with a major depressive disorder and a silent cerebrovascular disorder.

## Case Presentation

The subject is a 79-year-old female patient who was diagnosed as having recurrent major depressive disorder for 22 years on the basis of the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR); she showed deep white matter hyperintensities on a $T_2$-weighted magnetic resonance image (MRI), no microhemorrhage on $T_2$*-weighted MRI of the brain, and presented with persistent depressive symptoms for 3 years in the current episode. Somatic complications were excluded by regular physical examination and laboratory tests at the time of admission, and the patient was free from any other medications. Brain $^{99m}$Tc-ethyl cysteinate dimer single photon emission computed tomography ($^{99m}$Tc-ECD SPECT) images were obtained prior to cilostazol administration and five weeks after its initiation. The Hamilton Depression Rating Scale (HDRS) (Japanese version of the Structured Interview Guide for the HDRS (SIGH-D) [8]) was used to evaluate the symptoms. The Institutional Review Board of Gunma University Hospital approved this study. The subject gave her written consent to participate after procedures and possible side effects were explained to her.

In the current episode, the patient was hospitalized to our department after a suicide attempt. Despite antidepressant and anxiolytic medication, the patient continued to present with hypochondriacal complaints, hypobulia, and suicidal ideation, and adverse reactions often emerged due to abnormal activity of the metabolic liver enzyme cytochrome P450 2D6 (CYP2D6), which was discovered later. The drug treatment remained difficult and did not lead to remission. Although ECT relieved her depressed condition transiently, delirium arose and worsened, making it difficult to continue the ECT. After improvement of the delirium, hypochondriacal complaints, anxiety, restlessness, and diminished activity persisted. The doses of the drugs used at this point (15 mg/day, milnacipran; 250 mg/day, carbamazepine; 2.5 mg/day, olanzapine; and 50 mg/day, tiapride) were fixed 4 weeks prior to cilostazol administration, and brain MR imaging and $^{99m}$Tc-ECD SPECT studies were conducted. $T_2$-weighted brain MRI showed many deep white matter hyperintensities, and the pre-cilostazol $^{99m}$Tc-ECD SPECT showed a decrease in cerebral blood flow (**o Fig. 1**). The HDRS score 2 weeks prior to cilostazol administration was 21. The concomitant treatment with cilostazol at 50 mg/day reduced the hypochondriacal complaints, improved activity, and decreased the HDRS score from 21 (at treatment initiation) to 15 (1 week later), and further to 6 (5 weeks later); thus, the patient was discharged. Mean cerebral blood flow (mCBF: mL/100 g/min) measured by $^{99m}$Tc-ECD SPECT improved from 36.52 (2 weeks before cilostazol) to 40.14 (5 weeks after cilostazol). The plasma concentration of carbamazepine was 5.6 $\mu$g/mL (at treatment initiation) and 5.5 $\mu$g/mL (5 weeks later).

## Discussion

We report here a case with difficult-to-treat major depressive disorder with asymptomatic deep white matter hyperintensities on the brain $T_2$-weighted MRI. The concomitant administration of cilostazol was effective in this patient.

Cilostazol improves cerebral blood flow in patients with chronic cerebral infarction, and has been reported to improve cerebral function evaluated by P300 event-related potential [7], and the

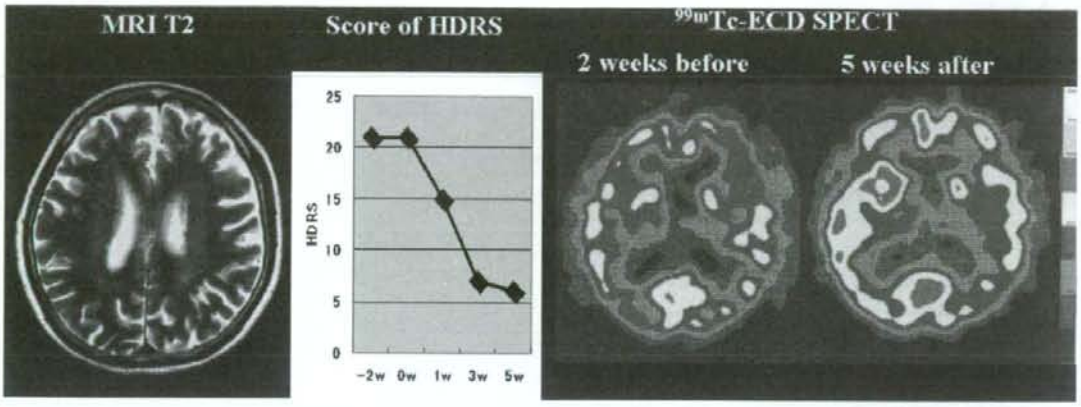


**Fig. 1** This case showed broad deep white matter hyperintensities on the $T_2$-weighted brain MRIs. The HDRS score cerebral blood flow as shown by $^{99m}$Tc-ECD SPECT, improved after cilostazol administration.

enhancing effect on cerebral blood flow may have led to the improvement of depressive symptoms in this case. Moreover, cilostazol inhibits PDE3; it has been confirmed in platelets [4] and vascular smooth muscle cells [11] that cilostazol inhibits the degradation of cAMP to AMP and increases the intracellular cAMP concentration. Moreover, in the white matter of rats with artificial chronic cerebral ischemia, increases in phosphorylated cAMP response element binding protein (CREB), Bcl-2, and COX-2 expression levels were reported in the cilostazol-treated group compared with the control group [12]. Furthermore, recent studies have revealed that chronic administration of antidepressants facilitates CREB phosphorylation [10] and transcription of target genes such as brain-derived neurotrophic factor (BDNF) [9] in the rat brain. Taken together, it may be postulated that the activation of these intracellular signaling molecules by cilostazol enhances neural activity, resulting in an increased cerebral blood flow and the eventual recovery from a depressive episode.

There is also a possibility that the plasma level of cilostazol may be affected by drugs like carbamazepine which induce liver enzymes, because cilostazol undergoes extensive hepatic metabolism. However, this is unlikely in the present case, as the plasma concentrations of carbamazepine were almost identical and low (5.5–5.6 μg/mL) before and after cilostazol treatment, and liver enzyme induction may be rather weak at this level.

In general, as in the case presented here, depressed patients with cerebrovascular lesions including asymptomatic lesions poorly respond to antidepressants and often develop adverse reactions, and the depressive symptoms tend to persist or recur [3]. For these major depressive disorder patients with cerebrovascular lesions, cilostazol may be promising as a new drug for augmentation therapy and merits further investigation.

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Research report

# Executive dysfunction in medicated, remitted state of major depression

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**Abstract**

*Background:* Past neuropsychological studies on depression have documented executive dysfunction and it has been reported that some dysfunction persists even after depressive symptoms disappear. Studies have shown a correlation between cerebrovascular lesions and executive dysfunction in depression among the elderly. The aim of the present study was to focus on executive functions in remitted major depressive disorder (MDD) patients, and to investigate whether remitted young and elderly patients show different patterns of executive dysfunction, and to ascertain the relationships with vascular lesions.
*Methods:* Subjects were 79 inpatients with MDD and 85 healthy controls. Each subject received Wisconsin Card Sorting Test (WCST), Stroop test, and Verbal Fluency Test (VFT) in a remitted state. Both the MDD and control groups were divided into young and elderly groups, and the performances between 4 groups were compared.
*Results:* For Stroop test, the scores of the MDD group were significantly lower than controls. In addition, as for VFT, the scores for the elderly MDD group were significantly lower than the other groups. Multiple regression analysis showed that VFT scores were affected by the presence of vascular lesions.
*Conclusions:* The results of the present study demonstrated that executive dysfunction remained even in a remitted state in MDD patients, but the patterns of impairment were different between young and elderly patients. The results also suggested that vascular lesions affect executive dysfunction, particularly in elderly depressive patients.


*Keywords:* Depression; Remission; Executive function; Elderly; Vascular

## 1. Introduction

It is well known that patients with major depressive disorder (MDD) present with cognitive deficits, in particular executive dysfunctions (Fromm and Schopflocher, 1984; Franke et al., 1993; Channon, 1996; Veiel, 1997; Degl'Innocenti et al., 1998). Lockwood et al. (2002) compared executive dysfunction between young

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and elderly MDD patients while on depressive episodes, and reported that selective and sustained attention exhibited no age and depression interaction whereas the elderly depressed patients alone demonstrated the slowed psychomotor speed and impaired performance on tasks requiring set sifting, problem solving and initiating novel responses. Recent studies have also documented that executive dysfunction may at least in part remain unresolved even after remission of depressive episodes (Trichard et al., 1995; Paradiso et al., 1997; Reischies and Neu, 2000; Neu et al., 2001; Biringer et al., 2005; Paelecke-Habermann et al., 2005).

Neural bases for residual executive dysfunction in remitted MDD patients are not well understood. One plausible account is existence of deep white matter vascular changes, which have reportedly been associated with executive dysfunction in elderly patients with MDD (Boone et al., 1992; Lesser et al., 1996; Aizenstein et al., 2002). White matter changes are more prevalent and severe in depressed elderly patients than in age-matched controls, and mainly occur in subcortical regions and their frontal white-matter projections (Coffey et al., 1990; Krishnan, 1993; Greenwald et al., 1996; Krishnan et al., 1997; Tupler et al., 2002; Taylor et al., 2003).

Although reports regarding executive dysfunction in remitted state of depression are increasing, few studies have investigated the possibly dissociable patterns between young and elderly patients. The aim of the present study was to explore differences in executive dysfunctions between young and elderly MDD patients in a remitted state and elucidate how cerebrovascular changes may affect these functions.

## 2. Subjects and methods

### 2.1. Subjects

Seventy-nine depressive inpatients (38 males and 41 females; mean age, 52.4 years; age range, 25–78 years) were recruited from Juntendo Koshigaya Hospital. All the patients previously met the Diagnostic and Statistical Manual for Mental Disorders, 4th edition (DSM-IV) criteria for MDD. Thirty-eight patients experienced a single episode, 32 patients experienced recurrent episodes. For remaining nine patients it was unable to determine the number of episodes. At the time of the study, however, the patients were considered in remission, which was confirmed by the fact that they no longer met the DSM-IV criteria for MDD. In addition, their Hamilton Rating Scale for Depression (HAM-D) scored below 7 points (Hamilton, 1960). Patients were excluded if they had a history of other psychiatric disorders including delusions, severe or acute medical illnesses, neurological disorders, or use of drugs that may cause depression. All the patients were on antidepressant medication at the time of the study (Table 1).

Detailed demographic and clinical features of the participants are shown in Table 1. Presumed IQ was computed using the Japanese Adult Reading Test (JART) (Matsuoka et al., 2006). The patients were examined using the brain computed tomography (CT) to assess low density areas indicating white matter cerebrovascular changes. The brain CT of each patient was read by two reading specialists independently of and blindly to the results of the neuropsychological tests. Patients with large organic changes that may directly affect psychiatric features were excluded. The CT data were assessed using the Fazekas criteria by Krishnan et al. (1997). These criteria introduced a modified Fazekas classification system (Greenwald et al., 1996), which provided a rough assessment of the extent of subcortical gray matter, deep white matter, and periventricular changes on brain CT. Patients were classified as having vascular lesions if a score of $\geq 2$ on either deep white matter low densities or subcortical gray matter ratings was obtained. Based on this criterion, 7% of young and 54% of elderly patients were classified as having vascular lesions.

Eighty-five healthy control participants (15 males and 70 females) matched for age and education were also recruited. All the controls were recruited from the employees of Genkikai Yokohama Hospital. Based on recent studies having reported individuals $\geq 60$ years as geriatric subjects (Lockwood et al., 2002; Murphy et al., 2006), we divided both the MDD patients and controls into two age groups (young, $<60$ years and elderly, $\geq 60$ years) and the neuropsychological performances were compared between the four groups. Presumed IQ was higher in the young MDD patients as compared to young controls ($p<0.05$). There were more females in the young controls than in the young MDD group ($p<0.001$). Subjects who had clinical evidence of dementia or whose Mini-Mental State Examination (MMSE) score $<24$ were excluded. Twelve MDD patients and 19 healthy subjects had hypertension.

The study was approved by the Medical Ethics Committee of Juntendo University, and was performed in accordance with the regulations outlined by Juntendo University. All the participants signed an informed consent form.

### 2.2. Executive function tests

Three executive function tests were given to each participant; the Wisconsin Card Sorting Test (WCST), Stroop and Verbal Fluency Test (VFT). For the WCST,

Table 1
Demographic of the subjects

| | Young (<60 years) | | Elderly (≥60) | |
|---|---|---|---|---|
| | Control ($N$=60) | MDD ($N$=55) | Control ($N$=25) | MDD ($N$=24) |
| | Mean (SD) | Mean (SD) | Mean (SD) | Mean (SD) |
| Age (years) | 44.0 (11.4) | 45.1 (8.3) | 66.6 (4.5) | 68.9 (4.5) |
| Sex (M/F) | 12/48 | 32/23 | 3/22 | 6/18 |
| Education (years) | 13.2 (3.0) | 14.2 (2.0) | 12.1 (2.9) | 11.5 (2.8) |
| Presumption IQ | 101.2 (11.6) | 105.5 (10.6) | 95.7 (13.1) | 97.3 (13.0) |
| On-set age (years) | | 40.5 (8.6) | | 63.0 (10.6) |
| Number of depressive episodes | | 1.8 (1.2) | | 2.1 (1.6) |
| Total duration of depressive phase (months) | | 25.8 (38.2) | | 43.0 (120.0) |
| Total duration of medication (months) | | 27.65 (42.1) | | 47.7 (116.4) |
| Vascular lesions | | 4(7%) | | 13(54%) |

two indices of Categories Achieved (CA) and Perseverative Errors (PE) were selected as measures. The PE were useful for documenting problems in forming concepts, profiting from correction, and conceptual flexibility (Lezak et al., 2004). A computerized version of the test was used in the present study. We used modified Japanese version of Golden's (1978) 'Stroop Color and Word Test'. In the Stroop test Part I, the participant read aloud color words printed in ink of different colors (congruent condition). Part II required the participant to name the incongruent printed color of the color words (incongruent condition). Time difference between Part I and II (Part II–Part I) was evaluated (Golden, 1978). This difference has been attributed to a response conflict and to a failure of response inhibition (Lezak et al., 2004). For the VFT, we used the Japanese characters "a," "ka," and "sa" as prompts, and the score was the sum of all acceptable words produced within three one-minute trials. Successful performance in this test depends in part on the participant's ability to "organize output in terms of clusters of meaningfully related words" (Lezak et al., 2004). Fluency tests requiring word generation according to an initial letter provide subjects with the greatest scope for seeking a strategy to guide the search for words (Estes, 1974; Lezak et al., 2004).

### 2.3. Data analysis

Statistical analysis was performed using 2 (diagnosis; MDD vs. controls)×2 (age group; young vs. elderly) analysis of variance (ANOVA) at a significance level of $p<0.05$. Performances on each executive function test between MDD and controls were compared using two-tailed unpaired Student's t-test for post hoc analysis within each age group. Bonferroni correction was applied, and 0.25% level of significance was adopted.

Multiple regression analysis was conducted using neuropsychological test scores as dependent variables and age, gender, education level, and vascular lesions as independent variables. Statistical procedures were performed using Japanese version of SPSS v15.1 (SPSS Japan Inc. Tokyo, Japan).

## 3. Results

The means and standard deviations of the executive function tests are presented in Table 2, along with the

Table 2
Results of the four groups on the neuropsychological tests

| | Young (<60) | | Elderly (≥60) | | Main effect of age | | Main effect of diagnosis | | Interaction between age×diagnosis | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Control ($N$=60) | MDD ($N$=55) | Control ($N$=25) | MDD ($N$=24) | | | | | | |
| | Mean (SD) | Mean (SD) | Mean (SD) | Mean (SD) | $F$ (1,160) | $p$ | $F$ (1,160) | $p$ | $F$ (1,160) | $p$ |
| *WCST* | | | | | | | | | | |
| CA | 3.3 (2.0) | 3.4 (2.1) | 1.96 (1.5) | 2.0 (1.9) | 14.84 | <0.001 | 0.08 | 0.782 | 0.27 | 0.869 |
| PE | 5.6 (5.0) | 6.0 (6.0) | 9.6 (5.0) | 7.9 (4.7) | 8.95 | 0.003 | 0.43 | 0.515 | 1.20 | 0.275 |
| Stroop test | 55.2 (20.6) | 78.5 (36.9)** | 82.6 (31.0) | 122.2 (77.8)* | 26.73 | <0.001 | 20.98 | <0.001 | 1.41 | 0.236 |
| VFT | 32.1 (8.9) | 32.7 (10.0) | 29.8 (12.9) | 22.2 (9.2)* | 14.24 | <0.001 | 4.19 | 0.042 | 5.73 | 0.018 |

Two-tailed paired Student's $t$-test for post hoc analysis within each age group. *$p<0.025$, **$p<0.001$.

Table 3
Results of multiple regression analysis

| | WCST | | | | Stroop test | | VFT | |
|---|---|---|---|---|---|---|---|---|
| | CA | | PE | | | | | |
| | β | p | β | p | β | p | β | p |
| Age | −0.27 | 0.129 | 0.05 | 0.780 | 0.54 | 0.001 | −0.17 | 0.225 |
| Sex | 0.13 | 0.347 | −0.21 | 0.153 | 0.11 | 0.370 | 0.09 | 0.398 |
| Education | 0.18 | 0.229 | −0.15 | 0.335 | −0.05 | 0.735 | 0.34 | 0.005 |
| Vascular lesions | 0.04 | 0.845 | −0.02 | 0.923 | −0.14 | 0.324 | −0.33 | 0.015 |

main effects of age and diagnosis, and the interaction between the two. For both CA and PE on the WCST, two-way ANOVA revealed a significant main effect for age, but not for diagnosis. The age × diagnosis interaction was not significant.

For the Stroop test, the main effects of both age and diagnosis were significant, indicating that MDD patients performed poorer than controls, irrespective of age. However, age × diagnosis interaction was not significant. Post hoc analysis confirmed that both young ($p<0.001$) and elderly ($p=0.024$) patients with MDD showed poorer performance in the Stroop test as compared to their age-matched counterpart.

For the VFT, significant main effects of age and diagnosis were obtained. In addition, an interaction between age and diagnosis reached significant level, suggesting that the elderly MDD group alone performed more poorly than the other three groups. This was confirmed by the post-hoc analysis which indicated that only elderly depressives performed poorer than elderly controls.

Multiple regression analysis showed that age had a significant effect on the Stroop test, while education level and the presence of vascular lesions had a significant effect on VFT scores (Table 3).

## 4. Discussion

Lockwood et al. (2002) investigated executive dysfunction in young and elderly MDD patients in depressed phase, and found that only elderly, but not young patients in current depression present with difficulty in set shifting as indexed by PE in the WCST. In contrast, Biringer et al. (2005) reported that the performance on the WCST of recovered patients with MDD were comparable to healthy controls. Our present results are in according with Biringer et al. (2005) study in that the two indices of the WCST were not affected by the previous episodes of depression. Although we did not directly compare WCST performance of MDD patients in depressed and remitted states, by combining previous findings and ours, one may speculate that deficient set shifting of elderly patients observed during depressive state resolves as depressed symptoms improve. One may further speculate that within the subdivisions of the prefrontal cortex (PFC), dorsolateral PFC activity which is most sensitively indexed by WCST set shifting abilities is recoverable after remission.

In the present study, performances on the Stroop test were affected both by age and diagnosis. For both young and elderly groups, remitted MDD patients performed more poorly than age-matched controls. The results were consistent with previous findings suggesting residual impaired performance on Stroop even after remission of MDD (Trichard et al., 1995; Paradiso et al., 1997; Paelecke-Habermann et al., 2005). Recent functional neuroimaging studies have suggested that the PFC and the anterior cingulate cortex (ACC) are particularly impaired in MDD patients (Bench et al., 1992; Ito et al., 1996; Mayberg, 1997; Mayberg et al., 1997; Davidson et al., 2002). In addition, studies documented that dysfunctions of PFC, ACC and amygdala may remain even in a remitted state, suggesting pathological influence within fronto-subcortical networks persist in remission (Drevets, 2000; Harrison, 2002; Holthoff et al., 2004). Within such functional abnormalities of amygdala-ACC-PFC projection, we consider that dysfunction of the ACC is most crucial in pathogenesis of MDD and reasonably persists in remitted stage (Ishizaki et al., 2008). Recent fMRI study using Stroop task paradigm demonstrated inefficiency of ACC and dorsolateral PFC in MDD (Wagner et al., 2006). Our findings, together with those of Paelecke-Habermann et al. (2005), are in line with such fMRI experiment suggesting residual pathophysiological dysfunction of the ACC in remitted MDD, which is most sensitively indexed by poor Stroop performance.

Concerning the VFT, we found a different pattern; i.e., performances of only elderly, but not young patients with MDD were impaired even in remitted state. Such dissociable findings between young and elderly patients with remitted depression were observed by Trichard

et al. (1995). Their MDD patients with an average age of 47.0 years, which approximated that of our young patients (45.2 years), showed severe impairment in the VFT on admission. However, such impairment in the VFT resolved to a comparable level to healthy controls at discharge. In addition, our study suggests that VFT scores were influenced by vascular lesions independent of age, gender, and education level. These vascular lesions associated with executive dysfunction are more prevalent and severe in depressed elderly individuals than age-matched controls, and mainly occur in subcortical regions and their frontal white-matter projections.

Neuropsychological and functional neuroimaging studies have documented that VFT is tightly linked with the left PFC, including Broca's and adjacent areas, together with the premotor cortex and insula (Tucha et al., 1999; Baldo et al., 2001). In addition, PET and fMRI studies have suggested that not only the left, but also the right frontal lobe, may play a crucial role in voluntary speech intention and/or attentional resources. Interconnected areas such as these are implicated in organizing a functional language network subserving word output (Perani et al., 2003). In the present study, VFT scores were found to be poor only for elderly depressives, and these poor results correlated to the presence of white matter lesions. This suggests that the vascular lesions associated with elderly depression may lead to impairment of the language-related frontal lobe functions.

## 5. Limitations

There are several limitations in the present study. First, the patients were treated with one or two different antidepressants, which may possibly have exerted deleterious effects on cognition. Executive dysfunction in our patients may have been influenced by an adverse effect of anticholinergic medication. Accordingly, drug-free euthymic patients should be investigated in future research.

Second, in the present study, neuroimaging information was obtained using brain CT only for MDD patients. In addition, CT images were assessed using rating scales, not with more quantified method. When compared to MRI, the detection rate of white-matter lesions is lower for CT, and in reality, more patients may have had vascular lesions in the present study than were detected. Additional research is warranted in order to investigate the relationship between executive dysfunction and the degree of white matter changes using MRI, with more quantified measurement. Also, direct comparison of vascular lesions between MDD patients and controls are warranted.

## 6. Conclusion

In MDD patients, executive dysfunction persisted even when in a remitted state; however, impairment patterns differed between young and elderly MDD. And the results suggest that vascular lesions affect executive dysfunction, particularly in elderly depressive.

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**Conflict of interest**

All authors declare that they have no conflicts of interest.

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# Altered expression of neurotrophic factors in patients with major depression

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**Abstract**

There is an abundance of evidence suggesting the involvement of altered levels of expression of neurotrophic factors in the pathophysiology of neuropsychiatric disorders. Although postmortem brain studies have indicated the alterations in the expression levels of neurotrophic factors in mood disorder patients, it is unclear whether these changes are state- or trait-dependent. In this study, we examined the expression levels of the members of the glial cell line-derived neurotrophic factor (GDNF) family (GDNF, artemin (ARTN), neurturin, and persephin), brain-derived neurotrophic factor, nerve growth factor, neurotrophin-3 (NT-3), and neurotrophin-4 mRNAs by using quantitative real-time PCR method in peripheral blood cells of patients with major depressive and bipolar disorders in both a current depressive and a remissive states. Reduced expression levels of GDNF, ARTN, and NT-3 mRNAs were found in patients with major depressive disorder in a current depressive state, but not in a remissive state. Altered expressions of these mRNAs were not found in patients with bipolar disorder. Our results suggest that the changes in the expression levels of GDNF, ARTN, and NT-3 mRNAs might be state-dependent and associated with the pathophysiology of major depression.


*Keywords:* Major depression; Bipolar disorder; Neurotrophic factor; GDNF; State marker

## 1. Introduction

Brain imaging studies have indicated the altered volumes of the hippocampus, prefrontal cortex, cingulate cortex, or amygdala in patients with major depression or bipolar disorder (Drevets et al., 1997; Sheline et al., 1999; Bremner et al., 2000; Caetano et al., 2006). Postmortem brain studies have also reported anatomical alterations such as neuronal/glial cell atrophy or reduction in cortical brain regions in depressed patients (Ongur et al., 1998; Rajkowska et al., 1999; Cotter et al., 2001). These studies suggest the alteration of structural and neuronal plasticity in mood disorders.

Neurotrophic factors have many effects on the nervous system such as neurogenesis, neuronal growth, differentiation, and plasticity. There is a growing body of evidence demonstrating that neurotrophic factors have crucial roles in stress response, the action of antidepressants, and pathophysiology of mood disorders (Smith et al., 1995; Nibuya et al., 1999; Karege et al., 2002; Nestler et al., 2002; Duman and Monteggia, 2006; Govindarajan et al., 2006). For example, the expression level of brain-derived neurotrophic factor (BDNF) was significantly decreased in animals subjected to social defeat, immobilization, and maternal deprivation stresses (Smith et al., 1995; Nibuya et al., 1999; Roceri et al., 2002; Tsankova et al., 2006). In addition, chronic antidepressant treatment increases the expression levels of BDNF and neurogenesis in rodents (Malberg et al., 2000; Duman and Monteggia, 2006; Tsankova et al., 2006). Furthermore, low levels of serum BDNF is

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suggested to be associated with major depression (Duman et al., 1997; Karege et al., 2002; Nestler et al., 2002). In addition to BDNF, the expression levels of other neurotrophic factors, including: nerve growth factor (NGF); glial cell line-derived neurotrophic factor (GDNF); neurotrophin-3 (NT-3); and neurotrophin-4 (NT-4) were also reported to be associated with stress response and neurogenesis in rodents and depression in humans (Smith et al., 1995; Ueyama et al., 1997; Aloe et al., 2002; Dwivedi et al., 2005; Shimazu et al., 2006). However, little is known about whether the changes in the expression levels of these molecules are state- or trait-dependent in mood disorders.

Another well known characteristic of the pathophysiology of mood disorders is the dysregulation of hypothalamic-pituitary–adrenal (HPA) axis (Arborelius et al., 1999; Holsboer, 2001; Nestler et al., 2002; de Kloet, 2003). Although the molecular mechanisms of the aberrant regulation of the HPA axis in mood disorders remains unclear, one candidate is the dysfunction of the glucocorticoid receptor (GR), which plays an important role in the negative feedback of the HPA axis and the adaptation to stress (Holsboer, 2000; Pariante and Miller, 2001). In response to stress, glucocorticoid hormone and GR are associated with stress-induced effects on the brain, including shrinkage of neural dendrites, suppressed neurogenesis, and reduced serotonin metabolism (Lopez et al., 1998; McEwen, 2000; Sapolsky et al., 2000; de Kloet, 2003). In addition, previous studies have indicated the reduced expression of GRα mRNA in the brain and peripheral white blood cells of patients with mood disorders (Webster et al., 2002; Knable et al., 2004; Perlman et al., 2004; Matsubara et al., 2006). Several lines of evidence have suggested that glucocorticoid hormone or GR affect the expression levels of neurotrophic factors (Barbany and Persson, 1992; Chao and McEwen, 1994; Smith et al., 1995; Mocchetti et al., 1996; Ridder et al., 2005; Schulte-Herbruggen et al., 2006). For example, adrenalectomy significantly decreased the mRNA levels of BDNF, NT-3, and NGF in the rat cerebral cortex and hippocampus (Barbany and Persson, 1992). In addition, GR knock-out heterozygous mice exhibited decreased levels of BDNF protein in the hippocampus, whereas GR overexpressing mice exhibited increased levels of BDNF protein in the amygdala and hippocampus (Ridder et al., 2005; Schulte-Herbruggen et al., 2006). These observations suggest that the reduced levels of GR may affect the expression levels of neurotrophic factors in patients with mood disorders.

In this study, we aimed to determine whether there are alterations in the expression levels of multiple neurotrophic factors, including GDNF family members (GDNF, artemin (ARTN), neurturin (NRTN), and persephin (PSPN)), BDNF, NGF, NT-3, and NT-4 mRNAs in peripheral white blood cells of mood disorder patients. Furthermore, to examine whether the altered expression of neurotrophic factor mRNAs are state- or trait-dependent, the mRNA levels of these factors were examined in both current depressive and remissive states.

## 2. Materials and methods

### 2.1. Subjects

Major depressive and bipolar disorder patients were diagnosed according to the criteria in the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV; American Psychiatric Association, 1994). These included both outpatients and inpatients of the Division of Neuropsychiatry of the Yamaguchi University Hospital. The extent of the depressive state was assessed by a 21-item "Hamilton Depression Rating Scale" (HDRS). Subjects were classified as under a current depressive state when they showed a score of more than 18 on HDRS and met the DSM-IV criteria for major depressive episode. Subjects were classified as being in remission when they showed a score of less than 6 on HDRS and did not show any symptoms of the major depressive episode in the DSM-IV criteria for more than 2 months. Individuals were excluded from the present study if they had abnormal physical examinations or abnormal results for routine medical laboratory tests such as a complete blood count, renal, liver or thyroid function. Female subjects who were pregnant or took oral contraceptives were also excluded. All healthy control subjects were screened to exclude significant current or past medical or neurological illnesses, significant alcohol or drug abuse and past or current Axis I psychiatric illnesses. This protocol was approved by the Institutional Review Board of Yamaguchi University Hospital. Informed written consent was obtained for all subjects.

### 2.2. Blood sample preparation, RNA isolation, and cDNA synthesis

Blood sample preparation, total RNA isolation and cDNA synthesis were performed as previously described (Matsubara et al., 2006). In brief, blood was obtained by venipuncture between 10:00 a.m. and 11:00 a.m. and processed for total RNA purification from peripheral blood cells by using QIAamp RNA Blood Mini Kit (Qiagen, Chatsworth, CA, USA) according to the manufacturer's manual. The quality of RNA was determined based on $A_{260}/A_{280}$ ratio, which was found as 1.7–2.0 for all RNA preparations. One microgram of total RNA was used for cDNA synthesis using random hexamer primers and Omniscript Reverse Transcriptase (Qiagen). The cDNA was stored at −80 °C until further use.

### 2.3. Serum cortisol determination

Serum cortisol concentration was measured via radioimmunoassay by the SRL Corporation (Tokyo, Japan).

### 2.4. Quantitative real- time polymerase chain reaction

Quantitative real-time polymerase chain reaction (qRT-PCR) was performed in an Applied Biosystems 7300 Fast

Real-Time PCR System with SYBR green PCR master mix (Applied Biosystems, CA, USA) according to the manufacturer's manual. PCR conditions were 15 min at 95 °C, 45 cycles of 15 s at 95 °C and 30 s at 60 °C. The primer sequences are described in Table 1. The amplification of a single PCR product was confirmed by monitoring the dissociation curve and electrophoresis on 1.5% agarose gels stained with ethidium bromide. Amplification curves were visually inspected to set a suitable baseline range and threshold level. To generate standard curves, various concentrations of cDNA, made from total RNA isolated from human lymphoma-derived Jurkat cells, were used in each PCR reaction. The number of cycles required to reach the threshold fluorescence level was scored and used for generating standard curves and interpolating mRNA concentration levels. The relative quantification method, in which the ratio between the amount of target molecule and a reference molecule within the same sample was calculated, was employed for quantifying target molecules according to the manufacturer's protocol. All measurements were performed at least in duplicate. Levels of GAPDH mRNA was used to normalize the relative expression levels of target mRNA. Expression values for mood disorder subjects were normalized by the mean of the value for control subjects.

### 2.5. Dex/CRH test

A few days after blood sampling for the RNA isolation and basal cortisol determination, Dexamethasone (Dex)/corticotropin-releasing hormone (CRH) test was performed as previously reported (Matsubara et al., 2006). Mood disorder patients were pretreated with an oral dose of 1 mg of Dex (Asahikasei Pharmaceutical Corporation, Tokyo, Japan) at 11:00 p.m. The next day intravenous cannulation was carried out at 12:30 p.m. and 100 μg of human CRH (hCRH, Mitsubishi Pharma Corporation, Tokyo, Japan) was administered intravenously at 1:00 p.m., immediately after the first blood collection. Blood specimens were drawn through the intravenous catheter 15 min, 30 min, 60 min and 120 min later. Blood samples were immediately centrifuged and stored at −20 °C. Serum level of cortisol and plasma level of ACTH were measured with radioimmunoassay (SRL). We defined non-suppressors as those individuals whose post-CRH plasma cortisol levels were more than 5 μg/dl.

### 2.6. Statistical analysis

Commercial software (SPSS version 11.5; Chicago, Illinois) was used to perform data analysis. All data are expressed as the mean ± standard error of the mean (SEM). Two group comparisons were performed using the Student's *t*-test. The other data were subjected to one-way analysis of variance (ANOVA) followed by post hoc analysis (Tukey test). In all cases, comparisons were considered statistically significant for $p < 0.05$.

## 3. Results

Table 2 shows the demographic and clinical characteristics of the subjects. The majority of the patients were on medications. The mean ages were not significantly different among major depressive disorder patients, bipolar depressive patients or healthy control subjects ($F = 1.228$, $df = 4.125$, $p = 0.303$).

We first examined the expression levels of the GDNF family members (GDNF, ARTN, NRTN, and PSPN) in patients with mood disorder in a current depressive state (Fig. 1). The qRT-PCR revealed that the expression of GDNF mRNA was significantly decreased in major depressive disorder patients compared with healthy control subjects ($F = 5.472$, $df = 2.58$, $p = 0.005$) (Fig. 1A). In addition, ARTN mRNA was significantly decreased in major depressive disorder patients compared with healthy control subjects and bipolar disorder patients ($F = 4.147$, $df = 2.58$, $p = 0.037$; $p = 0.049$) (Fig. 1B). There was no significant difference in the expression levels of GDNF and ARTN mRNA between patients with bipolar disorder in a current depressive state and healthy control subjects (GDNF: $F = 5.472$, $df = 2.58$, $p = 0.214$, ARTN: $F = 4.147$, $df = 2.58$, $p = 0.932$) (Fig. 1A and B). There was no significant difference in the expression levels of NRTN and PSPN mRNAs among major depressive, bipolar disorder patients and healthy control subjects (NRTN: $p = 0.67$; PSPN: $p = 0.282$) (Fig. 1C and D).

Next, we examined the expression levels of BDNF, NGF, NT-3 and NT-4 mRNAs in patients with mood

Table 1
Primer sequences used for quantitative real-time PCR

| | Forward primer (5′–3′) | Reverse primer (5′–3′) | Size (bp) |
|---|---|---|---|
| BDNF | TGGCTGACACTTTCGAACAC | AGAAGAGGAGGCTCCAAAGG | 145 |
| NGF | CAACAGGACTCACAGGAGCA | GTCTGTGGCGGTGGTCTTAT | 115 |
| NT-3 | GAAACGCGATGTAAGGAAGC | CCAGCCCACGAGTTTATTGT | 138 |
| NT-4 | CCTCCGCCAGTACTTCTTTG | TCAGATACCCAGTGCCTCCT | 114 |
| GDNF | CCAACCCAGAGAATTCCAGA | AGCCGCTGCAGTACCTAAAA | 150 |
| ARTN | CTCTCCACACGACCTCAGC | ATGAAGGAGACCGCTTGGTA | 123 |
| NRTN | ACGAGACGGTGCTGTTCC | GCAGCCCGAGGTCGTAGA | 72 |
| PSPN | CCGTAGGGAAGTTCCTGCT | CCTTTGCCACCTGTTCAGAC | 126 |
| GAPDH | CAGCCTCAAGATCATCAGCA | TGTGGTCATGAGTCCTTCCA | 106 |

Table 2
Demographic and clinical characteristics of subjects

| | Control | Patients | | | |
|---|---|---|---|---|---|
| | | MDD | | BPD | |
| | $n = 28$ | Depressive $n = 20$ | Remissive $n = 40$ | Depressive $n = 13$ | Remissive $n = 29$ |
| Age (years) | 50.0 ± 1.8 | 52.3 ± 3.5 | 57.1 ± 2.2 | 55.5 ± 3.5 | 53.6 ± 2.6 |
| Gender (Male/Female) | 15/13 | 10/10 | 15/25 | 2/11 | 5/24 |
| HDRS | | 25.9 ± 1.9 | 3.3 ± 0.2 | 24.6 ± 1.1 | 2.9 ± 0.2 |
| Serum cortisol (μg/dl) | 8.6 ± 0.8 | 10.3 ± 1.3 | 11.6 ± 1.1 | 10.9 ± 4.5 | 10.5 ± 1.0 |
| *Dex-CRH test* | | | | | |
| Suppressor | | 8 | 7 | 3 | 2 |
| Non-suppressor | | 8 | 3 | 7 | 5 |
| *Medication* | | | | | |
| No medication | 28 | 3 | 4 | 1 | 0 |
| Antidepressants | 0 | 17 | 36 | 9 | 14 |
| Mood stabilizers | 0 | 0 | 0 | 10 | 27 |

MDD, major depressive disorder; BPD, bipolar disorder; HDRS, Hamilton Depression Rating Scale.

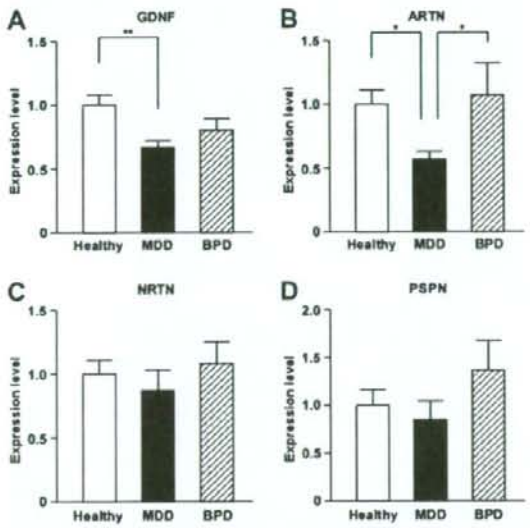


Fig. 1. The mRNA expression of GDNF family members in mood disorder patients in a depressive state. Quantitative real-time PCR revealed decreased GDNF and ARTN mRNA expression only in MDD patients ($n = 20$) compared with healthy control subjects ($n = 28$). MDD, major depressive disorder; BPD, bipolar disorder. Values are mean ± standard error. *: $p < 0.05$; **: $p < 0.01$.

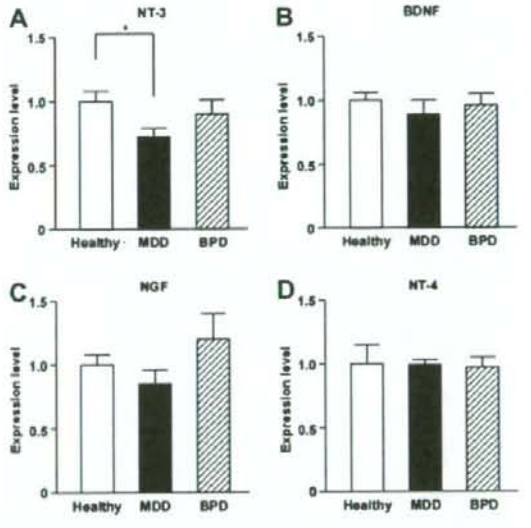


Fig. 2. The mRNA expression of NT-3, BDNF, NGF, and NT-4 in mood disorder patients in a depressive state. Quantitative real-time PCR revealed decreased NT-3 mRNA expression only in MDD patients ($n = 20$) compared with healthy control subjects ($n = 28$). There was no significant difference in the levels of BDNF, NGF, and NT-4 mRNAs among the three groups studied. MDD, major depressive disorder; BPD, bipolar disorder. Values are mean ± standard error. *: $p < 0.05$.

disorder in a current depressive state (Fig. 2). The expression levels of NT-3 mRNA was significantly decreased in major depressive patients compared with healthy control subjects ($F = 3.310$, df = 2.58, $p = 0.034$) (Fig. 2A). There was no significant difference in the expression level of NT-3 mRNA between patients with bipolar disorder in a current depressive state and healthy control subjects ($F = 3.31$, df = 2.58, $p = 0.675$) (Fig. 2A). There was no significant difference in the expression levels of BDNF, NGF and NT-4 mRNAs among the three groups (BDNF: $p = 0.615$; NGF: $p = 0.162$, NT-4: $p = 0.245$) (Fig. 2B–D).

We analyzed the effect of gender on the mRNA levels of GDNF, ARTN, and NT-3 in patients with major depressive disorder in a current depressive state and healthy controls. Gender difference did not produce a significant effect on GDNF, ARTN, and NT-3 mRNA expression of healthy control subjects (GDNF: $t = -0.970$, df = 26, $p = 0.341$, ARTN: $t = -1.187$, df = 26, $p = 0.246$, NT-3: $t = -0.386$, df = 26, $p = 0.703$) and those of major depressive disorder patients in a current depressive state (GDNF: $t = -0.919$, df = 18, $p = 0.370$, ARTN: $t = -0.984$, df = 18, $p = 0.338$, NT-3: $t = -0.659$, df = 18, $p = 0.519$).

To examine whether the altered expression levels of GDNF, ARTN, and NT-3 mRNAs observed in a current depressive state are state- or trait-dependent, the mRNA levels of these factors were also examined in a remissive state. The expression levels of GDNF, ARTN, and NT-3 mRNAs in major depressive disorder patients in a remissive state showed no significant differences compared with healthy control subjects or bipolar disorder patients (GDNF: $F = 2.296$, df = 2.94, $p = 0.106$; ARTN: $F = 2.619$, df = 2.94, $p = 0.078$; NT-3: $F = 0.980$, df = 2.94, $p = 0.379$) (Fig. 3A–C). In addition, the expression levels of GDNF, ARTN, and NT-3 mRNAs in bipolar disorder patients in a remissive state showed no significant differences compared with healthy control subjects or major depressive disorder patients (GDNF: $F = 2.296$, df = 2.94, $p = 0.997$; ARTN: $F = 2.619$, df = 2.94, $p = 0.063$; NT-3: $F = 0.980$, df = 2.94, $p = 0.522$) (Fig. 3A–C). We also examined the expression levels of GDNF, ARTN, and NT-3 mRNA in same patients with major depressive ($n = 15$) or bipolar disorder ($n = 9$) before and after remission. qRT-PCR revealed that the expressions of these three mRNA were significantly decreased in patients with major depressive disorder in a current depressive state compared with those in a current remissive state (GDNF: $t = -2.704$, df = 28, $p = 0.012$, ARTN: $t = -3.241$, df = 28, $p = 0.030$, NT-3: $t = -2.151$, df = 28, $p = 0.040$). There was no significant difference in the expression levels GDNF, ARTN, and NT-3 mRNA between patients with bipolar disorder in a current depressive and depressive state (GDNF: $t = -0.095$, df = 16, $p = 0.925$, ARTN: $t = -0.655$, df = 16, $p = 0.522$, NT-3: $t = 0.778$, df = 16, $p = 0.448$). Importantly, there was no significant difference in the treatment periods before remission between major depressive disorder ($2.93 \pm 0.36$ months) and bipolar disorder patients ($2.78 \pm 0.41$ months) ($t = 0.274$, df = 22, $p = 0.787$). In addition, there was no difference in the medication (imipramine equivalent) between patients with major depressive disorder in a current depressive and remissive state ($t = 0.120$, df = 28, $p = 0.906$). Furthermore, there was no correlation between expression levels of GDNF, ARTN, or NT-3 mRNA and medication (imipramine equivalent) in same patients with major depressive disorder (GDNF: $r = 0.186$, $p = 0.326$, ARTN: $r = -0.130$, $p = 0.493$, NT-3: $r = 0.048$, $p = 0.801$). These data suggest that the observed effects are likely to be due to the disease states.

There was no significant correlation between GDNF, ARTN, or NT-3 mRNA levels and the serum cortisol concentration for healthy control subjects, major depressive disorder patients or bipolar disorder patients (data not shown).

To examine the association between GDNF, ARTN or NT-3 mRNA levels and HPA axis activity; the mRNAs levels for these three genes of mood disorder patients in a current depressive state were compared between suppressors and non-suppressors of the Dex/CRH test. There was no significant difference in the expression level of the mRNAs for these three genes between suppressors and non-suppressors (data not shown).

## 4. Discussion

In the present study, we found the reduced expression of GDNF, ARTN, and NT-3 mRNAs in peripheral white blood cells of major depressive disorder patients in a current depressive state. In contrast, these reductions of GDNF, ARTN, and NT-3 mRNA expression were not observed in major depressive disorder patients in a remissive state. These results suggest that reduced expression of GDNF, ARTN, and NT-3 mRNAs are state-dependent.

Importantly, this represents the first study showing a reduced expression of ARTN mRNA in a depressive state, but not in a remissive state of major depressive disorder. ARTN is expressed in structures of the basal ganglia (subthalamic nucleus, putamen and the substantia nigra), thalamus and many peripheral adult human tissues (Baloh et al., 1998), and is a potent neurotrophic factor that may play an important role in the structural development and plasticity of ventral mesencephalic dopaminergic neurons (Zihlmann et al., 2005). It is known that the GDNF family ligands exert their effects through GDNF family receptor α (GFRα) and Ret (Airaksinen et al., 1999). Four distinct GFRαs (GFRα1-4) have been reported (Airaksinen et al., 1999). These molecules form a complex, containing the GDNF family ligand and GFRα homodimers, that

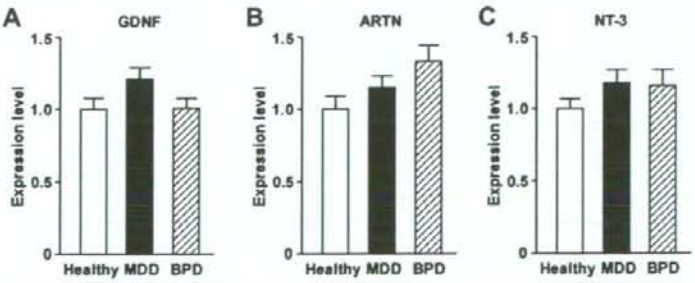


Fig. 3. The mRNA expression of GDNF, ARTN, and NT-3 in mood disorder patients in a remissive state. There was no significant difference in the levels of GDNF, ARTN, and NT-3 mRNAs among the three groups studied. MDD, major depressive disorder; BPD, bipolar disorder. Values are mean ± standard error.

brings two molecules of Ret together (Coulpier and Ibanez, 2004). Ret can then activate a variety of intracellular signaling pathways, including Ras/mitogen-activated protein kinase (MAPK); PI3K/AKT; RAC1/Jun NH2-terminal kinase (JNK); p38MAPK and phospholipase Cγ pathways (Ichihara et al., 2004; Kodama et al., 2005), resulting in cell survival, inflammation, differentiation and apoptosis. Concerning the relationship between ARTN and disease, the upregulation of ARTN mRNA expression is observed in smooth muscle cells of arteries, Schwann cells, and neural ganglia in chronic pancreatitis (Ceyhan et al., 2007), and in the auditory nerve of deafened rats (Wissel et al., 2006). To our knowledge, however, there is no report indicating the involvement of ARTN in the pathophysiology of neuropsychiatric disorders. It is important to note that both GDNF and ARTN bind to GFRα1 (Airaksinen et al., 1999). Our present data showed both GDNF and ARTN expression levels were reduced in patients with major depressive disorder, suggesting that dysfunctions of GFRα1-mediated pathways may be involved in the pathophysiology of major depression.

Our major finding is the reduced expression of two GDNF family gene (GDNF and ARTN) mRNAs in major depressive disorder. Recent postmortem brain studies have shown a reduction of glia in different brain structures of patients with mood disorders (Ongur et al., 1998; Rajkowska et al., 1999; Hamidi et al., 2004). Although there are no reports showing an altered expression of GDNF in the brains of patients with mood disorders, a report showed the decreased levels of whole blood GDNF in a remissive state of major depressive and bipolar disorder patients (Takebayashi et al., 2006). In contrast, increased serum GDNF immunocontent in bipolar disorders was observed (Rosa et al., 2006). The reason for this discrepancy is unclear, but one possible speculation is racial differences between the Japanese and Brazilian populations. Differences in the methodology used for sample preparation and determination of GDNF levels, such as ELISA (Takebayashi et al., 2006) and Western blotting (Rosa et al., 2006) may also account for this discrepancy.

A growing body of evidence has implicated a role for chronic or moderate oxidative stress in the pathogenesis of mood disorders. Previous studies showed that increased levels of malondialdehyde (a marker of lipid peroxidation) (Kuloglu et al., 2002; Khanzode et al., 2003; Ozcan et al., 2004), and decreased catalase levels (Ranjekar et al., 2003; Ozcan et al., 2004) were observed in the peripheral blood of mood disorder patients. Moreover, increased levels of oxidative DNA damage was reported in the peripheral blood of major depressive disorder patients (Forlenza and Miller, 2006). These results suggest an increased oxidative stress status in mood disorders. Importantly, GDNF blocked the DNA cleavage induced by bleomycin sulphate and L-buthionine-[*S*,*R*]-sulfoximine exposure in cultured mesencephalic neurons (Sawada et al., 2000), and reduced oxidative stress-induced cell death (Gong et al., 1999; Toth et al., 2002; Ugarte et al., 2003). Interestingly, increased levels of apoptosis have been observed in peripheral blood lymphocytes of depressive patients (Eilat et al., 1999; Ivanova et al., 2007). In addition, in a postnatal primary culture of mesencephalic dopaminergic neurons, GDNF supports their viability by suppressing apoptosis (Burke et al., 1998). Taken together, these data indicate that the reduced expression of GDNF mRNA may contribute to oxidative stress-induced cell death and apoptosis in peripheral blood cells of mood disorders, and this might be one of the mechanisms of immune inadequacy in mood disorders (Riccardi et al., 2002).

NT-3 mRNA and protein levels have been observed to be decreased in the hippocampus in a postmortem study (Dwivedi et al., 2005). In addition, increased levels of NT-3 in the cerebrospinal fluid of major depressive patients were reported (Hock et al., 2000). In peripheral blood, serum NT-3 levels were increased in bipolar disorder patients during a depressive state (Walz et al., 2007), and this data could not be replicated by our present study. Walz et al. (2007) speculated that the increased levels of NT-3 was due to a compensation to the decreased BDNF level. Our data showed decreased levels of NT-3 in a depressive state in major depressive disorder, but not for BDNF. The reason for this discrepancy is unclear.

A limitation of our study is that the majority of the patients were on medication, thus we can not exclude the influence of medication on the expression levels of the neurotrophic factors studied. A previous report indicated no alteration of the level of GDNF in the hippocampus of mice upon chronic treatment with antidepressants (Chen et al., 2001), whereas the increased expression of GDNF has been reported in cultured cells upon treatment with both antidepressants and mood stabilizers (Hisaoka et al., 2001; Castro et al., 2005). In addition, NT-3 levels have been reported to be decreased by chronic treatment with antidepressants which blocked norepinephrine uptake in the locus coeruleus of rats (Smith et al., 1995). In our study, however, expression levels of GDNF, ARTN, and NT-3 mRNA are only affected in the depressive state, but not in the remissive state. In addition, we found that there was significant difference in the expression levels of the GDNF, ARTN, and NT-3 mRNA in same patients with major depressive disorder before and after remission, although these two patient groups received similar medications. It is important to note that there was no correlation between expression levels of GDNF, ARTN, or NT-3 mRNA and medications in same patients with major depressive disorder before and after remission. These results suggest that there is no effect of medications on the expression of these three mRNAs, and the observed effects are likely to be due to the disease states.

Several lines of evidence have suggested that glucocorticoid hormone or GR affect the expression levels of GDNF and NT-3 mRNA (Barbany and Persson, 1992; Verity et al., 1998; Baecker et al., 1999; Hansson et al., 2000). It

is important to note that reduced expression of GRα has also been observed in the cerebral cortex, hippocampus, and amygdala in mood disorder patients (Webster et al., 2002; Knable et al., 2004; Perlman et al., 2004). Furthermore, we previously reported that the expression of GRα mRNA is also reduced in peripheral white blood cells of mood disorder patients (Matsubara et al., 2006). These observations raise the possibility that dysfunction of GR in peripheral blood plays a causal role in the aberrant GDNF and NT-3 expression in mood disorder patients. However, present our study indicated that there was no difference in the expression levels of GDNF and NT-3 mRNA between suppressors and non-suppressors of the Dex/CRH test or even in cortisol levels between mood disorders and healthy controls. Furthermore, it is important to mention that some antidepressants alter the expression of GR (Pariante and Miller, 2001). Thus, the involvement of GR in the aberrant transcriptional regulation of certain neurotrophic factors in patients with major depressive disorder remains unclear. Further studies are needed to clarify the aberrant transcriptional mechanisms of both GDNF and NT-3 expression in patients with major depressive disorder.

In conclusion, our results suggest that the changes in the expression levels of GDNF, ARTN, and NT-3 mRNAs in peripheral white blood cells might be state-dependent and associated with the pathophysiology of major depression.

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## 6. Conflict of interest

There are no conflicts of interest including any financial, personal, or other relationships with people for any of the coauthors related to the work described in the article.

## 7. Contributors

K. Otsuki, S. Uchida, and Y. Watanabe designated the research. K. Otsuki, T. Watanuki, Y. Wakabayashi, M. Fujimoto, H. Funato, and T. Matsubara performed the experiments. The manuscript was written by K. Otsuki, S. Uchida and Y. Watanabe. All authors discussed results and commented on the manuscript.

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