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ORIGINAL ARTICLE

Plasma Amino Acid Profiling in Major Depressive Disorder Treated With Selective Serotonin Reuptake Inhibitors

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Keywords
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SUMMARY

Aims: Amino acids are important body metabolites and seem to be helpful for understanding pathogenesis and predicting therapeutic response in major depressive disorder (MDD). We performed amino acid profiling to discover potential biomarkers in major depressive patients treated with selective serotonin reuptake inhibitors (SSRIs). **Methods:** Amino acid profiling using aTRAQ™ kits for Amino Acid Analysis in Physiological Fluids on a liquid chromatography–tandem mass spectrometry (LC-MS/MS) system was performed on 158 specimens at baseline and at 6 weeks after the initiation of SSRI treatment for 68 patients with MDD and from 22 healthy controls. **Results:** Baseline alpha-aminobutyric acid (ABA) discriminated the patients according to the therapeutic response. Plasma glutamic acid concentration and glutamine/glutamic acid ratio were different between before and after SSRI treatment only in the response group. Comparing patients with MDD with healthy controls, alterations of ten amino acids, including alanine, beta-alanine, beta-aminoisobutyric acid, cystathionine, ethanolamine, glutamic acid, homocystine, methionine, O-phospho-L-serine, and sarcosine, were observed in MDD. **Conclusion:** Metabolism of amino acids, including ABA and glutamic acid, has the potential to contribute to understandings of pathogenesis and predictions of therapeutic response in MDD.

Introduction

Major depressive disorder (MDD) is a common mental disorder that affects approximately 5–20% of the world population [1,2]. MDD has multifactored etiologies including environmental and genetic factors; however, a clear pathogenesis remains unrevealed [3,4]. The most frequently used antidepressants are selective serotonin reuptake inhibitors (SSRIs) due to their safety and efficacy [5,6]. They have been used for many years as first-line therapeutic agents as the first SSRI, fluoxetine, was introduced in the 1980s [7]. However, about half of patients with MDD show failure to respond to SSRIs and approximately 10–20% or higher of patients

treated with first- and second-generation antidepressants do not achieve complete recovery [8–13].

To understand the pathogenesis and to predict the prognosis of MDD, numerous studies have been conducted in diverse research fields, including genomics, transcriptomics, proteomics, and metabolomics [14–16]. In particular, the application of metabolomics has increased for the detection of changes according to the development of disease and treatment [17–20]. Metabolomics is on the terminal part of a series of processes from DNA to physical change; thus, it directly reflects alterations of body responses. Amino acids are a group of metabolites that may be an important part of human metabolome. Amino acids play a role as brain neurotransmitters and intermediates in the biosynthesis and metabolism of body compounds. Amino acids have been studied as candidates for biomarkers of diverse diseases, including metabolic syndrome, cancer, and psychiatric disorders including Alzheimer's disease, schizophrenia, and MDD [21–26].

While methodologies and results are not strictly consistent, findings of the disturbance of amino acids in MDD compared with healthy individuals were replicated in previous studies [21,26,27]. However, there is scant data on the changes and differences of amino acids according to the treatment including pharmacotherapy with SSRIs and therapeutic response in MDD [28,29]. Discovering alterations of amino acids in MDD would not only improve understandings of the disease but also help the selection of therapeutic agents and lead to the improvement of treatment efficiency. Recently, with the development of technology and medical knowledge, more-precise evaluations have been possible of the disturbance of amino acids in MDD through amino acid profiling using liquid chromatography–tandem mass spectrometry (LC-MS/MS) and isotope-labeled internal standards [30,31]. We performed profiling of 40 amino acids to identify differences according to the presence of disease in 68 patients with MDD and in 22 healthy individuals and according to the response of SSRI treatment in patients. We also assessed the significance for the changes of amino acids after SSRI treatment in total and subset (response and nonresponse) of patients and the association between the response of SSRI treatment and the changes of amino acids.

Experimental Procedures

Patients

Sixty-eight patients with MDD who were treated with SSRIs at Samsung Medical Center were included. Patients fulfilled the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, criteria for major depressive disorder, single episode or recurrent. Diagnoses were confirmed by a board-certified psychiatrist on the basis of the Samsung Psychiatric Evaluation Schedule, case review notes, and SCID (structured clinical interview for DSM-IV) to diagnose depression. A minimum baseline 17-item Hamilton Rating Scale for Depression (HAM-D) score of 15 was required [32]. Study participants were excluded for pregnancy, significant medical conditions, abnormal laboratory baseline values, unstable psychiatric features (e.g., suicidal attempt in current episode), history of alcohol or drug dependence, seizures, head trauma with loss of consciousness, or neurological illness. We also excluded patients with concomitant Axis I psychiatric disorder; however, patients with concomitant Axis II psychiatric disorder were not excluded in this study. All patients were treated with SSRIs, 10–30 mg/day for escitalopram, 20–40 mg/day for fluoxetine, 10–40 mg/day for paroxetine, and 100–125 mg/day for sertraline. Therapeutic response was defined as a 50% or more reduction of HAM-D score by 6 weeks after the initiation of antidepressant treatment. Twenty-two healthy individuals without family history of MDD were included as controls. This study was approved by the Samsung Medical Center institutional review board. Informed consent was obtained by all patients.

Amino Acid Analysis

We performed amino acid profiling using morning, overnight fasting plasma samples from patients at baseline and at 6 weeks after the initiation of antidepressant therapy as well as from healthy individuals. Protein precipitation using 200 μL of plasma was performed, and 40 amino acids in specimens using the aTRAQ™ kits for Amino Acid Analysis in Physiological Fluids (AB Sciex, Foster City, CA, USA) were labeled with isobaric tags that have distinguishable report ions to internal standards according to the manufacturer's instructions. The Agilent 1260 Infinity LC system (Agilent Technologies Inc., Santa Clara, CA, USA) with a reverse-phase C18 (5 μm, 4.6 mm×150 mm) column at 50°C was used to separate amino acids by a gradient mobile phase of 0.1% formic acid in water and 0.01% heptafluorobutyric acids in methanol at a flow rate of 0.45 mL/min. The amino acids were monitored using Agilent 6460 Triple Quadrupole MS/MS (Agilent Technologies Inc.) with positive electrospray ionization (ESI) in multiple-reaction monitoring (MRM) mode. Each amino acid was analyzed using a single transition. The analytical cycle time was 28 min. The amounts of amino acids were calculated by comparing the peak intensities of amino acids between specimens and internal standards at one to one.

Statistical Analysis

The comparisons of clinical variables between response and nonresponse groups were performed by the Wilcoxon rank-sum test or *t*-test for continuous variables and the Fisher's exact test for categorical variables. To discover amino acids at baseline that can be associated with therapeutic response, we analyzed using logistic regression model in univariable and multivariable analyses. For each amino acid, age, sex, antidepressant, and HAM-D score at baseline were included as covariates in the multivariable analysis. We also performed receiver operating characteristic (ROC) curve analysis for each amino acid at baseline to discriminate between response and nonresponse groups and obtained values of area under the curve (AUC). The association between the changes of amino acid concentrations and the therapeutic response after adjustment of covariates (age, sex, antidepressant, and HAM-D score at baseline) was investigated using partial Spearman

correlation analysis. The comparisons of amino acid concentrations between before and after SSRI treatment in total and subset of patients were performed by the Wilcoxon rank-sum test or t-test. Bonferroni's correction was applied to the results from subset analysis of response and nonresponse group due to multiple testing. Results were considered statistically signifi-

Table 1 The characteristics and plasma concentrations of amino acids between healthy controls (n = 22) and major depressive patients (n = 68) at baseline

Characteristics; Amino acid (μmol/L)	Controls		Patients		
	Median	Q_1–Q_3	Median	Q_1–Q_3	*P*-value[a]
Age	68.0	65.3–73.3	65.0	60.8–70.0	0.118[b]
Sex, M:F	4:18		16:52		0.771[c]
Alanine	406	345–412	446	351–540	0.043
Beta-alanine	23.5	9.00–62.2	11.0	7.80–17.5	0.024
Anserine	0.03	0.00–0.16	0.00	0.00–4.73	0.364
Aminoadipic acid	0.78	0.67–0.85	0.85	0.58–1.12	0.615
Alpha-aminobutyric acid	12.5	11.1–17.6	16.2	12.7–21.1	0.144
Gamma-aminobutyric acid	0.19	0.12–0.74	1.16	0.00–2.01	0.050
Beta-aminoisobutyric acid	3.89	1.02–6.58	1.12	0.27–1.92	<0.001
Arginine	58.7	39.1–75.2	69.7	49.0–94.1	0.099
Asparagine	53.5	47.1–64.6	50.9	41.3–70.3	0.662
Aspartic acid	4.30	3.14–5.49	3.51	2.60–5.73	0.411
Carnosine	0.02	0.00–0.08	0.00	0.00–0.00	0.055
Citrulline	30.3	25.7–36.6	34.0	25.2–45.6	0.194
Cystathionine	0.08	0.00–0.16	0.00	0.00–0.00	<0.001
Cystine	97.5	54.4–126	59.0	36.3–90.6	0.083
Ethanolamine	5.49	4.92–6.23	35.4	23.6–36.8	<0.001
Glutamic acid	51.8	35.1–61.6	59.6	39.1–76.9	0.047
Glutamine	600	554–686	621	495–794	0.796
Glycine	232	182–289	251	186–298	0.455
Histidine	82.4	74.7–92.3	73.8	63.1–96.5	0.304
Homocystine	0.02	0.00–0.06	0.00	0.00–0.00	<0.001
5-hydroxylysine	0.53	0.00–1.89	0.00	0.00–0.75	0.057
Hydroxyproline	6.12	4.14–8.79	7.94	5.08–14.3	0.088
Isoleucine	55.6	46.1–63.7	64.4	48.8–81.5	0.061
Leucine	114	88.6–121	117	89.4–148	0.478
Lysine	189	158–224	182	152–232	0.884
Methionine	21.0	19.3–25.6	27.3	21.6–40.6	0.004
1-methylhistidine	0.54	0.09–0.99	0.68	0.00–1.49	0.882
3-methylhistidine	4.86	3.78–5.52	4.23	2.48–5.49	0.109
Ornithine	109	88.2–132	98.1	70.5–129	0.242
Phenylalanine	53.0	49.0–57.1	59.3	47.7–74.9	0.072
O-phosphoethanolamine	1.49	0.93–1.94	1.64	1.10–2.27	0.242
O-phospho-L-serine	5.45	4.42–6.29	0.00	0.00–0.61	<0.001
Proline	142	127–185	162	123–231	0.258
Sarcosine	5.75	4.44–10.8	2.67	2.20–4.40	<0.001
Serine	119	103–143	114	92.8–150	0.870
Taurine	52.5	41.1–60.9	55.6	42.2–79.1	0.300
Threonine	118	102–150	111	88.3–150	0.450
Tryptophan	43.2	39.4–46.1	43.6	35.7–52.7	0.662
Tyrosine	56.6	50.1–61.6	61.4	49.6–74.6	0.258
Valine	215	196–251	237	206–311	0.144
Tyrosine/LNAA	0.13	0.12–0.15	0.12	0.11–0.14	0.270
Tryptophan/LNAA	0.10	0.09–0.11	0.09	0.08–0.10	0.176
Glutamine/glutamic acid	11.8	8.87–16.7	10.9	8.10–15.0	0.228
Serine/glycine	0.52	0.48–0.56	0.50	0.41–0.57	0.313

Q1, lower quartile; Q3, upper quartile; LNAA, large neutral amino acid. [a]*P*-values from Wilcoxon rank-sum test. [b]*P*-values from t-test. [c]*P*-values from Fisher's exact test.

cant with *P*-value less than 0.05. SAS version 9.3 (SAS Institute, Cary, NC, USA) was used for all statistical analyses.

Results

Comparison between patients with MDD and controls

We analyzed 40 amino acids in 158 specimens at baseline and at 6 weeks after the initiation of SSRI treatment, from 68 patients with MDD and from 22 healthy controls. Twenty male (22.2%) and 70 females (77.8%) with a median age of 64.5 years (range, 50–86 years) were included in this study. Forty-eight (70.6%) patients responded to the SSRI treatment, and median HAM-D scores were decreased from 18.5 at baseline to 7 at 6 weeks after treatment in the response group. Baseline plasma concentrations of alanine, beta-alanine, beta-aminoisobutyric acid, cystathionine, ethanolamine, glutamic acid, homocystine, methionine, O-phospho-L-serine, and sarcosine in patients with MDD showed differences from healthy controls (Table 1).

Comparison Between Response and Nonresponse Groups at Baseline

The demographic and clinical characteristics according to the therapeutic response are summarized in Table 2. Baseline alpha-aminobutyric acid (ABA) was higher in response group than in nonresponse group after adjustment of covariates ($P = 0.017$; odds ratio (OR), 1.12, 95% confidence interval (CI), 1.02–1.23; Figure 1). In ROC curve analysis of amino acids at baseline on the response, ABA (0.697) and aspartic acid (0.663) showed relatively high AUC of above 0.650 (Figure 1). In a combined ROC curve analysis of ABA and aspartic acid, the AUC value was 0.714.

Table 2 Characteristics of response (n = 48) and nonresponse (n = 20) depressive patients to antidepressant treatment

Characteristics	Patients (%)		*P*-value
	Response	Nonresponse	
Age, median (Q_1–Q_3), years	65.0 (62.0–69.3)	64.0 (58.5–71.0)	0.439[b]
Sex, M:F	12:36	4:16	0.762[b]
BMI, median (Q_1–Q_3)	23.8 (22.3–26.0)	24.4 (22.7–27.3)	0.369[c]
Onset age, median (Q_1–Q_3), years	59.5 (50.0–65.3)	57.5 (50.0–61.5)	0.839[c]
Episodes of MDD, median (Q_1–Q_3)	2 (1–3)	2 (1–2)	0.390[c]
Antidepressant used			
Fluoxetine	25 (52.1)	12 (60.0)	0.602[b]
Others[a]	23 (47.9)	8 (40.0)	
HAM–D score at baseline, median (Q_1–Q_3)	18.5 (17.0–21.0)	21.0 (19.0–22.3)	0.033[c]
Family history	11 (22.9)	2 (10.0)	0.317[c]
Comorbid conditions			
Hypertension	17 (35.4)	7 (35.0)	1.000[b]
Diabetes	9 (18.8)	4 (20.0)	1.000[b]
Hyperlipidemia	2 (4.2)	0 (0.0)	1.000[b]

Q1, lower quartile; Q3, upper quartile; BMI, body mass index; MDD, major depressive disorder; HAM-D, Hamilton rating scale for depression. [a]SSRIs including paroxetine, escitalopram, and sertraline. [b]*P*-values from Fisher's exact test. [c]*P*-values from Wilcoxon rank-sum test.

Changes of Amino Acid Concentrations and the Therapeutic Response

The ABA concentration was also decreased after antidepressant treatment in the response group, but not in the nonresponse group (median differences between before and after treatment: −3.8 vs. 2.2; $P = 0.010$). The association between the change of ABA concentration and the therapeutic response was significant when the effect of covariates (age, sex, antidepressant, and HAM-D score) is controlled ($P = 0.010$), and the change was larger in the negative direction for the response group than the nonresponse group ($\rho = -0.321$). The changes of isoleucine ($P = 0.024$, $\rho = -0.282$), 1-methylhistidine ($P = 0.033$, $\rho = -0.267$) and proline ($P = 0.014$, $\rho = -0.306$) concentrations, and tryptophan/large neutral amino acid (LNAA) ratio ($P = 0.025$, $\rho = 0.281$) showed the significant correlation with the therapeutic response according to antidepressant treatment after adjusting covariates.

Comparison between before and after SSRI treatment

Plasma concentrations of arginine ($P = 0.039$), asparagine ($P = 0.017$), cystine ($P = 0.039$), and glutamine/glutamic acid ($P = 0.039$) were increased at 6 weeks after the treatment, compared to the baseline in all patients (Table 3 and Figure 2). On the other hand, the median value of glutamic acid (59.81 vs. 48.02, $P = 0.045$ in response group; 57.30 vs. 55.52, $P = 1.000$ in nonresponse group) and the glutamine/glutamic acid ratio (10.5 vs. 12.8, $P = 0.014$ in response group; 13.1 vs. 13.2, $P = 1.000$ in nonresponse group) were significantly different between before and after treatment only in the response group.

Discussion

In this study, we performed amino acid profiling to identify the alteration of plasma amino acid concentrations in patients with MDD. ABA was differentially expressed at baseline between response and nonresponse groups. In comparisons between patients and healthy individuals, ten amino acids showed differences. Plasma glutamic acid concentration and glutamine/glutamic acid ratio were different between before and after SSRI treatment only in the response group.

Alpha-aminobutyric acid is a catabolic product derived from 2-ketobutyrate (2KB) which is a metabolite from the metabolism of methionine, threonine, serine, and glycine [1,33,34]. The increase of ABA has been considered as a general marker of various conditions including malnutrition, protein, catabolic status, sepsis, liver disease, and multiple organ failure [33,35]. In our study, the high ABA in patients with MDD compared to healthy individuals and the decreased concentration after the antidepressant treatment in the response group compared to nonresponse group may reflect

the chronic catabolic status in MDD caused by the poor appetite which is a common symptom of MDD. Another possibility is that the alteration of ABA may be caused by the impairment and restoration of the entrance of 2KB to tricarboxylic acid cycle, which would imply a disturbance and a normalization of energy metabolism, and the hypometabolic state. This hypothesis was previously suggested for pathogenesis in MDD [4,33,36–38]. An interesting finding is the differences in ABA concentrations at baseline between response and nonresponse groups. Although the discrimination power of ABA was modest, ROC curve analyses show the potential of ABA to discriminate responsive patients from nonresponsive patients. The significance of the effect of ABA on the therapeutic response was maintained after adjustment with clinical variables, including HAM-D score at baseline; thus, this finding is not caused by differences in severity of MDD. To clarify that ABA could be an indicator to predict therapeutic response in patients with MDD treated with SSRI, further study including patients treated with other types of antidepressants would be needed.

The phospholipid is an important constituent of mitochondrial membrane; it is a product from phosphoethanolamine that is produced by the phosphorylation of ethanolamine by ethanolamine kinase [38–40]. The increase of ethanolamine and phosphoethanolamine has been reported in psychiatric disorders including MDD, and the increase of these metabolites can be caused by enzyme defects such as ethanolamine-phosphate phosphorylase [38,40–42]. The mitochondrial dysfunction due to the disturbance of phospholipid metabolism might result in an alteration in energy production [38], which is correlated with the finding of elevated ABA in the present study. Brain is a highly energy-dependent tissue, in the same context, several studies have been reported that patients with mitochondrial disorders present with neuropsychiatric manifestations [43,44]. These findings and our study results support the previous hypothesis that the disturbance of energy metabolism caused by various etiologies, including mitochondrial dysfunction, may be involved in the development of MDD [38,45–47]. Alteration of excitatory neurotransmitter amino acids, including glutamic acid and aspartic acid, was also observed in the present study. Previous studies showed the increase of these excitatory amino acids in MDD and a positive correlation between glutamic acid concentration and HAM-D score [21,29,48,49]. Glutamatergic abnormalities in MDD have been reported to have a role in pathogenesis of MDD, and the efficacy of glutamatergic antidepressants including N-methyl-d-aspartate (NMDA) receptor antagonists has been demonstrated in MDD [50–52]. The alteration of glutamic acid in our study could reflect the glutamatergic abnormalities in MDD. Consistent with previous studies, we observed elevated concentration of glutamic acid in MDD and decreases after treatment in the present study. The glutamic acid and glutamine/glutamic acid ratio were recovered after antidepressant treatment only in the response group. Although these amino acids are not primary

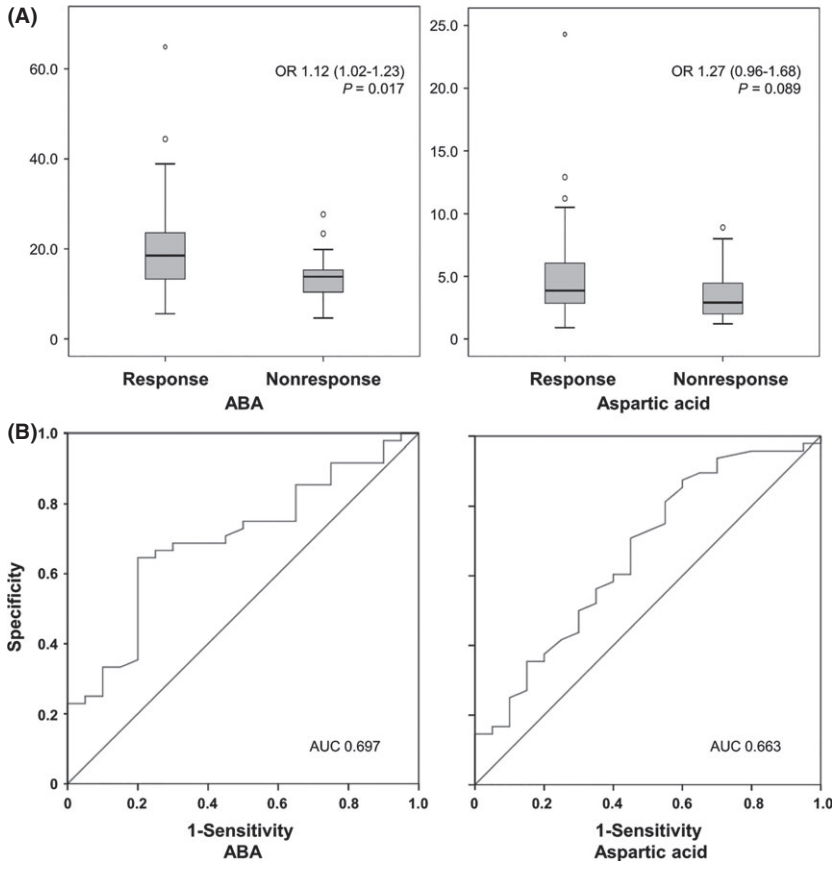

Figure 1 Differences of baseline amino acids between response and nonresponse groups in logistic regression analysis. (**A**) Alpha-aminobutyric acid (ABA) and aspartic acid are higher in the response group than in the nonresponse group. *P*-values are presented from multivariable analysis including clinical variables. (**B**) The receiver operating characteristic (ROC) curve analysis demonstrates the discrimination of the ABA and aspartic acid between response and nonresponse groups at baseline.

Table 3 Plasma concentrations of amino acids at baseline and at 6 weeks after the antidepressant treatment in depressive patients (n = 68)

	At baseline		At 6 weeks		
Amino acid (μmol/L)	Median	Q_1–Q_3	Median	Q_1–Q_3	*P*-value[a]
Alanine	446	351–540	434	357–599	0.851
Beta-alanine	11.0	7.80–17.5	11.9	8.32–17.7	0.383
Anserine	0.00	0.00–4.73	0.00	0.00–4.77	0.760
Aminoadipic acid	0.85	0.58–1.12	0.74	0.57–1.16	0.829
Alpha-aminobutyric acid	16.2	12.7–21.1	16.0	12.6–23.0	0.553
Gamma-aminobutyric acid	1.16	0.00–2.01	1.15	0.00–1.89	0.194
Beta-aminoisobutyric acid	1.12	0.27–1.92	1.07	0.00–1.96	0.795
Arginine	69.7	49.0–94.1	70.0	58.3–103	0.039
Asparagine	50.9	41.3–70.3	56.7	44.6–75.0	0.017
Aspartic acid	3.51	2.60–5.73	3.26	2.34–4.31	0.261
Carnosine	0.00	0.00–0.00	0.00	0.00–0.00	0.644
Citrulline	34.0	25.2–45.6	35.5	23.4–49.5	0.909
Cystathionine	0.00	0.00–0.00	0.00	0.00–0.00	0.570
Cystine	59.0	36.3–90.6	67.4	47.3–102	0.039
Ethanolamine	35.4	23.6–36.8	34.8	18.4–36.7	0.479
Glutamic acid	59.6	39.1–76.9	50.6	36.6–67.0	0.071
Glutamine	621	495–794	616	490–861	0.485[b]
Glycine	251	186–298	241	176–383	0.231
Histidine	73.8	63.1–96.5	72.8	58.1–106	0.467
Homocystine	0.00	0.00–0.00	0.00	0.00–0.00	0.500
5-hydroxylysine	0.00	0.00–0.75	0.25	0.00–0.77	0.222
Hydroxyproline	7.94	5.08–14.3	7.65	5.01–12.5	0.995
Isoleucine	64.4	48.8–81.5	62.3	53.5–80.1	0.483
Leucine	117	89.4–148	114	92.4–136	0.590
Lysine	182	153–232	188	135–251	0.926[b]
Methionine	27.3	21.6–40.6	29.0	23.1–42.8	0.832
1-methylhistidine	0.68	0.00–1.49	0.58	0.00–1.24	0.317
3-methylhistidine	4.23	2.48–5.49	3.49	2.50–5.20	0.718[b]
Ornithine	98.1	70.5–129	95.9	67.5–136	0.672
Phenylalanine	59.3	47.7–74.9	58.9	45.9–77.8	0.541
O-phosphoethanolamine	1.64	1.10–2.27	1.57	0.98–2.46	0.911
O-phospho-L-serine	0.00	0.00–0.61	0.00	0.00–0.71	0.666
Proline	162	123–231	174	133–242	0.229
Sarcosine	2.67	2.20–4.40	2.93	2.10–5.53	0.204
Serine	114	92.8–150	122	90.6–172	0.545
Taurine	55.6	42.2–79.1	54.3	35.1–77.0	0.376
Threonine	111	88.3–150	113	91.7–160	0.467[b]
Tryptophan	43.6	35.7–52.7	43.6	33.7–55.0	0.762
Tyrosine	61.4	49.6–74.6	61.2	47.6–80.7	0.431
Valine	237	206–311	245	196–296	0.957
Tyrosine/LNAA	0.12	0.11–0.14	0.13	0.11–0.14	0.400
Tryptophan/LNAA	0.09	0.08–0.10	0.09	0.08–0.10	0.603
Glutamine/glutamic acid	10.9	8.10–15.0	12.8	9.42–16.9	0.039
Serine/glycine	0.50	0.41–0.57	0.51	0.41–0.56	0.725[b]

Q1, lower quartile; Q3, upper quartile; LNAA, large neutral amino acid. [a]*P*-values from Wilcoxon rank-sum test. [b]*P*-values from t-test.

targets of SSRIs, these findings would imply that the metabolic disturbance in patients with MDD could be recovered by SSRIs treatment and support previous studies that SSRIs also influence other systems including glutamatergic neurons [53,54]. The disturbances of other amino acids were also observed in patients with MDD. Among these findings, we observed a low concentration of beta-aminoisobutyric acid in MDD, which was previously reported to have an inverse correlation with the severity of MDD [26]. We also observed a difference of alanine between patients and healthy individuals, which previously showed a positive correlation with HAM-D scores in patients with MDD and alterations in the brain in stressed rats [27,49].

We precisely measured 40 amino acids using internal standards for each compound in a relatively large number of patients comparing to previous studies. Our results showed alterations of amino acids on various pathways in patients with

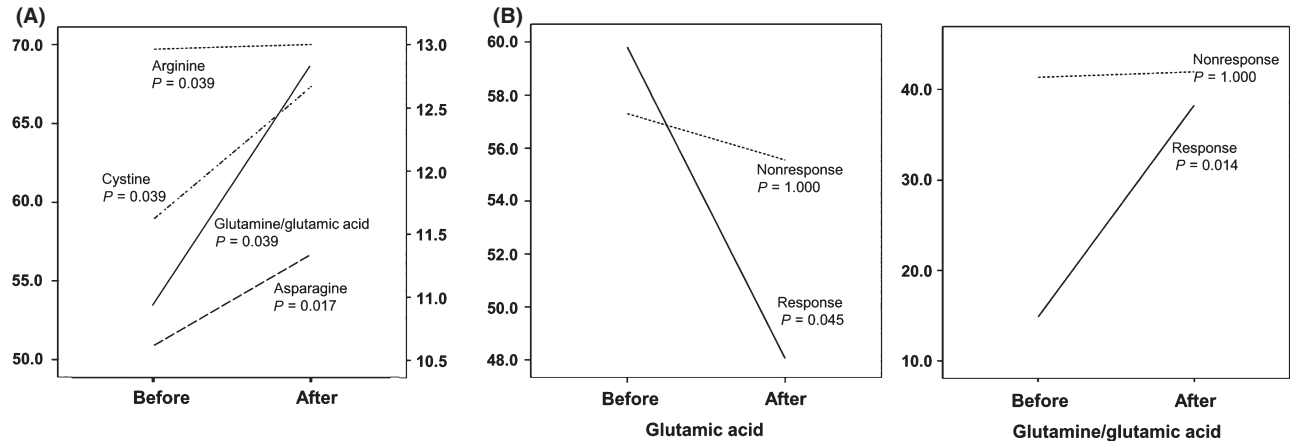

Figure 2 Comparison of amino acid concentrations between before and after SSRI treatment. (**A**) Arginine, asparagine, cystine (median value, left scale), and glutamine/glutamic acid ratio (median value, right scale) were increased after antidepressant treatment in Wilcoxon signed rank test. (**B**) Glutamic acid and glutamine/glutamic acid ratio were significantly changed after antidepressant treatment only in the response group, and not in the nonresponse group.

MDD compared to healthy individuals. This finding corresponds with previous literature reporting that MDD is a multifactorial disorder, and various etiologies, including energy metabolism and mitochondrial dysfunction, seem to be involved in the development of MDD [3,38,45,55]. However, we should acknowledge the limitations in this study. We could not identify amino acids that are consistently altered in all of the comparison and prediction analyses. Most findings correspond with previous results, but some were discordant. These inconsistencies could be caused by the limited sample size, and the differences of the analytic methods and the antidepressants. The high proportion of females among the participants in our study could act as a confounding factor. And identical to previous studies, it was not possible to define whether each metabolic disturbance is the cause or result of MDD. Although our study presents the disturbance of amino acid metabolism, we did not measure some of the metabolites and enzymes on interesting pathways because our study was designed to perform general amino acid profiling. Unfortunately, we did not measure the plasma or serum concentration of serotonin which is a key neurotransmitter on the pharmacological action of SSRI. Previous studies reported that the peripheral serotonin concentration was lower in patients with MDD than in healthy individuals, and it was drastically decreased after SSRI treatment [56–58]. Further in-depth analysis is needed of the metabolites and enzymes on the pathways on which alterations of amino acids are found. The measurement of serotonin concentration will contribute in a comprehensive understanding of the changes in amino acids including tryptophan which has a direct relationship with serotonin.

In conclusion, the significance of this study is that we not only have identified alterations of amino acids between patients with MDD and healthy individuals, but we also have demonstrated changes of amino acids after SSRI treatment and differences at baseline associated with responsiveness to antidepressant treatment. These findings indicate that disarrangement and restoration of systemic metabolic status are involved in the development and improvement of MDD. In particular, metabolites on various pathways of amino acids metabolism, including ABA and glutamic acid, have potential to increase understandings of pathogenesis and predictions of therapeutic response in MDD.

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Conflict of Interest

The authors declare no conflict of interest.

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