score decreased from 87.05 (SD = 29.40) to 54.00 (SD = 29.99) over the course of the study. The PHQ-9 and GAD-7 scores reduced from 11.11 (SD = 6.88) to 6.84 (SD = 5.07) and 9.32 (SD = 5.86) to 5.74 (SD = 4.74), respectively. A within-group *t*-test revealed significantly different scores between the pre- and post-CBT scores on the assessed scales: $t(1, 18) = 5.627$, $p < .001$, for the LSAS; $t(1, 18) = 3.338$, $p = .003$ for the PHQ-9; and $t(1, 18) = 2.486$, $p = .002$ for the GAD-7. The effect sizes between pre- and post-CBT were 1.124 (large), .620 (medium), and 0.611 (medium) for the LSAS, PHQ-9, and GAD-7, respectively.

Result of feedback from trainees

Ten trainees took part in the post-hoc survey about the training course. Regarding satisfaction with the length (i.e., one day a week for two years) of the training course, eight selected "satisfied," and two selected "very satisfied." As for the workshops, six selected "satisfied," three selected "very satisfied," and one selected "slightly satisfied." With respect to the frequency and the duration of the supervision, five selected "satisfied," three selected "slightly satisfied," and two selected "satisfied."

In response the question about the distinctive aspects of our training course compared to previous CBT training, five mentioned the continuity and practicality of our course, in contrast to classroom lectures for a short period. Additionally, four trainees appreciated the colleagues they had made through the training, and noted that they still support each other. Moreover, two pointed out that they obtained a wider perspective of CBT because both psychologists and psychiatrists were instructors in the training course. Regarding the difficulties trainees had during the training, three referred to their reluctance to record the sessions, although their clients usually agreed to be recorded. Finally, two noted that it was difficult to write clinical case reports.

Discussion

The purpose of this study was to report the preliminary outcomes of individual CBT for OCD, BN, and SAD delivered by the trainees of the Chiba CBT training course. We included patients with comorbid mood disorders if OCD, SAD, **or** BN was the principal diagnosis to reflect routine clinical practice. The results demonstrated that individual CBT for OCD, BN, and SAD in Japan led to significant reductions in symptom severity for these primary diagnoses. The effect size for OCD was comparable with those obtained in past trials involving psychological treatment for OCD (Rosa-Alcázar et al., 2008), and those for BN and SAD were large. Our study was designed not only to recruit patients similar to those seen in routine clinical practice but also to train clinicians who **will be** engaged in routine clinical practice; they were not fully trained therapists **before** this study.

Although it is difficult to directly compare our effect sizes with other published data due to a variety of factors (e.g., patient demographic and type/intensity of CBT), the overall effect sizes of 0.63 for PHQ-9 (medium) and 0.66 for GAD-7 (medium) were less than were those in other IAPT and other studies (Clark et al. 2009; Radhakrishnan et al. 2013; Richards & Borglin, 2011; Richards & Suckling, 2009; Westbrook & Hill, 1998; Westbrook & Kirk, 2005). It is possible that severity of depression and anxiety among our recruited patients was lower than that observed in previous reports, and thus resulted in a lower effect size. However, it is noteworthy that our results showed the lowest scores of PHQ-9 and GAD-7 at post-treatment (Table 4).

Table 4 about here

Our training course was highly evaluated by the trainees regarding the satisfaction with the length of the training course, the content of the workshops, and the frequency and duration of the supervision. This was confirmed by their comments suggesting that our course offered more comprehensive training than other courses. The trainees valued colleagues, probably because most of them do not have someone to

consult (even to talk) about CBT at their workplace. In order to address the difficulties in writing case reports, the supervisors addressed this issue by providing a special seminar about academic writing.

Dissemination of CBT across Japan

As noted in the Introduction, CBT is only covered by national health insurance for the treatment of mood disorders, primarily because the quantity of outcome research in CBT, particularly **using randomized control trials**, is exceptionally low in Japan. As Gunter and Whittal (2010) proposed, we need to conduct studies and evaluate more research-based data to obtain required funding and organizational support. The other issue hindering the dissemination of CBT in Japan is the paucity of training opportunities. Opportunities are limited for both pre- and post-qualification training. More than 160 universities **and** colleges provide postgraduate master's programs in clinical psychology, but only a few courses incorporate CBT in their curricula because of the scarcity of CBT experts. Our hope is that post-qualification training courses will be established in other areas of Japan so that more health professionals **can** attend workshops and benefit from regularly supervised practice.

Through the development and administration of the training course, the supervisors gained a wealth of professional knowledge concerning the dissemination of CBT in Japan. For example, approximately two years before the commencement of the training course, a survey was conducted with a number of psychiatric hospitals and clinics in Chiba province to identify the type of therapies that patients desired and clinicians would like to learn (Haraguchi et al., submitted for publication). The result of this survey revealed the strong need for CBT and provided rationale for establishing our training course. Additionally, as they ran the training course, supervisors had to identify and solve problems and difficulties as these arose. For instance, some trainees had difficulties with academic writing because they had completed their

undergraduate or postgraduate course many years ago. A special workshop was organized for the improvement of writing skills. To modify bias in their assessments, supervisors occasionally watched a video of a session together and compared each other's scores on the CTS-R. Moreover, they asked their supervisees to rate the Process Evaluation of Training and Supervision scale (Wilson, 2007) to assess the duration, frequency, supportive, and formative factors of supervision.

The concept of CBT as a Western therapy requiring major adaptation for effective use in Japanese culture must be considered further in on-going research. Compared to Western cultures, more emphasis is placed on interpersonal relationships than on self-fulfillment or self-development in Asian cultures. However, we believe that similar factors support the efficacy and utilization of CBT in Japan. **For example**, in a randomized trial, Nakatani et al. (2005) demonstrated that behavioral therapy is highly effective for Japanese patients with OCD. Matsunaga et al. (2008) elucidated the transcultural stability of the symptom structure of OCD, which is consistent with the hypothesis that OCD is mediated by universal psychobiological mechanisms.

The limitations of this study

Although the present study provided valuable information, it does have several limitations. This was a single-arm study without a concurrent control group. Moreover, our waiting period was not fixed, and scores were not obtained at a pre-treatment baseline point. Although these design factors reflect the real-world nature of mental health services, it remains unknown whether the observed improvements in symptom severity was related to the natural extinction of the disorders. More studies employing psychological placebo conditions to control for nonspecific factors, such as positive outcome expectancy and self-efficacy for problem management, are needed.

This study established the acute effectiveness of the treatment, but the lack of follow-

up data limits the generalizability of the study (e.g., long-term effects, relapse rates). Further, there was no control for the patients' use of medication, although our patient group had typically taken antidepressant medication for an extended period before referral to Chiba University Hospital. Again, this circumstance reflects the reality of the population of patients who access secondary mental health services in Japan. Further studies will need to include fixed waiting periods, control groups, and long-term follow-up to provide more insight into the implementation of CBT in routine practice in Japan. Currently, our research team is running a randomized control trial for SAD (Yoshinaga et al., in press: trial number: UMIN000007552) and single-arm trials for OCD and BN with fixed waiting periods. Changes in employment status, such as fewer days absent from work, should be examined after completion of the therapy. This would be a crucial test of whether increased access to psychological therapies would largely pay for itself by reducing other depression- and anxiety-related public costs (e.g., welfare benefits and medical costs) and increasing revenues (e.g., taxes, increased productivity).

This study focused on the effectiveness of CBT delivered by trainees to evaluate our training program. However, it remains unknown if the result of the CBT is due to the training or whether trainees had been already competent. Thus, other measures could also be employed to gain a better understanding of the ways training should be provided. Comparing scores on the cognitive therapy awareness scale (Sudak et al., 2003)— a multiple-choice questionnaire (Maunder et al., 2008; Myles & Milne, 2004)—between pre- and post-training would **reveal** how competent trainees felt as they progressed **through** training. A video assessment task (Myles & Milne, 2004) would provide a more objective perspective of the trainee competence.

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Tables

Table 1.

Demographic and clinical characteristics for OCD[a] (N = 14)

Variable		Value
Gender, female, n (%)		11 (79)
Age, years, mean (SD)		36.79 (9.88)
Comorbid Axis I diagnosis, N (%)	No comorbid condition (OCD only)	8 (57)
	With comorbidity	6 (43)
Age of onset, years, mean (SD)		31.57 (9.18)
Duration of OCD, years, mean (SD)		5.21 (4.67)
Employment status, N (%)	Employed full-time	2 (14)
	Part-time/homemaker	7 (50)
	Unemployed	5 (36)
Marital status, N (%)	Single	6 (43)
	Married	7 (50)
	Dating	1 (7)
Educational background, N (%)	High school	3 (22)
	Diploma	4 (28)
	Degree	7 (50)
Currently on medication, N (%)	AD and/or BZ	12 (86)

[a] Abbreviations: OCD = Obsessive-compulsive Disorder; BZ = Benzodiazepines; AD = Antipsychotics.

Table 2.

Demographic and clinical characteristics for BN[a] (N = 8)

Variable		Value
Gender, female, N (%)		8 (100)
Age, years, mean (SD)		31.3 (10.4)
BMI (kg/m^2)		23.5(5.7)
Comorbid Axis I diagnosis, N (%)	Without comorbidity (BN only)	5 (62.5)
	With comorbidity	3 (37.5)
Age of onset, years, mean (SD)		20.8 (7.1)
Duration of BN, years, mean (SD)		10.4 (8.9)
Employment status, N (%)	Employed full-time	1 (12.5)
	Full-time student	3 (37.5)
	Homemaker	2 (25.0)
	Unemployed	2 (25.0)
Marital status, N (%)	Single	5 (62.5)
	Married	3 (37.5)
	Divorced	2 (25.0)
Educational background, N (%)	Junior high school	0 (0)
	High school	1 (12.5)
	Diploma	3 (37.5)
	Degree	4 (50.0)
Currently on medication, N (%)	BZ and/or AD and/or MS	5 (62.5)

[a] Abbreviations: BN = Bulimia Nervosa; BZ = Benzodiazepines; AD = Antipsychotics; MS = Mood Stabilizers

Table 3.

Demographic and clinical characteristics for SAD[a] (N = 19)

Variable		Value
Gender, female, N (%)		14 (74)
Age, years, mean (SD)		32.3 (9.7)
Subtype, generalized, N (%)		16 (84)
Comorbid Axis I diagnosis, N (%)	Without comorbidity (SAD only)	11 (58)
	With comorbidity	8 (42)
Age of onset, years, mean (SD)		17.9 (8.8)
Duration of SAD, years, mean (SD)		14.3 (10.5)
Employment status, N (%)	Employed full-time	6 (32)
	Full-time student	5 (26)
	Part-time/homemaker	4 (21)
	Unemployed	4 (21)
Marital status, N (%)	Single	12 (63)
	Married	6 (32)
	Divorced	1 (5)
Educational background, N (%)	Junior high school	2 (13)
	High school	7 (37)
	Diploma	6 (32)
	Degree	4 (21)
Currently on medication, N (%)	AD and/or BZ	17 (87)

[a] Abbreviations: SAD = Social Anxiety Disorder; BZ = Benzodiazepines;

AD = Antidepressants

Table 4.

Comparison of effect sizes among various studies

Symptom	Data source	Intensity of CBT[a]	N (Dep , Anx)	Outcome	Pre Mean (SD)	Post Mean (SD)	ES[b]
Depression	Current data	High	45 (0%, 82%)	PHQ-9	10.6 (6.3)	6.6 (5.5)	0.63
	Westbrook (1988)	N/A	36 (27%, 36%)	BDI	18.2 (9.9)	10.9 (10.4)	0.79
	Westbrook (2005)	N/A	776 (19%, 56%)	BDI	16.9 (10.5)	9.8 (9.0)	0.68
	Clark (2009): Doncaster	High and low	1648 (95%, 5%)	PHQ-9	15.8 (6.2)	7.5 (6.9)	1.34
	Clark (2009): Newham	High and low	221 (46%, 43%)	PHQ-9	15.3 (6.2)	8.2 (7.2)	1.15
	Richards (2009)	High and low	1274 (N/A)	PHQ-9	16.0 (6.15)	8.1 (7.2)	1.28
	Richards (2011)	High and low	4183 (77%, 8%)	PHQ-9	16.2 (6.2)	9.0 (7.3)	1.17
	Radhakrishnan (2013)	High	2230 (N/A)	PHQ-9	14.4 (6.7)	9.2 (9.0)	0.79
		Low	4854 (N/A)	PHQ-9	12.5 (6.3)	8.0 (9.4)	0.72
Anxiety	Current data	High	45 (0%, 82%)	GAD-7	9.1 (5.8)	5.2 (4.6)	0.66
	Westbrook (1988)	N/A	36 (27%, 36%)	BAI	15.2 (10.4)	11.4 (11.1)	0.37
	Westbrook (2005)	N/A	473 (25%, 48%)	BAI	17.0 (11.8)	10.6 (8.9)	0.54
	Clark (2009): Doncaster	High and low	1648 (95%, 5%)	GAD-7	13.9 (5.2)	6.8 (6.2)	1.37
	Clark (2009): Newham	High and low	221 (46%, 43%)	GAD-7	13.7 (5.1)	6.8 (5.8)	1.35
	Richards (2009)	High and low	1274 (N/A)	GAD-7	14.0 (5.2)	7.2 (6.3)	1.07
	Richards (2011)	High and low	4183 (77%, 8%)	GAD-7	14.1 (5.1)	8.1 (6.4)	1.17
	Radhakrishnan (2013)	High	2230 (N/A)	GAD-7	12.9 (5.3)	8.2 (8.2)	0.89
		Low	4854 (N/A)	GAD-7	11/7 (5.4)	7.3 (9.0)	0.82

Abbreviations: CBT = Cognitive Behavioural Therapy; PHQ-9 = Patients Health Questionnaire-9 items; GAD-7 = Generalized Anxiety Disorder-7 items; BDI = Beck Depression Inventory;

BAI = Beck Anxiety Inventory; Dep = Depressive disorder; Anx = Anxiety disorder; ES = Effect Size.

[a] High = one-to-one, face-to-face psychological therapy; Low = guided self-help (e.g., using books, leaflets or computer support) and goup psychoeducation.

[b] Effect sizes (cohen's *d*) for each study were recalculated using same formula.

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Changes in plasma D-serine, L-serine, and glycine levels in treatment-resistant schizophrenia before and after clozapine treatment

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HIGHLIGHTS

- The plasma D-/L-serine ratio was lower in schizophrenia before clozapine treatment.
- The plasma D-/L-serine ratio increased in response to clozapine treatment.
- The plasma glycine/L-serine ratio increased in response to clozapine treatment.
- The glycine/L-serine ratio was higher in schizophrenia after clozapine treatment.

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ABSTRACT

Hypofunction of the N-methyl-D-aspartate (NMDA) subtype of glutamate receptors may be involved in the pathophysiology of schizophrenia. Many studies have investigated peripheral NMDA receptor-related glutamatergic amino acid levels because of their potential as biological markers. Peripheral D-serine levels and the ratio of D-serine to total serine have been reported to be significantly lower in patients with schizophrenia than in controls. Peripheral D-serine levels and the D-/L-serine ratio have also been reported to significantly increase in patients with schizophrenia as their clinical symptoms improve from the time of admission to the time of discharge. In this study, we examined whether peripheral NMDA receptor-related glutamatergic amino acids levels were altered in patients with treatment-resistant schizophrenia compared to controls and whether these peripheral amino acids levels were altered by clozapine treatment. Twenty-two patients with treatment-resistant schizophrenia and 22 age- and gender-matched healthy controls were enrolled. The plasma levels of D-serine, L-serine, glycine, glutamate, and glutamine were measured before and after clozapine treatment. We found that the plasma levels of D-serine and the D-/L-serine ratio were significantly lower in the patients before clozapine treatment than in the controls. The D-/L-serine ratio was significantly increased by clozapine treatment in patients, and no significant difference was observed in the plasma levels of D-serine and the D-/L-serine ratio between the patients after clozapine treatment and the controls. We also found that plasma glycine levels and the glycine/L-serine ratio were significantly increased following clozapine treatment in the patients, and the glycine/L-serine ratio was significantly higher in the patients after clozapine treatment than in the controls. There was no significant difference in the plasma levels of glutamate and glutamine both between the controls and

Abbreviations: CSF, cerebrospinal fluid; DSM-IV, Diagnostic and Statistical Manual of Mental Disorders, fourth edition; GAF, Global Assessment of Functioning; HPLC, high-performance liquid chromatography; NMDA, N-methyl-D-aspartate; PANSS, Positive and Negative Syndrome Scale.

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patients and between before and after clozapine treatment. This study firstly demonstrated changes of D-/L-serine and glycine/L-serine ratio between before and after clozapine treatment, suggesting that the plasma D-/L-serine ratio and glycine/L-serine ratio could be markers of therapeutic efficacy or clinical state in treatment-resistant schizophrenia.

1. Introduction

Recent investigations of schizophrenia have focused on hypofunction of N-methyl-D-aspartate (NMDA)-type glutamate receptors, in part, because of clinical evidence that phencyclidine, a non-competitive antagonist of the NMDA receptor, produces schizophrenia-like symptoms in normal controls [16].

A few studies investigated amino acids levels that are related to neurotransmission via the NMDA receptors; D-serine, L-serine, glycine, glutamate, and glutamine in postmortem brains of patients with schizophrenia [13,17,23]. No changes of these amino acids levels have been reported in postmortem brains of schizophrenia patients. Among these amino acids, glycine and glycine precursor, serine, co-agonists at NMDA receptors, and thus, increases glutamatergic neurotransmission, have drawn particular attention in schizophrenia research [7]. Because substantial quantities of D-serine have been found to be present in the mammalian brain [10] and because D-serine has a stronger affinity for the glycine site of NMDA receptors than does glycine [19], the importance of D-serine in the pathophysiology of schizophrenia has become the focus of the research field. Several studies have investigated CSF levels of these amino acids in patients with schizophrenia, and reduced D-serine levels and D-serine to total serine ratio in patients with schizophrenia has been reported [2,3,11,21].

There is evidence that the venous plasma and CSF levels of amino acids, including serine and glycine, are significantly correlated in human subjects [5], indicating that the plasma levels of these amino acids reflect, to some extent, those in the central nervous system. Serum/plasma glycine and serine levels have been investigated as biological markers for schizophrenia. First, total plasma serine and glycine levels have been found to be significantly higher in patients with schizophrenia than in controls [1]. An association between plasma glycine levels and negative symptoms in patients with schizophrenia has also been reported [24]. It has been reported that serum/plasma D-serine levels and the ratio of D-serine to total serine were significantly lower in patients with schizophrenia than in controls [4,12,25]. Many other studies have also investigated the serum/plasma glycine and serine levels in patients with schizophrenia, but these studies produced inconsistent results [3,21]. Moreover, only a few studies have investigated the plasma levels of these amino acids during the clinical course [22]. Ohnuma et al. reported that the D-serine level and the D-/L-serine ratio were significantly increased in patients with schizophrenia as their clinical symptoms improved from the time of admission to the time of discharge [22]. In addition, the increase in the plasma D-serine levels of drug-naïve patients was reported to be correlated with improvements in positive symptoms. In another study, it was reported that patients with schizophrenia taking clozapine had different serine and glycine metabolisms from the patients taking other antipsychotics [14]. The plasma levels of amino acids have not been investigated in treatment-resistant schizophrenia and the plasma levels of these amino acids have not been compared before and after clozapine treatment.

The aims of this study were to determine whether (1) plasma D-serine, L-serine, glycine, glutamate, and glutamine levels were altered in patients with treatment-resistant schizophrenia compared to controls and (2) these amino acids levels were altered by clozapine treatment.

2. Materials and methods

2.1. Subjects

Twenty-two patients with treatment-resistant schizophrenia who were treated with clozapine were included in this study. Twenty-two age- and gender-matched healthy controls also participated in this study. Detailed information is shown in Table 1. Blood samples were collected before and after clozapine treatment of the patients. Cases were recruited at the Osaka University hospitals. Each subject had been diagnosed and assessed by at least two trained psychiatrists according to the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) criteria based on a structured clinical interview. Treatment-resistant schizophrenia was defined according to the following criteria mentioned in the clozapine drug information in Japan: (1) no or little response to treatment from at least two adequately dosed antipsychotic trials for at least 4 weeks (including at least one second-generation antipsychotic, >600 mg/day of chlorpromazine equivalent) and Global Assessment of Functioning (GAF) scores that were never higher than 41, or (2) Intolerance to at least two second-generation antipsychotics because of extrapyramidal symptoms [26]. All subjects included in this study met the criterion of no or little response. All patients were inpatients when they start to take clozapine and were taking other antipsychotic drugs. Each patient was taking different drugs including typical and atypical antipsychotic drugs and average dosage and duration of treatment are shown in Table 1. The start dosage was 12.5 mg of once daily. The dosage was increased to 200 mg in 3 weeks or more. The dosage more than 50 mg was taken twice daily. Maintenance dosage was from 200 mg to 400 mg. The interval of dosage increase was 4 days or more and maximum dosage increase/day was 100 mg. Maximum dosage was 600 mg. Other antipsychotic withdrawal was performed within 4 weeks from the start of clozapine. Symptoms of schizophrenia were assessed using the Positive and Negative Syndrome Scale (PANSS). Patients with schizophrenia with comorbidities of substance-related disorders or mental retardation were excluded. Controls were recruited through local advertisements. Psychiatrically, medically and neurologically healthy controls were evaluated using the DSM-IV structured clinical interview, non-patient version. Subjects were excluded if they had neurological or medical conditions that could potentially affect the central nervous system, such as atypical headache, head trauma with loss of consciousness, chronic lung disease, kidney disease, chronic hepatic disease, thyroid disease, active stage cancer, cerebrovascular disease, epilepsy or seizures. Written informed consent was obtained from all subjects after the procedures had been fully explained. This study was conducted in accordance with the World Medical Association's Declaration of Helsinki and approved by the Research Ethical Committee of Osaka University, Tokushima University and Chiba University.

2.2. Determination of plasma levels of amino acids

Measurement of total, D- and L-serine levels in the plasma was carried out using a column-switching high performance liquid chromatography (HPLC) system (Shimadzu Corporation, Kyoto, Japan) as previously reported [9,25]. Measurement of glycine,

Table 1
Demographic variables for subjects.

Variables	Control ($n=22$)	Patients with schizophrenia ($n=22$)
Age (years)	38.1 ± 12.9	38.1 ± 13.2
Gender (male/female)	(12/10)	(12/10)
Schizophrenia type (paranoid/disorganized/catatonic/undifferentiated)	–	(15/7/0/0)
Outpatients/inpatients	–	(0/22)
Duration of illness (years)	–	17.2 ± 11.1
Duration of medication (years)	–	12.6 ± 7.8
Clozapine dose (mg)	–	448.6 ± 130.0
Antipsychotic dose before clozapine (CPZ equivalent doses) (mg)	–	1229 ± 642.9
Antipsychotic before clozapine (atypical only/atypical + typical)	–	(18/4)
PANSS positive (before/after clozapine treatment)	–	(29.8 ± 5.2/23.0 ± 4.6)
PANSS negative (before/after clozapine treatment)	–	(32.4 ± 7.7/25.5 ± 5.5)
PANSS general (before/after clozapine treatment)	–	(63.6 ± 13.0/52.9 ± 9.6)

Means ± SD are shown. CPZ, chlorpromazine.

glutamine, and glutamate was carried out using a HPLC system with fluorescence detection, as previously reported [11]. The researchers responsible for the measurements were blinded to the respective groups (controls and patients).

2.3. Statistical analysis

The statistical analyses were performed using SPSS 20.0J software (SPSS Japan Inc., Tokyo, Japan). Differences in the clinical characteristics between the patients and controls were analyzed using χ^2 tests for categorical variables. The groups did not differ with respect to age or gender (Table 1). Test of normality was performed by Shapiro–Wilk test and D-serine levels, Glycine levels in patients, D-/L-serine ratios in patients, and glycine/L-serine ratios in patients were not distributed normally and differences in the plasma amino acids levels between the patients and controls were analyzed using Mann–Whitney *U*-test. The differences in plasma amino acids levels and PANSS scores of the patients before and after treatment were analyzed by the Wilcoxon rank sum test. The positive, negative, and general symptom scores on the PANSS were significantly improved in the patients by clozapine treatment (Table 1). The Spearman rank order correlation test was performed to assess the possible correlation between the plasma levels of amino acids and clinical characteristics. The significance level for the statistical tests was set at $p < 0.05$.

3. Results

Plasma levels of D-serine, L-serine, glycine, glutamate, glutamine, and the D-/L-serine and glycine/L-serine ratios were compared between patients with treatment-resistant schizophrenia and controls (i.e., between controls and patients before clozapine treatment, and between controls and patients after clozapine treatment). The differences in the plasma levels of D-serine, L-serine, glycine, glutamate, glutamine, and the D-/L-serine and glycine/L-serine ratios before and after clozapine treatment were also compared.

The plasma levels of D-serine were significantly lower in the patients before clozapine treatment than in the controls (Fig. 1A and Table 2, Mann–Whitney *U*-test; $U = 141, Z = -2.4, p = 0.016$). No significant difference was observed in the plasma D-serine levels in the patients before and after clozapine treatment. The difference in the plasma D-serine levels between the controls and patients after clozapine treatment was not significant (Fig. 1A and Table 2). No significant difference was observed in the plasma levels of L-serine and glycine between the controls and patients before or after clozapine treatment (Fig. 1B and C, Table 2). The plasma levels of L-serine were significantly decreased in the patients after clozapine treatment (Fig. 1B and Table 2, Wilcoxon rank sum test; $Z = -2.8, p = 0.006$). The plasma levels of glycine were significantly increased in the patients after clozapine treatment (Fig. 1C and Table 2, Wilcoxon rank sum test; $Z = -2.3, p = 0.022$). There was no significant difference in the plasma levels of glutamate and glutamine between the controls and patients before or after clozapine treatment (Table 2). The plasma levels of glutamate and glutamine did not differ in the patients before and after clozapine treatment (Table 2).

The D-/L-serine ratio was significantly lower in the patients before clozapine treatment than in the controls (Fig. 2A and Table 2, Mann–Whitney *U*-test; $U = 123, Z = -2.8, p = 0.005$). The D-/L-serine ratio was significantly increased in the patients after clozapine treatment (Fig. 2A and Table 2, Wilcoxon rank sum test; $Z = -2.3$, $p = 0.02$), and the difference in the D-/L-serine ratio between the controls and patients after clozapine treatment was not significant (Fig. 2A and Table 2). The glycine/L-serine ratio did not differ between the controls and the patients before clozapine treatment (Fig. 2B and Table 2). The glycine/L-serine ratio was significantly increased in the patients after clozapine treatment (Fig. 2B and Table 2, Wilcoxon rank sum test; $Z = -3.8, p = 0.0002$) and was significantly higher in the patients after clozapine treatment than in the controls (Fig. 2B and Table 2, Mann–Whitney *U*-test; $U = 157$, $Z = -2.0, p = 0.046$).

The correlations between the plasma levels of these amino acids and clinical variables including duration of illness, clozapine dosage and positive, negative, and general symptom scores on the PANSS were also investigated; no significant correlation was observed (Supplementary Table 1).

4. Discussion

In this study, we measured the plasma amino acids levels before and after clozapine treatment in treatment-resistant schizophrenia; this is the first study, which investigated changes before and after clozapine treatment. We made the following findings: (1) The plasma levels of D-serine and the D-/L-serine ratio were lower in patients before clozapine treatment than in the controls, the D-/L-serine ratio increased in the patients in response to clozapine treatment and the plasma levels of D-serine and the D-/L-serine ratio in the patients after clozapine treatment were similar to those in the controls. (2) The plasma L-serine levels were decreased by clozapine treatment in the patients. (3) The plasma glycine levels and glycine/L-serine ratio were increased by clozapine treatment in the patients, and the glycine/L-serine ratio was higher in the patients after clozapine treatment than in the controls.

It has been reported that D-serine levels and the ratio of D-serine to total serine in CSF are lower in patients with schizophrenia than in controls [2,11]. Decreased serum D-serine levels and the ratio of D-serine to total serine in patients with schizophrenia were also reported [12,25]. We confirmed the lower plasma D-serine levels and D-/L-serine ratio in treatment-resistant schizophrenia

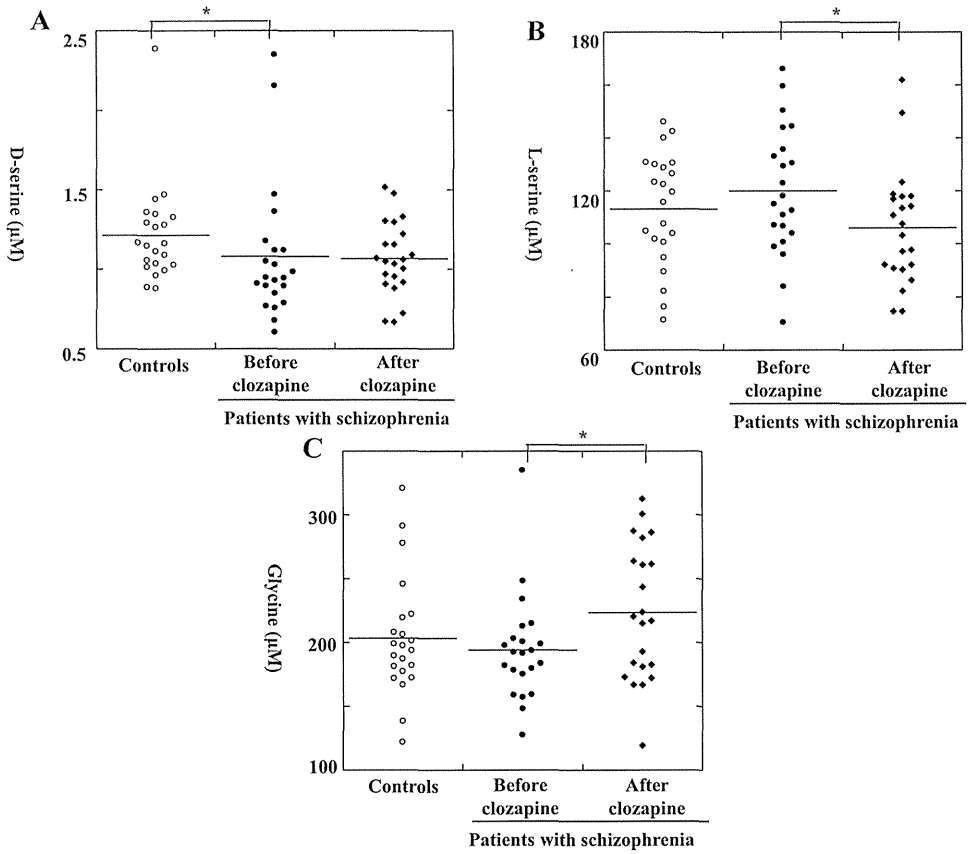

Fig. 1. Plasma levels of D-serine, L-serine, and glycine in treatment-resistant schizophrenia before and after clozapine treatment. The plasma levels of D-serine, L-serine, and glycine in the controls and patients with treatment-resistant schizophrenia before and after clozapine treatment (controls, $n = 22$; patients with schizophrenia, $n = 22$). The bars represent mean values. $^{*}p < 0.05$.

compared to controls. It has been reported that plasma D-serine levels and the D-/L-serine ratio increase during progression from the acute stage of schizophrenia to the remission stage and that L-serine levels decrease during this period [22]. Consistent with previous findings, we found that the D-/L-serine ratio increased and the L-serine levels decreased in response to clozapine treatment. Ohnuma et al. reported no significant difference in the plasma glycine levels between patients with different stages of schizophrenia, from the acute stage to the remission stage [22]. However, we found that the plasma glycine levels and the glycine/L-serine ratio increased in response to clozapine treatment and that the glycine/L-serine ratio was higher in patients after clozapine treatment. The increase in plasma glycine levels and the glycine/L-serine ratio may be specifically related to clozapine because clozapine was not used in the previous report by Ohnuma et al.

Many studies have investigated serum/plasma L-serine levels [21] and these studies have produced conflicting results. Several studies reported elevated L-serine levels in patients with schizophrenia [1,21], but other studies did not [8,21]. Serum/plasma glycine levels and glycine/serine ratio were also investigated by many studies because glycine acts as an

Table 2
Amino acids levels in patients with schizophrenia before and after clozapine treatment and in controls.

	Control ($n = 22$)	Patients with schizophrenia ($n = 22$)		*P* value		
		Before clozapine treatment	After clozapine treatment			
D-Serine (μM)	1.21 ± 0.31	1.08 ± 0.43	1.07 ± 0.23	0.018[a]	0.133[b]	0.485[c]
L-Serine (μM)	113.1 ± 21.5	120.0 ± 24.2	106.1 ± 21.8	0.385[a]	0.166[b]	0.006[c]
Glycine (μM)	203.3 ± 46.8	194.2 ± 41.8	223.4 ± 52.9	0.526[a]	0.260[b]	0.022[c]
Glutamate (μM)	35.8 ± 16.2	39.3 ± 13.5	33.8 ± 15.4	0.197[a]	0.907[b]	0.140[c]
Glutamine (μM)	510.9 ± 69.0	507.0 ± 75.3	475.0 ± 111.1	0.734[a]	0.348[b]	0.082[c]
D-/L-Serine ratio × 100	1.09 ± 0.23	0.90 ± 0.28	1.03 ± 0.24	0.005[a]	0.280[b]	0.020[c]
Glycine/L-serine ratio	1.82 ± 0.37	1.65 ± 0.32	2.15 ± 0.58	0.067[a]	0.046[b]	≤0.001[c]

Means ± SD are shown. Significant *p* values are underlined.

[a] The comparison between controls and patients before treatment with clozapine was performed by Mann–Whitney *U* test.

[b] The comparison between controls and patients after treatment with clozapine was performed by Mann–Whitney *U* test.

[c] The comparison between before and after treatment with clozapine was performed by Wilcoxon rank sum test.

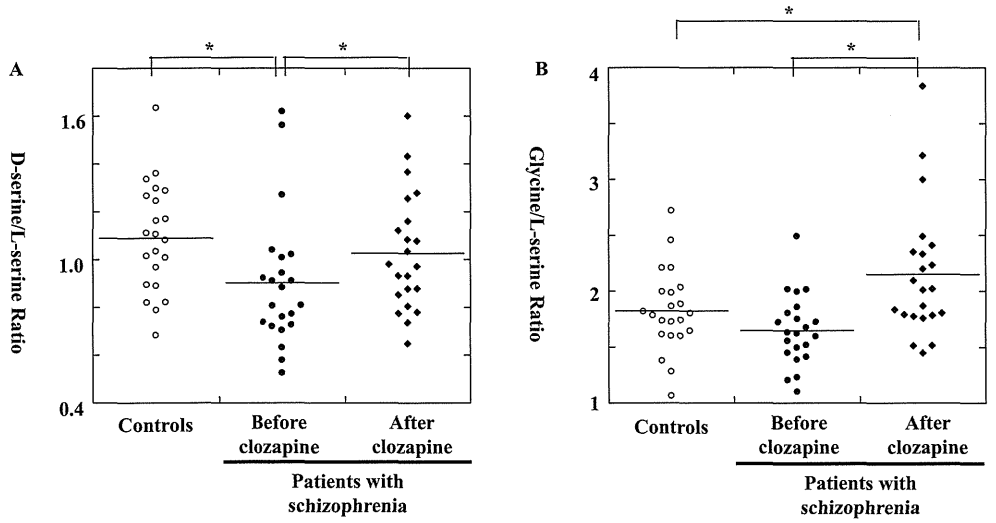

Fig. 2. Plasma D-/L-serine and glycine/L-serine ratio in treatment-resistant schizophrenia before and after clozapine treatment. The plasma D-/L-serine and glycine/L-serine ratios in the controls and patients with treatment-resistant schizophrenia before and after clozapine treatment (controls, $n=22$; patients with schizophrenia, $n=22$). The bars represent mean values. $^*p<0.05$.

endogenous, selective, full co-agonist at the glycine site of the NMDA receptor and modulates glutamatergic neurotransmission, and some studies found normal glycine levels [18,21], other studies reported increased concentrations in patients with schizophrenia [1,21] and other studies reported decreased levels in patients with schizophrenia [15,20,24]. In most of the previous studies, amino acids levels were measured at various times throughout clinical course, and the patients investigated were medicated with various antipsychotics or were medication-free. In this study, the only antipsychotic used in the treatment of patients was clozapine, and amino acids levels were measured before and after clozapine treatment, as the patients' clinical symptoms improved. We found no significant difference in the plasma L-serine and glycine levels in patients with schizophrenia, but we found significant change in the plasma L-serine and glycine levels in response to clozapine treatment. This change in the amino acids levels in response to treatment or clinical course may explain the inconsistencies between previous studies.

It has been reported that peripheral glutamate and glutamine levels were not changed in schizophrenia patients in comparison to controls [6,21]. Our result was consistent with previous studies.

Our study must be interpreted in light of its limitations. First, the sample size of the study is small. Second, only treatment-resistant patients with schizophrenia treated with clozapine were included, and patients treated with other antipsychotics or patients who were not treated with antipsychotics were not included in this study. Third, the antipsychotics used before clozapine treatment differ among the patients. Further studies are needed to evaluate the relationship between plasma amino acids levels, schizophrenia, and clozapine treatment.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.neulet.2014.08.052.

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