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Phosphoserine phosphatase activity is elevated and correlates negatively with plasma D-serine concentration in patients with schizophrenia

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ABSTRACT

The pathophysiology of schizophrenia may involve *N*-methyl-D-aspartate receptor (NMDAR) hypofunction. D-3serine and glycine are endogenous L-serine-derived NMDAR co-agonists. We hypothesized that the L-serine synthesis pathway could be involved in schizophrenia. We measured the activity of phosphoserine phosphatase (PSP), a rate-limiting enzyme in L-serine synthesis, in peripheral blood mononuclear cells of 54 patients with schizophrenia and 49 normal control subjects. Plasma amino acid (L-serine, D-serine, glycine, glutamine, and glutamate) levels were measured by high performance liquid chromatography. Peripheral blood mRNA expression levels of *PHGDH, PSAT1, PSP*, and *SR*, determined by quantitative real-time PCR were compared between patients and controls. PSP activity was higher in patients than in controls, especially in male patients. In male patients, the plasma L-serine concentration was higher than that in controls. In patients, PSP activity was negatively correlated with plasma D-serine and glycine levels. Furthermore, PSP activity was positively correlated with plasma L-serine concentration. These results were statistically significant only in male patients. *PSP*, *PSAT1*, and *PHGDH* mRNA levels were lower in patients than in controls, except when the *PHGDH* expression level was compared with *ACTB* expression. In summary, we found the L-serine synthesis system to be altered in patients with schizophrenia, especially in male patients.

1. Introduction

Schizophrenia is a chronic condition that generally appears in late adolescence or early adulthood, and may be related to *N*-methyl-D-aspartate receptor (NMDAR) hypofunction (Javitt and Zukin, 1991; Kantrowitz and Javitt, 2010; Nishikawa, 2011; Coyle et al., 2012; Gonzalez-Burgos and Lewis, 2012). D-serine and glycine are endogenous NMDAR co-agonists (Danysz and Parsons, 1998; Nishikawa, 2005), and some reports have indicated that the associated metabolic pathways can participate in the schizophrenia pathophysiology. Serine racemase (SR), D-amino acid oxidase (DAO), and their modifying factors (e.g., PICK 1 and G72) have been examined as key molecules in the pathophysiology of schizophrenia (Chumakov et al., 2002; Fujii et al., 2006; Kawazoe et al., 2007; Gonzalez-Burgos and Lewis, 2012). Moreover, a DAO inhibitor (Katane et al., 2013; Lane et al., 2013) and a glycine transporter1 inhibitor (Hashimoto, 2011; Javitt, 2012; Harvey and Yee, 2013; Umbricht et al., 2014), both of which can enhance NMDAR signaling, are promising candidate schizophrenia drugs.

Both D-serine and glycine are synthesized from L-serine (de Koning and Klomp, 2004) (Supplementary Figure S1); therefore, the L-serine synthesis pathway could be involved in schizophrenia pathophysiology. L-serine is biosynthesized from 3-phosphoglycerate, a glucose metabolite, via three enzymatic steps catalyzed by 3-phosphoglycerate dehydrogenase (PHGDH), phosphoserine aminotransferase 1 (PSAT1), and phosphoserine phosphatase (PSP) (de Koning and Klomp, 2004) (Supplementary Figure S1).

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We previously reported a case of a patient with schizophrenia and her son with a schizotypal personality, both of whom had low serum L-serine levels (Ozeki et al., 2011) and carried a balanced chromosomal translocation with breakpoints at 3q13.12 and 9q21.2. mRNA expression of *PSAT1*, which is located next to the breakpoint and encodes an enzyme in the L-serine synthesis cascade, was reduced in both individuals. Impaired *PSAT1* expression has a direct effect on decreased serum L-serine levels in rat astrocytes (Ozeki et al., 2011). In mature neuronal circuits of mice with a conditional deletion of *PHGDH*, L-serine availability determines the rate of D-serine synthesis in the forebrain and controls NMDAR function at least in the hippocampus (Yang et al., 2010).

Therefore, we propose that the L-serine synthesis cascade may be implicated in altered serine metabolism and schizophrenia. To test this hypothesis, we measured the activity of the PSP enzyme, the rate-limiting enzyme in L-serine synthesis (Wood et al., 1996), in patients with schizophrenia and normal control subjects and determined plasma concentrations of L-serine, D-serine, glycine, glutamine, and glutamate. Peripheral blood mRNA levels of *PHGDH*, *PSAT1*, *PSP*, and *SR*, encoding an enzyme that synthesizes D-serine from L-serine, and *DAO*, encoding an enzyme that catalyzes the oxidation of D-amino acids to the corresponding alpha-ketoacids, were measured. Data were compared between patients and controls, and amino acid concentrations were compared with clinical symptoms on the Positive and Negative Syndrome Scale (PANSS).

Table 1
Demographic of Participants.

	Schizophrenia	Normal healthy control
Number of participants	75	63
Age (years), mean ± SD	52.3 ± 13.7	47.6 ± 12.1
Sex	male: 44 female: 31	male: 39 female: 24
Antipsychotics dose (mg/day), mean ± SD (chlorpromazine equivalent dose)	831.3 ± 654.4	
Age at Onset of Illness (years), mean ± SD	24.5 ± 8.6	
Duration of treatment (years), mean ± SD	27.8 ± 13.5	
Number of smokers	31	19
PANSS score evaluated		
Number of participants	50	
sex	male: 31 female: 19	
PANSS total, mean ± SD	75.6 ± 17.0	
Positive, mean ± SD	15.0 ± 5.2	
Negative, mean ± SD	24.1 ± 5.7	
General, mean ± SD	36.5 ± 8.6	
Age (years), mean ± SD	53.8 ± 12.4	
Antipsychotics dose (mg/day), mean ± SD (chlorpromazine equivalent dose)	870.3 ± 721.6	
Age at Onset of Illness (years), mean ± SD	25.1 ± 8.8	
Disease duration (years), mean ± SD	28.7 ± 12.9	
Number of smorkers	26	

2. Methods

2.1. Participants

Seventy-five patients with schizophrenia and 63 controls were recruited between 2010 and 2013. All patients were treated at the Dokkyo Medical University Hospital, Mori Hospital, or Shimotsuga General Hospital. Demographic data of the participants are shown in Table 1. Typical antipsychotics were administered to 56 patients (haloperidol: 27, chlorpromazine: 14, bromperidol: 7, and levomepromazine: 31), and atypical antipsychotics were administered to 39 patients (olanzapine: 9, risperidone: 22, paliperidone: 2, quetiapine: 7, and aripiprazole: 6). The number of patients treated by monotherapy was 25. After the study procedures had been fully explained, written informed consent was obtained from all subjects. The study was approved by the ethics committee of the Dokkyo Medical University School of Medicine. All participants were assessed by two independent psychiatrists using DSM-IV-TR criteria. An inclusion criterion of this study was to satisfy the DSM-IV-TR criteria. Controls and their first degree relatives were confirmed to be free of mental illness. The PANSS scores were also measured in the 50 patients.

2.2. PSP enzyme assay

PSP enzyme activity was measured in peripheral blood mononuclear cells (PBMCs). Before noon, 8 mL whole blood samples were drawn from all individuals into BD Vacutainer® CPT™ cell preparation tubes containing sodium heparin (Becton, Dickinson Co., Plainfield, NJ, USA). Following the manufacturer's instructions, PBMCs were prepared, and cell pellets were stored at −80 °C for up to three months without loss of activity Jaeken et al. (1996). The separated plasma was also stored at −80 °C for measuring plasma amino acids.

PSP activity was measured as described by Jaeken et al. (Jaeken et al., 1996), as indicated by the release of [^{14}C]-serine from [^{14}C] 3-phosphoserine (American Radiolabeled Chemicals Inc., St Louis, MO, USA). One unit of enzyme was defined as the conversion of 1 μmol/min under the specific conditions of the assay.

2.3. Amino acid measurement

Plasma samples for high performance liquid chromatography (HPLC) were prepared using methods similar to those described previously (Long et al., 2001). Briefly, 150 μL of H_2O and 10 μL of 40% (w/v) of trichloroacetic acid (TCA) were added to 50 μL plasma and vortex-mixed. After centrifugation at 20,000*g* for 10 min at 4 °C, 150 μL of the supernatant was removed and added to 130 μL of H_2O, 100 μL of 200 mM borate buffer (pH 8.5), and 20 μL of 1 M NaOH, and vortex-mixed. Fluorescent derivatization was carried out by adding 40 μL of 50 mM borate buffer (pH 8.5) and 50 μL of 10 mM 4-fluoro-7-nitro-benzoxadiazole (NBD-F) to 20 μL of the solution described above, and the mixture was incubated at 60 °C for 5 min. The reaction was stopped by adding 890 μL of 2% TFA.

Separation and fluorometric detection of NBD-glutamine, NBD-glutamate, and NBD-glycine were carried out as previously reported (Hamase et al., 1997), except that TSKgel ODS-80Ts QA (250 × 4.6 mm, i.d.: 5 μm, Tosoh Corporation, Tokyo, Japan) was used as an ODS column, and the elution solvent and program were modified accordingly.

Separation and fluorometric detection of NBD-D- and L-serine were performed as previously reported (Long et al., 2001), except that InertSustain (250 × 4.6 mm, i.d.: 5 μm, GL Sciences Inc., Tokyo, Japan) and Sumichiral OA-3200 (250 × 4.6 mm, i.d.: 5 μm, Sumika Analytical Center, Osaka, Japan) were used as a reverseD-phase octyl silica column and a Pirkle-type chiral column, respectively. Separation on these columns was carried out with mobile phases of 1% methanol and 0.01% TFA in 15% acetonitrile (0.8 mL/min) and 1 mM citrate in methanol:acetonitrile (95:5) (1.0 mL/min), respectively. The chemicals were purchased from Sigma−Aldrich Japan (Tokyo, Japan), except when indicated above.