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Abnormality in glutamine–glutamate cycle in the cerebrospinal fluid of cognitively intact elderly individuals with major depressive disorder: a 3-year follow-up study

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INTRODUCTION
Late-life depression, one of the most common psychiatric disorders in older adults, is associated with significant functional impairment, variable treatment response, high recurrence rates, chronicity and high rates of medical comorbidity and mortality.1,2 Multiple lines of evidence suggest that late-life depression is a risk factor for the development of dementia, including Alzheimer’s disease (AD) and vascular dementia.2–4 However, the precise molecular mechanisms underlying the relationship between late-life depression and dementia risk remain unknown. Better understanding this relationship would likely contribute to improving preventive interventions in the elderly.

Glutamate (or L-glutamic acid) has an important role as the major excitatory neurotransmitter in the central nervous system. In the brain, glutamate is synthesized from glutamine by glutaminase, and metabolized to glutamine by glutamine synthetase (GS) in astrocytes.5–8 Accumulating evidence suggests that abnormalities in the glutamatergic neurotransmission via the N-methyl-D-aspartate receptor (NMDA-R) have a key role in the pathophysiology of depression. This study examined whether depression was associated with cerebrospinal fluid (CSF) levels of NMDA-R neurotransmission-associated amino acids in cognitively intact elderly individuals with MDD and age- and gender-matched healthy controls. CSF was obtained from 47 volunteers (MDD group, \(N = 28\); age- and gender-matched comparison group, \(N = 19\)) at baseline and 3-year follow-up (MDD group, \(N = 19\); comparison group, \(N = 17\)). CSF levels of glutamine, glutamate, glycine, L-serine and D-serine were measured by high-performance liquid chromatography. CSF levels of amino acids did not differ across MDD and comparison groups. However, the ratio of glutamine to glutamate was significantly higher at baseline in subjects with MDD than in controls. The ratio decreased in individuals with MDD over the 3-year follow-up, and this decrease correlated with a decrease in the severity of depression. No correlations between absolute amino-acid levels and clinical variables were observed, nor were correlations between amino acids and other biomarkers (for example, amyloid-\(\beta_{42}\), amyloid-\(\beta_{40}\), total and phosphorylated tau protein) detected. These results suggest that abnormalities in the glutamine–glutamate cycle in the communication between glia and neurons may have a role in the pathophysiology of depression in the elderly. Furthermore, the glutamine/glutamate ratio in CSF may be a state biomarker for depression.

ORIGINAL ARTICLE
Abnormality in glutamine–glutamate cycle in the cerebrospinal fluid of cognitively intact elderly individuals with major depressive disorder: a 3-year follow-up study

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Major depressive disorder (MDD), common in the elderly, is a risk factor for dementia. Abnormalities in glutamatergic neurotransmission via the N-methyl-D-aspartate receptor (NMDA-R) have a key role in the pathophysiology of depression. This study examined whether depression was associated with cerebrospinal fluid (CSF) levels of NMDA-R neurotransmission-associated amino acids in cognitively intact elderly individuals with MDD and age- and gender-matched healthy controls. CSF was obtained from 47 volunteers (MDD group, \(N = 28\); age- and gender-matched comparison group, \(N = 19\)) at baseline and 3-year follow-up (MDD group, \(N = 19\); comparison group, \(N = 17\)). CSF levels of glutamine, glutamate, glycine, L-serine and D-serine were measured by high-performance liquid chromatography. CSF levels of amino acids did not differ across MDD and comparison groups. However, the ratio of glutamine to glutamate was significantly higher at baseline in subjects with MDD than in controls. The ratio decreased in individuals with MDD over the 3-year follow-up, and this decrease correlated with a decrease in the severity of depression. No correlations between absolute amino-acid levels and clinical variables were observed, nor were correlations between amino acids and other biomarkers (for example, amyloid-\(\beta_{42}\), amyloid-\(\beta_{40}\), total and phosphorylated tau protein) detected. These results suggest that abnormalities in the glutamine–glutamate cycle in the communication between glia and neurons may have a role in the pathophysiology of depression in the elderly. Furthermore, the glutamine/glutamate ratio in CSF may be a state biomarker for depression.

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INTRODUCTION
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Glutamate (or L-glutamic acid) has an important role as the major excitatory neurotransmitter in the central nervous system. In the brain, glutamate is synthesized from glutamine by glutaminase, and metabolized to glutamine by glutamine synthetase (GS) in astrocytes.5–8 Accumulating evidence suggests that abnormalities in the glutamatergic neurotransmission via the N-methyl-D-aspartate receptor (NMDA-R) have a key role in the pathophysiology of major depressive disorder (MDD).5,6,9–12 Hashimoto et al.13 reported increased levels of glutamate in the prefrontal cortex in postmortem brain samples from MDD and bipolar disorder, suggesting a role of the glutamatergic system in mood disorders. A noninvasive in vivo proton magnetic resonance spectroscopy (\(^1\)H-MRS) study revealed increased levels of glutamate in the occipital cortex of patients with MDD,14 although other \(^1\)H-MRS studies found decreased levels of Glx (glutamate and glutamine) in the anterior cingulate cortex 3,5 and dorsomedial/dorsal anterolateral prefrontal cortex16 in patients with MDD. In contrast, there was no difference in glutamate levels in the occipital cortex between MDD patients and controls.17 A recent MRS study showed that young MDD (\(N = 90\)) and bipolar disorder (\(N = 75\)) groups (18–30 years) did not show different levels of hippocampal glutamate compared with controls (\(N = 40\)).18 A recent meta-analysis showed a significant reduction in Glx levels, but not glutamate levels alone, in the brains of depressed patients compared with controls across the studies included.19

Levine et al.20 reported higher cerebrospinal fluid (CSF) levels of glutamine in unmedicated patients with MDD. In contrast, a study showed low glutamate levels in the CSF of refractory patients with affective disorder.21 A recent study showed no significant differences between the control (\(N = 25\)) and the MDD groups (\(N = 18\)) in baseline CSF levels of glutamate (\(P = 0.761\)) and glutamine (\(P = 0.226\)).22 Thus, the results of studies on CSF levels of glutamine and glutamate in MDD patients are inconsistent. At present, there is no report that has evaluated the ratio of glutamine to glutamate in CSF of MDD patients although this ratio is an index of glutamine–glutamate cycle in glia–neuron

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communication.5,6,8,22,24 As the abnormalities in NMDA-R neuro-transmission by altered glutamate–glutamate cycle in the brain may be involved in the pathophysiology of MDD,5,6 it is of great interest to examine CSF levels of amino acids related with NMDA-R in elderly patients with MDD, and age- and gender-matched healthy controls.

In the present study, we measured CSF levels of amino acids (for example, glutamate, glutamine, glycine, δ-serine and L-serine) in elderly patients with MDD, and age- and gender-matched healthy controls. All these amino acids can affect glutamatergic neurotransmission via the NMDA-R.5,22 Furthermore, we also measured 3-year follow-up CSF samples in these subjects.

MATERIALS AND METHODS

Participants

This study was approved by the institutional review boards of the Nathan Kline Institute for Psychiatric Research and the New York University School of Medicine. Participants were volunteers who responded to advertisements in local newspapers and flyers, or were recruited from the Memory Education and Research Initiative Program.29 All participants provided informed consent before examination, and received up to $450.00 in compensation. A total of 133 participants completed the baseline evaluation, and 51 of these took part in the optional lumbar puncture procedure. Of these 51 participants, 3 were excluded because of evidence in their magnetic resonance imaging scans of confluent deep or periventricular white matter hyperintensities, defined as one or more hyperintense lesions measuring at least 10 mm in any direction. One individual was excluded because of a Mini-Mental State Examination (MMSE) score below 28. Of the 47 remaining participants, 28 were diagnosed with MDD by a board-certified psychiatrist, leaving 19 comparison subjects. The structural interview for DSM-IV disorders (SCID) was administered by a psychiatrist to establish an MDD diagnosis. Of the 28 individuals with MDD, 21 (75%) had recurrent episodes. Table 1 summarizes the demographic and clinical characteristics of the study participants at baseline.

Procedure

The study was conducted over four visits, usually 1 week apart. The first three visits were conducted at the Nathan Kline Institute for Psychiatric Research and the Clinical and Translational Science Institute, New York University Langone Medical Center. During the first visit, for the purpose of obtaining informed consent, study procedures were explained and participants were informed of their rights. Participants’ medical and psychiatric histories, including family history of AD, were also obtained, and their vital signs were measured. Participants then underwent a psychiatric evaluation, and their global cognitive status was assessed using the Mini-Mental State Examination (MMSE) score. The Hamilton Depression Rating Scale (HAM-D) was administered by a psychiatrist to establish an MDD diagnosis. Of the 28 participants with MDD, 21 (75%) had recurrent episodes. Table 1 summarizes the demographic and clinical characteristics of the study participants at baseline.

As reported previously,26 the two groups at baseline did not differ on any relevant clinical or demographic variable, with the exception of the mean HAM-D score, which was significantly higher in the MDD group (Table 1).4,5,11 First, Student t- and χ²-tests were used to compare the MDD and comparison groups with respect to dimensions not associated with depression (that is, MMSE score, years of education, body mass index, age, incidence of diabetes, gender, APOE status and reported family history of AD; Table 1). Second, Student t-test was used to compare the two diagnostic groups with respect to all remaining CSF variables. Third, 3-year follow-up data were analyzed using a 2x2 repeated-measures analysis of variance: the factors were time (baseline and follow-up) and MDD status (MDD and controls). All tests were two-tailed, and statistical significance was established at an α of 0.05, unless differently noted. All analyses were conducted using SPSS 22 (SPSS, Chicago, IL, USA).

RESULTS

As reported previously,26 the two groups at baseline did not differ on any relevant clinical or demographic variable, with the exception of the mean HAM-D score, which was significantly higher in the MDD group (Table 1). Of note, the proportion of participants with a reported family history of AD was slightly higher in the comparison group than in the MDD group. The CSF levels of amyloid-β42 in the MDD group at baseline were significantly lower than those of the comparison group, whereas differences in CSF levels of amyloid-β40 and total and phosphorylated tau protein at baseline did not reach statistical significance across conditions (Table 1).26
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between the MDD (N = 28) and the comparison groups (N = 19) at baseline (Table 2). However, the ratio of glutamine to glutamate in the MDD group was significantly higher than in the comparison group, whereas the ratio of glycine to L-serine and the ratio of D-serine to L-serine did not differ (Table 2). Furthermore, there were no correlations between CSF amino acids and typical AD biomarkers (amyloid-β42, amyloid-β40, and total and phosphorylated tau protein) at baseline in all subjects (data not shown).

At the 3-year follow-up, an interaction between time and MDD status was observed for the HAM-D scores. Post hoc comparisons confirmed that the severity of depression decreased over time in participants with MDD (P = 0.001), whereas no change was detected in controls (P = 0.366; Table 3 and Figure 1). When the same repeated-measures analysis of variance was applied to CSF levels of amino acids, no further interactions or main effects were observed when setting α at 0.006 (Sidak correction; eight tests), although a trend was observed for an interaction with the glutamine/glutamate ratio (Table 3 and Figure 1). Post hoc comparisons suggested that there was a decrease in this ratio in MDD subjects (P = 0.008), but no appreciable change in controls (P = 0.571). Finally, we observed a marginally significant negative correlation (r = −0.328, P = 0.051) between the change scores (baseline to follow-up) of the glutamine/glutamate ratio and of

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### Table 1. Demographic and clinical characteristics of cognitively intact individuals with MDD and age-matched comparison subjects at baseline

| Characteristic | Comparison group (N = 19) | MDD group (N = 28) | Statistical analysis |
|---------------|---------------------------|-------------------|----------------------|
| Age (years)   | 68.1 ± 7.3                | 66.5 ± 5.4        | t = 0.835, df = 45, P = 0.41 |
| Education (years) | 16.7 ± 2.7              | 16.5 ± 2.7        | t = 0.274, df = 44, P = 0.79 |
| Body mass index | 28.1 ± 4.7               | 28.8 ± 6.7        | t = 0.378, df = 45, P = 0.71 |
| 21-item HAM-D | 1.2 ± 1.9                 | 14.9 ± 8.8        | t = 8.02, df = 45, P < 0.001 |
| MMSE          | 29.5 ± 0.5                | 29.8 ± 0.6        | t = 1.56, df = 45, P = 0.13 |
| Total recall rating | 64.4 ± 12.3       | 64.9 ± 13.9       | t = 0.11, df = 45, P = 0.91 |
| Delayed recall rating | 8.5 ± 2.8        | 9.5 ± 2.5         | t = 1.258, df = 45, P = 0.22 |
| Trail-Making Test score |                     |                   |                      |
| Part A | 37.2 ± 12.4             | 36.0 ± 14.1       | t = 0.303, df = 45, P = 0.76 |
| Part B | 80.3 ± 31.5             | 86.1 ± 23.2       | t = 0.737, df = 45, P = 0.47 |
| Category fluency test | 41.7 ± 8.1        | 40.6 ± 8.2        | t = 0.45, df = 45, P = 0.66 |
| N (%) |                   |                   |                      |
| Diabetes      | 4 (21)                   | 5 (18)            | χ² = 0.08, df = 1, P = 0.79 |
| Female        | 12 (63)                  | 10 (36)           | χ² = 2.41, df = 1, P = 0.12 |
| Apolipoprotein ε level |                  |                   |                      |
| ε4           | 5 (26)                   | 11 (39)           | χ² = 0.369, df = 1, P = 0.54 |
| ε2           | 6 (32)                   | 7 (25)            | χ² = 0.03, df = 1, P = 0.87 |
| Family history of Alzheimer's disease | 6 (32)             | 3 (11)            | χ² = 3.3, df = 1, P = 0.07 |
| βAmyloid         | 335.4 ± 182.7           | 224.7 ± 125.1     | t = 2.471, df = 45, P = 0.02 |
| Amyloid-β40       | 65180.0 ± 2687.0        | 51460.0 ± 2369.0  | t = 1.845, df = 44, P = 0.07 |
| Total tau protein | 328.7 ± 151.7          | 273.0 ± 114.3     | t = 1.422, df = 44, P = 0.16 |
| Phosphorylated tau protein | 51.6 ± 20.9       | 48.9 ± 25.9       | t = 0.371, df = 45, P = 0.71 |

Abbreviations: df, degrees of freedom; 21-item HAM-D, 21-item Hamilton Depression Rating Scale; MDD, major depressive disorder; MMSE, Mini-Mental State Examination. The data are mean ± s.d. The data at baseline are from Pomara et al.20* Data for one control subject were not available. †Data for one MDD patient were not available.

### Table 2. CSF levels of amino acids and ratio of amino acids in subjects at baseline

| Characteristic | Comparison group at baseline (N = 19) | MDD group at baseline (N = 28) | Analysis |
|---------------|----------------------------------------|--------------------------------|-----------|
| Glutamine     | 568.17 ± 81.19                        | 591.04 ± 87.78                 | t = 0.903, df = 45, P = 0.371 |
| Glutamate     | 0.647 ± 0.82                          | 0.382 ± 0.11                   | t = 1.407, df = 18.5* P = 0.176 |
| Glycine       | 12.99 ± 5.95                          | 11.38 ± 2.67                   | t = 1.103, df = 23.0* P = 0.282 |
| L-Serine      | 25.45 ± 5.58                          | 22.36 ± 5.13                   | t = 1.955, df = 45, P = 0.057 |
| D-Serine      | 1.76 ± 0.38                           | 1.75 ± 0.43                    | t = 0.093, df = 45, P = 0.926 |
| Glutamine/glutamate | 1257.6 ± 458.4  | 1645.6 ± 434.7               | t = 2.938, df = 45, P = 0.005* |
| Glycine/L-serine | 0.496 ± 0.14                         | 0.526 ± 0.14                   | t = 0.7, df = 45, P = 0.488 |
| D-Serine/L-serine | 0.072 ± 0.021                       | 0.080 ± 0.019                  | t = 1.326, df = 45, P = 0.192 |

Abbreviations: CSF, cerebrospinal fluid; df, degrees of freedom; MDD, major depressive disorder. The data are mean ± s.d. *Equality of variance assumption not met. †Denotes significant finding at α = 0.006 after Sidak’s adjustment (eight tests).
**DISCUSSION**

In the present study, we found that older individuals with MDD showed an increased CSF glutamine/glutamate ratio compared with controls, although CSF levels of the individual amino acids were not different. To our knowledge, this is the first report in the literature of an increase in the glutamine/glutamate ratio in elderly MDD subjects, suggesting abnormalities in the glutamine–glutamate cycle in the brains of elderly depressed individuals. As the glutamine/glutamate ratio is an index for glutamine–glutamate cycle in the glia–neuron communication, it is likely that abnormalities in the glutamine–glutamate cycle in the brain have a role in the pathophysiology of late-life depression.

We previously reported a reduction of amyloid-β42 in the same MDD patients at baseline. Importantly, the glutamine/glutamate ratio in elderly MDD patients was no longer significantly different from controls after a 3-year period; the loss of significance coincided with reduction in the severity of depressive symptoms, suggesting that abnormalities in this ratio in depression may be state- and not trait-dependent. However, we found no significant correlations between the ratio or levels of the individual amino acids and AD biomarkers, or severity of depressive symptoms either at the baseline or follow-up. These results are generally consistent with previous findings of CSF studies that also reported a lack of significant correlations between these amino acids and severity of depressive symptoms. Garakani et al. reported no significant differences between controls and MDD patients in baseline CSF levels of glutamate (P = 0.761) or glutamine (P = 0.226). Furthermore, there was a significant positive correlation (r = 0.677, P = 0.016) between intensity of suicidal ideation and CSF glutamate, and a significant negative correlation (r = −0.558, P = 0.038) between baseline HAM-D score and the glutamate/glutamine ratio in adult patients (mean age of 40.4 years) with MDD. It is thus very difficult to reconcile the lack of significant correlations between the CSF ratio and severity of depressive symptoms with evidence that the increased CSF glutamine/glutamate ratio and other glutamatergic abnormalities associated with MDD might be state-dependent. Taken together, these findings highlight the limitations of static indices such as levels of glutamate or glutamine either in CSF or brain to reflect dynamic and brain region-specific alterations in the glutamatergic function. Using in vivo 13C-MRS and [1-13C]glucose in a single voxel spectroscopy study, Abdallah et al. found that measures of the neuronal tricarboxylic acid cycle in the occipital cortex from medication-free MDD patients was 26% lower compared with controls, suggesting mitochondrial dysfunction in depression. However, they reported no significant alterations in the

**Table 3.** HAM-D score and CSF levels of amino acids and ratio in subjects at baseline and 3-year follow-up

| Characteristic | Baseline (N = 17) | Baseline (N = 19) | 3-year follow-up (N = 17) | 3-year follow-up (N = 19) | Analyses (P-values) |
|---------------|-------------------|-------------------|--------------------------|--------------------------|---------------------|
| Comparison group | MDD group | MDD group | Comparison group | MDD group | ME time | ME MDD | Interaction |
| 21-item HAM-D | 1.118 ± 1.900 | 17.105 ± 10.493 | 2.235 ± 6.006 | 8.737 ± 8.096 | 0.005 | < 0.001 | < 0.001 |
| Glutamine | 567.0 ± 83.2 | 587.1 ± 85.1 | 604.8 ± 80.7 | 594.5 ± 90.4 | 0.175 | 0.835 | 0.359 |
| Glutamate | 0.680 ± 0.86 | 0.399 ± 0.116 | 0.653 ± 0.788 | 0.652 ± 0.658 | 0.482 | 0.355 | 0.395 |
| Glycine | 13.30 ± 6.18 | 11.96 ± 2.79 | 13.2 ± 4.51 | 13.57 ± 5.20 | 0.456 | 0.668 | 0.388 |
| L-Serine | 25.8 ± 5.68 | 23.23 ± 5.69 | 25.0 ± 5.66 | 25.4 ± 7.79 | 0.564 | 0.537 | 0.207 |
| o-Serine | 1.76 ± 0.40 | 1.75 ± 0.45 | 1.80 ± 0.41 | 1.91 ± 0.49 | 0.09 | 0.717 | 0.315 |
| Glutamine/ glutamate | 1221.98 ± 472.7 | 1571.21 ± 440.2 | 1311.06 ± 463.9 | 1230.63 ± 455.9 | 0.192 | 0.271 | 0.029 |
| Glycine/L-serine | 0.501 ± 0.147 | 0.536 ± 0.153 | 0.546 ± 0.208 | 0.535 ± 0.128 | 0.467 | 0.787 | 0.454 |
| o-Serine/L-serine | 0.071 ± 0.021 | 0.078 ± 0.021 | 0.074 ± 0.018 | 0.079 ± 0.021 | 0.277 | 0.41 | 0.673 |

Abbreviations: CSF, cerebrospinal fluid; 21 HAM-D, 21-item Hamilton Depression Rating Scale; MDD, major depressive disorder; ME MDD, main effect of MDD status (depressed and controls); ME time, main effect of time (baseline and follow-up). The data are mean ± s.d. The analysis method used was 2 × 2 repeated-measures analysis of variance.

**Figure 1.** Three-year follow-up of elderly MDD patients and control comparison. (a) The ratio of glutamine to glutamate in the cerebrospinal fluid (CSF) from major depressive disorder (MDD) patient group (N = 19) was significantly decreased at 3-year follow-up. In contrast, the ratio of glutamine to glutamate in the CSF from control comparison group (N = 17) was slightly increased at 3-year follow-up. (b) The Hamilton Depression Rating Scale (HAM-D) score in the MDD patient group (N = 19) was significantly decreased at 3-year follow-up. In contrast, HAM-D score in the control comparison group (N = 17) was slightly increased at 3-year follow-up.
glutamine–glutamate cycle and γ-aminobutyric acid (GABA) synthesis, inconsistent with our CSF findings. Furthermore, this study did not find any correlations with severity of depression. One possibility is that the investigators focused only on the occipital cortex, and they did not examine CSF or other brain regions in which glutamatergic abnormalities have been found. Glutamate is synthesized from glutamine by glutaminase, and metabolized to glutamine by the mainly astrocyte-located GS. In addition, glutamate is metabolized to GABA by glutamic acid decarboxylase (GAD).8 Released glutamate is taken up by surrounding astrocytes via the glutamate transporter, where it is converted to glutamine, transported back to presynaptic neurons and recovered to glutamate. Thus, the glutamine–glutamate–GABA cycle as part of glia–neuron communication has an important role in excitatory and inhibitory neurotransmission. The expression of GS mRNA was reduced in the prefrontal cortex, premotor cortex and amygdala from depressed suicide subjects, but not in suicide completers without depression.32,33 Furthermore, GS protein was decreased in the anterior cingulate cortex and orbitofrontal cortex from depressed patients.32,34,35 A recent study using postmortem brain samples showed that the density of GS-expressing astrocytes was significantly reduced in some cortical areas from MDD patients, suggesting a disturbance in the glutamine–glutamate cycle in MDD.36 Moreover, the immunoreactivity of EAAT2, one of the glutamate transporters in astrocytes, in the orbitofrontal cortex was significantly lower in MDD patients.35 In addition, increased immunoreactivity for GAD65/67 was demonstrated in neurons from several cortical regions, including orbitofrontal and dorsolateral prefrontal cortex, in depressed patients.37 By contrast, the amount of GAD67 in the dorsolateral prefrontal cortex of MDD patients was significantly reduced as compared with matched controls.38 These findings suggest a dysregulation of the glutamate–GABA cycle in depression. Taken together, it is likely that abnormalities in the glutamine–glutamate–GABA cycle in the brain have a role in the pathophysiology of depression.

In this study, we found that the glutamine/glutamate ratio in MDD patients was significantly decreased after 3-year follow-up in concert with improvement in depressive symptoms. Given the role of glutamatergic neurotransmission in the mechanisms of antidepressants, it is noteworthy that symptom improvement caused by antidepressants may be mediated by effects on glutamine–glutamate cycle in the brains from depressed patients. In contrast, HAM-D score and the glutamine/glutamate ratio in healthy subjects were slightly increased after 3-year follow-up, although these increases were not statistically significant. CSF levels of glutamine in individuals with AD were reported to be lower than in controls, whereas CSF levels of glutamate were higher.40,41 Although the glutamine/glutamate ratio was not measured in either study, and depression was not a focus of either investigation, these results do not support a link between the abnormalities in the glutamine–glutamate cycle in depression with those in AD. Increased CSF levels of GS in individuals with AD compared with controls also has been reported.42,43 In contrast, a recent study using a quantitative ELISA system showed that CSF levels of total GS in AD patients were not different from those observed in healthy controls,44 suggesting that total CSF levels of GS may not be a suitable biomarker for AD, or that further work needs to be done with this biomarker to clarify whether GS abnormalities exist in AD that are associated with the apparent abnormalities in mood disorders.

The NMDA-R antagonist ketamine is known to produce a rapid and sustained antidepressant effect in treatment-resistant patients with MDD. A recent MRS study demonstrated that a single infusion of ketamine (0.5 mg kg$^{-1}$) caused an increase of Glx and GABA in the medial prefrontal cortex of MDD patients in ~26 min, indicating that ketamine has a rapid effect on the glutamine–glutamate–GABA cycle in the prefrontal cortex in depressed patients. In contrast, earlier studies showed that a single infusion of ketamine (0.5 mg kg$^{-1}$) did not produce changes in glutamate and glutamine concentrations in occipital cortex in MDD patients or in the anterior cingulate/medial prefrontal cortex in healthy subjects, suggesting that changes in occipital amino-acid levels are not correlated with ketamine’s antidepressant action. Thus, it would be of great interest to measure CSF amino acids in MDD patients at baseline and after a single ketamine infusion.

Finally, there are some limitations to this study that should be noted. The main limitation was small sample size, and similar, future studies in geriatric depression would likely benefit from larger sample sizes. Another limitation was the inability to measure CSF GABA in the subjects of this study. We could not measure CSF levels of GABA because these were below the lower limit of detection of our HPLC system. Given the key role of glutamine–glutamate–GABA cycle in glia–neuron communication, it is of great interest to measure CSF GABA levels using a high-sensitive analytical system (for example, LC/MS/MS).

In conclusion, we found that the CSF ratio of glutamine/glutamate levels in elderly patients with MDD was significantly higher than that of age-matched healthy controls, and that the increased ratio in patients was significantly decreased after 3-year follow-up by medication in conjunction with decreased depression symptoms over this time period. These findings suggest that abnormalities in the glutamine–glutamate cycle in the glia–neuron communication have a role in the pathophysiology of elderly depression. Further studies measuring CSF levels of amino acids (for example, glutamine, glutamate and GABA) using larger cohorts, particularly cohorts of antidepressant-naïve patients, will be of great interest.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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