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Expression of Ca^{2+}-dependent activator protein for secretion 2 is increased in the brains of schizophrenic patients

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ABSTRACT

Ca^{2+}-dependent activator protein for secretion 2 (CADPS2), a secretory granule associate protein, mediates monoamine transmission and the release of neurotrophins including brain-derived neurotrophic factor (BDNF) which have been implicated in psychiatric disorders. Furthermore, the expression of CADPS2deltaExon3, a defective splice variant of CADPS2, has been reported to be associated with autism. Based on these observations, we examined whether expression levels of CADPS2 and CADPS2deltaExon3 are altered in psychiatric disorders. Quantitative polymerase chain reaction analysis was performed for postmortem frontal cortex tissues (BA6) from 15 individuals with schizophrenia, 15 with bipolar disorder, 15 with major depression, and 15 controls (Stanley neuropathology consortium). The mean CADPS2 expression levels normalized to human glyceraldehyde-3phosphate dehydrogenase (GAPDH) or TATA-box binding protein levels was found to be significantly increased in the brains of the schizophrenia group, compared to the control group. On the other hand, the ratio of CADPS2deltaExon3 to total CADPS2 was similar in the 4 diagnostic groups. We then analyzed CADPS2 expression in blood samples from 121 patients with schizophrenia and 318 healthy controls; however, there was no significant difference between the two groups. Chronic risperidone treatment did not alter the expression of CADPS2 in frontal cortex of mice. The observed increase in the expression of CADPS2 may be related to the impaired synaptic function in schizophrenia.

1. Introduction

Ca^{2+}-dependent activator protein for secretion (CADPS) family, which consists of two members, CADPS1 and CADPS2, is a secretory granule-associated proteins involved in Ca^{2+}-dependent exocytosis of large dense-core vesicles containing diverse array of modulators including neurotrophines, monoamines and neuropeptides (Liu et al., 2008; Sadakata et al., 2004). CADPS2 mediates the release of neurotrophins such as brain-derived neurotrophic factor (BDNF) and neurotrophin-3. Mouse CADPS2 protein is associated with BDNF-containing secretory vesicles and promotes activity-dependent release of BDNF (Sadakata et al., 2004). BDNF release is significantly reduced in cultured neurons prepared from the brain of CADPS2 deficient mice (Sadakata et al., 2007a,b).

A number of findings suggest that BDNF action is impaired in psychiatric disorders including schizophrenia, bipolar disorder and depression. Several studies have shown decreased levels of BDNF or its receptor, TrkB, in the postmortem brains of patients with schizophrenia (Hashimoto et al., 2005; Iritani et al., 2003; Weickert et al., 2003), although there are contradictive reports (Chen et al., 2001; Dunham et al., 2009; Durany et al., 2001; Takahashi et al., 2000). The contribution of BDNF in depression has been suggested from animal studies that demonstrated stressful environments decrease, and antidepressive treatments increase BDNF levels in the brain (Duman and Monteggia, 2006; Martinowich et al., 2007). Also, centrally administered BDNF has an antidepressant-like effect in rat models (Siuciak et al., 1997). Thus, the molecules that contribute to the trafficking and release of BDNF may be a culprit of these disorders.

CADPS family also mediate monoamine transmission. Both CADPS1 and CADPS2 mediate the refilling of catecholamine to the releasable vesicles, and catecholamine secretion is significantly suppressed in the CADPS1/2 double deficient cells. (Liu et al., 2008). Another study supports that CADPS family are involved in monoamine storage as antibodies against CADPS1 or 2 inhibit monoamine

Abbreviations: ANCOVA, Analysis of covariance; BDNF, Brain-derived neurotrophic factor; CADPS2, Ca^{2+}-dependent activator protein for secretion 2; CCK, Cholecystokinin; DSM–IV, Diagnostic and Statistical Manual of Mental Disorders, 4th edition; FST, Freezer storage time; M.I.N.I., Mini-International Neuropsychiatric Interview; NT, Neurotensin; PCR, Polymerase chain reaction; PMI, Postmortem interval; SD, Standard deviation; TBP, TATA-box binding protein.

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sequestration by synaptic vesicles from mice brain (Brunk et al., 2009).

Dysregulation of monoamine neurotransmission has been hypothesized to play a central role in the etiology of psychiatric disorders including schizophrenia and mood disorders. In schizophrenia, not only classical evidence that dopamine agonists induce and dopamine D2 receptor antagonists ameliorate psychoses but also brain imaging studies on drug naïve patients have suggested that dopamine transmission is affected in this disorder (Lyon et al., 2011). In major depression, reduced monoamine transmission hypothesis was derived from the finding that most anti-depressants increase monoamine levels in the synaptic cleft and that reserpine, a monoamine-depleting drug, worsen depressive symptoms in a subset of patients with mood disorder (Krishnan and Nestler, 2008), although imaging, postmortem, or cerebrospinal fluid studies have yet to find the definitive evidence for altered monoamine neurotransmission in this disorder (Belmaker and Agam, 2008; Nikolaus et al., 2009).

While, to our knowledge, CADPS2 expression in schizophrenia or mood disorders have not yet been examined, aberrant splicing of CADPS2 mRNA was reported in autism (Sadakata et al., 2007b). In this study, an exon-3 skipped isoform, CADPS2ΔExon3, was detected in the bloods of several autistic patients but not in those of healthy controls. They also showed that CADPS2ΔExon3 was deficient in proper axonal transport, which results in the loss of local synaptic BDNF release. Though the CADPS2ΔExon3 expression in the brains of patients with autism is unclear, the aberrant splicing of CADPS2 could contribute to susceptibility to autism by affecting neurotrophin release.

Based on above findings, the present study was aimed to examine whether the expression of CADPS2 transcripts is altered in the frontal cortex of patients with psychiatric disorders including schizophrenia, major depression and bipolar disorder. The CADPS2 expression levels in the blood of schizophrenia were also examined.

2. Materials and methods

2.1. Brain samples

Frozen postmortem samples of frontal cortex (BA6) were obtained from the Stanley Foundation Neuropathology Consortium (Torrey et al., 2000). The collection consists of 60 subjects: 15 with schizophrenia, 15 bipolar disorder, 15 major depression and 15 unaffected controls. All groups were matched for age, sex, race, pH and hemispheric side (Table 1), although postmortem interval (PMI) and freezer storage time differed across the groups. The brain tissues obtained were coded. Once our blind study was complete, we sent the data to the Stanley Foundation who then returned the codes, demographic and clinical data. In a cold-room, each frozen brain tissue was broken into powder in the plastic bag using dry-ice block and dry-ice-cold hammer. The powder was then transferred and kept in dry-ice-cold tubes. Temperature of the tubes and instruments that directly contacted to the samples was frequently measured by infrared-thermometer (AD-5613A, A&D Company, Japan) and kept under −20 °C. Then, 30 to 40 mg of brain powder was used for cDNA synthesis. RNA was extracted using RNAqueous (Applied biosystems, Foster City, CA) according to manufacturer's instructions with a slight modification, i.e., after homogenization, samples were washed twice with 500 μl of chloroform, and then applied to the spin-column. Extracted RNA was quantified by optical density reading at 260 nm using NanoDrop ND-1000 (Thermo Scientific, Rockford, IL). Then, the obtained RNA (14 μl) was used for cDNA synthesis using SuperScript VILO cDNA Synthesis Kit (Invitrogen, Carlsbad, CA).

Table 1
Demographic information on brain specimens of Stanley Neuropathology Consortium.

	Control	Schizophrenia	Bipolar disorder	Major depression
Age (years)	48.1 (29–68)	44.2 (25–62)	42.3 (25–61)	46.4 (30–65)
Gender (M/F)	9/6	9/6	9/6	9/6
Race	14 C, 1 AA	13 C, 2 A	14 C, 1 AA	15 C
PMI (hours)	23.7 (8–42)	33.7 (12–61)	32.5 (13–62)	27.5 (7–47)
pH	6.3 (5.8–6.6)	6.1 (5.8–6.6)	6.2 (5.8–6.5)	6.2 (5.6–6.5)
Side of brain frozen (R/L)	7/8	6/9	8/7	6/9
Freezer storage time (months)	11.3 (1–26)	20.7 (2–31)	20.7 (7–28)	14.5 (3–31)

AA, African American; A, Asian; C, Caucasian; F, female; M, male; and PMI, postmortem interval.

2.2. Blood samples

Subjects were 121 patients with schizophrenia (84 males and 37 females; age 44.1 ± 13.7 (mean ± SD) years) and 318 controls (90 males and 228 females; age 43.1 ± 15.3 years). All subjects were biologically unrelated Japanese and recruited from the same geographical area (Western part of Tokyo Metropolitan). Consensus diagnosis by at least two psychiatrists was made for each patient according to the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM–IV) criteria (American Psychiatric Association, 1994) on the basis of unstructured interviews and information from medical records. The controls were healthy volunteers recruited from the community, through advertisements in free local magazines and our website announcement. Control individuals were interviewed by the Japanese version of the Mini-International Neuropsychiatric Interview (M.I.N.I.) (Otsubo et al., 2005; Sheehan et al., 1998) and those who had a current or past history of psychiatric treatment were not enrolled in the study. After the nature of the study procedures had been fully explained, written informed consent was obtained from all subjects. The study was conducted in accordance with the Declaration of Helsinki and approved by the ethics committee of the National Center of Neurology and Psychiatry, Japan.

Blood collection and RNA isolation were performed using the PAXgene blood RNA system (Qiagen, Valencia, CA). Blood samples were collected around 11 A.M. Extracted RNA was quantified as described above. Samples that contained more than 40 ng/μl of total RNA were used for analysis; 8 μl from each sample was reverse transcribed using SuperScript VILO cDNA Synthesis Kit (Invitrogen, Carlsbad, CA).

2.3. Chronic risperidone treatment to mice

C57BL/6J male mice aged 10 weeks were purchased from Crea Japan. Chronic oral risperidone treatment was performed according to Belforte et al., (Belforte et al., 2010). In brief, 2.5 mg/kg/day of risperidone (Rispadal liquid, Janssen Pharmaceutical, Tokyo, Japan) in drinking water freshly made every 72 h had been administered continuously for 3 weeks. Control mice received solvent (1.4 mM tartaric acid neutralized to pH 6–7). All experimental procedures were in accordance with the guidelines of the United State's National Institutes of Health (1996) and were approved by the Animal Care Committee of the National Institute of Neuroscience, NCNP.

2.4. Quantitative real-time polymerase chain reaction

Polymerase chain reaction (PCR) amplifications were performed in triplicate (5 μl volume) on 384-well plates using ABI prism 7900HT (Applied Biosystems, Foster City, CA). Each reaction contained 0.28 μl of cDNA sample, qPCR QuickGoldStar Mastermix Plus (Eurogentec, Seraing, Belgium) and a primer of the target, i.e. human CADPS2 (Hs01095968_m1 at Exon 4–5 on NM_017954.9), mouse CADPS2 (Mm00462577_m1), human CADPS2ΔExon3 (Forward primer: GTAGCTGACGAAGCATTTTGCA,

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

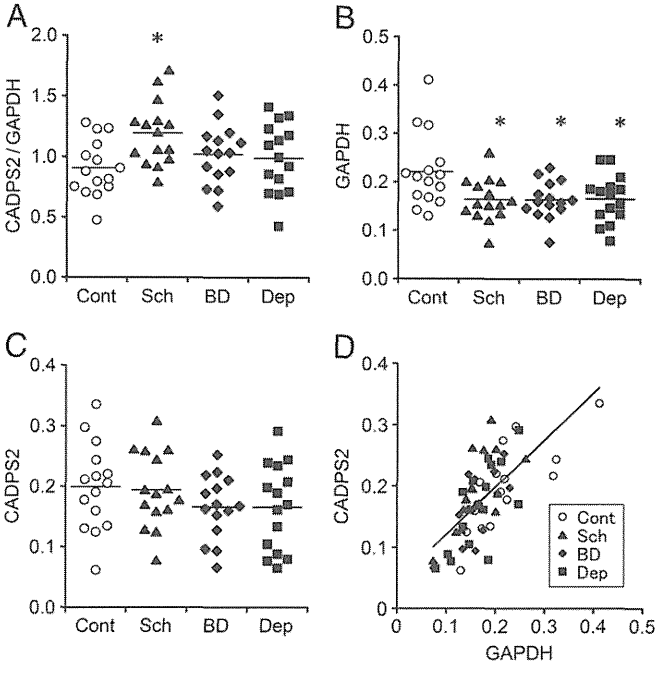

Fig. 1. CADPS2 expression levels in the postmortem brains of psychiatric disorder. (A) CADPS2 expression levels normalized by GAPDH levels. Scatter plots display the variability and differences in the CADPS2 mRNA expression levels normalized by each GAPDH expression levels. A crossbar on each scatter 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).

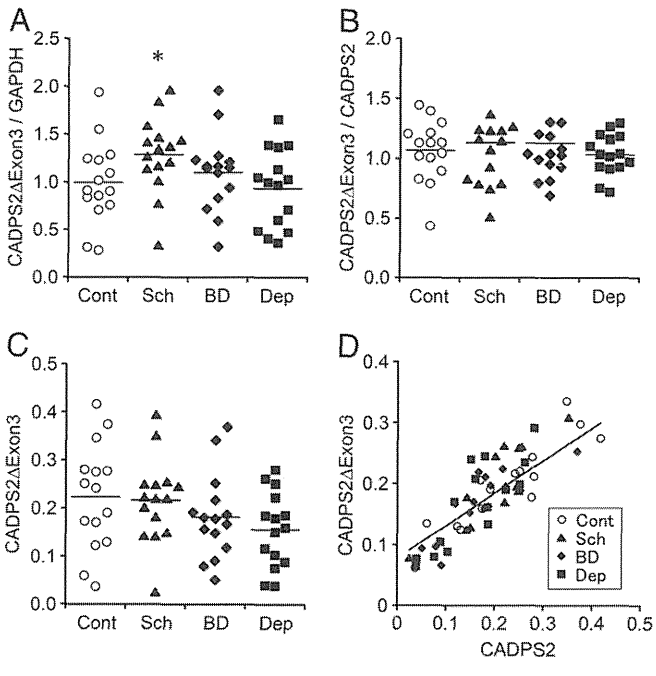

Fig. 2. CADPS2ΔExon3 expression levels in the postmortem brains of psychiatric disorder. (A) CADPS2ΔExon3 expression levels normalized by GAPDH levels. Scatter plots display the variability and differences in the CADPS2ΔExon3 mRNA expression levels normalized by each GAPDH expression levels. A crossbar 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 l0 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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Possible association between *Interleukin-1beta* gene and schizophrenia in a Japanese population

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Abstract

Background: Several lines of evidence have implicated the pro-inflammatory cytokine interleukin-1beta (IL-1β) in the etiology of schizophrenia. Although a number of genetic association studies have been reported, very few have systematically examined gene-wide tagging polymorphisms.

Methods: A total of 533 patients with schizophrenia (302 males: mean age ± standard deviation 43.4 ± 13.0 years; 233 females; mean age 44.8 ± 15.3 years) and 1136 healthy controls (388 males: mean age 44.6 ± 17.3 years; 748 females; 46.3 ± 15.6 years) were recruited for this study. All subjects were biologically unrelated Japanese individuals. Five tagging polymorphisms of *IL-1β* gene (rs2853550, rs1143634, rs1143633, rs1143630, rs16944) were examined for association with schizophrenia.

Results: Significant difference in allele distribution was found between patients with schizophrenia and controls for rs1143633 ($P = 0.0089$). When the analysis was performed separately in each gender, significant difference between patients and controls in allele distribution of rs1143633 was observed in females ($P = 0.0073$). A trend towards association was also found between rs16944 and female patients with schizophrenia ($P = 0.032$).

Conclusions: The present study shows the first evidence that the *IL-1β* gene polymorphism rs1143633 is associated with schizophrenia susceptibility in a Japanese population. The results suggest the possibility that the influence of *IL-1β* gene variations on susceptibility to schizophrenia may be greater in females than in males. Findings of the present study provide further support for the role of IL-1β in the etiology of schizophrenia.

Background

Several lines of evidence suggest that pro-inflammatory cytokine interleukin-1beta (IL-1β) is implicated in the etiology and pathophysiology of schizophrenia. Although studies investigating peripheral levels of IL-1β in schizophrenic patients have reported inconsistent results [1-6], a study examining the cerebrospinal fluid has shown a marked elevation of IL-1β in patients with first-episode schizophrenia compared to healthy controls [7]. Kowalski et al [8] reported that the release of IL-1β by peripheral monocytes was increased before treatment and then normalized by antipsychotic medication in patients with schizophrenia. Recently, Liu et al. [9] showed that IL-1β in the peripheral blood mononuclear cells was overexpressed not only in schizophrenia patients but also in their siblings, suggesting the involvement of the hereditary factors. Furthermore, previous findings suggested that IL-1β may be involved in the possible link between prenatal exposure to infection and schizophrenia [10,11].

The *IL-1β* gene is located in a region on 2q14. This region has consistently shown positive linkage findings in schizophrenia. Many studies have reported this region among their largest results [12,13]. Furthermore, Lewis et al [14] have shown in their meta-analysis of 20 genome scans that 2p12-q22.1 was associated with a genomewide significant *P* value. Linkage of this region with schizophrenia in an Asian population has also been reported [15].

A number of genetic association studies have suggested that genetic variation of the *IL-1β* gene might confer susceptibility to schizophrenia. Three studies in Caucasian populations reported a significant association of schizophrenia with an *IL-1β* gene polymorphism

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rs16944 [16-18]. However, this association was not confirmed in other studies [19,20]. Furthermore, none of the previous studies in Asian populations have obtained evidence for an association between *IL-1β* gene and schizophrenia [21-23]. All of the aforementioned association studies, except for that of Shirts, et al. [19], examined only rs16944 and/or rs1143634. Therefore, the role of other *IL-1β* gene polymorphisms remains to be determined. We here examined 5 tagging polymorphisms of the *IL-1β* gene for an association with schizophrenia in a Japanese sample.

Methods

Subjects

Subjects were 533 patients with schizophrenia (302 males: mean age ± standard deviation 43.4 ± 13.0 years; 233 females; mean age 44.8 ± 15.3 years) and 1136 healthy controls (388 males: mean age 44.6 ± 17.3 years; 748 females; 46.3 ± 15.6 years). The mean age at onset was 23.9 ± 8.0 and 25.8 ± 9.8 years for male and female patients, respectively. All subjects were biologically unrelated Japanese individuals, based on their self-reports, 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 two psychiatrists was made for each patient according to the Diagnostic and Statistical Manual of Mental Disorders, 4^{th} edition criteria [24], on the basis of unstructured interviews and information from medical records. The controls were healthy volunteers with no current or past history of psychiatric treatment, and were screened using the Japanese version of the Mini International Neuropsychiatric Interview (M.I.N.I.) [25,26] by a research psychiatrist to rule out any axis I psychiatric disorders. Participants were excluded if they had prior medical histories of central nervous system disease or severe head injury, or if they met the criteria for substance abuse or dependence, or mental retardation. The study protocol was approved by the ethics committee at the National Center of Neurology and Psychiatry, Japan. After description of the study, written informed consent was obtained from every subject. Most of the subjects had participated in our previous genetic association studies [27,28]. Some of the control subjects had also participated in our previous studies which examined *IL-1β* gene polymorphisms [29,30].

Genotyping

Five tagging single nucleotide polymorphisms (SNPs) (rs2853550, rs1143634, rs1143633, rs1143630, rs16944) in a region 1 kilobase (kb) upstream to 1 kb downstream of the *IL-1β* gene (chromosome 2: 113,302,808 - 113,311,827 bp) were selected by Haploview 4.2 [31] using Japanese and Chinese population in the HapMap SNP set (version 22), at an r^2 threshold of 0.80 with a minor allele frequency greater than 0.1. Genomic DNA was prepared from the venous blood according to standard procedures. The SNPs were genotyped using the TaqMan 5'-exonuclease allelic discrimination assay. Thermal cycling conditions for polymerase chain reaction were 1 cycle at 95°C for 10 minutes followed by 50 cycles of 92°C for 15 seconds and 60°C for 1 minute. The allele-specific fluorescence was measured with ABI PRISM 7900 Sequence Detection Systems (Applied Biosystems, Foster city, CA, USA). Genotype data were read blind to the case-control status. Ambiguous genotype data were not included in the analysis. The call rates for each SNP ranged from 97.7% to 98.6%. The genotyping failure rate for all SNPs combined was < 2%. In 92 subjects, all 5 SNPs were genotyped in duplicate to ensure genotyping accuracy, and the concordance rate of called genotypes was over 99%.

Statistical analysis

Deviations of genotype distributions from the Hardy-Weinberg equilibrium (HWE) were assessed with the exact test described by Wigginton et al [32]. Genotype and allele distributions were compared between patients and controls by using the χ^2 test for independence or with Fisher's exact test. The above statistical analyses were performed using PLINK version 1.07 [33].

Haploview 4.2 [31] was used to estimate haplotype frequencies and linkage disequilibrium (LD) coefficients. Haplotypes with frequencies > 1% were included in the association analysis. Permutation procedure (10,000 replications) was used to determine the empirical significance.

Statistical tests were two tailed and statistical significance was considered when $P < 0.05$. Significance level corrected for multiple comparisons of 5 SNPs was set at $P < 0.013$ by a method proposed by Li et al [34], which was calculated using SNPSpD (SNP Spectral Decomposition) software [35].

Power calculations were performed using the Power Calculator for Two Stage Association Studies (http://www.sph.umich.edu/csg/abecasis/CaTS/). Power was calculated under prevalence of 0.01 using an allelic model with an alpha level of 0.05. Assuming disease allele frequencies of 0.20 and 0.40, our sample had 80% statistical power to detect relative risks of 1.28 and 1.23, respectively. Similarly, we had 90% power to detect relative risks of 1.33 and 1.27.

Since several aspects of immunity have marked sex differences [36], analyses were performed not only for the entire sample but also for each gender separately. Assuming allele frequency of 0.40, male and female samples each had 80% statistical power to detect relative risks of 1.35 and 1.34, respectively.

Table 1 Association analysis of the 5 SNPs in both genders combined

SNP name	Allele 1/2		Males							
			N	Genotype			Allele		P-value		HWE P-value
				1/1	1/2	2/2	1	2	Genotype	Allele	
rs2853550	A/G	Schizophrenia	531	9	128	394	146	916	0.23	0.088	0.86
				(0.02)	(0.24)	(0.74)	(0.14)	(0.86)			
		Controls	1115	14	232	869	260	1970			0.88
				(0.01)	(0.21)	(0.78)	(0.12)	(0.88)			
rs1143634	A/G	Schizophrenia	525	1	41	483	43	1007	0.97[(a)]	0.90	0.59
				(0.00)	(0.08)	(0.92)	(0.04)	(0.96)			
		Controls	1121	2	90	1029	94	2148	1.00		
				(0.00)	(0.08)	(0.92)	(0.04)	(0.96)			
rs1143633	C/T	Schizophrenia	524	111	249	164	471	577	0.035	0.0089	0.38
				(0.21)	(0.48)	(0.31)	(0.45)	(0.55)			
		Controls	1123	188	525	410	901	1345			0.38
				(0.17)	(0.47)	(0.37)	(0.40)	(0.60)			
rs1143630	T/G	Schizophrenia	520	13	140	367	166	874	0.88	0.66	1.00
				(0.03)	(0.27)	(0.71)	(0.16)	(0.84)			
		Controls	1119	24	296	799	344	1894			0.65
				(0.02)	(0.26)	(0.71)	(0.15)	(0.85)			
rs16944	A/G	Schizophrenia	521	123	253	145	499	543	0.18	0.060	0.54
				(0.24)	(0.49)	(0.28)	(0.48)	(0.52)			
		Controls	1111	226	534	351	986	1236			0.39
				(0.20)	(0.48)	(0.32)	(0.44)	(0.56)			

(a) Calculated using Fisher's exact test.

SNP: single nucleotide polymorphism; HWE: Hardy-Weinberg Disequilibrium

Numbers in parentheses represent the frequencies of genotypes and alleles.

Results

Genotype and allele distributions of the examined SNPs for the entire sample, males, and females are shown in Table 1, 2, and 3, respectively. The genotype distributions did not significantly deviate from the HWE in any of the SNPs examined. Significant differences in genotype and allele distributions were found between the patients with schizophrenia and controls for rs1143633. The C allele was significantly more common in patients than in controls (odds ratio 1.22, 95% confidence interval (CI) 1.05 to 1.41, $P = 0.0089$). This association remained significant after correcting for multiple testing of 5 SNPs (corrected $P = 0.013$). When the analysis was performed separately in each gender, significant difference between patients and controls in allele distribution of rs1143633 was observed only in females (odds ratio 1.34, 95% CI 1.08 to 1.66, $P = 0.0073$). The A allele of rs16944 also showed a trend towards association with schizophrenia in female subjects (odds ratio 1.26, 95% CI 1.02 to 1.56, $P = 0.032$).

Linkage disequilibrium (LD) coefficients (D' and r^2) and haplotype blocks are shown in Figure 1. Results of the haplotype association analyses are shown in Table 4. No significant difference in haplotype distribution was found between patients with schizophrenia and controls (all $P > 0.05$ by permutation test).

Discussion

To our knowledge, the present study is the largest study to date that examined the *IL-1β* gene polymorphisms for association with schizophrenia. The results provide the first evidence suggesting that the C allele of rs1143633 is associated with schizophrenia.

The study in a United States population by Shirts et al [19] was the only one that previously examined the association of schizophrenia with rs1143633, in which no significant difference was found in allele frequencies between patients and controls. Although Watanabe et al [23] have also examined 9 SNPs of the IL-1 gene complex in Japanese subjects, none of the SNPs examined in their study was in remarkable linkage disequilibrium with rs1143633 or rs16944 (all $r^2 < 0.1$ based on HapMap Japanese and Han Chinese population data, release 22). The inconsistent results regarding the effect of rs1143633 between Shirts, et al [19] and our study may be attributable to ethnic difference. Indeed, a recent meta-analysis has shown a significant association of the G allele of rs16944 and the G allele carrier status of rs1143634 with a risk of schizophrenia in Caucasian, but not in Asian, populations [37]. Our samples provided sufficient power to detect relatively small relative risks, and therefore suggest that rs16944 and rs1143634 have no major effect on

Table 2 Association analysis of the 5 SNPs in males

SNP name	Allele 1/2		Males								
			N	Genotype			Allele		P-value		HWE P-value
				1/1	1/2	2/2	1	2	Genotype	Allele	
rs2853550	A/G	Schizophrenia	300	4	74	222	82	518	0.68[a]	0.69	0.62
				(0.01)	(0.25)	(0.74)	(0.14)	(0.86)			
		Controls	383	7	85	291	99	667			0.82
				(0.02)	(0.22)	(0.76)	(0.13)	(0.87)			
rs1143634	A/G	Schizophrenia	298	0	24	274	24	572	0.81[a]	0.82	1.00
				(0.00)	(0.08)	(0.92)	(0.04)	(0.96)			
		Controls	383	1	27	355	29	737			0.42
				(0.00)	(0.07)	(0.93)	(0.04)	(0.96)			
rs1143633	C/T	Schizophrenia	299	59	145	95	263	335	0.43	0.47	0.81
				(0.20)	(0.48)	(0.32)	(0.44)	(0.56)			
		Controls	383	77	168	138	322	444			0.059
				(0.20)	(0.44)	(0.36)	(0.42)	(0.58)			
rs1143630	T/G	Schizophrenia	295	7	81	207	95	495	0.75	0.73	1.00
				(0.02)	(0.27)	(0.70)	(0.16)	(0.84)			
		Controls	383	6	106	271	118	648			0.32
				(0.02)	(0.28)	(0.71)	(0.15)	(0.85)			
rs16944	A/G	Schizophrenia	295	66	143	86	275	315	0.92	0.67	0.64
				(0.22)	(0.48)	(0.29)	(0.47)	(0.53)			
		Controls	385	82	186	117	350	420			0.61
				(0.21)	(0.48)	(0.30)	(0.45)	(0.55)			

(a) Calculated using Fisher's exact test.

SNP: single nucleotide polymorphism; HWE: Hardy-Weinberg Disequilibrium

Numbers in parentheses represent the frequencies of genotypes and alleles.

Table 3 Association analysis of the 5 SNPs in females

SNP name	Allele 1/2		Males								
			N	Genotype			Allele		P-value		HWE P-value
				1/1	1/2	2/2	1	2	Genotype	Allele	
rs2853550	A/G	Schizophrenia	231	5	54	172	64	398	0.18	0.096	0.78
				(0.02)	(0.23)	(0.74)	(0.14)	(0.86)			
		Controls	732		7	147	578		161	1303	0.57
				(0.01)	(0.20)	(0.79)	(0.11)	(0.89)			
rs1143634	A/G	Schizophrenia	227	1	17	209	19	435	0.46[a]	0.84	0.32
				(0.00)	(0.07)	(0.92)	(0.04)	(0.96)			
		Controls	738	1	63	674	65	1411			1.00
				(0.00)	(0.09)	(0.91)	(0.04)	(0.96)			
rs1143633	C/T	Schizophrenia	225	52	104	69	208	242	0.013	**0.0073**	0.29
				(0.23)	(0.46)	(0.31)	(0.46)	(0.54)			
		Controls	740	111	357	272	579	901			0.76
				(0.15)	(0.48)	(0.37)	(0.39)	(0.61)			
rs1143630	T/G	Schizophrenia	225	6	59	160	71	379	0.97	0.83	0.80
				(0.03)	(0.26)	(0.71)	(0.16)	(0.84)			
		Controls	736	18	190	528	226	1246			0.89
				(0.02)	(0.26)	(0.72)	(0.15)	(0.85)			
rs16944	A/G	Schizophrenia	226	57	110	59	224	228	0.11	0.032	0.69
				(0.25)	(0.49)	(0.26)	(0.50)	(0.50)			
		Controls	726	144	348	234	636	816			0.50
				(0.20)	(0.48)	(0.32)	(0.44)	(0.56)			

(a) Calculated using Fisher's exact test.

SNP: single nucleotide polymorphism; HWE: Hardy-Weinberg Disequilibrium

Numbers in parentheses represent the frequencies of genotypes and alleles. Significant P-values (< 0.013) are shown in boldface.

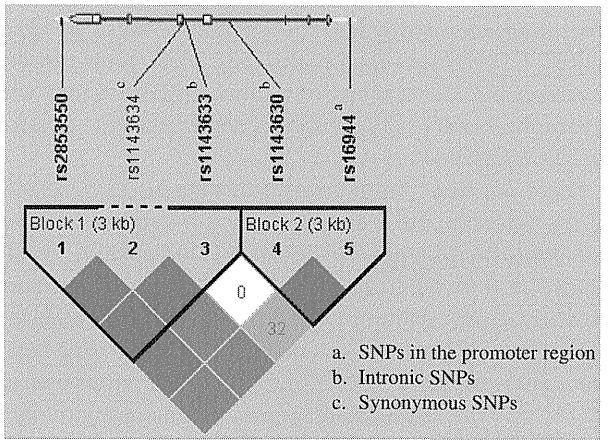

Figure 1 Haplotype block structure of the *IL-1β* gene. Genomic organization and linkage disequilibrium (LD) structure of the *IL-1β* gene are shown. Exons are shown as boxes. Shades of red represent extent of LD (darker red denotes D' = 1). Numbers in squares give r^2 values multiplied by 100.

schizophrenia susceptibility in Asian populations, which is consistent with the previous Asian findings [21-23]. However, there was a trend of association of rs16944, in the opposite direction to that of the Caucasians, with schizophrenia susceptibility in female subjects. Therefore, there remains a possibility that a larger study would yield a significant difference between Japanese female schizophrenic patients and controls in the allele frequency of rs16944.

A number of genome-wide association studies (GWAS) have searched for polymorphisms associated with schizophrenia [38-43]. Although no evidence of association with *IL-1β* gene has been reported, common risk alleles in the major histocompatibility region on chromosome 6, which is involved in the immune response, have shown statistically significant evidence of association [38-40]. Furthermore, a genome-wide pharmacogenomic study has shown that *IL-1α* rs11677416, which is in weak LD with rs1143633 (r^2 = 0.094, D' = 0.809 based on HapMap Japanese and Han Chinese population data, release 22), was associated with response of neurocognitive symptoms to antipsychotic treatment [44]. These findings, together with ours, suggest genetic influence on immune alterations in schizophrenia.

A shift towards the T helper type 2 (Th2) system has been indicated in schizophrenia [45-47]. IL-1β stimulates

Table 4 Haplotype analysis of *IL-1β* gene polymorphisms

			Males					Females				
Block	Haplotype	Diagnosis	Carrier	Non-carrier	χ2	Nominal P value	Permutation P value	Carrier	Non-carrier	χ2	Nominal P value	Permutation P value
	GT	Schizophrenia	336.3	265.7	0.557	0.456	0.957	251.0	213.0	6.240	0.0125	0.118
			(0.559)	(0.441)				(0.541)	(0.459)			
		Controls	447.9	326.1				901.0	585.0			
			(0.579)	(0.421)				(0.606)	(0.394)			
1	GC	Schizophrenia	183.1	418.9	0.216	0.642	0.995	149.0	315.0	2.298	0.130	0.691
			(0.304)	(0.696)				(0.321)	(0.679)			
		Controls	226.4	547.6				422.5	1063.5			
			(0.293)	(0.707)				(0.284)	(0.716)			
	AC	Schizophrenia	82.6	519.4	0.215	0.643	0.995	63.7	400.3	3.281	0.0701	0.461
			(0.137)	(0.863)				(0.137)	(0.863)			
		Controls	99.6	674.4				158.5	1327.5			
			(0.129)	(0.871)				(0.107)	(0.893)			
	GG	Schizophrenia	321.4	280.6	0.154	0.694	0.996	231.2	228.8	5.012	0.0252	0.207
			(0.534)	(0.466)				(0.503)	(0.497)			
		Controls	422.6	353.4				837.4	652.6			
			(0.545)	(0.455)				(0.562)	(0.438)			
2	GA	Schizophrenia	183.5	418.5	0.040	0.841	1.00	156.4	303.6	5.326	0.0210	0.178
			(0.305)	(0.695)				(0.340)	(0.660)			
		Controls	232.7	543.3				422.8	1067.2			
			(0.300)	(0.700)				(0.284)	(0.716)			
	TA	Schizophrenia	97.1	504.9	0.081	0.776	0.999	72.4	387.6	0.027	0.869	1.00
			(0.161)	(0.839)				(0.157)	(0.843)			
		Controls	120.7	655.3				229.8	1260.2			
			(0.156)	(0.844)				(0.154)	(0.846)			

Numbers in parentheses represent the frequencies of haplotypes. Permutation P values were based on 10,000 permutations.

the production of prostaglandin E2, which is an important cofactor for the induction of T-helper lymphocyte activity towards Th2 direction. Significant increase in circulating mRNA expression levels of IL-1β has been observed in schizophrenic patients [9]. The changes in mRNA levels may reflect the genetic variation in *IL-1β* gene. The findings on biological roles of *IL-1β* polymorphisms, however, have not been consistent across studies. A/A genotype of rs16944 has been associated with higher gastric mucosa IL-1β levels in H. pylori positive population [48]. On the other hand, subjects with G/G genotype showed an increased release of IL-1β from mononuclear cells after stimulation with lipopolysaccharide [49]. Recent studies suggest that the functional role of rs16944 may depend on the *IL-1β* promoter region haplotypes including rs16944 and rs1143627 [50-53]. Although the findings are inconsistent, these previous studies suggest that rs16944 could affect the expression levels of IL-1β. On the other hand, the influence of rs1143633 on IL-1β expression levels has not been previously reported.

Intriguingly, rs1143633 and rs16944 have also been associated with cortisol response to dexamethasone in healthy subjects [30]. Alleles associated with increased cortisol response to dexamethasone were shown to be associated with schizophrenia in the present study. Higher rates of non-suppression to dexamethasone compared to healthy subjects have been reported in schizophrenia [54] and schizotypy [55]. On the other hand, Ismail et al [56] reported that less than 2% of their schizophrenic patients were non-suppressors. Although the findings are inconsistent, these studies indicate that schizophrenia may be associated with alteration in hypothalamic- pituitary- adrenal (HPA) axis. Taken together, our findings suggest that *IL-1β* gene polymorphisms may play a role in the HPA axis alteration in schizophrenic patients.

Our results showed significant association of rs1143633 with schizophrenia in only females. Although our male sample was not large enough to detect a small relative risk, our data suggest that susceptibility to schizophrenia is more influenced by the *IL-1β* gene variation in females. To our knowledge, no previous studies have examined the gender differences in the association between *IL-1β* gene polymorphisms and schizophrenia. However, gender differences have been reported in the association between schizophrenia and RELA gene [27] encoding the major component of NF-κB, which is activated by IL-1β. Taken together with our results, the influence of IL-1β on susceptibility to schizophrenia may differ between genders. Indeed, gender differences in immunity have been reported in previous studies [36]. IL-1 release from mononucleated cells has been shown to be menstrual phase dependent in females and lower in males [57]. Furthermore, in vitro stimulation of lymphocytes with phytohemagglutinin has shown that females produce more Th2 cytokines than males [58]. Thus, future studies investigating associations of immune-related genes with schizophrenia should take into consideration the possible gender differences.

There are some limitations to this study. The ethnicity of the participants was based on self-reports and was not confirmed by genetic analyses. Our positive results might be derived from sample bias due to population stratification, although the Japanese are a relatively homogeneous population. Furthermore, structured interview such as SCID (Structured Clinical Interview for DSM) was not used for diagnosis in this study. Finally, the function of the *IL-1β* gene SNPs are unclear. Future studies are necessary to elucidate the function and its relationship with the pathogenesis of schizophrenia.

Conclusions

Our results suggest that rs1143633 of *IL-1β* gene is associated with schizophrenia susceptibility in a Japanese population and that the influence of *IL-1β* gene variations on susceptibility to schizophrenia may be greater in females than in males. We obtained no significant evidence for a well-studied polymorphism rs16944 being associated with schizophrenia, which is consistent with previous studies in Asian populations. However, a trend of higher A allele frequency of rs16944 in female patients with schizophrenia leaves open a possibility that a larger study may yield a significant difference. The results of the present study provide further support for the role of IL-1β in the etiology of schizophrenia. Future studies are warranted to replicate the present findings and to reveal the functional role of *IL-1β* gene in pathophysiology of schizophrenia.

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Authors' contributions

DS and HK designed the study and DS wrote the draft of the manuscript. DS, HH, TT, KH, MO, MT, and HK made the diagnosis according to DSM-IV criteria. DS, HH, TT, KH, MO, and HK screened the healthy participants using the Mini International Neuropsychiatric Interview (M.I.N.I.). DS and YI performed the genotyping. HK supervised the data analysis and writing of the paper. TH and NA also supervised the writing of the paper and gave critical comments on the manuscript. All authors contributed to and have approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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

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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, $p = 0.009$).

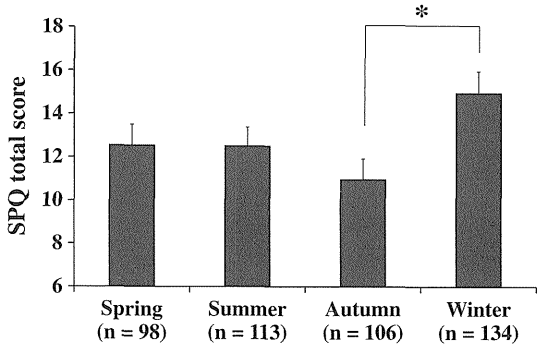

Fig. 1. Comparisons of the total SPQ score between individuals born in each of the four seasons (based on the Traditional criterion). *$p < 0.05$ (according to the ANCOVA with post-hoc analysis with Bonferroni correction).

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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