Reverse Primer: TGATCTGGGCTGCTTGTTCAT, Reporter: CTGCGTTATC-CAGCTCAT) and a primer of the housekeeping gene human glyceraldehyde-3phosphate dehydrogenase (GAPDH, 4326317E), mouse GAPDH (4352339E) and human TATA-box binding protein (TBP, Hs99999910_ml) all purchased from Applied Biosystems (Foster City, CA). Negative control reactions were carried out with "no RNA" samples. The real time PCR reactions ran at 50 °C for 2 min, at 95 °C for 10 min and in 40 or 45 cycles changing between 95 °C for 15 s and 60 °C for 1 min. A standard amplification curve was made by serial dilution of a "standard" pooled cDNA sample in each plate. The mean value of triplicate of each sample was normalized to the standard curve. Then, the values of CADPS2 and CADPS2ΔExon3 from each sample were normalized to those of GAPDH.

2.5. Statistical analyses

Data analyses were performed with SPSS software (Version 11, SPSS Japan, Tokyo, Japan). Effect of age, brain pH, postmortem interval (PMI), and freezer storage time on each brain analysis was assessed by Pearson's correlations (Table 2). Variables showing significant correlations were included as covariates in the main analysis. Levene's test was used to assess the equality of variances across diagnostic group. Analysis of covariance (ANCOVA) was used to identify overall effects of diagnosis and significant main effects of diagnosis were investigated by planned post hoc contrasts. In the blood sample analyses, CADPS2 expression levels were converted to 10-log scale before statistical analysis in order to obtain a normal distribution (Castensson et al., 2005). The effect of diagnosis on blood CADPS2 expression was assessed by ANCOVA with sex and age as covariates after Levene's test. The effect of diagnosis on blood CADPS2ΔExon3 expression was assessed by logistic regression, controlling for sex and age as covariates. The effect of risperidone on CADPS2 expression in mice brain was assessed by student's *t*-test after F-test.

3. Results

3.1. CADPS2 expression levels in the postmortem brain (BA6)

We first analyzed the effects of age, brain pH, postmortem interval (PMI), and freezer storage time (FST) on each expression analysis (Table 2). Brain pH was significantly correlated with GAPDH expression levels or raw CADPS2 expression levels. PMI also tended to be correlated with GAPDH expression levels or raw CADPS2 expression levels. If the effects were analyzed separately within each diagnostic group, no significant correlation was detected.

CADPS2 expression levels normalized to GAPDH expression levels (CADPS2/GAPDH) in each sample are shown in Fig. 1A. ANCOVA with brain pH as covariates detected a significant effect of diagnosis on CADPS2/GAPDH levels ($F=3.4$, $df=3$, $p=0.025$) and post hoc test detected a significant difference between schizophrenia and control groups ($p=0.03$). Even if PMI was added as another covariate, the difference was significant ($p=0.002$). There was no significant difference between bipolar disorder and controls or between depression and controls. There was no significant correlation between CADPS2/GAPDH levels and lifetime dose of antipsychotic drugs (data not shown). There was a significant effect of diagnosis on GAPDH expression levels ($F=3.4$, $df=3$, $p=0.023$, Fig. 1B). GAPDH levels in the control group was significantly higher than that of schizophrenia ($p=0.012$), bipolar disorder ($p=0.009$) or major depression group ($p=0.013$). Raw CADPS2 levels did not differ among the diagnostic groups ($F=1.0$, $df=3$, $p=0.38$, Fig. 1C). There was a significant correlation between GAPDH expression levels and raw CADPS2 expression levels (Pearson's correlation 0.69, $p<0.001$, Fig. 1D).

We compared relative CADPS2 expression levels among diagnostic groups using another endogenous control, TATA-box binding protein (TBP), and obtained similar result (Fig. S1, this experiment was done after uncode the sample). ANCOVA with brain pH as covariates detected a significant effect of diagnosis on CADPS2/TBPlevels ($F=3.3$, $df=3$, $p=0.027$) and post hoc test detected a significant

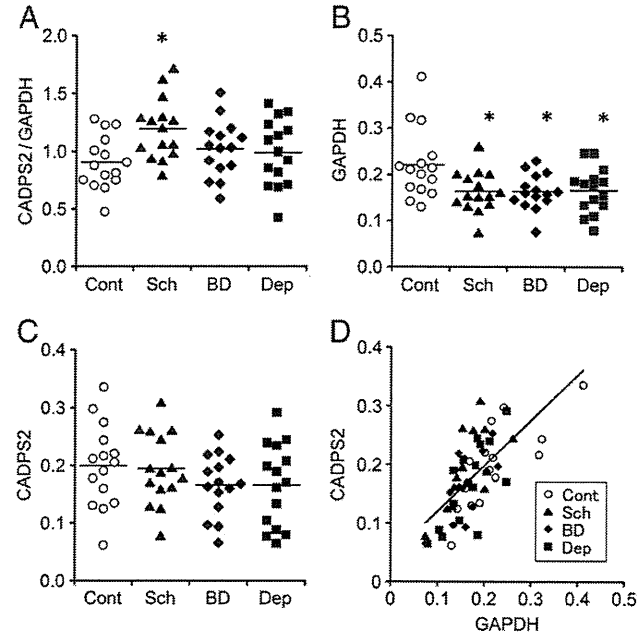

Fig. 1. CADPS2 expression levels in the postmortem brains of psychiatric disorder. (A) CADPS2 expression levels normalized by GAPDH levels. Scatter plots display the variability and differences in the CADPS2 mRNA expression levels normalized by each GAPDH expression levels. A crossbar on each scatter plot represents mean expression levels for each group. (B) GAPDH expression levels (C) Raw CADPS2 expression levels. (D) Correlation between GAPDH levels and raw CADPS2 levels. Cont, control; Sch, schizophrenia; BD, Bipolar Disorder; and Dep, Depression. *, statistically significant difference ($p<0.05$).

Table 2
The effect of age, pH, postmortem interval, and freezer storage time on each brain expression analysis.

		GAPDH	CADPS2	ΔExon3	CADPS2/GAPDH	ΔExon3/G APDH	ΔExon3/C ADPS2
Age	Pearson's	0.013	−0.13	0.19	−0.18	0.088	0.27
	P	0.92	0.34	0.37	0.16	0.51	0.041
pH	Pearson's	0.36	0.26	0.25	0.031	0.12	0.090
	p	0.005	0.048	0.058	0.81	0.38	0.50
Post mortem Pearson's interval (hours)	−0.23	−0.22	−0.13	−0.040	0.039	0.15	
	P	0.076	0.098	0.30	0.76	0.77	0.25
Freezer storage time (months)	Pearson's	−0.22	−0.034	−0.041	0.21	0.12	0.052
	P	0.092	0.80	0.75	0.11	0.36	0.69

ΔExon3, CADPS2ΔExon3; and Pearson's, Pearson's correlation.

difference between schizophrenia and control groups ($p=0.019$). Even if PMI was added as another covariate, the difference was significant ($p=0.012$).

With respect to CADPS2ΔExon3/GAPDH level (Fig. 2A), the effect of age was detected in the control group (Pearson's correlation 0.58, $p=0.023$) and the effect of pH was detected in the bipolar disorder group (Pearson's correlation 0.60, $p=0.018$). ANCOVA with age and brain pH as covariates detected the marginal effect of diagnosis ($F=2.8$, $df=3$, $p=0.050$) and the mean expression level was significantly increased in the schizophrenia group, compared to the control group ($p=0.030$). When the ratio of CADPS2ΔExon3 to raw (total) CADPS2 expression levels was compared, the ratio was similar in the 4 diagnostic groups ($F=1.1$, $df=3$, $p=0.36$, Fig. 2B). Neither the effect of diagnosis on raw CADPS2ΔExon3 levels was observed ($F=1.9$, $df=3$, $p=0.15$, Fig. 2C). There was a significant correlation between GAPDH expression levels and raw CADPS2ΔExon3 expression levels (Pearson's correlation 0.66, $p<0.001$, Fig. 2D).

3.2. Cortical CADPS2 expression after chronic antipsychotic treatment in mice

To see whether antipsychotics alter the mRNA expression of CADPS2, we measured the CADPS2 levels in the frontal cortex of mice, following chronic treatment with an antipsychotic risperidone. Oral administration of risperidone (2.5 mg/kg, $n=15$ for the controls and 16 for the risperidone group) for 3 weeks did not alter CADPS2 expression ($F=1.5$, $df=29$, $p=0.61$).

3.3. CADPS2 expression in blood sample

Since we observed increased expression of CADPS2 in postmortem brains of schizophrenia patients, we then examined whether such an alteration exists in peripheral blood samples. The CADPS2/GAPDH expression levels were converted to 10-logarism before statistical analyses to obtain normal distribution. The mean (Standard deviation) CADPS2 expression level was 0.17 (1.29) in the control group and 0.32 (1.46) in the schizophrenia group. ANCOVA controlling for age and sex did not detect the significant effect of diagnosis on CADPS2/GAPDH level ($F=1.67$, $df=1$, $p=0.20$). We also measured CADPS2ΔExon3 levels in the blood samples. Compared to brain samples, the expression levels were quite low and could not detect in the majority of samples. Thus, we defined "expressed" when at least 2 tubes in triplet analyses of each sample were detected until 45 cycles. CADPS2ΔExon3 expression was detected in 36 of 318 control samples (ratio = 0.11), and 21 of 121 schizophrenia samples (ratio = 0.17). There was no significant effect of diagnosis on CADPS2ΔExon3 expression by the logistic regression analysis controlling for age and sex (odds ratio 1.51, [95% CI 0.80–2.86], $p=0.21$). Even when men and women were examined separately, there was no significant difference between the patients and controls for each sex (data not shown).

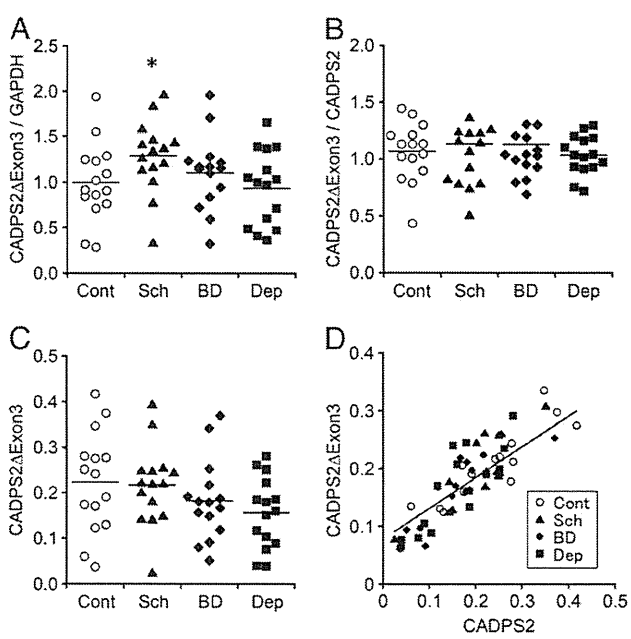

Fig. 2. CADPS2ΔExon3 expression levels in the postmortem brains of psychiatric disorder. (A) CADPS2ΔExon3 expression levels normalized by GAPDH levels. Scatter plots display the variability and differences in the CADPS2ΔExon3 mRNA expression levels normalized by each GAPDH expression levels. A crossbar on each scatter plot represents mean expression levels for each group. (B) CADPS2ΔExon3 levels normalized to each total CADPS2 expression levels. (C) Raw CADPS2ΔExon3 expression levels. (D) Correlation between GAPDH expression levels and raw CADPS2ΔExon3 expression levels. Cont, control; Sch, schizophrenia; BD, Bipolar Disorder; and Dep, Depression. *, statistically significant difference ($p<0.05$).

4. Discussion

4.1. Main findings

In the present study, we analyzed the expression of CADPS2 mRNA in the postmortem brains (BA6) of psychiatric patients (schizophrenia, major depression and bipolar disorder) and controls. A significant increase in the CADPS2 expression was detected in the brains of the schizophrenia group, compared to the control group. No change was detected in other disease groups. While a CADPS2 splice variant, CADPS2ΔExon3 showed a non-significant increase in the schizophrenia group, its ratio to the total CADPS2 levels was not different from the control group. Chronic risperidone treatment did not alter the CADPS2 levels in mice brain. We also analyzed CADPS2 or CADPS2-ΔExon3 expression levels in the blood samples of schizophrenia and control subjects; however, the levels were not significantly different between the two groups.

4.2. Brain analysis

4.2.1. Drug effect

A large number of gene expressions in the brain are affected by antipsychotic treatments (Girgenti et al.,; Mehler-Wex et al., 2006; Thomas, 2006). Therefore, the observed increase in CADPS2 mRNA in the schizophrenia group could be the result of antipsychotic treatment. However, our results did not support this assumption because the CADPS2 levels did not correlate to life-time antipsychotic dose and chronic risperidone treatment in mice did not alter CADPS2 expression on their cortices, although caution is required for the interpretation of those results because we don't have data for the latest dose before death and other drugs such as chlorpromazine, haloperidol and clozapine might be used in the patients.

4.2.2. Possible relevance to BDNF secretion, dopamine transmission, and neuropeptide release

Considering that defective BDNF signaling has been suggested in schizophrenia and mood disorders (Angelucci et al., 2005) and that CADPS2 mediates BDNF release in neurons (Sadakata et al., 2004), we initially expected that CADPS2 levels would be decreased in frontal cortex in patients with these psychiatric disorders. However, in our results, CADPS2 levels were not altered in mood disorders but increased in schizophrenia. In addition, the relative levels of defective CADPS2 isoform, CADPS2ΔExon3 were not altered in those disorders. Thus, it is unlikely that altered CADPS2 expression might be a cause of BDNF deficits in schizophrenia. It may be rather a compensatory consequence of reduced BDNF signaling.

CADPS2 also promotes monoamine storage in neurons (Brunk et al., 2009; Liu et al., 2008). CADPS2 is highly expressed in the dopamine-rich brain areas such as ventral tegmental area and substantia nigra of mice brain (Sadakata et al., 2006) and it is reported to interact with dopamine D2 receptor (Binda et al., 2005). Growing evidence has demonstrated increased presynaptic dopamine levels in the striatum of schizophrenia patients (Lyon et al., 2009). If the observed increase in the expression of CADPS2 occurs in the subcortical regions including striatum and midbrain as well as frontal cortex, it might be the cause of hyper-dopamine transmission that reflects psychotic state (Howes et al., 2009).

Furthermore, large dense-core vesicles contain not only neurotrophins and monoamines but also neuropeptides (Salio et al., 2006). Neuropeptides such as endorphins, cholecystokinin (CCK), neurotensin (NT), somatostatin, Neuropeptide Y and neuregulin 1 have been implicated in schizophrenia (Caceda et al., 2007). Especially reduced levels of CCK and NT have been repeatedly reported in the disorder (Caceda et al., 2007), which may have caused compensatory increase in the CADPS2 expression in schizophrenia.

4.3. CADPS2 expression in the blood

4.3.1. CADPS2 expression and diagnosis

Following the report that 4 of 16 patients with autism expressed CADPS2ΔExon3 in peripheral bloods but none in 24 normal subjects (Sadakata et al., 2007b), another group reported that they detected CADPS2ΔExon3 in some control subjects (Eran et al., 2009). Thus we assumed that the ratio of CADPS2ΔExon3 to total CADPS2 rather than whether CADPS2ΔExon3 exists or not is important and therefore we applied quantitative real-time PCR to measure their expression. The pilot experiment in the present study indicated that our quantification method using SuperScript VILO and random-hexamer, was 4 to 8 fold more sensitive than one step real-time PCR using gene specific primers and could detect 10 to 100 clones of CADPS2 or CADPS2-ΔExon3 sequence-containing vector. Compared with the brains, CADPS2 expression was 32 to 128 fold lower in the blood. Unlike in the brain, CADPS2ΔExon3 could not be detected in most blood samples. So we performed qualitative analysis for each subject. As a result, we didn't detect any significant difference in the expression of CADPS2ΔExon3 in the blood between patients with schizophrenia and controls. The CADPS2ΔExon3 was abundantly expressed in the brain and the levels were unchanged across the diagnostic groups. Thus, it is unlikely that the expression or the splicing balance should relate to diseases we analyzed.

5. Conclusion

In conclusion, we found increased mRNA expression of CADPS2 in the postmortem frontal cortex of schizophrenia patients which might have some relevance to dysregulation in the release of dopamine, neurotrophins, and/or neuropeptides in the disorder. This increase was unlikely to be attributable to antipsychotic medication. We also analyzed the CADPS2ΔExon3 in human brains and found that it is abundantly present in the frontal cortex in any diagnostic group. We obtained no evidence for the specific role of the splice variant in schizophrenia or mood disorders. Future research should include the evaluation of CADPS2 expression in other brain areas, and basic studies on the cause and consequence of increased CADPS2 expression.

Supplementary materials related to this article can be found online at doi:10.1016/j.pnpbp.2011.05.004.

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Association of plasma IL-6 and soluble IL-6 receptor levels with the Asp358Ala polymorphism of the IL-6 receptor gene in schizophrenic patients

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ABSTRACT

Recent studies indicate a role of excessive interleukin-6 (IL-6) signaling in the pathogenesis of schizophrenia. A previous study reported a significant association of schizophrenia with the IL-6 receptor (IL-6R) gene Asp358Ala polymorphism, which is known to regulate circulating IL-6 and soluble IL-6R (sIL-6R) levels in healthy subjects. To further examine the influence of the polymorphism in schizophrenic patients, we compared the plasma levels of IL-6 and sIL-6R between schizophrenic patients and healthy controls for each genotype of the Asp358Ala polymorphism. Asp358Ala genotyping and plasma IL-6 level measurements were performed in 104 patients with schizophrenia and 112 healthy controls. Of these participants, 53 schizophrenic patients and 49 controls were selected for the measurement of plasma sIL-6R levels. A two-way factorial analysis of covariance was performed with the transformed plasma levels as the dependent variable, diagnosis and genotype as independent variables, and sex and age as covariates. No significant diagnosis × genotype interaction was observed for IL-6 and sIL-6R levels. The Ala allele of Asp358Ala was significantly associated with higher levels of both IL-6 and sIL-6R. IL-6 levels were significantly elevated in schizophrenic patients compared to those in controls, whereas no significant difference in sIL-6R levels was observed between schizophrenic patients and controls. Our findings suggest that the presence of schizophrenia is associated with elevated IL-6 levels, whereas sIL-6R levels are mainly predetermined by the Asp358Ala genotype and are not associated with the disease status. Increased IL-6 levels without alterations in sIL-6R levels may result in excessive IL-6 signaling in schizophrenia.

1. Introduction

Inflammatory immune processes have been strongly implicated in the etiology of schizophrenia (Watanabe et al., 2010). Elevated serum or plasma levels of interleukin-6 (IL-6) is observed in patients with schizophrenia (Potvin et al., 2008), suggesting a role of excessive IL-6 signaling in the pathogenesis of this disorder. IL-6 binds to the soluble IL-6 receptor (sIL-6R) to form an IL-6/sIL-6R complex that is capable of binding to gp130 in the cellular membrane to mediate intracellular signaling. As membrane-bound IL-6R is expressed selectively on monocytes, neutrophils, T and B lymphocytes, and hepatocytes, other cells require the IL-6/sIL-6R complex for IL-6 signaling. Therefore, it could be inferred that sIL-6R plays an important part in the pathogenesis of schizophrenia.

An increased IL-6 level is one of the most robust findings in the study of inflammatory markers in schizophrenia, as evidenced by a meta-analysis of 19 studies comprising 1219 subjects (Potvin et al., 2008). Furthermore, one study showed a positive correlation between the severity of symptoms and plasma IL-6 levels in antipsychotic-free schizophrenic patients (Pae et al., 2006). However, findings regarding changes in the circulating levels of sIL-6R in patients with schizophrenia have been equivocal. Some studies reported increased sIL-6R levels in patients with schizophrenia (Lin et al., 1998; Maes et al., 1997), whereas one study reported lower sIL-6R levels (Maes et al., 1994). Others reported no significant differences in sIL-6R levels between patients and controls (Maes et al., 1995; Muller et al., 1997; O'Brien et al., 2008). Non-significant effect size estimates were obtained for sIL-6R in a meta-analysis of 7 studies (Potvin et al., 2008).

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Table 1
Subject characteristics.

	Patients with schizophrenia				Healthy controls			
	Asp/Asp	Asp/Ala	Ala/Ala	Statistical test results	Asp/Asp	Asp/Ala	Ala/Ala	Statistical test results
All subjects								
Gender (males/females)	15/17	26/23	14/9	$\chi2 = 1.1$, $p = 0.59$	14/24	31/29	11/3	$\chi2 = 7.3$, $p = 0.03$
Age (years)	38.8 ± 13.9	40.8 ± 12.3	35.3 ± 8.5	$F = 1.64$, $p = 0.20$	41.0 ± 13.0	38.4 ± 13.1	39.2 ± 11.2	$F = 0.48$, $p = 0.62$
Duration of illness (years)	16.7 ± 11.0	17.7 ± 10.7	15.4 ± 8.8	$F = 0.39$, $p = 0.68$				
Duration of treatment (years)	13.0 ± 8.6	15.1 ± 10.1	12.3 ± 7.8	$F = 0.91$, $p = 0.41$				
CP equivalent dose	852 ± 685	993 ± 883	1030 ± 667	$F = 0.44$, $p = 0.65$				
Subjects selected for the measurement of sIL-6R levels								
Gender (males/females)	9/9	9/9	8/9	$\chi2 = 0.04$, $p = 0.98$	6/12	7/10	11/3	$\chi2 = 7.1$, $p = 0.03$
Age (years)	41.2 ± 10.2	40.6 ± 12.1	36.1 ± 7.6	$F = 1.28$, $p = 0.29$	43.5 ± 10.1	37.8 ± 13.3	39.2 ± 11.2	$F = 1.15$, $p = 0.32$
Duration of illness (years)	17.0 ± 7.3	18.1 ± 11.2	16.3 ± 7.7	$F = 0.17$, $p = 0.84$				
Duration of treatment (years)	14.8 ± 8.1	16.4 ± 10.8	12.2 ± 6.2	$F = 1.02$, $p = 0.37$				
CP equivalent dose	922 ± 688	1020 ± 788	1094 ± 746	$F = 0.24$, $p = 0.79$				

Continuous values are shown as mean ± standard deviation, CP: chlorpromazine.

sIL-6R is generated by shedding of the membrane-bound IL-6R. This process is influenced by the single nucleotide polymorphism (SNP) Asp358Ala of the IL-6R gene (rs8192284), which results in an amino acid substitution in the proteolytic cleavage site. The Ala allele of this polymorphism in healthy subjects is known to be strongly associated with higher levels of circulating sIL-6R (Galicia et al., 2004; Rafiq et al., 2007; Reich et al., 2007) and IL-6 (Jiang et al., 2010; Rafiq et al., 2007; Reich et al., 2007). Therefore, possession of the Ala allele may result in constitutively elevated IL-6 signaling.

A previous genetic association study reported a significant association of the Ala allele of the IL-6R Asp358Ala polymorphism with schizophrenia (Sun et al., 2008). It can be hypothesized that the excessive IL-6 signaling associated with Ala alleles may increase the susceptibility to schizophrenia. However, the increased IL-6 levels without significant change in sIL-6R levels in schizophrenic patients (Potvin et al., 2008) could not be explained solely by the increased Ala allele frequency in schizophrenia.

To our knowledge, the possible associations of the IL-6R Asp358Ala polymorphism with circulating sIL-6R and IL-6 levels in patients with schizophrenia have not yet been examined. Further investigation of the influence of this polymorphism in schizophrenia is necessary to elucidate the roles of IL-6 and sIL-6R in this disorder. Thus, we compared the plasma levels of IL-6 and sIL-6R between patients with schizophrenia and healthy controls for each genotype of the Asp358Ala polymorphism.

2. Materials and methods

2.1. Subjects

Asp358Ala genotyping and plasma IL-6 level measurements were performed in 104 patients with schizophrenia (55 men and 49 women; mean age ± standard deviation: 39.0 ± 12.2 years), and 112 healthy controls (56 men and 56 women; age: 39.4 ± 12.8 years), frequency-matched for sex and age. Of these participants, 53 schizophrenic patients (26 men and 27 women; age: 39.4 ± 10.2 years) and 49 controls (24 men and 25 women; age: 40.3 ± 11.6 years), matched for the number of cases and controls for each genotype, were selected for the measurement of plasma sIL-6R levels. All subjects were biologically unrelated Japanese individuals and were recruited from the outpatient clinic of the National Center of Neurology and Psychiatry Hospital, Tokyo, Japan or through advertisements in free local information magazines and by our website announcement. Consensus diagnosis by at least 2 psychiatrists was made for each patient according to the Diagnostic and Statistical Manual of Mental Disorders, 4th edition criteria (American Psychiatric Association, 1994), on the basis of unstructured interviews and information from medical records. All patients were under treatment with antipsychotic medication, of which 25 were receiving inpatient treatment. The average chlorpromazine equivalent dose converted from daily doses of antipsychotics using published guidelines (American Psychiatric Association, 1997; Inagaki et al., 1999) was 957.0 ± 776.7 mg/day (typical antipsychotics, 528.8 ± 747.7 mg/day; atypical antipsychotics, 428.2 ± 458.0 mg/day). The mean age at onset was 21.9 ± 7.1 years, and the mean durations of illness and antipsychotic treatment were 16.9 ± 10.3 and 13.8 ± 9.2 years, respectively. The controls were healthy volunteers with no current or past histories of psychiatric treatment and were screened using the Japanese version of the Mini International Neuropsychiatric Interview (Otsubo et al., 2005; Sheehan et al., 1998) by a research psychiatrist to eliminate the possibility of any axis I psychiatric disorders. Participants were excluded if they had prior medical histories of central nervous system diseases or severe head injury or if they met the criteria for substance abuse or dependence or mental retardation. The self-reports indicated that none of the participants suffered from any inflammatory or infectious diseases at the time of assessment. The study protocol was approved by the ethics committee at the National Center of Neurology and Psychiatry, Japan. After describing the study, written informed consent was obtained from every subject.

2.2. Genotyping

Genomic DNA was prepared from venous blood according to standard procedures. The Asp358Ala polymorphism was genotyped using the TaqMan 5′-exonuclease allelic discrimination assay.

Table 2
Distribution of the Asp358Ala variants.

	Genotype count (frequency)						Allele count (frequency)					HWE	
	N	Asp/Asp	Asp/Ala	Ala/Ala	$\chi2$ (df = 2)	*P* value	N	Asp	Ala	$\chi2$ (df = 1)	*P* value	$\chi2$ (df = 1)	*P* value
Patients	104	32 (0.31)	49 (0.47)	23 (0.22)	3.52	0.17	208	113 (0.54)	95 (0.46)	1.80	0.18	1.69	0.19
Controls	112	38 (0.34)	60 (0.54)	14 (0.13)			224	136 (0.61)	88 (0.39)			0.27	0.61

HWE: Hardy–Weinberg equilibrium.

Table 3
IL-6 and sIL-6R levels in patients with schizophrenia and healthy controls.

		Patients with schizophrenia			Healthy controls			Mann–Whitney's test
		N	Mean	S.D.	N	Mean	S.D.	
IL-6 (pg/ml)	Both genders	104	1.80	0.99	112	1.43	0.56	$U = 4296$, $P = 0.00087$
	Males	55	1.87	1.00	56	1.53	0.64	$U = 1261$, $P = 0.10$
	Females	49	1.73	0.99	56	1.33	0.44	$U = 879$, $P = 0.0015$
sIL-6R (pg/ml)	Both genders	53	453	127	49	463	136	$U = 1273$, $P = 0.86$
	Males	26	461	124	24	511	137	$U = 244$, $P = 0.18$
	Females	27	446	132	25	416	120	$U = 279$, $P = 0.28$

IL-6: interleukin-6; sIL-6R: soluble interleukin-6 receptor, S.D.: standard deviation.

The thermal cycling conditions for polymerase chain reaction were as follows: 1 cycle at 95 °C for 10 min followed by 50 cycles of 92 °C for 15 s and 60 °C for 1 min. The allele-specific fluorescence was measured with ABI PRISM 7900 Sequence Detection Systems (Applied Biosystems, Foster City, CA). Ambiguous genotype data were not included in the analysis.

2.3. Laboratory methods

Plasma samples were collected between 1100 and 1200 h in tubes containing ethylenediaminetetraacetic acid. The samples were stored at −80 °C until they were assayed. Plasma levels of IL-6 were determined by the BD™ Cytometric Bead Array system using the BD FACSCanto II system (BD Biosciences, San Jose, CA), according to the manufacturer's instructions. Data analysis was performed using the FCAP Array software (BD Biosciences). Plasma levels of sIL-6R were measured using a commercially available immunoassay kit (Quantikine; R&D Systems, Inc., Minneapolis, MN), according to the manufacturer's instructions.

2.4. Statistical analysis

Deviations of genotype distributions from Hardy–Weinberg equilibrium (HWE) were assessed using the χ^2 test for goodness of fit. Genotype and allele distributions were compared between patients and controls by using the χ^2 test for independence. Comparison of continuous variables was analyzed using one-way analysis of variance or Mann–Whitney's test, according to the data distribution. Non-continuous variables were analyzed using χ^2 tests. To determine the possible interaction effects between diagnosis and Asp358Ala genotype, two-way factorial analysis of covariance (ANCOVA) was performed with the transformed plasma levels as the dependent variable, diagnosis and genotype as independent variables, and sex and age as covariates. Because the plasma IL-6 and sIL-6R levels were not normally distributed, the aligned rank transformation method was used to transform the data prior to conducting ANCOVA (Wobbrock et al., 2011). Post-hoc comparisons between genotypes were Bonferroni-corrected for multiple comparisons. Correlations between continuous values were assessed using Spearman's correlation coefficient. Statistical analyses were performed using the Statistical Package for the Social Sciences version 11.0 (SPSS Japan, Tokyo, Japan). All statistical tests were two-tailed, and $P < 0.05$ indicated statistical significance.

3. Results

The subject characteristics for each genotype of the IL-6R Asp358Ala polymorphism are shown in Table 1. Table 2 shows the genotype and allele frequencies of the Asp358Ala polymorphism in the patients and controls. The genotype distribution did not significantly deviate from HWE in the patient or control group. No significant differences were found in the Asp358Ala genotype or allele distribution between the patients and controls. However, analysis under the recessive genetic model suggested a trend of higher frequencies of the Ala/Ala genotype in schizophrenic patients ($\chi^2 = 3.51$, $P = 0.061$). Table 3 shows the overall mean plasma IL-6 and sIL-6R levels. IL-6 levels were significantly higher in patients with schizophrenia compared to those in the controls. In contrast, no significant difference in sIL-6R levels was observed between patients and controls. The associations of IL-6 and sIL-6R levels with clinical characteristics are shown in Table 4. The IL-6 and sIL-6R levels were significantly higher in healthy men than in healthy women. Age exhibited significant correlations with IL-6 levels in the patients and controls. The duration of illness and treatment also exhibited significant correlations with IL-6 levels in the patients; however, after controlling for age, these correlations with IL-6 levels were not significant any more (duration of illness: $\rho = 0.10$, $P = 0.30$; duration of treatment: $\rho = 0.16$, $P = 0.11$). The chlorpromazine equivalent dose was not significantly correlated with IL-6 or sIL-6R levels.

The plasma IL-6 and sIL-6R levels of patients with schizophrenia and healthy controls in each genotype of the Asp358Ala polymorphism are shown in Fig. 1. Table 5 presents the results of the two-way ANCOVA performed with the transformed plasma levels

Table 4
Associations of IL-6 and sIL-6R levels with clinical characteristics.

	Statistical test results			
	Patients with schizophrenia		Healthy controls	
	IL-6 ($N = 104$)	sIL-6R ($N = 53$)	IL-6 ($N = 112$)	sIL-6R ($N = 49$)
Gender (males/females)	$U = 1264$, $p = 0.59$	$U = 328$, $p = 0.68$	$U = 1170$, $p = 0.020$[a]	$U = 169$, $p = 0.009$[a]
Age (years)	$\rho = 0.32$, $p < 0.0009$[b]	$\rho = -0.19$, $p = 0.18$	$\rho = 0.32$, $p = 0.0006$[b]	$\rho = -0.15$, $p = 0.32$
Duration of illness (years)	$\rho = 0.33$, $p = 0.0008$[b]	$\rho = 0.029$, $p = 0.84$		
Duration of treatment (years)	$\rho = 0.33$, $p = 0.0007$[b]	$\rho = 0.012$, $p = 0.94$		
CP equivalent dose	$\rho = 0.042$, $p = 0.67$	$\rho = 0.039$, $p = 0.78$		

IL-6: interleukin-6; sIL-6R: soluble interleukin-6 receptor.
Age, duration of illness, and duration of treatment were significantly correlated with IL-6 levels in patients with schizophrenia (Spearman's rank correlation test).
[a] The IL-6 and sIL-6R levels were significantly higher in healthy males compared to healthy females (Mann–Whitney's test).
[b] Age was significantly correlated with IL-6 levels in healthy controls (Spearman's rank correlation test).

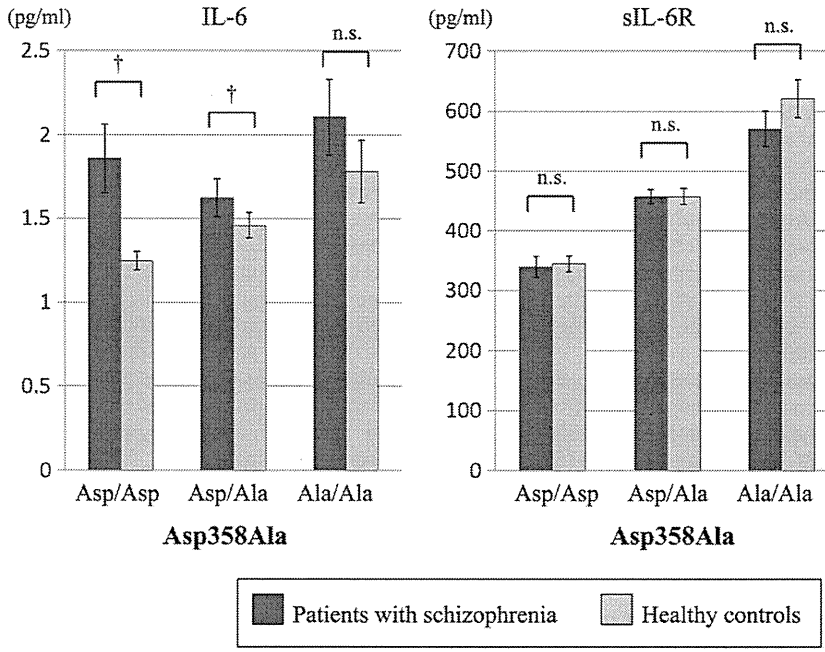

Fig. 1. The mean plasma levels of IL-6 and sIL-6R in patients with schizophrenia and controls are shown for each genotype of the IL-6R Asp358Ala. Error bars indicate the standard error of the means. †Significant difference between schizophrenia and controls (Mann–Whitney's test, $p < 0.05$); n.s.: no significant difference; IL-6: interleukin-6; sIL-6R: soluble interleukin-6 receptor.

as the dependent variable, diagnosis and the genotype as independent variables, and sex and age as covariates. Because the recessive genetic model suggested a trend of higher frequencies of the Ala/Ala genotype in schizophrenic patients, we used the recessive model as well as the co-dominant model in the ANCOVA analysis. Regarding IL-6 levels, two-way ANCOVA revealed significant effect of diagnosis and of Asp358Ala genotype with no significant diagnosis × genotype interaction. In the co-dominant model, Bonferroni's post hoc tests revealed a significant difference between Asp/Ala and Ala/Ala genotypes and a trend toward significance between Asp/Asp and Ala/Ala genotypes. Regarding sIL-6R levels, two-way ANCOVA demonstrated a significant effect of the genotype but not of the diagnosis and no significant diagnosis × genotype interaction. Post hoc tests revealed significant differences across genotypes.

4. Discussion

The results show that the Ala allele of the IL-6R Asp358Ala polymorphism is associated with higher plasma levels of IL-6 and sIL-6R in both patients with schizophrenia and healthy controls. The overall IL-6 levels were elevated in schizophrenic patients with no significant change in sIL-6R levels. Consistent with our findings, previous studies of healthy subjects also showed that higher levels of sIL-6R and IL-6 levels were associated with the Ala allele of Asp358Ala (Galicia et al., 2004; Rafiq et al., 2007; Reich et al., 2007). A two-way factorial ANCOVA revealed no significant diagnosis × genotype interaction for IL-6 and sIL-6R levels. Taken together, our findings suggest that the IL-6 levels of schizophrenic patients are elevated compared to those of their healthy counterparts irrespective of the Asp358Ala genotype and that the increase in IL-6 levels without alterations in sIL-6R levels results in excessive IL-6 signaling in schizophrenic patients.

Consistent with the previous finding that the Ala allele was associated with susceptibility to schizophrenia (Sun et al., 2008), our results indicated a trend of higher frequencies of the Ala/Ala genotype in schizophrenic patients. Taken together with the finding that the Ala allele was associated with higher IL-6 and sIL-6R levels, our results lend support to the evidence that excessive IL-6 signaling could cause neurodevelopmental abnormalities

Table 5
The results of the two-way ANCOVA.

	Diagnosis		Genotypes of Asp358Ala		Interaction		Post hoc tests between genotypes					
							Asp/Asp vs Asp/Ala		Asp/Ala vs Ala/Ala		Ala/Ala vs Asp/Asp	
	F value	*P* value	*F* value	*P* value	*F* value	*P* value	*F* value	*P* value[a]	*F* value	*P* value[a]	*F* value	*P* value[a]
Co-dominant model (Asp/Asp vs Asp/Ala vs Ala/Ala)												
Plasma IL-6 levels	7.03	0.01	3.96	0.02	1.07	0.35	0.74	1.0	6.59	0.03	5.14	0.08
Plasma sIL-6R levels	0.36	0.55	105	<0.0001	0.25	0.78	65.4	<0.0001	40.4	<0.0001	237	<0.0001
Recessive model (Asp/Asp + Asp/Ala vs Ala/Ala)												
Plasma IL-6 levels	6.74	0.010	8.11	0.0048	1.00	0.32						
Plasma sIL-6R levels	0.03	0.86	82.5	<0.0001	0.63	0.43						

ANCOVA was performed with the transformed plasma levels as the dependent variable, the diagnosis and the genotype as independent variables, and sex and age as covariates.

ANCOVA: analysis of covariance; IL-6: interleukin-6; sIL-6R: soluble interleukin-6 receptor.

[a] Bonferroni-corrected *P* values.

associated with schizophrenia (Gilmore et al., 2004; Marx et al., 2001). To conclude the possible genetic association between the Asp358Ala polymorphism and schizophrenia, further studies in a larger sample size are required.

Recent studies have shown that IL-6 signaling functions as a risk factor for the development of schizophrenia. Behrens et al. (2008) reported that IL-6 production by neurons induces NAPDH oxidase and subsequently leads to the degeneration of parvalbumin, the dysfunction of which is considered one of the key features in the brain pathology of schizophrenia (Lewis et al., 2005). In an animal study, injection of IL-6 alone into mothers was sufficient to cause schizophrenia-like behavioral abnormalities in the offspring, whereas anti-IL-6 antibody blocked the development of such abnormalities (Smith et al., 2007).

The two-way ANCOVA revealed that IL-6 levels in schizophrenic patients were elevated compared to those in controls with no significant diagnosis × genotype interaction, suggesting that some schizophrenia-related factors other than the Asp358Ala polymorphism are associated with elevated IL-6 levels. Conversely, similar sIL-6R levels between schizophrenic patients and the controls suggest that sIL-6R levels are mainly predetermined by the IL-6R Asp358Ala polymorphism and are unrelated to the disease status.

Because IL-6 levels are affected by a number of environmental and genetic factors, various conditions may be attributed to increased IL-6 levels in schizophrenic patients. For example, acute mental stress could induce a significant increase in plasma IL-6 levels (von Kanel et al., 2006). Therefore, stressful life events triggering the exacerbation of psychotic symptoms, as well as psychological stress caused by the onset of the disease, may have contributed in the elevation of IL-6 levels. Stimulation of the peripheral immune system can result in activation of microglia in the central nervous system. According to the recent microglia hypothesis of schizophrenia (Monji et al., 2009), activated microglia release pro-inflammatory cytokines and free radicals, thereby causing neuronal degeneration, white matter abnormalities, and decreased neurogenesis associated with the pathophysiology of schizophrenia. Genetic variations other than the one examined in this study may also play a role in the regulation of IL-6 levels in schizophrenic patients. For example, a well-known functional polymorphism, −174G/C of the IL-6 gene, known to affect the circulating levels of IL-6 (Bonafe et al., 2001; Fishman et al., 1998; Olivieri et al., 2002), was found to be associated with schizophrenia in a Caucasian population (Paul-Samojedny et al., 2010). Although this SNP is reported as monomorphic in the HapMap Japanese population, there may still be other unknown genetic polymorphisms attributable to higher IL-6 levels in patients with schizophrenia.

Previous studies have suggested the influence of antipsychotic treatment on the IL-6 and sIL-6R levels in schizophrenic patients. Xu et al. (1994) reported higher plasma IL-6 levels in schizophrenic patients taking antipsychotic medication than in neuroleptic-free patients. Loffler et al. (2010) reported that treatment with clozapine increased plasma IL-6 levels. In line with this, van Kammen et al. (1999) reported that exacerbation after haloperidol withdrawal resulted in decreased plasma IL-6 levels. In contrast, a larger study by Zhang et al. (2004) demonstrated no significant influence of risperidone or haloperidol on serum IL-6 levels in their schizophrenic patients. Regarding sIL-6R, one study reported a significant decrease of the serum levels after neuroleptic treatment (Muller et al., 1997). The present study obtained no evidence of association between the antipsychotic dose and the levels of IL-6 and sIL-6R, which is consistent with Zhang et al. (2004). Although the duration of antipsychotic treatment and the duration of illness correlated significantly with IL-6 levels, which is consistent with some previous studies (Ganguli et al., 1994; Kim et al., 2000), these significant correlations appeared to reflect the influence of age, as the correlations disappeared after controlling for age. Further studies are required to draw conclusions as to the possible influence of antipsychotic medication on IL-6 and sIL-6R levels.

Some limitations must be considered when interpreting the results of this study. First, the cross-sectional design did not allow for any definitive conclusions regarding whether the increased IL-6 levels in schizophrenic patients were premorbid or the result of illness onset. Secondly, only the IL-6R Asp358Ala polymorphism was examined in the present study. Future studies should examine gene-wide tagging polymorphisms of IL-6 and IL-6R genes and their associations with the circulating levels of IL-6 and sIL-6R in schizophrenic patients. Thirdly, we did not assess inflammation markers such as C-reactive protein. As the presence or absence of inflammatory diseases was based only on self-reports, the results may have been affected by unrecognized inflammatory processes in some participants.

In conclusion, the Ala allele of the IL-6R Asp358Ala polymorphism was found to be associated with higher plasma levels of both IL-6 and sIL-6R in schizophrenic patients and controls. The overall IL-6 levels were elevated in schizophrenic patients with no significant change in sIL-6R levels, supporting the role of excessive IL-6 signaling in schizophrenia. The finding that the IL-6 levels in schizophrenic patients were elevated with no significant diagnosis × genotype interaction suggests that some schizophrenia-related factors, other than the effects of the polymorphism, are associated with increased IL-6 levels in schizophrenic patients. In contrast, the sIL-6R levels are mainly predetermined by the polymorphism and are not influenced by the disease status.

Contributors

D.S., C.W., and H.K. designed the study and D.S. wrote the draft of the manuscript. D.S., H.H., T.T., K.H., and M.O. screened the study participants using the Mini International Neuropsychiatric Interview (M.I.N.I.). C.W. measured the IL-6 levels and D.S. measured the sIL-6R levels. D.S. performed the genotyping. D.S. and H.H. undertook the statistical analysis. H.K. supervised the data analysis and writing of the paper. M.I., K.A., T.H., and N.A. also supervised the writing of the paper and gave critical comments on the manuscript. All authors contributed to and have approved the final manuscript.

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Conflict of interest

The authors report no conflicts of interest.

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Relationships between season of birth, schizotypy, temperament, character and neurocognition in a non-clinical population

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Character

ABSTRACT

While schizophrenia has been associated with a slight excess of winter/early spring birth, it is unclear whether there is such an association in relation to schizotypal personality traits. Season of birth has also been reported to relate to temperament and character personality dimensions and cognitive functioning. Moreover, non-clinical schizotypy has been shown to be associated with mild cognitive impairment, although its precise nature is yet to be elucidated. Here we examined the relationships between season of birth, schizotypal traits, temperament and character, and cognitive function. Four hundred and fifty-one healthy adults completed the Schizotypal Personality Questionnaire (SPQ). The Temperament and Character Inventory (TCI) and a neuropsychological test battery consisting of full versions of the Wechsler Memory Scale-Revised and the Wechsler Adult Intelligence Scale-Revised, and the Wisconsin Card Sorting Test, were also administered to most of the participants. The total SPQ score of those born in winter was significantly higher than that of the remaining participants. Season of birth was not significantly associated with any of the TCI dimensions or cognitive test results. Significant but mild relationships between higher SPQ scores and lower scores on some aspects of IQ were observed. These results support the notion that schizotypy and schizophrenia are neurodevelopmental conditions on the same continuum.

1. Introduction

Schizotypy refers to latent personality organization that indicates an individual's proneness to psychosis and, in particular, to schizophrenia (Raine et al., 1995). The dimensional model of schizotypal personality posits that the degree of schizotypal traits varies on a continuum where normality lies on one extreme, non-clinical to clinical schizotypy in the middle, and clinically expressed schizophrenia on the opposite extreme (Claridge, 1985, Kendler et al., 1991). In accord with this view, several lines of evidence have demonstrated a range of abnormalities in relation to schizotypal personality that are intrinsically similar to those seen in schizophrenia (Siever and Davis, 2004), such as neurophysiological (O'Driscoll et al., 1998; Kiang and Kutas, 2005), neurocognitive (Lenzenweger and Korfine, 1994; Gooding et al., 2006), neuroendocrinological (Mitropoulou et al., 2004; Hori et al., 2011) and neuroimaging (Folley and Park, 2005; Hori et al., 2008a) abnormalities.

On the other hand, little has been done to identify early-life origins that predict greater schizotypal traits in adulthood, although it is now well established that schizophrenia is to some extent associated with such origins. Among a variety of factors that originate early in life, season of birth has been extensively studied in the epidemiology of schizophrenia, with most studies reporting a slight (approximately 10%) excess of winter/early spring birth in patients with schizophrenia (reviewed in Torrey et al., 1997; Davies et al., 2003). To better understand the etiology of and risk factors for schizophrenia in light of the dimensional model of this disorder, it would be of importance to investigate the possible effect of season of birth on schizotypal traits. To our knowledge, there have been four studies that shed light on this topic. Reid and Zborowski (2006), using a sample of undergraduate students from the Northeast United States, found significantly higher scores on the Perceptual-Aberration and Magical-Ideation scale, developed by Chapman and his colleagues, in individuals born in spring than those born in the other seasons. Kirkpatrick et al. (2008) found in undergraduates from the middle-eastern United States that June/July birth was associated with a proxy measure for the deficit syndrome, which was defined by combining the Chapman's Social Anhedonia Scale and the Beck Depression Inventory. Lahti et al. (2009), using a large cohort consisting of approximately 5000 people born in Northern Finland in 1966, showed that winter/autumn birth, in addition to several other early-life characteristics, predicted augmented negative schizotypal traits (as assessed with the Chapman's Physical Anhedonia Scale) in women. More recently, Cohen and Najolia (2011) screened more than 25,000 university students from the southern United States and showed that season of birth of individuals who scored extremely high on the Schizotypal Personality

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Questionnaire (SPQ, Raine, 1991) did not differ from that of those whose scores were less than one standard deviation above the mean. Taken together, findings to date on the association between season of birth and schizotypal traits have not been consistent and thus await further investigations.

Season of birth has also been reported to be associated with other personality traits, including temperament/character dimensions (Chotai et al., 2001, 2002, 2009; Kamata et al., 2009). Studies including ours have shown that temperament and character of patients with schizophrenia are markedly different from those of healthy controls, such that these patients, compared to healthy controls, exhibit lower novelty seeking, self-directedness and cooperativeness and higher harm avoidance (Guillem et al., 2002; Hori et al., 2008b). In addition, temperament and character personality dimensions have been shown to correlate with schizotypal personality (Daneluzzo et al., 2005; Bora and Veznedaroglu, 2007).

There also exists evidence that certain aspects of cognitive function could be affected by season of birth (Martin et al., 2004; McGrath et al., 2006; Gobet and Chassy, 2008). Interestingly, some studies have found an association between superior cognitive performance and winter/spring birth (McGrath et al., 2006; Gobet and Chassy, 2008), which might be counterintuitive to the fact that neurocognitive impairments are a core feature of schizophrenia. Another line of research has looked at the relationships between personality traits and neurocognitive functioning. As for the association between schizotypy and neurocognition, a number of studies in psychometrically identified schizotypes have, albeit not entirely unequivocally, found this condition to be associated with compromised functioning in various cognitive domains, including sustained attention (Lenzenweger, 2001; Gooding et al., 2006), spatial working memory (Park and McTigue, 1997), and executive function as assessed with the Wisconsin Card Sorting Test (WCST, Lenzenweger and Korfine, 1994; Daneluzzo et al., 1998). Although the relationship between temperament/character and cognition is less well studied, several studies have examined this relation among various psychiatric conditions. For instance, Bergvall et al. (2003) reported that higher self-directedness and cooperativeness were significantly associated with less errors in an attentional set-shifting task in a sample consisting of incarcerated offenders, correctional officers and medical aides. Similarly, Smith et al. (2008) showed that self-directedness and cooperativeness were strongly positively correlated with working memory and crystallized intelligence in non-psychotic siblings.

In this context, the present study sought to explore the associations of season of birth with schizotypal personality traits, temperament/character dimensions and cognitive function. In addition, we also attempted to clarify the relationships between schizotypal traits, temperament/character and cognitive functioning.

2. Methods

2.1. Participants

Participants were 451 Japanese adults (age range: 19–73 years) who resided in the western part of Tokyo. They were recruited between 2006 and 2010 from the community through advertisements in free local magazines and our website announcement, and also from hospital staff and their associates through flyers and by word of mouth. At the first visit, participants were interviewed using the Japanese version of the Mini-International Neuropsychiatric Interview (Sheehan et al., 1998; Otsubo et al., 2005) by a research psychiatrist, and only those who demonstrated no current Axis I psychiatric disorders were enrolled in the study. In addition, those individuals who demonstrated one or more of the following conditions in a non-structured interview performed by an experienced psychiatrist were excluded: past or current regular contact to psychiatric services, having a history of regular use of psychotropics or substance abuse/dependence, presenting other obvious self-reported signs of past primary psychotic and mood disorders, and having a prior medical history of central nervous system disease or severe head injury. The present experiments on our participants were conducted in accordance with the Declaration of Helsinki. After the nature of the study procedures had been fully explained, written informed consent was obtained from all participants. The study was approved by the ethics committee of the National Center of Neurology and Psychiatry, Japan.

2.2. Personality assessment

Personality was assessed with two self-report questionnaires, which were distributed to each participant at our laboratory. He/she was allowed to take as much time as needed to complete the questionnaires, then returned them to us by mail or by hand.

2.2.1. Schizotypal Personality Questionnaire (SPQ)

The SPQ (Raine, 1991) is a 74-item validated self-report questionnaire with a "yes/no" response format that incorporates DSM-III-R (American Psychiatric Association, 1987) criteria for a diagnosis of schizotypal personality disorder (SPD). All items endorsed "yes" are scored 1 point. The questionnaire consists of nine subscales, which have been found to load onto three factors: cognitive–perceptual (comprising the "ideas of reference," "odd beliefs/magical thinking," "unusual perceptual experiences" and "suspiciousness/paranoid ideation" subscales), interpersonal ("social anxiety," "no close friends," "constricted affect," and "suspiciousness/paranoid ideation"), and disorganized ("eccentric/odd behavior and appearance" and "odd speech") factors (Raine et al., 1994). We employed the Japanese version of the SPQ (Fujiwara, 1993), reliability and validity of which had been demonstrated to be similar to those of the original version (Raine, 1991). All participants completed this questionnaire.

2.2.2. Temperament and Character Inventory (TCI)

TCI (Cloninger et al., 1993) is a 240-item (including 14 items which are not analyzed) self-report questionnaire; each item requires a true/false answer. The term temperament refers to automatic emotional reactions to subjective experiences that may be genetically transmitted and therefore stable over time. Four dimensions of temperament are distinguished: novelty seeking, harm avoidance, reward dependence, and persistence. Novelty seeking, harm avoidance, and reward dependence were assumed to relate to dopaminergic, serotonergic, and noradrenergic neurotransmission, respectively (Cloninger, 1987). The term character refers to concepts pertaining to the individual, focusing on personal differences in intentions, decisions and values. Three dimensions of character are distinguished: self-directedness, cooperativeness, and self-transcendence. The reliability and validity of the original American version of the TCI have been established (Cloninger et al., 1993; Svrakic et al., 1993). The Japanese version of the TCI translated and validated by Kijima et al. (1996, 2000) was used in the present study. Of the total 451 participants, 443 (98.2%) completed the TCI.

2.3. Cognitive test battery

A neurocognitive test battery, comprising full versions of the Wechsler Memory Scale-Revised (WMS-R, Wechsler, 1987; Sugishita, 2001) and the Wechsler Adult Intelligence Scale-Revised (WAIS-R, Wechsler, 1981; Shinagawa et al., 1990), and the Wisconsin Card Sorting Test (WCST, Heaton, 1981; Kashima et al., 1987), was administered. Using these tests, we measured verbal memory, visual memory, general memory, attention/concentration, delayed recall (WMS-R), verbal intelligence quotient (IQ) and its six subtests, performance IQ and its five subtests, full-scale IQ (WAIS-R), and executive function (WCST). Outcome measures of the WCST comprised the number of categories achieved, total errors, and perseverative errors. Perseverative errors included two types of perseveration: inappropriate repetitions of a response that sticks to the previously achieved category (i.e., perseverative errors of Milner type; PEM) and repetitions of the immediately preceding incorrect response (i.e., perseverative errors of Nelson type; PEN). The WMS-R, WAIS-R and WCST were completed by 415 (92.0%), 379 (84.0%) and 408 (90.5%) of the total 451 participants, respectively.

2.4. Analysis

Information on the date of birth was obtained for all participants. Based on this information, two types of the four seasons were considered; one criterion ("Traditional criterion") was the traditional Japanese definition of the four seasons (i.e., March, April and May into spring; June, July and August into summer; September, October and November into autumn; December, January and February into winter), and the other criterion ("Astronomical criterion") was the definition of the four seasons taking account of the equinoxes (i.e., March 22–June 21 into spring; June 22–September 21 into summer; September 22–December 21 into autumn; December 22–March 21 into winter). We herein considered these two definitions of the four seasons because previous studies investigating the association between season of birth and schizophrenia/schizotypy have employed different definitions, such that some have used the Traditional criterion (e.g., Takei et al., 1995; Kunugi et al., 1997) while others the Astronomical criterion (e.g., Reid and Zborowski, 2006; Cohen and Najolia, 2011). In the present report, the Traditional criterion was used unless otherwise specified and the Astronomical criterion was used for confirmation purpose.

Averages are reported as means ± Standard deviation (S.D.). Categorical variables were compared using the χ^2 test. The *t*-test or analysis of variance (ANOVA) was used to examine differences between groups. Pearson's *r* was used to examine correlations. Partial correlation analysis was used to examine correlations, controlling for potentially confounding variables. The analysis of covariance (ANCOVA) was performed to compare scores controlling for confounders as defined below. Statistical significance was set at two-tailed $p<0.05$. Analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 18.0 (SPSS Japan, Tokyo).

3. Results

3.1. Demographic characteristics and schizotypal personality

Of the 451 participants, 112 were male and 339 female. Mean age of the participants was 45.2 ± 15.2 years. Mean years of education was 14.9 ± 2.6. Numbers of participants who were born in spring, summer, autumn and winter (based on the "Traditional criterion") were 98, 113, 106 and 134, respectively. There were no significant differences in age [$F(3,447) = 0.10$, $p = 0.96$] or sex [$\chi^2(3) = 6.02$, $p = 0.11$] distribution depending on season of birth. Numbers of participants who were born in January, February, March, April, May, June, July, August, September, October, November and December were 42, 53, 41, 31, 26, 38, 32, 43, 32, 46, 28 and 39, respectively. There were no significant differences in age [$F(11,439) = 1.52$, $p = 0.12$] or sex [$\chi^2(11) = 12.5$, $p = 0.12$] distribution depending on month of birth. Mean scores of the cognitive–perceptual factor, interpersonal factor, disorganized factor, and the total SPQ score were 4.0 ± 4.2, 6.5 ± 5.6, 3.4 ± 3.2, and 12.9 ± 10.0, respectively. Males scored significantly higher than females in the interpersonal factor ($t = 2.4$, d.f. = 162, $p = 0.016$), but they did not differ in the other two factors or the total SPQ score (all $p > 0.3$). Age showed a significant negative correlation with the disorganized factor ($r = -0.22$, $p < 0.001$) and total SPQ score ($r = -0.12$, $p = 0.009$), while years of education did not show any significant correlations with the SPQ indices (all $p > 0.3$). Therefore, we decided to control for age and sex in the ANCOVA model as these variables could potentially confound the association between season of birth and schizotypal traits.

3.2. Correlations between schizotypal traits and temperament/character dimensions

Table 1 shows the partial correlations, controlling for age and sex, between the SPQ indices and seven dimensions of the TCI. The SPQ indices were positively correlated with harm avoidance and self-transcendence while they were negatively correlated with novelty seeking, reward dependence, self-directedness and cooperativeness.

3.3. Association of season of birth with schizotypal trait, temperament/character, and cognitive function

Fig. 1 shows the relationship between birth season (based on the "Traditional criterion") and the total SPQ score in the whole sample. Mean total SPQ scores of those born in spring, summer, autumn, and winter were 12.6 ± 9.3, 12.5 ± 8.9, 11.0 ± 9.7, and 15.0 ± 11.4, respectively. The ANCOVA, controlling for age and sex, revealed that the total SPQ score was significantly different between these four seasonal groups [$F(3,445) = 3.6$, $p = 0.014$]; post-hoc analysis with Bonferroni correction revealed that the total SPQ score was significantly higher in those individuals born in winter than those born in autumn (estimated mean difference = 4.09, 95% confidence interval = 0.69 to 7.50, $p = 0.009$). In addition, the total SPQ score was significantly higher in the winter-born individuals than in the remaining individuals (15.0 ± 11.4 vs. 12.0 ± 9.3; $t = 2.66$, d.f. = 211, $p = 0.008$). Taking into account that Lahti et al. (2009) found the significant association between winter/autumn birth and schizotypal traits only in women, the similar analyses, including the ANCOVA with age as a covariate and the t-test, were repeated separately for males and females. For males, the ANCOVA revealed that the main effect of season of birth was not significant [$F(3,107) = 0.86$, $p = 0.46$]. The total SPQ score was not significantly different between winter-born men and the remaining men (15.1 ± 11.4 vs. 13.1 ± 10.7; $t = 0.85$, d.f. = 110, $p = 0.40$). For females, the ANCOVA revealed that the total SPQ score was significantly different between these four seasonal groups [$F(3,334) = 3.9$, $p = 0.009$]; post-hoc analysis with Bonferroni correction revealed that the total SPQ score was significantly higher in those individuals born in winter than those born in autumn (estimated mean difference = 4.64, 95% confidence interval = 0.86 to 8.43, $p = 0.007$). The total SPQ score was significantly higher in the winter-born women than in the remaining women (14.9 ± 11.4 vs. 11.6 ± 8.7; $t = 2.94$, d.f. = 162.5, $p = 0.009$).

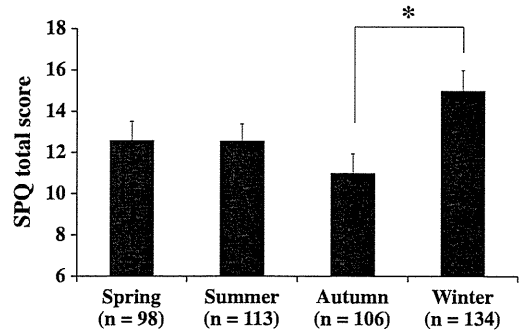

Fig. 1. Comparisons of the total SPQ score between individuals born in each of the four seasons (based on the Traditional criterion). *$p < 0.05$ (according to the ANCOVA with post-hoc analysis with Bonferroni correction).

When the Astronomical criterion was used, mean total SPQ scores of those born in spring, summer, autumn, and winter were 12.3 ± 8.3, 12.4 ± 9.7, 11.6 ± 9.0, and 14.8 ± 11.8, respectively. The similar ANCOVA again revealed the significant difference in the total SPQ score between these four seasonal groups [$F(3,445) = 3.0$, $p = 0.029$]; post-hoc analysis with Bonferroni correction revealed that the total SPQ score was significantly higher in those individuals born in winter than those born in autumn (estimated mean difference = 3.53, 95% confidence interval = 0.15 to 6.91, $p = 0.035$). The total SPQ score was significantly higher in the winter-born individuals than in the remaining individuals (14.8 ± 11.8 vs. 12.1 ± 9.0; $t = 2.65$, d.f. = 206, $p = 0.018$).

Supplementary Fig. 1 shows the relationship between birth month and the total SPQ score in the whole sample. This figure also presents the deviance of temperature of each month from the annual average temperature in Tokyo (calculated as |"average temperature of individual month" − "annual average temperature"|) averaged between 1970 and 2000 according to statistics reported by the Japan Meteorological Agency; thus, peaks and valleys of this line graph indicate severe and mild climates, respectively. In this figure we can

Table 1
Partial correlations between schizotypal personality and temperament/character dimensions, controlling for age and sex ($n = 443$).

	Novelty seeking	Harm avoidance	Reward dependence	Persistence	Self-directedness	Cooperativeness	Self-transcendence
Cognitive–perceptual factor	−0.08	0.15**	−0.19***	0.11*	−0.38***	−0.19***	0.37***
Interpersonal factor	−0.24***	0.53***	−0.37***	−0.03	−0.58***	−0.37***	0.02
Disorganized factor	−0.03	0.28***	−0.24***	0.01	−0.47***	−0.24***	0.14**
Total SPQ score	−0.16**	0.40***	−0.31***	0.02	−0.56***	−0.30***	0.21***

Each figure represents partial correlation coefficient (r) (d.f. = 439).
* $p < 0.05$ (2-tailed).
** $p < 0.01$ (2-tailed).
*** $p < 0.001$ (2-tailed).

Table 2
Partial correlations of SPQ three factors and total score with neurocognitive test results, controlling for age and sex.

	Verbal memory[a]	Visual memory[a]	General memory[a]	Attention/ concentration[a]	Delayed recall[a]	Information[b]	Digit span[b]	Vocabulary[b]	Arithmetic[b]	Comprehension[b]
Cognitive–perceptual factor	−0.05	−0.06	−0.03	−0.08	−0.03	−0.05	0.01	0.00	−0.02	−0.06
Interpersonal factor	−0.08	−0.02	−0.07	−0.02	−0.02	−0.05	−0.02	−0.04	−0.06	−0.14**
Disorganized factor	−0.05	−0.06	−0.06	−0.08	−0.04	−0.03	−0.06	−0.01	−0.08	−0.11*
Total SPQ score	−0.07	−0.06	−0.07	−0.06	−0.03	−0.06	−0.02	−0.02	−0.07	−0.13*

Each figure represents partial correlation coefficient (r).
*$p<0.05$; **$p<0.01$ (2-tailed).
[a] $n=415$, d.f. = 411.
[b] $n=379$, d.f. = 375.
[c] $n=408$, d.f. = 404.

see the general tendency that the higher bars correspond to the peaks of the line graph, indicating that those born in more severe climates, i.e., January, August and December, demonstrated greater schizotypal traits. Indeed, correlation between the temperature deviance from the annual average and the SPQ total score was significant ($r=0.10$, $p=0.028$).

Supplementary Figs. 2–5 show the relationships of four birth seasons (based on the "Traditional criterion") with seven dimensions of the TCI (Fig. S2), five memory indices as measured by the WMS-R (Fig. S3), three IQ indices as measured by the WAIS-R (Fig. S4), and four indices of executive function as measured by the WCST (Fig. S5). The ANCOVA controlling for age and sex showed that the four seasonal groups did not differ in any of the seven TCI dimensions (all $p>0.1$) or in any of the neurocognitive test results (i.e., indices of the WMS-R, WAIS-R, and WCST) (all $p>0.05$). These relationships remained non-significant when the same analyses were repeated using the "Astronomical criterion" of season.

3.4. Relationships of schizotypal trait with cognitive function

Table 2 presents correlations between the SPQ indices and cognitive test results, controlling for age and sex. Comprehension and digit symbol subtests of the WAIS-R showed significant, albeit weak (correlation coefficients ranging from −0.11 to −0.14), negative correlations with the interpersonal and disorganized factors and the total SPQ score, while there were no significant correlations of memory indices of the WMS-R or executive function indices of the WCST with any indices of the SPQ.

3.5. Relationships of temperament/character with cognitive function

Table 3 shows correlations between the seven TCI dimensions and cognitive test results, controlling for age and sex. Harm avoidance was significantly negatively, while self-directedness and cooperativeness were significantly positively, associated with several memory and IQ measures including verbal memory, general memory, and a number of IQ subtests, especially those belonging to verbal IQ. Persistence was associated with better performance on the WCST.

4. Discussion

We can summarize the main findings as follows. A significant association of schizotypal traits with winter birth was found, while season of birth was not significantly associated with any of the TCI dimensions or neurocognitive test results. We also found significant but mild relationships between higher SPQ scores and lower performance in two subtests of IQ.

The majority of studies in schizophrenia, in particular those conducted in the Northern Hemisphere, have reported that there is a winter (or winter/early spring) birth excess (Torrey et al., 1997; Davies et al., 2003), and such birth seasonality has also been found in Japanese patients (Kunugi et al., 1997; Tatsumi et al., 2002). However, this season of birth effect has not been replicated in the Southern Hemisphere (reviewed in McGrath and Welham, 1999). Taking into account that the countries in the Southern Hemisphere where these studies have been conducted are closer to the equator than those in the Northern Hemisphere, one of the possible explanations for this discrepancy is considered to relate to the latitude, i.e., the higher the latitude, the greater the season of birth effect (McGrath and Welham, 1999; Davies et al., 2003). The observed association between winter birth and greater SPQ score in our non-clinical population is compatible with the evidence in schizophrenia obtained in the Northern Hemisphere, supporting the dimensionality between schizotypal personality and schizophrenia. The present result of the significant association in women also accords with a recent study in Finland showing that winter/autumn birth in women predicts augmented schizotypal traits in adulthood (Lahti et al., 2009). For men, however, the absence of a significant association between winter birth and SPQ score in the present study might be due to the type II error since the mean total SPQ score was higher in winter-born men than in the remaining men (i.e., 15.1 ± 11.4 vs. 13.1 ± 10.7). On the other hand, as mentioned earlier, findings from the three other precedent studies that have investigated the season of birth effect on

Table 3
Partial correlations between TCI seven dimensions and neurocognitive test results, controlling for age and sex.

	Verbal memory[a]	Visual memory[a]	General memory[a]	Attention/ concentration[a]	Delayed recall[a]	Information[b]	Digit span[b]	Vocabulary[b]	Arithmetic[b]	Comprehension[b]
Novelty seeking	0.06	0.00	0.07	−0.09	−0.01	0.05	−0.05	0.07	−0.01	0.14**
Harm avoidance	−0.15**	0.01	−0.14**	−0.01	−0.09	−0.04	−0.01	−0.05	−0.08	−0.14**
Reward dependence	0.03	0.05	0.03	−0.04	0.03	−0.10	0.04	−0.08	−0.05	−0.02
Persistence	−0.01	−0.04	0.02	0.00	0.00	−0.01	0.04	0.04	0.01	0.06
Self-directedness	0.13**	0.02	0.12*	0.06	0.12*	0.08	0.07	0.09	0.14**	0.20***
Cooperativeness	0.12*	0.03	0.10*	0.01	0.07	0.03	0.09	0.09	0.00	0.13*
Self-transcendence	−0.03	−0.07	−0.01	−0.01	−0.02	−0.06	0.04	0.03	−0.04	−0.04

Each figure represents partial correlation coefficient (r).
*$p<0.05$; **$p<0.01$; ***$p<0.001$ (2-tailed).
[a] $n=409$, d.f. = 405.
[b] $n=376$, d.f. = 372.
[c] $n=402$, d.f. = 398.

Similarities[b]	Picture completion[b]	Picture arrangement[b]	Block design[b]	Object assembly[b]	Digit symbol[b]	Verbal IQ[b]	Performance IQ[b]	Full-scale IQ[b]	WCST category[c]	WCST total errors[c]	WCST PEM[c]	WCST PEN[c]
−0.10	0.02	0.02	−0.04	−0.04	−0.08	−0.06	−0.03	−0.05	0.01	−0.03	0.02	−0.02
−0.09	−0.03	0.02	−0.06	0.02	−0.11*	−0.10	−0.03	−0.08	0.00	−0.02	0.02	−0.05
−0.07	−0.03	0.00	−0.01	0.01	−0.14**	−0.08	−0.04	−0.07	−0.01	−0.02	0.02	0.01
−0.10	−0.02	0.02	−0.04	0.00	−0.13**	−0.10	−0.04	−0.08	0.00	−0.03	0.02	−0.02

schizotypy are not in line with the present one; two studies (Reid and Zborowski, 2006; Kirkpatrick et al., 2008) reported different relationships between season of birth and schizotypy from the present one, and one study (Cohen and Najolia, 2011) did not find any such significant association although this study observed that no less than 60% of individuals within the schizotypy group reporting a diagnosis of schizophrenia or prior hospitalization had been born during winter months. Given that the four studies (Reid and Zborowski, 2006; Kirkpatrick et al., 2008; Lahti et al., 2009) including the present one that found any significant relationship between season of birth and schizotypy were conducted in higher latitude regions of 35° to 60° whereas the study by Cohen and Najolia (2011) was conducted in a subtropical region with relatively mild winters, this discrepancy may have stemmed, at least partly, from the differences in latitude, as in the aforementioned schizophrenia literature.

Possible mechanisms underlying the association between season of birth and schizophrenia have been speculated, and a variety of seasonal factors, such as ambient temperature, viral infections (e.g., influenza) and vitamins, have been implicated (reviewed in Tochigi et al., 2004). Among these, the ambient temperature might be a promising factor that explains the winter/early spring birth excess in schizophrenia patients (Tochigi et al., 2004). We found that January, August and December, when the ambient temperature is either extremely hot or cold, were the top three months in terms of the increased likelihood of giving birth to those with greater schizotypal traits. In keeping with this, a summer birth excess in patients with deficit schizophrenia has been reported (Kirkpatrick et al., 2002; Messias et al., 2004).

Concerning the association between season of birth and temperamental/character personality dimensions, previous studies have reported significant effects of season of birth on certain temperament and/or character dimensions. Chotai et al. (2001) found in a Swedish adult cohort that those individuals born during February to April were significantly more likely than those born from October to January to have high novelty seeking among women and to have high persistence among men. Chotai et al. (2009) obtained a similar finding in a Finnish adult sample that women born in summer had significantly higher novelty seeking than women born in winter. By contrast, Chotai et al. (2002) observed in Swedish adolescents that novelty seeking was significantly higher for females born from October to January as compared to females born otherwise. Kamata et al. (2009) found in a Japanese adult sample that higher ambient temperature at birth month was related to higher scores of self-directedness and persistence in females. These previous findings, though somewhat variable, suggest that certain temperamental/character dimensions could be affected by season of birth. In the present study, however, we did not find any significant association between season of birth and temperament/character dimensions. These varied findings may be due to sample characteristics (e.g., age, ethnicity, and geographic area), and more studies are therefore required to clarify this association.

Several lines of evidence have suggested that season of birth is also associated with cognitive function, although the findings are again mixed. Some studies reported that winter/spring birth was associated with better scores on the Wechsler Intelligence performance and full-scale IQs at age 7 (McGrath et al., 2006) and with higher proportion of expert chess players (Gobet and Chassy, 2008), whereas others found superior intellectual functioning in individuals born in summer (Gotoda, 1995; Bibby et al., 1996). In contrast, we did not observe any significant associations between season of birth and cognitive functioning. It should be noted, however, that the previous studies and the present one employed quite different samples and cognitive measures to assess participants' cognitive abilities.

We examined the correlation between schizotypal personality and temperament/character, and found a number of significant correlations between the three factors pertaining to schizotypy and temperament/character dimensions (Table 1). The results are largely consistent with those from two previous studies (Daneluzzo et al., 2005; Bora and Veznedaroglu, 2007), both of which demonstrated that cognitive–perceptual and disorganized factors of the SPQ correlated negatively with self-directedness and positively with self-transcendence, and that interpersonal factor of the SPQ correlated positively with harm avoidance and negatively with self-directedness.

With respect to the association between personality and cognitive function, we found significant but weak negative correlations between SPQ scores and comprehension and digit symbol subtests

Similarities[b]	Picture completion[b]	Picture arrangement[b]	Block design[b]	Object assembly[b]	Digit symbol[b]	Verbal IQ[b]	Performance IQ[b]	Full-scale IQ[b]	WCST category[c]	WCST total errors[c]	WCST PEM[c]	WCST PEN[c]
0.00	0.07	0.11*	0.01	0.09	−0.03	0.04	0.07	0.06	0.01	0.01	0.04	0.06
−0.03	−0.07	0.04	−0.07	−0.03	−0.08	−0.09	−0.05	−0.09	−0.06	0.06	0.02	−0.02
0.02	0.01	0.09	0.01	−0.02	0.00	−0.04	0.02	−0.01	0.00	0.02	0.04	0.04
0.04	0.08	0.01	0.02	−0.10*	0.07	0.05	0.00	0.03	0.12*	−0.13**	−0.11*	−0.08
0.16**	0.01	−0.06	0.10	−0.02	0.18***	0.18***	0.06	0.15**	0.05	−0.04	−0.03	0.02
0.15**	0.11*	0.02	0.11*	0.08	0.05	0.12*	0.09	0.13*	0.00	0.00	0.04	0.04
−0.02	0.06	0.03	0.06	−0.02	−0.01	−0.01	0.00	0.01	0.03	−0.02	0.00	0.03

of the WAIS-R. As described earlier, previous studies have demonstrated that schizotypal traits, even at a non-clinical level, are associated with mild impairments in cognitive domains including sustained attention, spatial working memory, and executive functioning. What is more relevant to the present finding is that recent studies have reported the association between psychometrically defined schizotypy and mild impairments in some aspects of IQ (Matheson and Langdon, 2008; Noguchi et al., 2008). In addition, the present finding may correspond well to the evidence that processing speed as measured by the digit symbol coding task is the most severely impaired cognitive domain in schizophrenia (Dickinson et al., 2007). In the present study, temperament/character dimensions, in particular harm avoidance, self-directedness and cooperativeness, were associated with performances on several cognitive domains including memory and IQ. In line with the present finding, previous studies have reported significant associations between higher self-directedness and cooperativeness on the one hand and better cognitive performance on the other (Bergvall et al., 2003; Smith et al., 2008).

Findings reported here should be considered in the context of a number of limitations. First, the present finding on the association between season of birth and schizotypy obtained in Japan may not be easily extrapolated to another location, particularly to the southern hemisphere. Second, given the high number of the correlational analyses between personality measures and cognitive indices (Tables 2 and 3), some stringent measures may have been needed to correct for the multiple testing. Nevertheless, we would like to note that our significant findings on the associations between specific personality traits and mildly impaired cognitive functioning were generally in harmony with previous ones (Bergvall et al., 2003; Matheson and Langdon, 2008; Noguchi et al., 2008; Smith et al., 2008), as discussed above. Third, since women were overrepresented in our sample, it is possible that the present findings are applicable only to women. The fourth limitation could be the relatively low mean total SPQ score of 12.9 (S.D. = 10.0) of our sample, given that the mean total SPQ scores of non-clinical populations have been reported to be around 20 in the majority of prior studies, most of which were conducted in Western countries. This discrepancy is likely to have been derived from ethnic differences (Western vs. Japanese/Asian). Indeed, mean total SPQ scores in healthy Japanese populations have been consistently shown to be relatively low, ranging from 8.1 to 12.9 (Someya et al., 1994; Wang et al., 2004; Hori et al., 2008a; Noguchi et al., 2008; Takahashi et al., 2010). Another plausible explanation for the discrepancy in SPQ scores between these samples might be the difference in mean age of participants, i.e., college students in most of the prior studies vs. adults in the present study. In a study of Chen et al. (1997), for example, mean total SPQ scores of Taiwanese adolescents and adults were 20.6 and 12.9, respectively. Thus, the present sample could be deemed representative of non-clinical Asian adults. Finally, although we targeted the adult population, most of the previous studies investigating the correlates of non-clinical schizotypy have targeted students. From the standpoint of the risk of developing schizophrenia, students may be a better suited population; however, we believe that our dimensional approach has its own merits. To further explore the dimensional model and to identify the risk population for the development of psychosis, future studies that investigate the association between season of birth and schizotypal traits among clinical populations (e.g., SPD patients with and without family history of schizophrenia) as well as student populations are needed.

In summary, the present study found that schizotypal traits in a non-clinical population were associated with an excess of winter birth, whereas season of birth was not significantly associated with temperament/character personality dimensions or neurocognition. Schizotypal traits were also associated with mild impairments in some aspects of intellectual functioning. These results point to the etiological similarity between schizotypal personality and schizophrenia-spectrum disorders, potentially supporting the notion that these conditions are on the same continuum. From the perspective of public health, such attempts to identify early signs that confer vulnerability to schizotypy as well as schizophrenia-spectrum disorders might provide an important clue to prevention of and early intervention for schizophrenia.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.psychres.2011.07.028.

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A polymorphism of the *ABCA1* gene confers susceptibility to schizophrenia and related brain changes

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ABSTRACT

Objective: The ATP-binding cassette transporter A1 (ABCA1) mediates cellular cholesterol efflux through the transfer of cholesterol from the inner to the outer layer of the cell membrane and regulates extracellular cholesterol levels in the central nervous system. Several lines of evidence have indicated lipid and myelin abnormalities in schizophrenia.
Method: Initially, we examined the possible association of the polymorphisms of the ABCA1 gene (*ABCA1*) with susceptibility to schizophrenia in 506 patients with schizophrenia (DSM-IV) and 941 controls. The observed association was then subject to a replication analysis in an independent sample of 511 patients and 539 controls. We further examined the possible effect of the risk allele on gray matter volume assessed with magnetic resonance imaging (MRI) in 86 patients with schizophrenia (49 males) and 139 healthy controls (47 males).
Results: In the initial association study, the 1587 *K* allele (rs2230808) was significantly more common in male patients with schizophrenia than in male controls. Although such a significant difference was not observed in the second sample alone, the increased frequency of the 1587 K allele in male patients remained to be significant in the combined male sample of 556 patients and 594 controls. Male schizophrenia patients carrying the 1587 *K* allele had a smaller amount of gray matter volume than those who did not carry the allele.
Conclusion: Our data suggest a male-specific association of the 1587 *K* allele of *ABCA1* with susceptibility to schizophrenia and smaller gray matter volume in schizophrenia.

1. Introduction

The ATP-binding cassette transporter A1 (ABCA1) mediates cellular cholesterol efflux through transfer of cholesterol from the inner to the outer layer of the cell membrane, enabling the binding of cholesterol to apolipoproteins (Knight, 2004). It plays a critical role in the regulation of extracellular cholesterol levels in the central nervous system (CNS). Mice lacking the ABCA1 gene (*ABCA1*) had significantly reduced cholesterol levels in the cerebrospinal fluid (Wahrle et al., 2004). Moreover, *ABCA1* polymorphisms are reported to be associated with serum cholesterol concentration. For instance, the 219*K* (rs2230806) allele was associated with high plasma levels of low-density lipoprotein (LDL) cholesterol (Katzov et al., 2004), and the 771*M* (rs2066718) and the 1587*K* (rs2230808) alleles were associated with low plasma levels of high-density lipoprotein (HDL) cholesterol (Clee et al., 2001; Frikke-Schmidt et al., 2004). Cholesterol is required for myelination (Saher

Abbreviations: ABCA1, ATP-binding cassette transporter A1; ANCOVA, analysis of covariance; ANOVA, analysis of variance; CNS, central nervous system; DNA, deoxyribonucleic acid; DSM-IV, Diagnostic and Statistical Manual of Mental Disorders, 4th edition; FDR, false discovery rate; FWE, familywise error rate; GWAS, genome-wide association study; HDL, high-density lipoprotein; HWE, Hardy–Weinberg equilibrium; IL1β, interleukin-1 β; LDL, low-density lipoprotein; MINI, Mini-International Neuropsychiatric Interview; MRI, magnetic resonance imaging; mRNA, messenger ribonucleic acid; PCR, polymerase chain reaction; SNP, single nucleotide polymorphisms; SPM, Statistical Parametric Mapping; TE, echo time; TR, repetition time; VBM, voxel-based morphometry

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et al., 2005), dendrite differentiation (Goritz et al., 2005) and synaptogenesis (Mauch et al., 2001). Therefore ABCA1 expressed in neurons and glial cells plays an important role in the regulation of synaptic development (Karasinska et al., 2009). Estrogen administration is also known to increase *ABCA1* messenger ribonucleic acid (mRNA) (Srivastava, 2002), and a sex difference in the activity of cholesterol transport has been observed (Catalano, 2008). Disturbances in CNS cholesterol homeostasis have been implicated in neurodegenerative diseases including Alzheimer's (Vance et al., 2005) and Huntington's diseases (Valenza et al., 2005). Previous studies have examined the association between polymorphisms of *ABCA1*, particularly the non-synonymous single nucleotide polymorphisms (SNPs) of rs2230806 (*R219K*), rs2066718 (*V771M*), and rs2230808 (*R1587K*) and risk for Alzheimer's disease. Some of these studies have shown a significant association (Katzov et al., 2004; Sundar et al., 2007; Shibata et al., 2006), although this association demonstrated a sex difference (Sundar et al., 2007). Several studies have demonstrated myelin abnormalities in schizophrenia (Thomas et al., 2001; Hakak et al., 2001; Garver et al., 2008; Tkachev et al., 2003; Huang and Chen, 2005), and the relationship between schizophrenia and ABCA1 was also noted (Chen et al., 2009). To date, sterol-regulatory-element binding protein-2 (SREBP-2), that regulates the ABCA1 (Wong et al., 2006), was suggested to be associated with schizophrenia (Le Hellard et al., 2010). Recent genetic studies also have revealed that the interleukin-1 β (IL1β) gene or the IL1 gene complex is associated with schizophrenia (Xu and He, 2010), and it is also suggested that change in IL1β levels in cerebrospinal fluid and serum may play a role in the pathophysiology of schizophrenia (Barak et al., 1995). IL-1β has been shown to down-regulate ABCA1 (Chen et al., 2007). However, to our knowledge, no study has thus far focused on the association between *ABCA1* polymorphisms and risk of schizophrenia. To our knowledge, no genome-wide association study (GWAS) has suggested that this chromosomal region contains a susceptibility locus for schizophrenia yet. However, some GWASs for bipolar disorder have reported this locus as a candidate region. Data from GWASs are also beginning to provide strong support for shared genetic risk across the disorders (Venken et al., 2005; Park et al., 2004; Liu et al., 2003; Badenhop et al., 2002). Interestingly, a recent study using data from GWASs strongly supported the hypothesis of shared genetic risk between schizophrenia and bipolar disorder (Moskvina et al., 2009). Thus we examined the possibility of association between the *ABCA1* variants and schizophrenia.

Previous magnetic resonance imaging (MRI) studies in schizophrenia have shown gray matter volume reduction, particularly in the insula, anterior cingulate cortex, medial frontal cortex, and hippocampal area (Fornito et al., 2009; Glahn et al., 2008). Furthermore, studies have shown the effect of disease-associated genes on such structural abnormalities in the brain (Mata et al., 2009). Deviations in brain morphology potentially reflecting genetic risk have been ubiquitous in the literature, and quantitative measures of brain structure using various neuroimaging techniques have a long history as effective endophenotype (Honea et al., 2008). In this study, we examined whether genetic variations of *ABCA1* are associated with the development of schizophrenia. We also investigated the potential influence of the disease-associated genotype of *ABCA1* on the regional cerebral gray matter volume measured with MRI.

2. Methods

2.1. Subjects

2.1.1. Initial study (Tokyo sample)

Subjects were 506 patients with schizophrenia (278 males, mean age 44.3 ± 14.1 years), diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) (American Psychiatric Association, 1994), and 941 healthy controls (334 males, 44.8 ± 16.3 years). All patients and controls were biologically unrelated Japanese who resided in the same geographical area (the western part of Tokyo). Consensus diagnosis by at least two psychiatrists was made for each patient based on all the available information obtained from interviews and medical records. Healthy controls were interviewed for enrollment by research psychiatrists using the Japanese version of the Mini-International Neuropsychiatric Interview (MINI; Otsubo et al., 2005; Sheehan et al., 1998). Those who demonstrated no history of psychiatric illness or contact with psychiatric services were enrolled as controls in this study. Participants were excluded if they had a prior medical history of CNS disease or severe head injury. Among the subjects, 86 (49 males) schizophrenia patients and 139 healthy controls (47 males) underwent brain MRI.

2.1.2. Replication study (Tokai sample)

For the replication analysis, we used an independent Japanese sample comprising 511 cases (283 males, mean age 43.8 ± 14.9 years) and 539 controls (267 males, 36.3 ± 14.2 years). All subjects were unrelated, living in the Tokai area of the mainland of Japan, and self-identified as Japanese. Control subjects were members of the general public who had no personal history of mental disorders. This was ascertained in face-to-face interviews where subjects were asked if they had suffered an episode of depression, mania, or psychotic experiences or if they had received treatment for any psychiatric disorder. Patients were entered into the study if they 1) met DSM-IV criteria for schizophrenia; 2) were physically healthy and had normal routine laboratory tests; and 3) had no mood disorders, substance abuse, neurodevelopmental disorders, epilepsy, or known mental retardation. Consensus diagnoses were made by at least two experienced psychiatrists according to DSM-IV criteria on the basis of unstructured interviews with patients and families and review of medical records.

After description of the study, written informed consent was obtained from each subject. This study was approved by institutional ethics committees.

2.2. SNP selection and genotyping

Since genetic variations that result in an amino acid change are most likely to alter function, we searched for non-synonymous polymorphisms of *ABCA1* in the NCBI dbSNP database (http://www.ncbi.nlm.nih.gov/sites/entrez?db=snp). We also searched the literature for polymorphisms of *ABCA1* previously reported to be associated with CNS diseases. We found only four well-validated SNPs with a heterozygosity value of >0.10 in Asian populations: rs2230806 (*R219K*), rs2066718 (*V771M*), rs2066714 (*I883M*), and rs2230808 (*R1587K*). Venous blood was drawn from the subjects and genomic deoxyribonucleic acid (DNA) was extracted from whole blood according to the standard procedures. The four SNPs were genotyped using the TaqMan 5′-exonuclease allelic discrimination assay; the assay IDs were C___2741051_1_ for rs2230806, C__11720789_10 for rs2066718, C___2741083_1_ for rs2066714, and C___2741104_1_ for rs2230808 (Applied Biosystems, Foster City, CA). Thermal cycling conditions for polymerase chain reaction (PCR) were 1 cycle at 95 °C for 10 min followed by 50 cycles of 92 °C for 15 s and 60 °C for 1 min. After amplification, the allele-specific fluorescence was measured on ABI PRISM 7900 Sequence Detection (Applied Biosystems). The genotypes were scored using the software SDS2.1. Failed reactions were called as 'undetermined' by this one and these data were not included in the analysis. Genotype data were read blind to the case–control status.

2.3. MRI data acquisition and processing

All MR studies were performed on a 1.5 Tesla Siemens Magnetom Vision plus system. A three-dimensional (3D) volumetric acquisition of a T1-weighted gradient echo sequence produced a gapless series of 144 sagittal sections using an MPRAGE sequence (echo time (TE)/repetition time (TR): 4.4/11.4 ms; flip angle: 15°; acquisition

matrix: 256×256; 1NEX, field of view: 31.5 cm; slice thickness: 1.23 mm). The raw 3D T1-weighted volume data were transferred to a workstation, and structural images were analyzed using an optimized, voxel-based morphometry (VBM) technique. Data were analyzed using Statistical Parametric Mapping 5 (SPM5) software (Welcome Department of Imaging Neuroscience, London, UK) running on MATLAB 7.0 (Math Works, Natick, MA). Images were processed using an optimized VBM script. The details of this process are described elsewhere (Li and Ji, 2005). First, each individual 3D-T1 image was normalized with the optimized VBM method. Normalized segmented images were modulated by multiplication with Jacobian determinants of the spatial normalization function to encode the deformation field for each subject as tissue density changes in normal space. Images were smoothed using an 8-mm full-width at half-maximum of an isotropic Gaussian kernel.

2.4. Statistical analysis

Deviations of genotype distributions from the Hardy–Weinberg equilibrium (HWE) were assessed with the χ^2 test for goodness of fit. First, genotype distributions were compared between patients and controls using the χ^2 test for independence. Since some animal studies showed the gender specific findings (Koldamova et al., 2005; Kuivenhoven et al., 2003), and estrogen has functional relevance to the *ABCA1*-mediated pathway (Srivastava, 2002) and a sex difference in the activity of cholesterol transport has been observed (Catalano et al., 2008), analysis for each sex was also performed. These tests were performed with SPSS software ver. 11 (SPSS Japan, Tokyo, Japan). For multiple analyses, we applied the spectral decomposition method of SNPSpD software (http://gump.qimr.edu.au/general/daleN/SNPSpD/) (Nyholt, 2004; Li and Ji, 2005), which considers marker linkage disequilibrium information and generates an experiment-wide significance threshold required to keep the type I error rate at 5%. As a result, the critical P value was corrected as 0.0128. Then, the observed association was subject to a replication analysis in an independent Tokai sample using the χ^2 test for independence.

Second, we then evaluated the differences in regional gray matter volume across the clusters sorted by the genotype distributions of the SNP that showed a statistically significant difference between the patients and healthy subjects. Statistical analyses were performed using Statistical Parametric Mapping 2 (SPM2) software (Welcome Department of Imaging Neuroscience, London, UK). Since the regional cerebral gray matter volume is influenced by age (Good et al., 2001), we examined the differences in regional gray matter volume by the analysis of covariance (ANCOVA), controlling for age. Only associations that met the following criteria were deemed statistically significant for the first analysis: familywise error rate (FWE)<0.05, and for the *post hoc* analyses: a voxel level of $p<0.001$ (uncorrected) and a cluster level of $p<0.05$ (uncorrected). We also evaluated the differences across the groups according to age using one-way analysis of variance (ANOVA) and the differences between two groups of schizophrenia patients categorized according to duration of illness and daily dose of antipsychotic drugs using a two-sample *t*-test.

3. Results

3.1. ABCA1 polymorphisms and susceptibility to schizophrenia

First, genotype and allele distributions of the 4 SNPs in the initial sample (Tokyo sample) are shown in Table 1. The genotype distribution for rs2230806 in the female control group deviated significantly from the HWE, thus was excluded from further analysis. In the total sample, the genotype or allele distribution did not differ significantly between the cases and controls for any SNP. However, when men and women were examined separately, a nominally significant difference in the genotype distribution for rs2230808 (*R1587K*) was observed in men ($p=0.014$), but not in women ($p=0.674$). Difference in allele frequency was observed at a trend level in men ($p=0.055$), but not in women ($p=0.440$). When the observed difference in the genotype distribution for rs2230808 was further analyzed based on the recessive and dominant models, there was a significant difference in the dominant model ($p=0.006$; odds ratio (OR) 1.60, 95% confidential interval (CI): 1.14–2.24), but not in the recessive one ($p=0.96$), in male subjects. There was no significant difference in genotype or allele distribution of the other 3SNPs even when subjects were stratified by sex.

Table 2 shows genotype and allele distributions for rs2230808 in the replication sample (Tokai sample). There was no significant difference in genotype or allele distribution between the patients and controls. When men and women were examined separately, there was no significant difference for either sex. We also analyzed based on the dominant model; however, no statistically significant differences in genotype distribution were found in total subjects or each sex. However, the initial and replication samples were combined, the frequency of male patients carrying the 1587K allele remained to be increased than male controls at nominally significant level (OR 1.30, 95% CI 1.02 –1.65, $p=0.032$).

3.2. ABCA1 polymorphism and MRI volumetry

Since carrying the *1587K* allele was found to be significantly more common in male patients with schizophrenia than in male controls in the genetic association study, the subjects with MRI data were grouped into four groups for each sex based on the case–control status and whether the subject carried the *1587K* allele or not. The demographic and clinical characteristics of the groups are presented in Table 3. For both men and women, the analyses showed no significant difference in duration of illness or daily dose of antipsychotics between the two genotype-based groups of patients with schizophrenia (men: duration of illness: $t(47)=-0.15$, $p=0.88$, daily dose of drug: $t(47)=-1.58$, $p=0.12$; female: duration of illness: $t(34)=-0.40$, $p=0.69$, daily dose of drug: $t(34)=-0.20$, $p=0.85$). Further, for both men and women, there was no significant difference in mean age across the healthy subjects and two schizophrenia groups (men: $df=2$, $F=1.54$, $p=0.22$; women: $df=2$, $F=1.16$, $p=0.32$).

Initially, we evaluated the difference in gray matter volume between the two genotype-based healthy groups for each sex using ANCOVA, controlling for age. There were no significant differences related to genotype for either sex, respectively. We therefore combined the healthy groups with and without the *1587K* allele for each sex in the following analyses. When the group effect was assessed using ANCOVA with F-test (FWE<0.05), we found statistically significant volume differences in thalami, medial temporal regions, and nearly all the circumferential cortical regions in males (Fig. 1A). Male patients with schizophrenia carrying the *1587K* allele showed significant small gray matter volume in the bilateral occipital regions and posterior cingulate cortices compared with those who did not carry the *1587K* allele (Fig. 1B). Male patients with schizophrenia who did not carry the *1587K* allele showed significant small volume only in bilateral orbitofrontal, insulae, and left parahippocampus, compared with all male controls (Fig. 1C). However, the male schizophrenia patients carrying the *1587K* allele showed smaller volume across almost the whole gray matter, than all male controls (Fig. 1D). When we re-analyzed these *post hoc* statistics using rigorous criteria (false discovery rate (FDR) $p<0.05$, cluster level of $p<0.05$), results indicated with Fig. 1C and D showed almost the same as the previous ones, the statistics using the relatively small sample size indicated with the Fig. 1B showed no statistically significant difference between the schizophrenic groups.

In women, in contrast, there were no significant differences in gray matter volume between schizophrenia patients with and without the *1587K* allele or between controls with and without the allele (data not