in the expressions of the heterochromatin protein 1, binding protein 3 (*HP1BP3*) gene ($P = 7.8 \times 10^{-6}$), which lies 3′ to *EIF4G3*, as a cis-acting effect (< 200 kb), and the calpain 14 (*CAPN14*) gene ($P = 6.3 \times 10^{-6}$), as a trans-acting effect (> 200 kb; Supplementary Table S4). Both *HP1BP3* and *CAPN14* are expressed in moderate-to-high levels throughout the adult human SFG (Supplementary Figures S4 and Supplementary Figure S5), as visualized in the Allen Institute Human Brain Atlas Explorer 2 software (http://human.brain-map.org/static/brainexplorer).

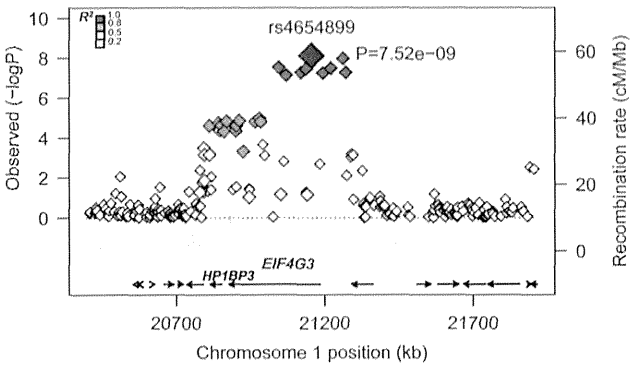

Figure 2. The strongest association with the right superior frontal gyrus was found for rs4654899. *P*-values ($-\log_{10}$) are shown in regions peripheral to rs4654899 (±750 kb).

DISCUSSION

To date, it remained unclear whether there were genetic variants strongly related to SFG volume in patients with schizophrenia and healthy subjects. This study is the first GWAS to identify the SNPs associated with the SFG, which have an important role in schizophrenia-related social functions and is reduced in patients with schizophrenia. We revealed that there were associations at the genome-wide significant level between SFG and genetic variants of the *EIF4G3* gene on 1p36.12. Individuals with minor A-allele of the most significant variant rs4654899 had smaller right SFG volumes compared with those with major C-allele in both patients and controls. Bioinformatical data indicate that the rs3767248 proxy SNP for rs4654899 has important roles in the expression of the *HP1BP3* and *CAPN14* genes, which are expressed in human adult SFG. The *HP1BP3* and *CAPN14* gene expressions of the minor G-allele of the rs3767248 polymorphism were significantly lower than those of the major A-allele. However, whether the expression levels of these genes in the brains or serums of patients with schizophrenia are lower or higher than those in healthy subjects is unknown. Further study is needed to investigate the difference of the expressions between patients and controls.

Table 1. TOP 10 SNPs for the right superior frontal gyrus

Rank	*SNP*	*Chr*	*Bp*	*m*	*M*	*MAF*	*Combined subjects*			*Schizophrenia*			*Controls*			*Closest gene*
							N	*β*	P	*N*	*β*	P	*N*	*β*	P	
1	rs4654899	1	21410231	A	C	0.33	509	−969.5	**7.52E−09**	153	−1164	3.71E−04	356	−935.9	1.86E−06	*EIF4G3*
2	rs6702110	1	21515906	G	A	0.32	526	−965.3	**1.07E−08**	155	−1255	1.79E−04	371	−902.3	4.34E−06	*EIF4G3*
3	rs6700718	1	21299363	A	C	0.33	537	−895.1	**2.89E−08**	159	−1110	4.68E−04	378	−847.7	6.81E−06	*EIF4G3*
4	rs10218584	1	21474480	G	C	0.33	537	−891.8	**3.21E−08**	159	−1110	4.68E−04	378	−842.7	7.68E−06	*EIF4G3*
5	rs1354792	1	21391875	C	T	0.33	535	−892.7	**3.46E−08**	157	−1114	5.35E−04	378	−847.7	6.81E−06	*EIF4G3*
6	rs6703227	1	21374810	C	T	0.33	537	−879.3	5.28E−08	159	−1056	9.55E−04	378	−847.7	6.81E−06	*EIF4G3*
7	rs1609558	1	21525228	C	T	0.30	533	−899.8	5.36E−08	158	−1031	1.18E−03	375	−870.9	7.67E−06	*EIF4G3*
8	rs12402486	1	21447935	A	G	0.34	527	−880.9	5.58E−08	159	−1110	4.68E−04	368	−830	1.20E−05	*EIF4G3*
9	rs2874367	1	21324491	A	C	0.33	531	−874.5	7.19E−08	157	−1092	6.24E−04	374	−829	1.25E−05	*EIF4G3*
10	rs6945071	7	26122423	G	A	0.16	537	−1035	8.46E−07	159	−1120	3.25E−03	378	−983.2	1.02E−04	*NFE2L3*

Abbreviations: Chr, chromosome; Bp, nucleotide location; m, minor allele; M, major allele; MAF, minor allele frequency; SNP, single-nucleotide polymorphism. Genome-wide significant *P*-values are shown as bold font and are underlined.

Table 2. TOP 10 SNPs for the left superior frontal gyrus

Rank	*SNP*	*Chr*	*Bp*	*m*	*M*	*MAF*	*Combined subjects*			*Schizophrenia*			*Controls*			*Closest gene*
							N	*β*	P	*N*	*β*	P	*N*	*β*	P	
1	rs4574391	4	27212223	C	T	0.25	533	−880	7.63E−07	158	−693	4.27E−02	375	−965	3.51E−06	*STIM2*
2	rs4654899	1	21410231	A	C	0.33	509	−787	1.51E−06	153	−691	3.81E−02	356	−863	4.22E−06	*EIF4G3*
3	rs2046701	4	27211040	C	A	0.25	532	−826	2.22E−06	158	−697	3.87E−02	374	−887	1.28E−05	*STIM2*
4	rs1609558	1	21525228	C	T	0.3	533	−763	2.33E−06	158	−524	1.11E−01	375	−885	1.67E−06	*EIF4G3*
5	rs6702110	1	21515906	G	A	0.32	526	−769	3.37E−06	155	−714	3.90E−02	371	−834	9.21E−06	*EIF4G3*
6	rs10218584	1	21474480	G	C	0.33	537	−704	8.32E−06	159	−566	8.48E−02	378	−791	1.05E−05	*EIF4G3*
7	rs1354792	1	21391875	C	T	0.33	535	−704	8.69E−06	157	−625	5.92E−02	378	−776	1.59E−05	*EIF4G3*
8	rs2623384	3	99064220	G	A	0.39	525	−724	9.15E−06	157	−894	6.79E−03	368	−642	5.92E−04	*COL8A1*
9	rs2292343	17	45455670	C	G	0.34	524	−721	9.74E−06	155	−836	1.22E−02	369	−666	3.45E−04	*EFCAB13*
10	rs3883317	17	45484111	A	G	0.34	534	−703	1.03E−05	158	−743	2.70E−02	376	−675	1.72E−04	*EFCAB13*

Abbreviations: Chr, chromosome; Bp, nucleotide location; m, minor allele; M, major allele; MAF, minor allele frequency; SNP, single-nucleotide polymorphism.

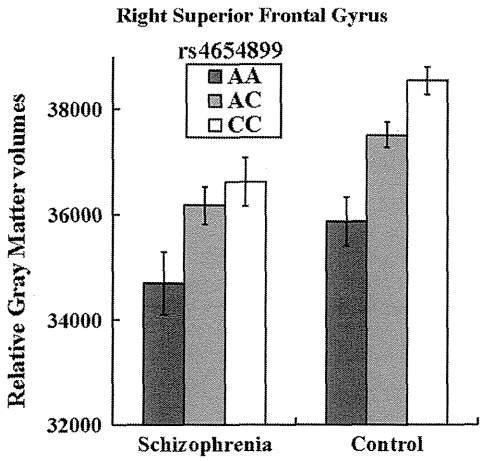

Figure 3. Impact of the rs4654899 genotype of the *EIF4G3* gene on the right superior frontal gyrus. Each column shows relative gray matter volumes of the right superior frontal gyrus. Error bars represent the standard error.

To our knowledge, no study has reported associations between these genes and schizophrenia, although the chromosomal region (1p36.12) related to the risk of schizophrenia has been reported.[30] The exact functions of these two genes are unknown; however, *HP1BP3* is predicted to bind to DNA and have a role in nucleosome assembly. *CAPN14*, which belongs to the calpain large subunit family, is a cytosolic calcium-activated cysteine protease involved in a variety of cellular processes, including apoptosis, cell division, modulation of integrin–cytoskeletal interactions and synaptic plasticity.

In this study, we examined the effects of genotypes on SFG volumes in a combined sample of patients and controls, and found similar effects of genotypes in patients and controls. Susceptibility genes for schizophrenia do not directly encode for their clinical syndrome/behaviors. The syndrome/behaviors observed in schizophrenia are produced by intermediate steps that occur between genes and syndrome/behaviors; and intermediate steps, such as changes of brain volumes, underlie the syndrome/behavior of schizophrenia. The intermediate phenotypes are located on the pathogenesis path, and are likely associated with a more basic and proximal etiological process rather than pathogenesis of disease itself.[5,6] Therefore, each genetic variant is related to controls as well as patients, and accumulations of each genetic variant could contribute to pathogenesis of schizophrenia through intermediate steps.

To date, although abnormal brain lateralization in schizophrenia causing a failure of left hemisphere dominance has been reported,[31] there is no evidence of SFG lateralization in schizophrenia. In addition, there is no report for developmental/functional differences between the right and left SFG. We found genome-wide significant variants related to right SFG volumes, whereas these variants were not related to left SFG volumes at genome-wide significant level. The difference of significance between right and left SFG was due to a difference of genotype effects in patients (for example, rs4654899, right: $P=3.71\times10^{-4}$, left: $P=3.81\times10^{-2}$) but not in controls (right: $P=1.86\times10^{-6}$, left: $P=4.22\times10^{-6}$). As it has been reported that gray matter volume deficits were more extensive in individuals with first-episode schizophrenia and neuroleptic naive than that of their neuroleptic-treated counterparts in left SFG,[9] confounding factors, such as duration of antipsychotic treatment or dose of antipsychotics, might affect our results.

In this study, we provide new insights into the genetic architecture of a brain structure closely linked to schizophrenia. It is still unclear whether and to what extent the effects of the genetic variant on SFG volumes observed here might be associated with an increased risk for schizophrenia. We suggest that the variant may have a role in the impairments of self-awareness and emotion noted in patients with schizophrenia through volumetric vulnerability of the SFG.

There were several limitations to this study. We recruited a relatively large sample with an only Japanese ethnicity to avoid population stratification. However, the existence of a false-positive association cannot be excluded as an explanation for our results. Further investigations of other samples with much larger sample sizes and/or with different ethnicities are needed to confirm our findings. It is unclear whether our results are directly/indirectly linked to the rs4654899 SNP, to other SNPs in high linkage disequilibrium with this SNP or to interactions between this SNP and other SNPs. To determine whether rs4654899 is the most strongly associated variant for SFG volume in the chromosomal region, an extensive search such as sequencing for other functional variants at this locus could provide further information underlying the genomic mechanism for this variant.

In conclusion, we found that genetic variants of the *EIF4G3* gene could be associated with structural vulnerability of the SFG. Further replication studies are necessary to confirm our findings. Identification of causal variants and the functional effects of these genes may help to reveal additional genetic variables involved in the neurodevelopment and pathogenesis of schizophrenia.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

We thank all the individuals who participated in this study. This work was supported by research grants from the Japanese Ministry of Health, Labor and Welfare (H22-seishin-ippan-001); KAKENHI, 22390225-Grant-in-Aid for Scientific Research (B), 23659565-Grant-in-Aid for Challenging Exploratory Research and Grant-in-Aid for Scientific Research on Innovative Areas (Comprehensive Brain Science Network) from the Japanese Ministry of Education, Culture, Sports, Science and Technology (MEXT) and the Japan Foundation for Neuroscience and Mental Health.

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Supplementary Information accompanies the paper on the Translational Psychiatry website (http://www.nature.com/tp)

Neuroscience Letters 582 (2014) 93–98

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

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.

ARTICLE INFO

Article history:
Received 10 April 2014
Received in revised form 31 July 2014
Accepted 30 August 2014
Available online 10 September 2014

Keywords:
Clozapine
Glycine
Plasma
Serine
Treatment-resistant schizophrenia

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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http://dx.doi.org/10.1016/j.neulet.2014.08.052

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

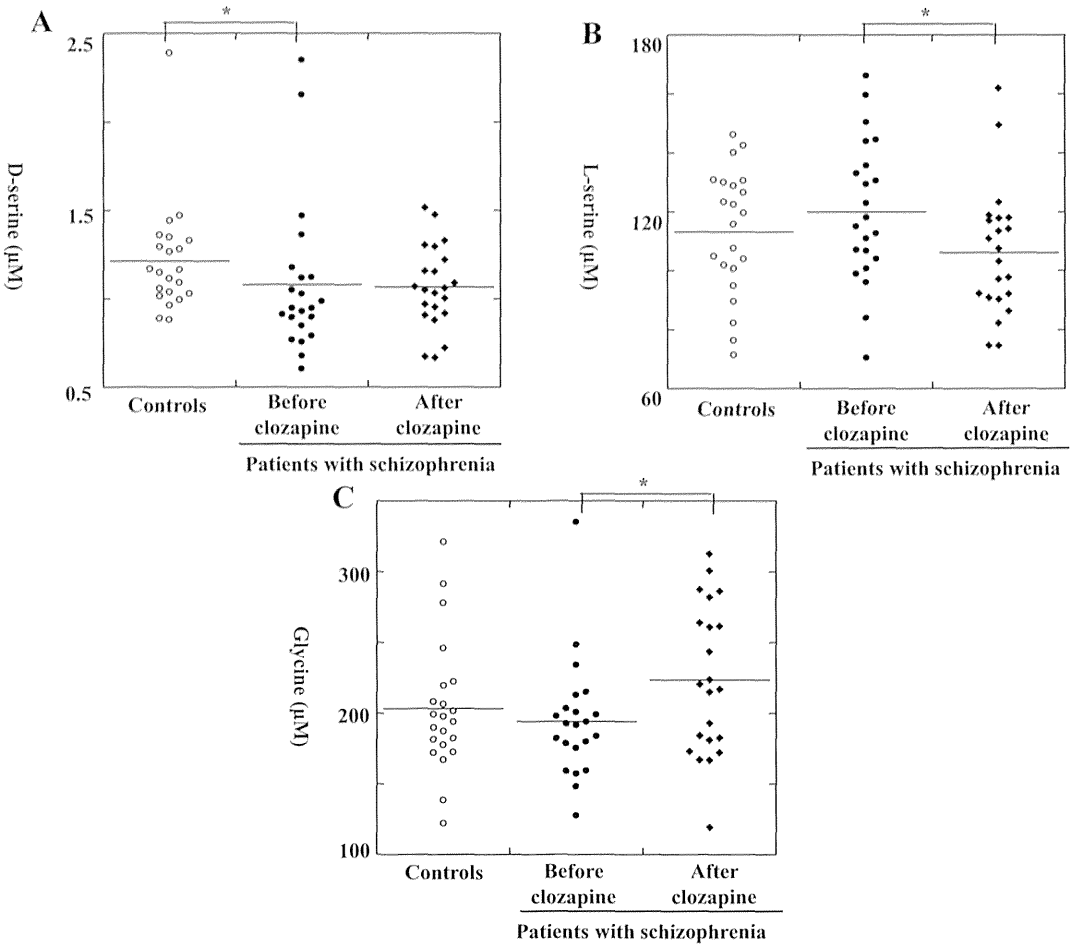

Fig. 1. Plasma levels of D-serine, L-serine, and glycine in treatment-resistant schizophrenia before and after clozapine treatment. The plasma levels of D-serine, L-serine, and glycine in the controls and patients with treatment-resistant schizophrenia before and after clozapine treatment (controls, $n = 22$; 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.

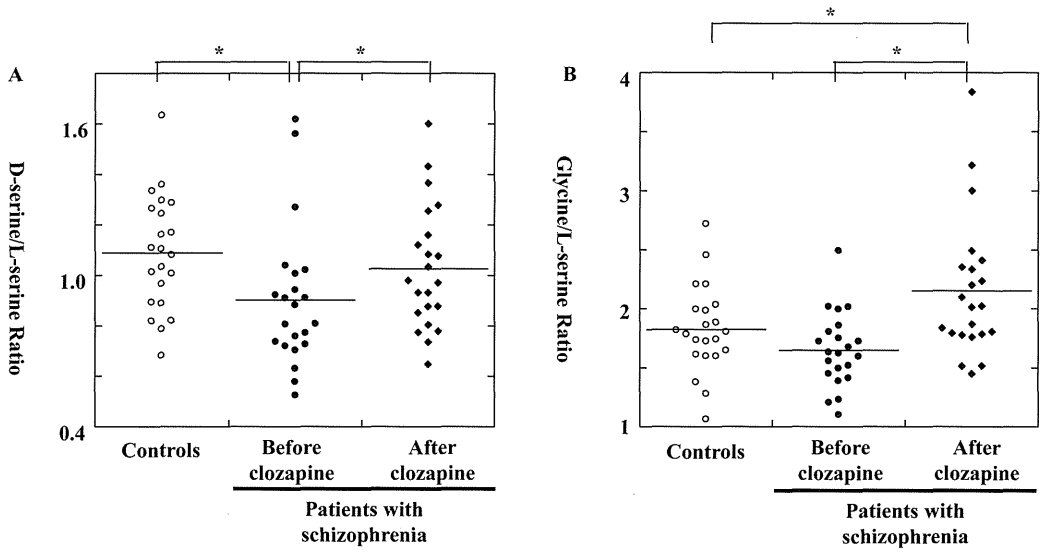

Fig. 2. Plasma D-/L-serine and glycine/L-serine ratio in treatment-resistant schizophrenia before and after clozapine treatment. The plasma D-/L-serine and glycine/L-serine ratios in the controls and patients with treatment-resistant schizophrenia before and after clozapine treatment (controls, $n=22$; 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.

Acknowledgments

We thank all of the individuals who participated in this study. This work was supported in part by research grants from the Japanese Ministry of Education, Culture, Sports, Science and Technology (MEXT) KAKENHI [25461730-Grant-in-Aid for Scientific Research (C), 22390225-Grant-in-Aid for Scientific Research (B), 23659565-Grant-in-Aid for Challenging Exploratory Research and 221S0003-Grant-in-Aid for Scientific Research on Innovative Areas (Comprehensive Brain Science Network)]; and the Japan Foundation for Neuroscience and Mental Health. The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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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Psychiatry and Clinical Neurosciences 2014; **68**: 534–541 doi:10.1111/pcn.12165

Regular Article

Performance on the Wechsler Adult Intelligence Scale-III in Japanese patients with schizophrenia

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Aim: Patients with schizophrenia have been reported to perform worse than non-schizophrenic populations on neuropsychological tests, which may be affected by cultural factors. The aim of this study was to examine the performance of a sizable number of patients with schizophrenia on the Japanese version of the Wechsler Adult Intelligence Scale-III (WAIS-III) compared with healthy controls.

Methods: Performance on the WAIS-III was evaluated in 157 Japanese patients with schizophrenia and in 264 healthy control subjects.

Results: All IQ scores and four indices from the WAIS-III were impaired for patients with schizophrenia compared with healthy controls. Processing Speed was markedly disturbed, approximately 2 SD below that of the healthy control group. Among the 13 subtests, Comprehension ($z = -1.70$, $d = 1.55$), Digit Symbol Coding ($z = -1.84$, $d = 1.88$), and Symbol Search ($z = -1.85$, $d = 1.77$) were profoundly impaired relative to the healthy controls.

Conclusion: These results indicate that the pattern and degree of impairment, as evaluated by the WAIS-III, in Japanese patients are similar to those previously reported in English-speaking patients and that the deficits of some neuropsychological domains relevant to functional outcomes are universally characteristic of schizophrenia.

Key words: cognitive impairment, cross-national difference, functional outcome, schizophrenia, Wechsler Adult Intelligence Scale-III.

SCHIZOPHRENIA IS CHARACTERIZED by positive (e.g., delusions and hallucinations) and negative (e.g., blunted affect and withdrawal) symptoms as well as cognitive deficits.[1] Specifically, various domains of cognitive function, such as several types of memory, attention, executive function, fluency, and information processing, are impaired in patients with schizophrenia.[2–8] Cognitive disturbances are regarded as a core feature of schizophrenia and have been reported to profoundly alter everyday social functioning.[9] Most patients with schizophrenia fail to reach their expected level of cognitive functioning estimated from educational level and premorbid intelligence.[10] Cognitive impairment appears to be independent of other aspects of the symptomatology

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Received 13 March 2013; revised 7 January 2014; accepted 15 January 2014.

of schizophrenia, for example, positive symptoms.[11] Although other psychiatric disorders, such as bipolar disorder, also present with cognitive degradation to some degree, patients with schizophrenia exhibit more global and more severe impairment.[12–14]

Cognitive impairments in schizophrenia are also known to affect everyday functioning and social abilities.[15] For these reasons, efforts have been made to enhance cognitive functioning, such as by cognitive rehabilitation, to encourage the patients' social adjustment.[16–18]

The Wechsler Adult Intelligence Scale-III (WAIS-III)[19] has been developed to assess intellectual ability and is frequently used in studies of cognitive abilities in patients with schizophrenia.[20–22] Several modifications have been incorporated in the update from the WAIS-Revised (previous version of the WAIS-III) into the WAIS-III. In addition to Full-Scale IQ (FSIQ), Verbal IQ, and Performance IQ, four new indices have been included: Verbal Comprehension (VC), Perceptual Organization (PO), Working Memory (WM), and Processing Speed (PS). VC is a measure of the ability to understand and provide oral explanations corresponding to certain tasks and to identify abstract concepts. PO measures non-verbal thinking, such as the manipulation of designs and pictures, fluid reasoning, and spatial processing. WM measures the ability to retain information temporarily to perform a designated task. PS measures the ability to perform given tasks quickly and efficiently based on the presented information. Upon adopting these four indices, new subtests have been included in the WAIS-III. Brief descriptions of these tasks and other tasks are provided in Table S1. The reliability of the Japanese version of the WAIS-III was confirmed through a standardization study. The reliability coefficients of the Japanese version of the WAIS-III were calculated using the split-half method and test–retest reliabilities. The mean reliability coefficients across all age groups were 0.81–0.92 for all subtests and 0.91–0.97 for indices and IQs, which showed good reliabilities.[23]

Despite the accumulation of studies, performance on the WAIS-III has only been grossly reported, and no study has clarified the comprehensive profile of the WAIS-III in populations of patients with schizophrenia. The aim of this study was to examine the performance on the Japanese version of the WAIS-III in patients with schizophrenia compared with healthy controls.

METHODS

Subjects

A total of 157 patients with schizophrenia (97 male, 60 female) and 264 healthy control subjects (153 male, 111 female) were included in this study. The patients consisted of inpatients and outpatients treated at the Department of Psychiatry, Osaka University Hospital. A consensus diagnosis, according to the DSM-IV[1] criteria, was made by experienced senior psychiatrists using the Structured Clinical Interview for DSM-IV (SCID) for schizophrenia. Psychotic symptoms (positive symptoms, negative symptoms, and general psychopathology) were evaluated using the Positive and Negative Syndrome Scale (PANSS).[24]

Healthy control subjects were recruited from the community through local advertisements at Osaka University. The healthy control subjects were evaluated psychiatrically, medically, and neurologically, and the DSM-IV-Non-Patient version of the SCID was used to exclude individuals who had current or past contact with psychiatric services or had previously received psychiatric medications.

Subjects were excluded from this study if they had neurological or medical conditions that could 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, cancer in an active stage, cerebrovascular disease, epilepsy, seizures, substance-related disorders, and mental retardation.

All participants provided written informed consent after the study procedures were fully explained. All procedures were conducted according to the Declaration of Helsinki and approved by the Ethical Committee of Osaka University.

Measures

The Japanese version of the WAIS-III[23] was used. It comprises three IQ categorical scores: Full Scale IQ (FSIQ), Verbal IQ, and Performance IQ. Verbal IQ consists of seven subtests: Vocabulary, Similarities, Arithmetic, Digit Span, Information, Comprehension, and Letter–Number Sequencing. Performance IQ consists of six subtests: Picture Completion, Digit Symbol Coding, Block Design, Matrix Reasoning, Picture Arrangement, and Symbol Search. The subtests of the WAIS-III are grouped into four index scores as

follows: VC (Vocabulary, Similarities, and Information), PO (Picture Completion, Block Design, and Matrix Reasoning), WM (Arithmetic, Digit Span, and Letter–Number Sequencing), and PS (Digit Symbol Coding and Symbol Search) (Table S1). The raw scores of these subtests were converted to scaled scores.

Premorbid IQ was estimated using the Japanese Adult Reading Test (JART).[25,26]

Statistical analysis

Statistical analyses were performed using SPSS 17.0 (SPSS Japan, Tokyo, Japan). Group comparisons of demographic variables were performed using *t*-tests or χ^2-tests, as appropriate. The effects of a psychiatric diagnosis on IQ scores and the WAIS-III indices were analyzed using *t*-tests. To control for confounding factors, analyses of covariance (ANCOVA) were performed with sex and JART scores as covariates (the significance level was set at two-tailed $P < 0.05$). A strong correlation is known to exist between JART and years of education; thus, only the former, the index of premorbid intelligence, was included as a covariate.

The premorbid and WAIS-III IQ scores and the scores of the four indices and subtests of the WAIS-III were transformed to standardized *z*-scores on the basis of the means and SD of the healthy control group. In addition, effect size (Cohen's *d*) was calculated; the mean difference between the healthy controls' scores and the patients' scores was divided by the pooled SD (weighted by *n* of each group).

Pearson's correlations were calculated for the patients to examine the correlations between clinical variables and the performance on the WAIS-III.

RESULTS

Table 1 summarizes the demographic and clinical information of the subjects. Patients and healthy controls did not differ significantly in age, sex, and education years (*P*s > 0.05).

Table 2 presents premorbid IQ, FSIQ, Verbal IQ, Performance IQ, the four indices, and the scaled scores of the WAIS-III subtests. All the IQ scales and scores of the four indices were significantly lower for the patients with schizophrenia than for the healthy controls (all *P*s < 0.001). In contrast to a relatively small decline in premorbid IQ, the patients' FSIQ, Verbal IQ, and Performance IQ fell between 1.34 and 1.90 SD below normal values (Fig. 1, upper). Performance IQ was 8.4 points below Verbal IQ in the patient group. PS was markedly disturbed, being approximately 2 SD below the healthy control group ($z = -2.16$, $d = 2.06$). In contrast, the three other index scores demonstrated a decline of approximately 1 SD (Fig. 1, lower).

Performance on the subtests revealed that scores fell significantly below normal (*P*s < 0.001). Among the 13 subtests, the deficits of Information ($z = -0.62$, $d = 0.61$) and Digit Span ($z = -0.49$, $d = 0.50$) were

Table 1. Comparisons of healthy controls and schizophrenia patients on demographic variables

	Healthy controls ($n = 264$)		Schizophrenia patients ($n = 157$)			
	Mean	SD	Mean	SD	Statistics	*P*-value
Age	37.5	12.9	35.9	12.2	$t_{342.9} = 1.231$	0.219
Sex (Male/Female)	153/111		97/60		$\chi^2_{(1)} = 0.598$	0.439
Education (years)	14.1	2.0	13.9	2.5	$t_{267.9} = 0.895$	0.372
Age at onset	–	–	24.1	9.4	–	–
Duration of untreated psychosis (years)	–	–	2.0	4.1	–	–
Duration of illness (years)	–	–	11.8	9.7	–	–
CPZeq (mg/day)	–	–	589.7	537.4	–	–
PANSS positive	–	–	19.6	5.6	–	–
PANSS negative	–	–	20.1	5.9	–	–
PANSS general psychopathology	–	–	42.7	10.3	–	–

CPZeq, chlorpromazine equivalent of total antipsychotics; PANSS, Positive and Negative Syndrome Scale.

relatively mild, whereas Comprehension ($z = -1.70$, $d = 1.55$), Digit Symbol Coding ($z = -1.84$, $d = 1.88$), and Symbol Search ($z = -1.85$, $d = 1.77$) were markedly impaired relative to the healthy controls (Fig. 2).

Correlations between clinical variables and WAIS-III indices are shown in Table 3. Age at onset, duration of untreated psychosis, and duration of illness were not correlated with the indices. Correlations between antipsychotic medications and indices were mild to moderate. Positive symptoms were partly correlated with the indices. Correlations between negative symptoms and the indices were moderately correlated. General psychopathology was mildly correlated with the indices. Correlations between clinical variables and the WAIS-III subtests are presented in Table S2 (Supporting Information), representing similar results of the indices.

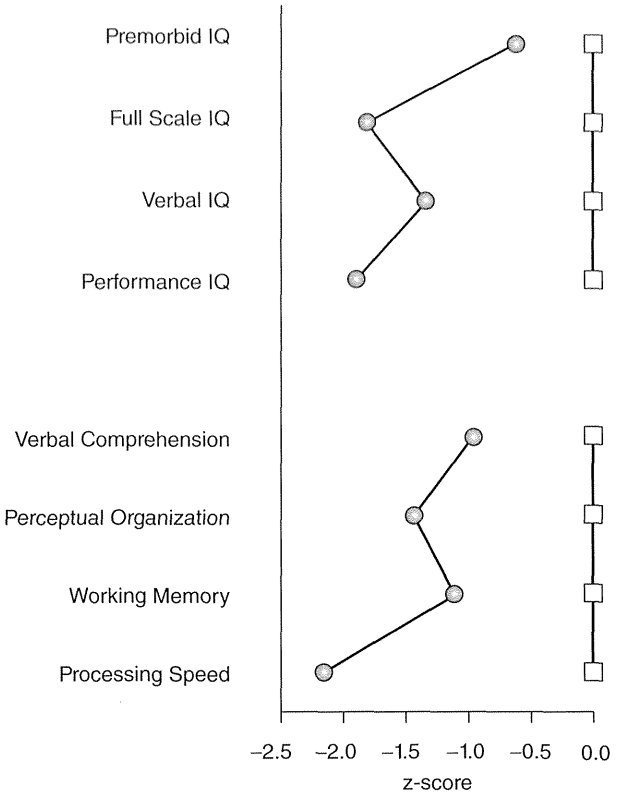

Figure 1. Scores of premorbid IQ and the Wechsler Adult Intelligence Scale-III IQ and four indices. (-□-) Controls, (-○-) Patients. Each score was transformed to standardized *z*-scores based on the means and SD of the healthy controls. Therefore, the means of the healthy controls are represented by the zero line (SD = 1).

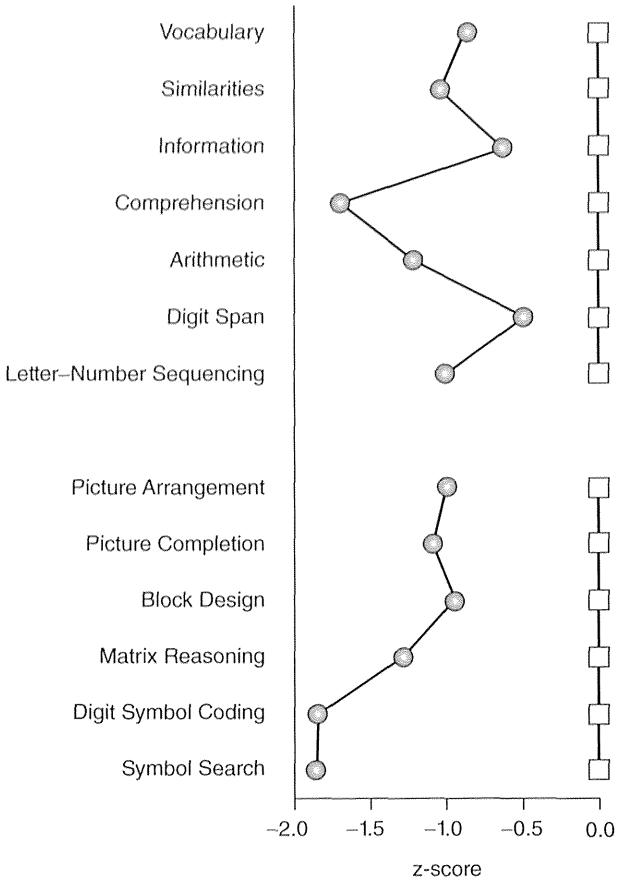

Figure 2. Wechsler Adult Intelligence Scale-III subtests scores. (-□-) Controls, (-○-) Patients. The subtest scores were transformed to standardized *z*-scores based on the means and SD of the healthy controls.

DISCUSSION

This study evaluated the complete profile of the performance of a sizable number of subjects with schizophrenia on the Japanese version of WAIS-III. Performances on the subtests associated with functional outcome and sensitivity to language systems were closely examined.

Profile of the Japanese version of the WAIS-III

Generally, patients with schizophrenia performed significantly worse on the Japanese WAIS-III, as was reported by previous studies with the English version of the WAIS-III,[20,27] even though the patients

Table 2. Comparison of healthy controls and schizophrenia patients on premorbid IQ, WAIS-III IQ, indices, and subtests

	Healthy controls ($n = 264$)		Schizophrenia patients ($n = 157$)		*t*-test			ANCOVA			
	Mean	SD	Mean	SD	t	d.f.	*P*-value	$F_{1, 419}$	*P*-value	z	d
Premorbid IQ	106.7	8.0	101.7	10.2	5.23	269.1	3.46×10^{-7}	NA	NA	−0.62	0.56
Full Scale IQ	108.0	12.4	85.5	16.6	14.77	259.8	2.90×10^{-36}	246.5	5.61×10^{-44}	−1.82	1.60
Verbal IQ	108.4	13.4	90.5	16.1	11.77	282.1	2.84×10^{-26}	136.3	1.92×10^{-27}	−1.34	1.24
Performance IQ	105.8	12.4	82.1	16.3	15.67	263.5	1.54×10^{-39}	238.8	6.45×10^{-43}	−1.90	1.69
Indices											
Verbal Comprehension	106.1	13.4	93.2	15.7	8.57	287.3	6.47×10^{-16}	49.6	7.94×10^{-12}	−0.96	0.90
Perceptual Organization	105.3	13.2	86.3	17.9	11.56	256.7	3.77×10^{-25}	121.4	6.02×10^{-25}	−1.44	1.26
Working Memory	105.7	15.6	88.2	16.5	10.75	314.5	3.74×10^{-23}	90.1	1.77×10^{-19}	−1.12	1.10
Processing Speed	106.8	13.1	78.4	14.8	19.80	297.1	3.18×10^{-56}	356.4	6.61×10^{-58}	−2.16	2.06
Subtests											
Vocabulary	11.4	3.0	8.8	3.2	8.13	310.5	1.06×10^{-14}	36.5	3.36×10^{-9}	−0.86	0.83
Similarities	11.0	2.4	8.5	3.1	8.75	272.0	2.26×10^{-16}	51.4	3.46×10^{-12}	−1.03	0.94
Information	10.8	2.9	9.0	3.0	5.97	314.0	6.58×10^{-9}	11.5	7.60×10^{-4}	−0.62	0.61
Comprehension	12.2	2.8	7.5	3.4	14.56	275.8	5.07×10^{-36}	191.8	3.75×10^{-36}	−1.70	1.55
Arithmetic	11.6	3.0	8.0	3.2	11.60	315.0	3.76×10^{-26}	113.8	1.18×10^{-23}	−1.21	1.19
Digit Span	10.8	3.2	9.3	3.0	5.00	342.2	9.10×10^{-7}	12.2	5.29×10^{-4}	−0.49	0.50
Letter–Number Sequencing	10.7	3.2	7.5	3.3	9.71	319.1	1.08×10^{-19}	68.1	2.03×10^{-15}	−1.00	0.99
Picture Arrangement	10.5	3.4	7.2	3.7	9.22	304.9	5.01×10^{-18}	62.1	2.91×10^{-14}	−0.98	0.95
Picture Completion	10.4	2.6	7.6	3.4	8.82	261.5	1.70×10^{-16}	64.8	8.80×10^{-15}	−1.08	0.95
Block Design	11.0	3.1	8.2	3.7	8.18	281.2	9.89×10^{-15}	53.3	1.48×10^{-12}	−0.94	0.86
Matrix Reasoning	11.4	2.8	7.8	3.5	10.84	271.4	5.66×10^{-23}	101.4	1.69×10^{-21}	−1.28	1.16
Digit Symbol Coding	11.3	2.8	6.1	2.7	18.95	343.2	2.49×10^{-55}	294.1	2.86×10^{-50}	−1.84	1.88
Symbol Search	11.3	2.8	6.2	3.1	17.10	299.4	3.33×10^{-46}	262.1	4.37×10^{-46}	−1.85	1.77

Scores of subtests were scaled scores.
ANCOVA (covariate = sex, premorbid IQ [Japanese Adult Reading Test]).
WAIS-III, Wechsler Adult Intelligence Scale-III.

Table 3. Correlations between clinical variables and WAIS-III indices in patients with schizophrenia

	FSIQ	VIQ	PIQ	VC	PO	WM	PS
Age at onset	0.079	0.084	0.054	0.045	0.016	0.010	0.025
Duration of untreated psychosis	0.018	−0.020	0.054	−0.070	0.030	−0.034	−0.015
Duration of illness	0.058	0.044	0.059	0.034	0.027	0.023	0.123
CPZeq (mg/day)	−0.311***	−0.267***	−0.325***	−0.218**	−0.320***	−0.352***	−0.251**
PANSS positive	−0.184*	−0.151	−0.199*	−0.094	−0.204*	−0.152	−0.084
PANSS negative	−0.434***	−0.428***	−0.379***	−0.381***	−0.327***	−0.352***	−0.312***
PANSS general psychopathology	−0.290***	−0.268***	−0.271***	−0.207**	−0.249**	−0.239**	−0.190*

*$P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (2 tailed).
CPZeq, chlorpromazine equivalent of total anti psychotics; FSIQ, Full-Scale IQ; PANSS, Positive and Negative Syndrome Scale; PIQ, Performance IQ; PO, Perceptual Organization; PS, Processing Speed; VC, Verbal Comprehension; VIQ, Verbal IQ; WAIS-III, Wechsler Adult Intelligence Scale-III; WM, Working Memory.

were demographically equivalent to the normal controls. Impairment of the patients' premorbid IQ was relatively mild ($z = -0.62$, $d = 0.56$), whereas the performance on the FSIQ was severely disturbed ($z = -1.82$, $d = 1.60$), indicating a large decrement from the expected level of cognitive functioning.[10] Consistent with previous studies,[22,28,29] Performance IQ tended to be below Verbal IQ in patients with schizophrenia. VC was most preserved ($z = -0.96$, $d = 0.90$), followed by WM ($z = -1.12$, $d = 1.10$) and PO ($z = -1.44$, $d = 1.26$). PS was markedly impaired in schizophrenic patients ($z = -2.16$, $d = 2.06$). Previous studies have reported that VC and PS were associated with community functioning and vocational functioning.[27,30] Specifically, PS is one of the most negatively affected cognitive abilities in schizophrenia.[6,31,32]

Generally, scores of the 13 subtests were slightly lower than those of a previous study;[33] however, the profile pattern in the patients studied here was similar to those reported in the literature. Among the 13 subtests, Comprehension ($z = -1.70$, $d = 1.55$), Digit Symbol Coding ($z = 1.84$, $d = 1.88$), and Symbol Search ($z = -1.85$, $d = 1.77$) were severely impaired, as was shown in studies using the WAIS-R.[6] Comprehension and Digit Symbol Coding were closely associated with functional outcome in patients with schizophrenia and first-episode psychosis.[27,30,34] The Information ($z = -0.62$, $d = 0.61$) and Digit Span ($z = -0.49$, $d = 0.50$) subtests were relatively preserved, consistent with a previous study.[21,35] The former subtest is considered to reflect crystallized intelligence, which may not be largely affected by the illness.

Negative symptoms and general psychopathology were moderately correlated with all indices of WAIS-III, as reported in WAIS-R.[36] Although the association between cognitive performance and psychiatric symptoms are consistent in a number of studies, whether there is a causal link between these variables remains unclear.[37] Further investigation may provide a comprehensive model for key linkages to cognition and symptoms.

In summary, the results obtained in this study are consistent with previous studies of the English version of the WAIS-III in patients with schizophrenia.[20,27,33]

Performance in letter–number sequencing

Generally, the WAIS-III subtests are designed in a culture- or language-independent manner and thus require some minor adjustments in translations. Letter–Number Sequencing needs a relatively large modification to equalize the cognitive demands among different language users. The cognitive demand for the manipulation of letters is considered to be more complex in the Japanese *kana* (a two-dimensional structure by vowels and consonants, approximately 50 letters) than in the English alphabet (a one-dimensional sequence of phonemes, 26 letters). Consequently, the Japanese version of Letter–Number Sequencing was modified to reduce the level of difficulty by adding extra practice tasks and restricting the types of letters used in the task.[23] In the current study, the degree of deficits in Letter–Number Sequencing was similar to that reported in a previous study for English users.[33] This result indicates that the Japanese version of Letter–Number Sequencing is adequately adjusted, in terms of difficulty level, compared with the English version.

In addition to the WAIS-III, the Letter–Number Sequencing task is also included in the Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) Consensus Cognitive Battery (MCCB). Although the method of Letter–Number Sequencing in the Japanese version of the MCCB partially differs from that of the Japanese version of the WAIS-III, Japanese *kana* have also been adopted in the MCCB, and the letters have been selected in a similar manner.[38] Kaneda *et al.* preliminarily examined the validity of the Japanese version of the MCCB.[39] Further investigation will be needed to adequately validate the Japanese WAIS-III version of Letter–Number Sequencing.

Limitations

The present study had several limitations that should be noted. Although all of the patients had FSIQ of 70 or more to exclude the possibility of mental retardation, some patients may have had difficulty completing the WAIS-III. Therefore, the results of the current study might only be relevant to a subgroup of patients with relatively higher-level capacities. Cognitive impairment in patients with schizophrenia can be affected by combining multiple antipsychotic medications, negatively influencing cognition in a dose-dependent manner.[40] However, cognitive impairments were also observed in drug-naïve patients with schizophrenia.[41] Therefore, cognitive impairment could be a core feature of patients with schizophrenia. This study was cross-sectional, and treatment response and temporal changes in perfor-

mance were not considered. Future longitudinal studies will be needed to investigate the role of cognitive functioning for the treatment outcome and social functioning. Despite these limitations, this study was the first to examine the performance of schizophrenic patients on the Japanese version of WAIS-III and compare that performance with that of normal controls.

The current study has reported the profile of the performance on the Japanese version of WAIS-III in a sizable number of patients with schizophrenia and in healthy control subjects. Analyses of the results indicate that the profile and degree of cognitive impairment of Japanese patients are similar to those of English-speaking patients.

ACKNOWLEDGMENTS

We would like to thank all of the individuals who participated in this study. This work was supported in part by research grants from the Japanese Ministry of Health, Labor and Welfare (H22-seishin-ippan-001); the Japanese Ministry of Education, Culture, Sports, Science and Technology (MEXT) KAKENHI (22390225-Grant-in-Aid for Scientific Research (B), 23659565-Grant-in-Aid for Challenging Exploratory Research and 221S0003-Grant-in-Aid for Scientific Research on Innovative Areas [Comprehensive Brain Science Network]); and the Japan Foundation for Neuroscience and Mental Health. Additionally, this work was supported by the Strategic Research Program for Brain Sciences (Development of Biomarker Candidates for Social Behavior) of the Ministry of Education, Culture, Sports, Science, and Technology, Japan. No additional external funding was received for this study. The funders had no role in the study design, data collection and analyses, decision to publish, or preparation of the manuscript. All authors declare that they had no conflict of interest.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Subtests descriptions and indices of the Wechsler Adult Intelligence Scale-III.
Table S2. Correlations between clinical variables and Wechsler Adult Intelligence Scale-III subtests in patients with schizophrenia.

CORTEX 58 (2014) 23–26

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Letter to the editor

Genetic risk variants of schizophrenia associated with left superior temporal gyrus volume

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ARTICLE INFO

Article history:
Received 11 January 2014
Revised 4 April 2014
Accepted 21 May 2014
Published online 4 June 2014

Schizophrenia is a common and complex psychiatric disease with a high estimated heritability of approximately 80% (Sullivan, Kendler, & Neale, 2003), and hundreds of common single-nucleotide polymorphisms (SNPs) are weakly implicated in the pathogenesis of schizophrenia (Purcell et al., 2009). Gray matter volume (GM) in brain also has an estimated heritability of approximately 60–90% in healthy subjects (Thompson et al., 2001) and reduced GM volumes in patients with schizophrenia have been frequently reported (Chan, Di, McAlonan, & Gong, 2011). A single polygenic

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http://dx.doi.org/10.1016/j.cortex.2014.05.011

schizophrenia score (PSS), which was calculated by combining the additive effects of thousands of common independent SNPs weakly associated with schizophrenia explained approximately 3% of the variance in liability for schizophrenia in independent subjects (Purcell et al., 2009). A recent study found that PSS predicts the total brain (TB) and white matter volumes (WM) but not the GM, explaining approximately 5% of the variance in the TB and WM (Terwisscha van Scheltinga et al., 2013). However, it remains unclear whether PSS affects variation in specific GM. Therefore, we investigated the effect of PSS on GM, using (i) voxel-based morphometry (VBM) and (ii) VBM-based region of interest (ROI) methods.

For PSS, the odds ratios for genome-wide SNP data were calculated in a discovery Japanese genome-wide association study (JPN_GWAS) sample including 560 patients with schizophrenia and 548 healthy subjects (Ikeda et al., 2011). On the basis of the genomic-control adjusted *p*-values in an allele-wise association analysis from the discovery sample, nominally associated alleles at the following liberal significance threshold (P_T) were selected: $P_T \leq .1$, $P_T \leq .2$, $P_T \leq .3$, $P_T \leq .4$, and $P_T \leq .5$. Of 67,315 independent SNPs remained after pruning, the numbers of SNPs at each P_T are as follows; $P_T \leq .1$ ($n = 7{,}332$), $P_T \leq .2$ ($n = 14{,}294$), $P_T \leq .3$ ($n = 21{,}205$), $P_T \leq .4$ ($n = 27{,}921$), and $P_T \leq .5$ ($n = 34{,}523$). These data were used to calculate individual PSSs in our target sample of 160 patients with schizophrenia and 378 healthy subjects. The structural images in the target sample were acquired using a 1.5T GE Magnetic Resonance Imaging (MRI) scanner, and the MRI images were processed using the VBM8 toolbox in Statistical Parametric Mapping 8 (SPM8). Detailed information regarding the subjects and methods is provided in the Supplementary Materials and Methods and Table S1. Written informed consent was obtained from all subjects after the procedures had been fully explained. This study was performed in accordance with the World Medical Association's Declaration of Helsinki and was approved by the Research Ethical Committee of Osaka University.

First, to identify brain regions related to PSS based on each threshold, we conducted a whole-brain search in patients with schizophrenia and healthy subjects using a multiple regression model in SPM8. Age, gender and diagnosis were included as covariates. As we found a marginal interaction between diagnosis and PSS on the left superior temporal gyrus (STG), an area of the brain reported to have reduced GM in high-risk individuals and first-episode and chronic schizophrenia patients (Chan et al., 2011) (a maximum of $T = 4.44$ and $P_{FWE} = .075$ at $P_T \leq .5$) (Fig. S1), we next performed separate whole-brain searches to examine the effects of PSS in patients with schizophrenia and healthy subjects. In the patients, PSS was significantly negatively correlated with the local GM in the left STG at the different P_T-values at the whole-brain corrected level ($P_{FWE} < .05$, a maximum of $T = 5.04$ and $P_{FWE} = .012$ at $P_T \leq .3$) (Fig. 1). Higher PSSs were associated with smaller left STG volumes. The STG was the only region showing the association. Such effects were similarly found at the $P_T \leq .2$ ($T = 4.75$, $P_{FWE} = .037$) to $P_T \leq .5$ ($T = 4.73$, $P_{FWE} = .040$) threshold levels, indicating that many more SNPs based on threshold levels more lenient than $P_T \leq .2$ are predictive of

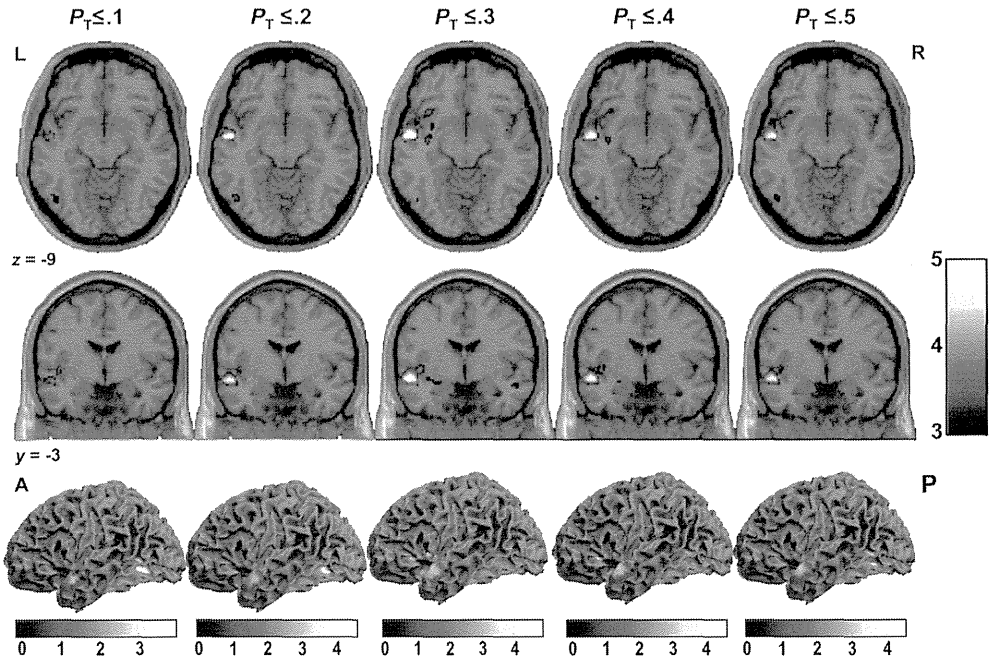

Fig. 1 – Impacts of polygenic scores on gray matter volume in patients with schizophrenia. The effects of PSS based on each threshold ($P_T \leq .1$, $P_T \leq .2$, $P_T \leq .3$, $P_T \leq .4$, and $P_T \leq .5$) on the gray matter volume are shown according to the t values showed by the colored bars. The most significant region of PSS association was in the left superior temporal gyrus (Talairach coordinates of peak voxel: −50, −3, −9). The anatomical localizations are displayed on the axial (upper line) and coronal (middle line) sections of a normal MRI spatially normalized to the Montreal Neurological Institute template. *Z* and *y* represent the *z* and *y* coordinates in Talairach space. The surface-rendered view (lower line) of the brain region correlating with PSS is shown. L, left; R, right; A, anterior; P, posterior.

reduced STG volumes. When including the number of nonmissing SNPs, PANSS scores, duration of illness, or antipsychotic dosage as covariates in the VBM analysis, the effects of PSS on the region remained significant ($P_{FWE} < .05$). In contrast, there was no effect of the score on the GM in healthy subjects ($P_{FWE} > .05$).

The STG is involved in auditory processing, the perception of emotions in facial stimuli, and social cognition (Bigler et al., 2007; Radua et al., 2010). To confirm whether the effect at voxel level on the initial VBM analyses is accepted in the larger structural and functional region, we secondly investigated the effects of PSS on calculated total left STG volumes in patients with schizophrenia and healthy subjects using a multiple linear regression model, with the number of nonmissing SNPs as a covariate using PASW18.0 software. Consistent with the VBM results and expected from them, the ROI analysis revealed that the PSS were significantly negatively correlated with the total left STG volume at all different P_T-values (a maximum $R^2 = .032$, $p = .0090$, at $P_T \leq .2$) in the patients (Fig. S2), whereas there was no effect of the score on the region in the controls ($p > .13$). The PSS explained approximately 3.2% of the variance in the total left STG in the patients with schizophrenia, and the effects of PSS on the region reached a peak at $P_T \leq .3$ in the VBM and $P_T \leq .2$ in the ROI analyses. To examine whether there is a strong association of SNPs with the total left STG, we subsequently conducted a GWAS of the region in the same target samples of patients with schizophrenia. We did not observe any association at a widely used benchmark for genome-wide significance ($p > 5.0 \times 10^{-8}$, Figs. S3–S4 and Table S2).

Although Ikeda et al. (2011) reported that there was a significant correlation of the PSSs between the Japanese and UK samples, there are likely many unique risk variants included in the PSS derived in the Japanese dataset. As the genes comprising the PSS in this study are not identical to those in the MRI study of Terwisscha van Scheltinga et al. (2013), we additionally attempted to replicate the association between PSS and TB and WM (Fig. S5). The PSS were marginally negatively correlated with the TB at the $P_T \leq .5$ ($R^2 = .0035$, $p = .049$) and GM at different P_T-values (a maximum $R^2 = .0073$, $p = .015$, at $P_T \leq .5$), whereas there was no effect of the score on the WM ($p > .05$). The reason why we failed to replicate the associations may be caused by false-negative results due to a small number of discovery samples and/or difference of ethnicities between present and previous studies. We used liberal thresholds ($P_T = .1$ to .5) to obtain PSS according to prior studies (Ikeda et al., 2011; Purcell et al., 2009). However, it was more liberal compared to previous study of Terwisscha van Scheltinga et al. (2013) ($P_T = .002$ to .4). The thresholds we used were so liberal as to likely include a large number of false positives.

Substantially larger controls participated in this study. However, there was a lack of the association in the group. As demographic variables in our samples did not match between healthy subjects and patients with schizophrenia, we matched controls to patients for age and sex and additionally performed the VBM analysis, by removing healthy subjects from the total samples. However, the lack of association in the control group did not change. We considered two reasons for the lack of association; 1) The PSS may be related to a genetic architecture of patients with schizophrenia but not controls because the PSS were scores derived from risk of schizophrenia. 2) The association that we detected in patients may result from a false-positive finding due to small samples.

Our findings suggest that a set of SNPs weakly associated with schizophrenia may have an accumulative effect on the brain structure of patients, but not controls. However, our findings should be carefully interpreted because there has not been enough evidence for the heritability of brain structures in unaffected siblings (Birnbaum & Weinberger, 2013). It is interesting to note that the STG is the only brain region showing significant association with the PSS in our schizophrenia dataset, even though structural imaging studies of patients with schizophrenia have identified other brain regions that show volume differences between patients and controls, and that no associations were found in normal subjects, a considerably larger sample. This selective association with the STG and only in the patients was not expected and must be viewed as preliminary pending further replication.

Acknowledgments

The authors report no conflict of interest. We would like to thank all the individuals who participated in this study. This work was supported by research grants from the Japanese Ministry of Health, Labor and Welfare (H22-seishin-ippan-001), KAKENHI, 22390225-Grant-in-Aid for Scientific Research (B), 23659565-Grant-in-Aid for Challenging Exploratory Research and Grant-in-Aid for Scientific Research on Innovative Areas (Comprehensive Brain Science Network) from the Japanese Ministry of Education, Culture, Sports, Science and Technology (MEXT), and the Japan Foundation for Neuroscience and Mental Health. The authors declare no competing financial interests.

Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.cortex.2014.05.011.

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