administration of METH significantly ($p<0.001$) increased METH (1 mg/kg)-induced hyperlocomotion in mice previously treated with METH, compared with the control group. The post hoc analysis also showed that pretreatment with SFN (10 mg/kg) significantly [F (3, 27)=6.13, $p<0.05$] attenuated the development of sensitization after the administration of METH. In contrast, locomotion in the SFN (10 mg/kg)+vehicle group did not differ from that of the control (vehicle+vehicle) group (Fig. 2).

Effects of SFN on hyperthermia induced by the administration of METH

Two-way ANOVA analysis revealed significant differences among the four groups [F (3, 9)=38.51, $p<0.0001$]. Repeated injections of METH (3 mg/kg×3 at 3-h intervals) produced significant hyperthermia in these mice (Fig. 3). However, pretreatment with SFN (10 mg/kg) did not affect the induction of METH-induced hyperthermia in the mice (Fig. 3). Furthermore, SFN (10 mg/kg) alone did not alter rectal temperatures in these mice.

Effects of SFN on the reduction in DA and DOPAC levels in the striatum by the repeated administration of METH

One-way ANOVA analysis revealed that striatal DA [F (3, 38)=12.95, $p<0.0001$] and DOPAC [F (3, 38)=5.60, $p=0.0028$] levels were significantly different among the four groups studied. Pretreatment and a subsequent dose of SFN (10 mg/kg) significantly attenuated the reduction of DA and DOPAC in the striatum typically observed after repeated administration of METH (Fig. 4). Furthermore, treatment

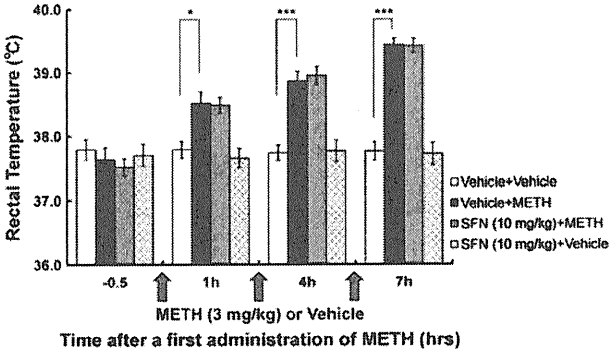

Fig. 3 Effect of SFN on METH-induced hyperthermia in mice. Mice received three injections of vehicle (10 ml/kg, 3-h intervals, s.c.) or METH (3 mg/kg, 3-h intervals, s.c). Vehicle (10 ml/kg, i.p.) or SFN (10 mg/kg, i.p.) was injected into the mice 30 min prior the first injection of METH or vehicle. Rectal temperature was recorded 30 min before the first injection of METH or vehicle and 1, 4, or 7 h after the first METH (or vehicle) injection. Each value is the mean± SEM (n=8–9 per group). *$p<0.05$, ***$p<0.001$ as compared to the vehicle+METH group (Bonferroni/Dunn method)

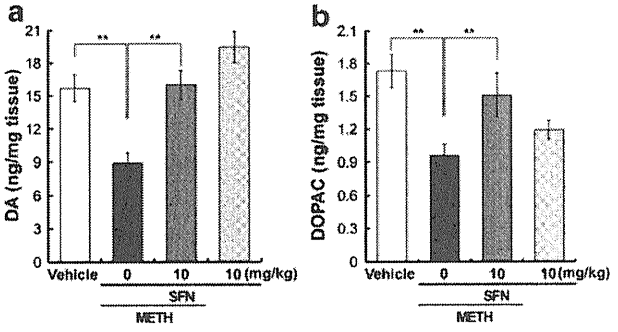

Fig. 4 Effects of SFN on DA (**a**) and DOPAC (**b**) levels in the mouse striatum after the repeated administration of METH. Thirty minutes after i.p. injection of SFN (10 mg/kg) or vehicle (10 ml/kg), mice received three injections of METH (3 mg/kg, s.c.) or vehicle (10 ml/kg, s.c.) at 3-h intervals (day 1). Then, vehicle (10 ml/kg, i.p.) or SFN (10 mg/kg, i.p.) was administered to the mice 12 h after the first administration of vehicle or SFN (day 1). Mice received two daily (12-h intervals) injections of SFN (10 mg/kg, i.p.) or vehicle (10 ml/kg, i.p.) for two consecutive days (days 2 and 3). Mice were sacrificed 3 days after the administration of METH (day 4). Values are the mean±SEM (n=10–11 per group). **$p<0.01$ as compared to the vehicle+METH group (Bonferroni/Dunn method)

with SFN (10 mg/kg) alone did not alter levels of DA and DOPAC in the mouse striatum (Fig. 4).

DAT immunohistochemistry

Repeated administration of METH (3 mg/kg×3 at 3-h intervals) markedly decreased the density of DAT in the mouse striatum (Fig. 5). One-way ANOVA analysis showed significant differences in DAT immunoreactivity in the striatum [F (3, 30)=55.93, $p<0.0001$] among the four groups. The post hoc analysis indicated that pretreatment and subsequent administration of SFN (10 mg/kg) significantly ($p<0.0001$) attenuated the reduction of DAT immunoreactivity in the mouse striatum usually seen after repeated administration of METH (Fig. 5). The administration of SFN (10 mg/kg) alone did not alter the density of DAT immunoreactivity in the mouse striatum (Fig. 5).

MAC1 immunohistochemistry

Three days after the repeated dose of METH (3 mg/kg×3 at 3-h intervals), MAC1 immunoreactivity in the mouse striatum was markedly increased (Fig. 6). One-way ANOVA analysis revealed significant differences among the four groups [F (3, 31)=277.41, $p<0.0001$], and the post hoc analysis showed that pretreatment and a subsequent administration of SFN (10 mg/kg) significantly ($p<0.001$) attenuated the increase in MAC1 immunoreactivity in the striatum associated with the administration of METH. Treatment with SFN (10 mg/kg) plus vehicle had no effect on MAC1 immunoreactivity in the mouse striatum (Fig. 6).

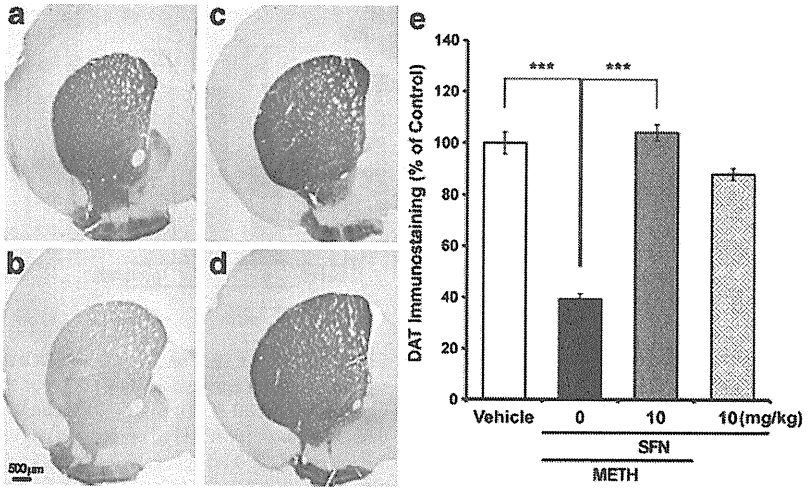

Fig. 5 Effects of SFN on the reduction of DAT density in the mouse striatum after repeated administration of METH. **a** Vehicle+vehicle, **b** vehicle+METH, **c** SFN+METH, and **d** SFN+vehicle. Thirty minutes after i.p. injection of SFN (10 mg/kg) or vehicle (10 ml/kg), the mice received three injections of METH (3 mg/kg, s.c.) or vehicle (10 ml/kg, s.c.) at 3-h intervals (day 1). Then, vehicle (10 ml/kg, i.p.) or SFN (10 mg/kg, i.p.) was administered to the mice 12 h after the first administration of vehicle or SFN (day 1). The mice received two daily (12-h intervals) injections of SFN (10 mg/kg, i.p.) or vehicle (10 ml/kg, i.p.) for two consecutive days (days 2 and 3). The mice were perfused 3 days after the administration of METH (day 4). **a–d** Representative photomicrographs showing the DAT immunoreactivity in the striatum of the mice. *Scale bar* was 500 μm. The mean value for DAT immunoreactivity staining was determined for each group and was expressed as a percentage of that of matched control group (**e**). Each value is the mean±SEM (n=7–9 per group). ***p<0.001 as compared to the vehicle+METH group (Bonferroni/Dunn method)

Discussion

A major finding of this study is that SFN ameliorated behavioral changes such as acute hyperlocomotion and the development of behavioral sensitization typically induced by the administration of METH. It also showed that SFN protects against METH-induced dopaminergic neurotoxicity in the mouse striatum. SFN occurs naturally in cruciferous vegetables like broccoli, cabbage, watercress, and Brussels sprouts, in its precursor form, glucosinolate. On chewing, the glucose moiety of this glucosinolate precursor is hydrolyzed by myrosinase into the corresponding isothiocyanate (Fenwick et al. 1983). Interestingly, dietary SFN-rich sprouts reduce colonization and attenuate gastritis in *Helicobacter pylori*-infected humans (Yanaka et al. 2009; Yanaka 2011). In addition, a phase II study of SFN (200 μmol (35 mg) daily) in patients with recurrent prostate cancer is now in progress (NCT01228084). Taken together, our results suggest that SFN could be a promising, therapeutic drug for the treatment of multiple signs associated with METH abuse in humans, particularly as it is safe for human consumption.

The acute pharmacological effects of amphetamines such as METH are linked to their capacity to increase extracellular DA levels via the release of DA from presynaptic terminals and by the inhibition of DA re-uptake (Seiden et al. 1993). In this study, we found that pretreatment with SFN attenuated acute hyperlocomotion in mice, induced by a single dose of METH. This study does not identify the precise cellular mechanisms for the actions of SFN on METH-induced behavioral effects; however, the findings at least in part suggest that SFN acts by decreasing extracellular DA levels in the mouse striatum. SFN is widely reported to induce Nrf2-dependent gene expression, although its molecular targets have not been fully characterized (Juge et al. 2007; Cheung and Kong 2010; Kwak and Kensler 2010). It remains unclear as to whether activation of the Nrf-2-electrophile-responsive element/ARE pathway accounts for the ability of SFN to diminish the acute behavioral effects induced by METH in mice. Further studies on the ability of this agent to ameliorate METH-associated acute neurochemical and behavioral effects will be necessary.

Repeated exposure to METH results in a progressively enhanced and enduring behavioral response to the drug, a phenomenon known as behavioral sensitization. A number of behavioral, neurochemical, biochemical, and molecular studies have shown that the initiation of this complex process involves the interaction of several neurotransmitters, neuropeptides, neurotrophic factors, and their associated receptor signaling pathways (Robinson and Becker 1986; Pierce and Kalivas 1997; White and Kalivas 1998; Licata and Pierce 2003; Vanderschuren and Kalivas 2000; Scholl et al. 2009). Determining the full interplay of these factors remains elusive. Several studies propose the involvement of the mesolimbic dopamine system, including the ventral tegmental area, nucleus accumbens, and associated brain regions such as the striatum in the development of behavioral sensitization. Previously, we reported that antioxidants

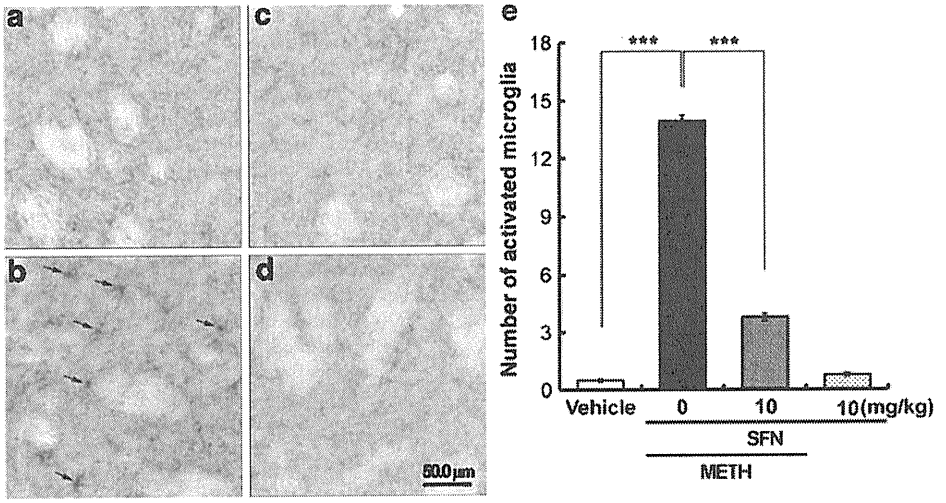

Fig. 6 Effects of SFN on microglial activation in the mouse striatum after the repeated administration of METH. **a** Vehicle+vehicle, **b** vehicle+METH, **c** SFN+METH, and **d** SFN+vehicle. Thirty minutes after i.p. injection of SFN (10 mg/kg) or vehicle (10 ml/kg), the mice received three injections of METH (3 mg/kg, s.c.) or vehicle (10 ml/kg, s.c.) at 3-h intervals (day 1). Then, vehicle (10 ml/kg, i.p.) or SFN (10 mg/kg, i.p.) was administered to the mice 12 h after the first administration of vehicle or SFN (day 1). The mice received two daily (12-h intervals) injections of SFN (10 mg/kg, i.p.) or vehicle (10 ml/kg, i.p.) for two consecutive days (days 2 and 3). Mice were perfused 3 days after the administration of METH (day 4), and MAC1 immunohistochemistry was performed. **a–d** Representative photomicrographs depicting MAC1 immunoreactivity (activated microglia) in the striatum of mice. Resting state microglia was observed in the striatum of the control mice (**a**). Activated microglia was observed in the striatum of METH-treated mice (**b**). *Scale bar* was 50 μm. The mean value representing the staining of activated microglia was determined for each group and was expressed as a percentage of that of matched control mice (**c**). The number of activated microglia was counted based on one microscopic view (×200) (**e**). Each value is the mean±SEM (n=8–9 per group). ***p<0.001 as compared to vehicle+METH group (Bonferroni/Dunn method)

such as *N*-acetyl-L-cysteine and minocycline attenuate the development of METH-induced behavioral sensitization in rodents (Fukami et al. 2004; Zhang et al. 2006). It is therefore likely that the potent antioxidant properties of SFN play a role in its actions, although further detailed studies are needed to confirm this.

In this study, we found that pretreatment and subsequent administration of SFN (10 mg/kg) significantly attenuated the METH-induced reduction of DA and DOPAC levels as well as DAT immunoreactivity in the mouse striatum. These protective mechanisms may block the neurotoxic effects on DA neurons. Further studies will still be necessary to fully define these mechanisms. In the treatment paradigm of METH-induced neurotoxicity (3 mg/kg×3 at 3-h intervals), we performed behavioral evaluations in mice after three injections of METH. We found that behavioral abnormalities in mice after the first administration of METH are similar to the results (Fig. 1) of acute METH administration (Supplemental Fig. 1). In contrast, we found that behavioral abnormalities in mice after the third administration of METH were similar between the groups, vehicle+METH and SFN+METH, indicating that the effect of SFN was not detectable in behavioral abnormalities after the third administration of METH (Supplemental Fig. 1). Thus, it is unlikely that the biochemical effects of SFN on METH-induced neurotoxicity are similar to the behavioral effects of SFN on METH-induced behavioral abnormalities.

A number of studies show that the METH-induced neurotoxic effect on DA nerve endings within the striatum is associated with microglial activation (Escubedo et al. 1998; Pubill et al. 2003; Guilarte et al. 2003; LaVoie et al. 2004; Thomas et al. 2004; Thomas and Kuhn 2005; Zhang et al. 2006). The temporal and dosage relationships between METH-induced neurotoxicity and microglial activation suggest that this activation might contribute to METH-induced neurotoxicity in the striatum (Thomas et al. 2004). Interestingly, SFN reduces lipopolysaccharide-induced microglial activation in the mouse brain, suggesting that SFN is a potent inhibitor of microglial activation (Innamorato et al. 2008). We conclude that, in part, SFN reduces METH-induced neurotoxicity in the mouse striatum by inhibiting microglial activation. Additional studies on the role of microglial activation in METH-induced dopaminergic neurotoxicity are warranted.

A number of studies indicate that neurotoxic doses of METH cause hyperthermia and that hypothermia can suppress METH-induced neurotoxicity, suggesting a role for body temperature in METH-induced dopaminergic neurotoxicity (Albers and Sonsalla 1995; Ali et al. 1996). However, we found no evidence that SFN altered METH-induced hyperthermia in mice. Therefore, it is unlikely that body temperature plays a role in the protective effect of SFN on METH-induced neurotoxicity in mice.

Recently, it has been reported that SFN increases Nrf2 protein levels in the mouse striatum and protects against MPTP-induced death of nigral dopaminergic neurons in a cell culture model of Parkinson's disease (Jazwa et al. 2011). In the neonatal hypoxia–ischemia rat model, pretreatment with SFN increases the expression of Nrf2 immunoreactivity, while decreasing the number of TUNEL-positive neurons and microglial activation in the rat brain (Ping et al. 2010), suggesting that its neuroprotective effect is mediated through increased Nrf2 expression. It is likely that SFN protects against dopaminergic neurotoxicity in the mouse striatum by increasing Nrf2 expression, although this needs to be confirmed.

In conclusion, this study demonstrated that in mice, SFN inhibited METH-induced behavioral changes such as acute hyperlocomotion and the development of behavioral sensitization and dopaminergic neurotoxicity in mice. This makes SFN a promising therapeutic agent for the treatment of multiple signs associated with METH abuse, since it is a naturally occurring compound found in cruciferous vegetables.

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Conflicts of interest All authors had no potential conflict of interest.

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Associations of serum brain-derived neurotrophic factor with cognitive impairments and negative symptoms in schizophrenia

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ABSTRACT

Brain-derived neurotrophic factor (BDNF) may be involved in the pathophysiology of schizophrenia. The aim of this study was to examine the associations of serum BDNF levels with the cognition and clinical characteristics in patients with schizophrenia. Sixty-three patients with schizophrenia and 52 age- and sex-matched healthy controls were examined with neuropsychological tests. Serum BDNF levels were determined by enzyme-linked immunosorbent assay (ELISA). There were no significant differences in serum BDNF levels between normal controls and patients with schizophrenia. Serum BDNF levels of normal controls showed negative correlations with verbal working memory, but this was not the case with schizophrenic patients. Meanwhile, serum BDNF levels of schizophrenic patients showed positive correlations with the scores of the Scale for the Assessment of Negative Symptoms (SANS) and the Information subtest scores of Wechsler Adult Intelligence Scale Revised (WAIS-R). Serum BDNF levels are related with the impairment of verbal working memory and negative symptoms in patients with schizophrenia.

1. Introduction

Schizophrenia is characterized by three distinct symptom clusters: positive symptoms, negative symptoms, and cognitive impairments. Negative symptoms, an important and enduring component of the psychopathology of schizophrenia (Stahl and Buckley, 2007), include blunted affect, alogia, asociality, anhedonia and avolition (Andreasen, 1982; Kirkpatrick et al., 2006). Negative symptoms predict quality of life, social functioning and overall outcome measures in patients with schizophrenia (Bow-Thomas et al., 1999; Dickerson et al., 1999; Milev et al., 2005). Negative symptoms and cognitive impairments are involved in the prefrontal cortex (Ingvar and Franzen, 1974; Weinberger, 1988) and share many features, but are separable domains of illness (Harvey et al., 2006). While positive symptoms are greatly improved with atypical antipsychotic medication, negative symptoms and cognitive impairments are not sufficiently improved (Erhart et al., 2006; Keefe et al., 2007).

The cognitive impairments are the core features of schizophrenia, with both working memory and attention being characteristically impaired in patients with schizophrenia (Elvevag and Goldberg, 2000; Reichenberg, 2010). Cognitive deficits are related to community outcome, social problem solving and skill acquisition (Green, 1996), and therefore might predict the functional outcome in schizophrenic patients (Green et al., 2004).

Accumulating evidence suggests that brain-derived neurotrophic factor (BDNF) plays a role in the pathophysiology of psychiatric diseases, including depression and schizophrenia (Angelucci et al., 2005). It is well documented that BDNF is involved in neuronal survival, differentiation and outgrowth during brain development (Numakawa et al., 2010). Recently, a meta-analysis study showed that blood levels of BDNF were reduced in medicated and drug-naïve patients with schizophrenia (Green et al., 2010). However, the significant heterogeneity across the study results remained unexplained.

Abbreviations: BDNF, Brain-derived neurotrophic factor; BMI, Body-mass index; BPRS, Brief psychiatry rating scale; DIEPSS, Drug induced extrapyramidal symptoms scale; DSDT, Digit span distraction test; ELISA, Enzyme-linked immunosorbent assay; IQ, Intelligence quotient; PANSS, Positive and negative syndrome scale; SANS, Scale for the assessment of negative symptoms; WAIS-R, Wechsler adult intelligence scale revised; WCST, Wisconsin card sorting test.

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In this study, we examined the associations of serum BDNF levels with negative symptoms and cognitive impairments in patients with schizophrenia. To assess the cognitive functioning of the prefrontal cortex, 5 neuropsychological tests, verbal fluency, Wisconsin card sorting test (WCST), Stroop test, digit span distraction test (DSDT), and trail making test were administered. The rationale for choosing these tests stems from the hypothesis that each test works on a region-dominant part (medial or dorsolateral portions) of the brain and could examine the region-related functions.

2. Methods

2.1. Subjects

Sixty-three Japanese patients with schizophrenia (age: mean, 35.9 [SD, 8.2]; education: mean, 13.8 [SD, 2.3]; 26 men and 37 women) were recruited from the outpatients of the Chiba University Hospital and its affiliated hospitals, Chiba, Japan. Fifty-two age- and sex-matched healthy Japanese subjects also participated in this study as normal controls. Characteristics of the subjects are shown in Table 1. All subjects provided written informed consent for participation in the study after the procedure had been fully explained. The ethics committee of Chiba University Graduate School of Medicine approved the present study.

All patients were diagnosed according to the DSM-IV criteria for schizophrenia, and had no other psychiatric disorders, assessed by two senior level psychiatrists. Of the patients, 44 were diagnosed as the residual type and 19 were the paranoid type. They had been clinically stable for at least 3 months. All patients had been receiving monotherapy with a stable dose of a second-generation antipsychotic drug for at least 8 weeks prior to entry into the study. The antipsychotic drugs were risperidone (n = 25), olanzapine (n = 18), quetiapine (n = 8), perospirone (n = 2), aripiprazole (n = 9), and bronanserine (n = 1). The chlorpromazine-equivalent dose was 306 ± 240 (means ± SD) mg/day (Woods, 2003). Normal controls were recruited from the local community around the Chiba University Hospital. None of the normal controls presented with a personal history of psychiatric or neurological disorder, assessed by two senior level psychiatrists.

2.2. Clinical assessments

Clinical symptoms were assessed by using the Brief Psychiatry Rating Scale (BPRS) (Overall and Gorham, 1962) and the Scale for the Assessment of Negative Symptoms (SANS) (Andreasen, 1982). Drug-induced extrapyramidal symptoms were evaluated by using the Drug Induced Extrapyramidal Symptoms Scale (DIEPSS), because cognitive functions are influenced by extrapyramidal motor side effects (Inada et al., 2002). Intelligence quotient (IQ) scores were estimated by using the short version of the Japanese Wechsler Adult Intelligence Scale Revised (WAIS-R) (Misawa et al., 1993; Nakamura et al., 2000), which consisted of the Information, Digit Span, and the Picture Completion subtests. Age at onset, duration of illness and duration of untreated psychosis were evaluated.

2.3. Enzyme immunoassay

Blood samples of the participants were collected between 10:00 and 13:00 h. Serum was then separated by centrifugation at 3000 rpm for 7 min and stored at −80 °C until assay. Serum BDNF levels were measured by using a BDNF Emax Immunoassay System kit (Promega, Madison, WI).

2.4. Neuropsychological assessments

In the Verbal Fluency Test (letter, category), the number of words produced in 1 min for each trial was recorded for evaluation (Sumiyoshi et al., 2005). In the WCST, the number of achieved categories and perseverative errors were assessed (Shad et al., 2006). We used the short version of the WCST (Keio version; 48 cards) to shorten the procedural time (Hori et al., 2006; Igarashi et al., 2002). In the Trail Making Test Part A and Part B, the time taken to complete each part of the test was assessed in seconds (Reitan and Wolfson, 1993). In the Stroop Test, a list of 24 colored dots (Part D) and 24 colored words incongruent with the color (Part C) were used (Carter et al., 1995; Chan et al., 2004). The reaction time taken to complete each part of the test was assessed in seconds. In the DSDT, subjects were asked to remember a tape-recorded string of digits read by a female voice while ignoring the digits read by a male voice (distractor) (Green et al., 1997; Oltmanns and Neale, 1975). The percentages of digits correctly recalled under the condition with and without a distractor were assessed separately.

2.5. Statistical analysis

All statistical analyses were performed by using SPSS software (SPSS version 18.0J; SPSS, Tokyo, Japan). For the comparisons

Table 1
Demographic characteristics and serum BDNF levels of subjects.

	Controls	Patients				Controls vs patients
	n = 52	n = 63	Subtype		Residual vs paranoid	
			Residual	Paranoid		
			n = 44	n = 19	p	p
Gender (male/female)	25/27	26/37	19/25	7/12	NS [a]	NS [a]
Age, year	34.9 (7.3)	35.9 (8.2)	36.7 (8.3)	34.1 (8.1)	NS [b]	NS [c]
Education, year	14.7 (2.7)	13.8 (2.3)	13.8 (2.4)	13.7 (2.1)	NS [b]	NS [b]
Smoking (Non-smoker/smoker)	43/9	45/18	33/11	12/7	NS [a]	NS [a]
Age at onset of illness, year	–	26.8 (7.0)	27.5 (7.3)	25.2 (6.1)	NS [c]	–
Duration of illness, year	–	9.1 (7.3)	9.2 (6.8)	9.0 (8.6)	NS [b]	–
Duration of untreated psychosis, month	–	8.1 (13.4)	7.3 (9.6)	9.8 (19.9)	NS [b]	–
BPRS	–	25.5 (7.5)	23.7 (7.1)	29.6 (6.9)	< 0.05 [b]	–
SANS	–	70.4 (11.8)	68.1 (12.0)	75.7 (9.5)	< 0.05 [b]	–
DIEPSS	–	2.7 (2.7)	2.5 (2.5)	3.3 (3.3)	NS [b]	–
BDNF, ng/ml	14.6 (4.4)	15.3 (3.8)	14.9 (3.6)	16.2 (4.2)	NS [c]	NS [b]

Values represent mean (SD). NS, not significant.
Abbreviation: BPRS, Brief Psychiatric Rating Scale; SANS, Scale for the Assessment of Negative Symptoms; DIEPSS, Drug Induced Extra-Pyramidal Symptoms Scale; BDNF, Brain-Derived Neurotrophic Factor.
[a] χ^2 test.
[b] Mann–Whitney *U*-test.
[c] Student's *t*-test.

between patients and normal controls, the Chi-squared test was used for the categorical variables, and Student's *t*-test and the Mann–Whitney *U* test were employed for the continuous variables. Pearson's and Spearman's correlation coefficients were examined to identify any correlations of serum BDNF levels with the clinical variables of patients and with the scores of neuropsychological tests of all subjects. The Shapiro–Wilk test was used to assess normal distribution. Significance for the results was set at $p<0.05$.

3. Results

3.1. Serum BDNF levels between schizophrenic patients and healthy controls

There was no significant difference in serum BDNF levels between normal controls (mean, 14.6 ng/ml [SD, 4.4]) and patients with schizophrenia (mean, 15.3 ng/ml [SD, 3.8]) ($U=1870.5$, $p>0.05$). Furthermore, we found no differences in BDNF levels between the residual and paranoid types of schizophrenia (Table 1), and no differences in serum BDNF levels among the four drug groups, i.e., the risperidone, olanzapine, quetiapine and the aripiprazole groups (Table 2).

3.2. Correlation between serum BDNF levels and cognitive functions

As shown in Table 3, significant differences were observed between patients with schizophrenia and normal controls in all neuropsychological tests.

Then, we examined the correlations between serum BDNF levels and scores of the cognitive function tests. Interestingly, we found significant negative correlations between serum BDNF levels and scores of DSDT in normal controls, but not in patients with schizophrenia (Table 4). There were no other correlations between serum BDNF levels and the scores of neuropsychological tests in patients or controls. Additionally, we found significant positive correlations between the Information subtest scores of WAIS-R and the scores of the DSDT in normal controls; without distractor ($r=0.332$, $p=0.016$) and with distractor ($r=0.259$, $p=0.064$) respectively, but not in patients with schizophrenia.

3.3. Correlation between serum BDNF levels and clinical variables

There was a significant positive correlation between serum BDNF levels and SANS total scores in patients with schizophrenia (Table 4). Of the five subscale symptom groups in SANS, significant positive correlations with the serum BDNF level were detected in two subscales (S1 affective flattering–blunting, S4 anhedonia-asociality), but not in the S2 alogia, S3 avolition-apathy or S5 attention impairment subscales (Table 4).

Table 3
Cognitive data of subjects.

	Controls	Patients	p
	n=52	n=63	
Estimated IQ	110.2 (12.0)	102.4 (13.9)	<.01[a]
Information	11.1 (2.6)	10.1 (2.7)	<.05
Digit span	11.7 (2.9)	10.6 (2.9)	<.05
Picture completion	11.0 (1.9)	10.5 (2.2)	NS
Letter fluency test, words	35.2 (9.0)	28.0 (8.9)	<.001
Category fluency test, words	49.1 (6.8)	39.9 (6.9)	<.001[a]
WCST, accomplished categories	4.9 (1.5)	3.3 (2.2)	<.001
WCST, perseverative errors	0.9 (1.8)	4.5 (6.7)	<.001
Trail making test A, sec	27.2 (7.7)	33.8 (10.1)	<.001
Trail making test B, sec	52.9 (16.0)	80.5 (27.1)	<.001
Stroop test part D, sec	12.7 (2.5)	14.2 (2.6)	<.01
Stroop test Part C,sec	18.6 (5.3)	22.7 (5.9)	<.001
DSDT without distractor, %	83.0 (14.9)	74.8 (17.5)	<.01
DSDT with distractor, %	92.4 (9.8)	82.8 (17.7)	<.001

Values represent mean (SD). NS, not significant.
Abbreviations: WCST, Wisconsin Card Sorting Test; DSDT, Digit Span Distraction Test.
[a] Student's *t*-test. The other p values are by Mann–Whitney *U*-test.

In patients with schizophrenia, among the three subtests in the short version of the WAIS-R, a significant correlation with serum BDNF levels was detected in the Information subtest scores, but not in the Digit Span or Picture Completion subtests (Table 4). Furthermore, we found no correlation between the Information subtest scores and SANS scores ($r=-0.009$, $p=0.944$). Therefore, the Information subtest scores and SANS scores were independently correlated with the serum BDNF levels.

The serum BDNF levels in patients were not correlated with the age of illness onset ($r=0.186$, $p=0.143$), the duration of illness ($r=0.069$, $p=0.590$) or the duration of untreated psychosis ($r=-0.059$, $p=0.646$). There were no significant correlations between serum BDNF levels and BPRS scores ($r=-0.001$, $p=0.991$) or DIEPSS scores ($r=0.054$, $p=0.673$). There was no significant correlation between serum BDNF levels and antipsychotic dosages ($r=0.066$, $p=0.606$).

4. Discussion

The primary finding of the present study is a positive correlation of serum BDNF levels with the SANS scores in the patients with schizophrenia. Furthermore, serum BDNF levels correlated with only negative dimensions (affective flattening, anhedonia) of the SANS subscales, but not with cognitive dimensions (alogia, attention) of the SANS subscales in the schizophrenic patients. This finding was in a good agreement with a previous study which demonstrated a positive correlation between serum BDNF levels and the negative subscale of the Positive and Negative Syndrome Scale (PANSS) in chronic patients with schizophrenia (Reis et al., 2008). Also, we

Table 2
Characteristics of the patients by the type of atypical antipsychotics.

Type of atypical antipsychotics	Total					p
	n=60	Risperidone	Olanzapine	Quetiapine	Aripiprazole	
		n=25	n=18	n=8	n=9	
Age, year	36.2 (8.2)	38.4 (7.0)	34.9 (10.1)	34.8 (7.4)	33.7 (7.5)	NS[a]
Onset, year	26.9 (7.1)	25.9 (5.6)	26.3 (9.3)	30.6 (5.9)	27.4 (7.1)	NS
Duration of illness, year	9.3 (7.4)	12.5 (7.9)	8.6 (7.4)	4.1 (2.1)	6.2 (5.1)	<0.01[b]
Antipsychotic drug dose,[c] mg/day	313.7 (244.0)	228.0 (154.2)	230.6 (121.4)	791.7 (267.7)	293.3 (80.0)	<0.001[d]
BDNF, ng/ml	15.4 (3.9)	16.6 (3.9)	15.3 (4.2)	14.6 (3.2)	12.7 (3.0)	NS

Values represent mean (SD). NS, not significant. Perospirone (n=2) and bronanserine (n=1) groups are excluded.
Abbreviation: BDNF, Brain Derived Neurotrophic Factor.
[a] P values are by ANOVA. The other p values are by Kurskal–Wallis test.
[b] Risperidone vs Quetiapine is significant by post hoc Bonferroni correction ($p<0.05$).
[c] Chlorpromazine equivalent dose.
[d] Risperidone vs Quetiapine and Olanzapine vs Quetiapine are each significant by post hoc Bonferroni correction ($p<0.05$).

Table 4
Correlation coefficients with serum BDNF levels.

	Controls	Patients
	n = 52	n = 63
Subtests of WAIS-R		
Information	−0.205	0.291*
Digit span	−0.037	0.185
Picture completion	−0.005	0.074
Neuropsychological tests		
Letter fluency test, words	−0.045	0.020
Category fluency test, words	−0.128	0.043[a]
WCST, accomplished categories	−0.177	0.002
WCST, perseverative errors	0.088	0.197
Trail making test A, sec	0.096	0.128
Trail making test B, sec	−0.052	0.048
Stroop test part D, sec	0.180	0.087
Stroop test part C, sec	−0.006	0.152
DSDT without distractor, %	−0.309*	0.048
DSDT with distractor, %	−0.353*	0.060
SANS total score	–	0.291[a,*]
S1 affective flattening	–	0.300[a,*]
S2 alogia	–	0.133[a]
S3 avolition apathy	–	−0.005
S4 anhedonia asociality	–	0.303*
S5 attention	–	0.108

No other clinical values significantly correlated with serum BDNF levels.
[a] Pearson's correlation coefficients. The other values are Spearman's correlation coefficients.
* $p<0.05$.

found a positive correlation between serum BDNF levels and the Information subtest scores of WAIS-R in patients with schizophrenia, but not in normal controls. This finding may further extend a recent study suggesting that measurement of serum BDNF levels may be of use to monitor for a successful training for cognitive enhancement in patients with schizophrenia (Vinogradov et al., 2009).

However, we found no differences in serum BDNF levels between normal controls and patients with schizophrenia. A recent meta-analysis demonstrated a reduction in blood BDNF levels in schizophrenic patients compared to healthy controls (Green et al., 2010). While some studies reported that BDNF levels in the serum of schizophrenic patients were not different from those of healthy volunteers (Huang and Lee, 2006; Jockers-Scherubl et al., 2004; Shimizu et al., 2003), other studies found a significant decrease (Grillo et al., 2007; Pirildar et al., 2004; Tan et al., 2005; Toyooka et al., 2002) or even an increase (Gama et al., 2007). Since the present study showed significant correlations of serum BDNF levels with the SANS scores and Information scores of WAIS-R in schizophrenia, the causes of heterogeneity across study results might include the SANS scores and Information scores of WAIS-R. Previous studies demonstrated a relationship between intelligence and serum BDNF in schizophrenia (Adcock et al., 2009; Vinogradov et al., 2009) and a significant association between BDNF levels and positive symptoms (Buckley et al., 2007, Rizos et al., 2008, 2010a, 2010b).

Additional causes of the heterogeneity across study results may include the different stages of illness, duration of untreated psychosis, subtype (hebephrenic vs paranoid) or influences of antipsychotic medication. In support, a recent study showed association of serum BDNF and duration of untreated psychosis in first episode schizophrenic patients (Rizos et al., 2010a, 2010b). A previous study reported associations between serum BDNF levels and clinical phenotypes in schizophrenic patients (Huang and Lee, 2006), although no differences in BDNF levels were found between the residual and paranoid types of schizophrenia in the present study (Table 1). Recent studies of schizophrenia reported that chronic treatment with olanzapine, lurasidone and risperidone increase BDNF levels (Chen and Huang, 2011; Czubak et al., 2009; Fumagalli et al., 2011) and that serum BDNF increase during antipsychotic treatment (Lee et al., 2011), although no differences in serum BDNF levels were found among the four drug groups (risperidone, olanzapine, quetiapine and aripiprazole groups) in the current study (Table 2).

Since previous studies suggested that serum BDNF levels correlate with gender, age, dietary profile or BMI (Green et al., 2010; Guimaraes et al., 2008; Zhang et al., 2007), these factors may influence the serum BDNF levels in subjects. The lack of these factors' information is considered as limitation.

Finally, the scores of the DSDT were negatively correlated with serum BDNF levels in normal controls, but not in patients with schizophrenia. The DSDT reflects verbal working memory (Green et al., 1997; Oltmanns and Neale, 1975). Thus, serum BDNF levels may associate with verbal working memory in healthy subjects. When we see a rule in normal controls but not in the patients, it is natural to think that the rule plays a role in the pathophysiology of the disease. Thus, the lack of association of serum BDNF levels with cognitive functions of schizophrenic patients in the present results might be related with the dysfunction of working memory, which is different from previous studies (Vinogrado et al., 2009; Carlino et al., 2011). Future study will be needed to elucidate this point.

We did not assess the proteolytic isoforms of BDNF (pro-BDNF, truncated BDNF, mature BDNF) in the serum. BDNF detected by an ELISA assay includes pro-BDNF and truncated-BDNF as well as mat-BDNF (Carlino et al., 2011). Thus, the alteration in serum BDNF in the present result might well include pro-BDNF, truncated-BDNF and mat-BDNF. Furthermore, the study by Carlino et al. (2011) demonstrated that mat-BDNF and pro-BDNF were increased whereas truncated-BDNF was decreased in the schizophrenic patients (Carlino et al., 2011). Therefore, the heterogeneity of serum BDNF examined by an ELISA assay might be due to the differential alteration of mat-BDNF, truncated-BDNF and pro-BDNF. The same study showed that cognitive impairments in patients with schizophrenia correlated with low serum truncated-BDNF isoform levels (Carlino et al., 2011). In the present study, the proteolytic isoforms of BDNF might influence the results of cognitive impairments. The question of why serum BDNF correlates positively with the autistic features of schizophrenia, and correlates negatively with cognitional tests might be answered by the differential alteration of the proteolytic isoforms of BDNF. Future study will be needed to elucidate these points.

5. Conclusion

In conclusion, there was a significant negative correlation between serum BDNF levels and verbal working memory in normal controls, but not in patients with schizophrenia. Meanwhile, the scores of negative symptoms and the scores of a verbal comprehension test each positively correlated with serum BDNF levels in chronic patients with schizophrenia. These findings suggest that the heterogeneity of serum BDNF levels may associate with that of negative symptoms and cognitive impairments in chronic patients with schizophrenia.

Declaration of interest

All authors declare that they have no conflicts of interest.

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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

Table 1
Genotype and allelic distributions of the ABCA1 SNPs in patients with schizophrenia and controls.

db SNP ID and aminoacid change	Position*	Inter-SNP distance (bp)	Gender	Group	N	Genotype distribution (frequency)			χ^2	P	Allele count (frequency)				χ^2	P	HWE of Controls (df = 1)
						R/R	*R/K*	*K/K*			*R*		*K*				
rs2230806 Arg219Lys	107620867 exon 7	(–)	All	Schizophrenia	497	119 (0.24)	241 (0.48)	137 (0.28)	2.77	0.250	479	(0.48)	515	(0.52)	0.01	0.897	$\chi^2 = 3.73$
				Controls	932	204 (0.22)	495 (0.53)	233 (0.25)			903	(0.48)	961	(0.52)			P = 0.053
			M	Schizophrenia	274	63 (0.23)	137 (0.50)	74 (0.27)	0.47	0.789	263	(0.48)	285	(0.52)	0.45	0.503	$\chi^2 = 0.05$
				Controls	330	71 (0.22)	162 (0.49)	97 (0.29)			304	(0.46)	356	(0.54)			P = 0.827
			F	Schizophrenia	223	56 (0.25)	104 (0.47)	63 (0.28)			216	(0.48)	230	(0.52)			$\chi^2 = 6.81$
				Controls	602	133 (0.22)	333 (0.55)	136 (0.23)			599	(0.50)	605	(0.50)			P = 0.009
						V/V	*V/M*	*M/M*			*V*		*M*				
rs2066718 Val771Met	107589255 exon 16	31,612	All	Schizophrenia	494	438 (0.89)	54 (0.11)	2 (0.00)	1.09	0.580	930	(0.94)	58	(0.06)	0.99	0.319	$\chi^2 = 0.04$
				Controls	936	812 (0.87)	120 (0.13)	4 (0.00)			1744	(0.93)	128	(0.07)			P = 0.847
			M	Schizophrenia	273	242 (0.89)	29 (0.11)	2 (0.01)	1.70	0.428	513	(0.94)	33	(0.06)	0.50	0.480	$\chi^2 = 0.30$
				Controls	333	287 (0.86)	45 (0.14)	1 (0.00)			619	(0.93)	47	(0.07)			P = 0.582
			F	Schizophrenia	221	196 (0.89)	25 (0.11)	0 (0.00)	1.32	0.518	417	(0.94)	25	(0.06)	0.60	0.437	$\chi^2 = 0.03$
				Controls	603	525 (0.87)	75 (0.12)	3 (0.00)			1125	(0.93)	81	(0.07)			P = 0.856
						I/I	*I/M*	*M/M*			*I*		*M*				
rs2066714 Ile883Met	107586753 exon 18	34,114	All	Schizophrenia	487	208 (0.43)	212 (0.44)	67 (0.14)	3.86	0.145	628	(0.64)	346	(0.36)	1.75	0.186	$\chi^2 = 0.91$
				Controls	917	345 (0.38)	446 (0.49)	126 (0.14)			1136	(0.62)	698	(0.38)			P = 0.339
			M	Schizophrenia	266	115 (0.43)	116 (0.44)	35 (0.13)	3.23	0.199	346	(0.65)	186	(0.35)	0.87	0.335	$\chi^2 = 2.40$
				Controls	330	122 (0.37)	168 (0.51)	40 (0.12)			412	(0.62)	248	(0.38)			P = 0.122
			F	Schizophrenia	221	93 (0.42)	96 (0.43)	32 (0.14)	1.23	0.542	282	(0.64)	160	(0.36)	0.62	0.430	$\chi^2 = 0.002$
				Controls	587	223 (0.38)	278 (0.47)	86 (0.15)			724	(0.62)	450	(0.38)			P = 0.966
						R/R	*R/K*	*K/K*			*R*		*K*				
rs2230808 Arg1587Lys	107562804 exon 35	58,063	All	Schizophrenia	491	174 (0.35)	252 (0.51)	65 (0.13)	4.05	0.132	600	(0.61)	382	(0.39)	0.63	0.427	$\chi^2 = 0.50$
				Controls	923	367 (0.40)	422 (0.46)	134 (0.15)			1156	(0.63)	690	(0.37)			P = 0.478
			M	Schizophrenia	273	87 (0.32)	148 (0.54)	38 (0.14)	8.51	0.014	322	(0.59)	224	(0.41)	3.68	0.055	$\chi^2 = 1.17$
				Controls	327	140 (0.43)	141 (0.43)	46 (0.14)			421	(0.64)	233	(0.36)			P = 0.278
			F	Schizophrenia	218	87 (0.40)	104 (0.48)	27 (0.12)	0.79	0.674	278	(0.64)	158	(0.36)	0.60	0.440	$\chi^2 = 0.01$
				Controls	596	227 (0.38)	281 (0.47)	88 (0.15)			735	(0.62)	457	(0.38)			P = 0.945

HWE; Hardy–Weinberg equilibrium.
* Chromosome position was determined from the dbSNP database.

Table 2
Genotype and allelic distributions of rs2230808 in independent replication sample.

db SNP ID and aminoacid change	Gender	Group	N	Genotype distribution (frequency)			$\chi 2$	P	Allele count (frequency)				$\chi 2$	P	HWE of controls (df = 1)
				R/R	*R/K*	*K/K*			*R*		*K*				
rs2230808	All	Schizophrenia	539	211 (0.39)	252 (0.47)	76 (0.14)	0.25	0.88	676	(0.63)	404	(0.37)	0.03	0.85	$\chi^2 = 0.49$
Arg1587Lys		Controls	511	201 (0.39)	233 (0.46)	77 (0.15)			635	(0.62)	387	(0.38)			P = 0.48
	M	Schizophrenia	283	109 (0.39)	133 (0.47)	41 (0.14)	0.15	0.93	351	(0.62)	215	(0.38)	0.14	0.71	$\chi^2 = 0.01$
		Controls	267	106 (0.40)	125 (0.47)	36 (0.13)			337	(0.63)	197	(0.37)			P = 0.92
	F	Schizophrenia	256	102 (0.40)	119 (0.46)	35 (0.14)	0.97	0.62	323	(0.63)	189	(0.37)	0.43	0.51	$\chi^2 = 1.17$
		Controls	244	95 (0.39)	108 (0.44)	41 (0.17)			298	(0.61)	190	(0.39)			P = 0.28

HWE; Hardy–Weinberg equilibrium.

shown). We evaluated the difference between the all controls and all cases using ANCOVA. The female schizophrenia patients showed smaller gray matter volume in the bilateral insulae, anterior cingulate cortex, and orbitofrontal cortex, than all female controls (Fig. 1E).

We also we evaluated the difference in gray matter volume between the schizophrenic groups with and without the 1587*K* allele for each sex using ANCOVA, controlling for age, duration of illness, educational period, and medication. There were no statistically significant differences between the groups for each sex, however, male patients with schizophrenia carrying the 1587*K* allele showed small gray matter volume in the left occipital region and bilateral posterior cingulate cortices, almost the same as Figure (B), compared with those who did not carry the 1587*K* allele at nominal trend level (F) ($P<0.01$ uncorrected). There were no differences between the female schizophrenic patients with or without the 1587*K* allele using loose criteria ($P<0.01$ uncorrected, data not shown).

4. Discussion

We found that the 1587*K* allele of *ABCA1* was significantly more common in male patients with schizophrenia than in male controls. However, such a difference was not observed in women. Furthermore, our results showed that male schizophrenic patients who carried the 1587*K* allele have smaller gray matter volume than in those who did not, but this difference did not extend to women. To our knowledge, this is the first study that reports the possible association of *ABCA1* with susceptibility to schizophrenia and related brain abnormalities.

4.1. ABCA1 polymorphisms and susceptibility to schizophrenia

The 1587*K* allele was reported to increase cerebrospinal fluid tau level and brain amyloid beta load (Katzov et al., 2004). It was also associated with low plasma levels of apolipoprotein A1 (Tregouet et al., 2004) and HDL-cholesterol (Clee et al., 2001; Frikke-Schmidt et al., 2004), suggesting functional differences between the *R*1587 and 1587*K* alleles, which may explain our results.

The present study showed gender-specific association between *R*1587*K* (rs2230808) and schizophrenia in our population. Serum from men displays an enhanced free cholesterol efflux capacity via the ABCA1 transporter pathway compared with that from perimenopausal women (Catalano et al., 2008). Estradiol was known to modulate a wide range of functions of the brain. From the onset of menopause, declining levels of estradiol can cause cognitive disturbances and changes in behavior that can be counterbalanced by hormone replacement. Studies in mice have suggested that the atheroprotective effects of estrogen may occur partly via the ABCA1-mediated pathway (Srivastava, 2002). Another study found that ABCA1 was up-regulated by estradiol (Sárvári et al., 2010). Taking these previous findings into consideration, the observed sex difference in our study may be explained, at least in part, by the fact that estrogen is involved in the regulation of ABCA1 activity. The role of CNS cholesterol in synaptic function and neurodegenerative disorders has recently been appreciated, but the mechanisms regulating its transport and homeostasis are only partially understood. Therefore, further studies that focused on the sex difference should be needed to reveal the function of the ABCA1.

In the initial 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 1587K allele in male patients remained to be significant in the combined male sample. Though there was the association of *ABCA1* with susceptibility to schizophrenia, it is suggested that this relationship may be fairly weak.

4.2. ABCA1 polymorphism and MRI volumetry

Our results showed that male schizophrenia patients carrying the 1587*K* allele showed smaller gray matter volume than those who did not carry the allele. Schizophrenia has been associated with volume reductions in the limbic, paralimbic, frontal, and temporal cortical regions (Glahn et al., 2008; Ellison-Wright et al., 2008; Shenton et al., 2001; Wright et al., 2000), although some previous studies did not detect disturbances in such regions (Kanaan et al., 2005; Kubicki et al.,

Table 3
Characteristics of the subjects who underwent MRI.

		Genotype distribution of rs2230808	N	Age	Duration of illness	Drug dose (chlorpromazine equivalent)
Men	Control	*R/R*	21	39.8 ± 12.4 (20–71)		
		K carrier	26	38.9 ± 11.9 (25–69)		
		All	47	39.3 ± 12.0 (20–71)		
	Schizophrenia	*R/R*	16	44.7 ± 16.7 (22–76)	21.0 ± 16.9	770.0 ± 636.0
		K carrier	33	43.7 ± 13.0 (27–72)	21.7 ± 12.9	1183.6 ± 945.7
Women	Control	*R/R*	39	48.2 ± 13.5 (25–74)		
		K carrier	53	39.9 ± 11.6 (22–71)		
		All	92	43.4 ± 13.0 (22–74)		
	Schizophrenia	*R/R*	16	47.5 ± 12.3 (22–67)	17.2 ± 13.2	691.6 ± 566.3
		K carrier	21	46.3 ± 15.0 (23–75)	19.0 ± 13.2	731.2 ± 623.9

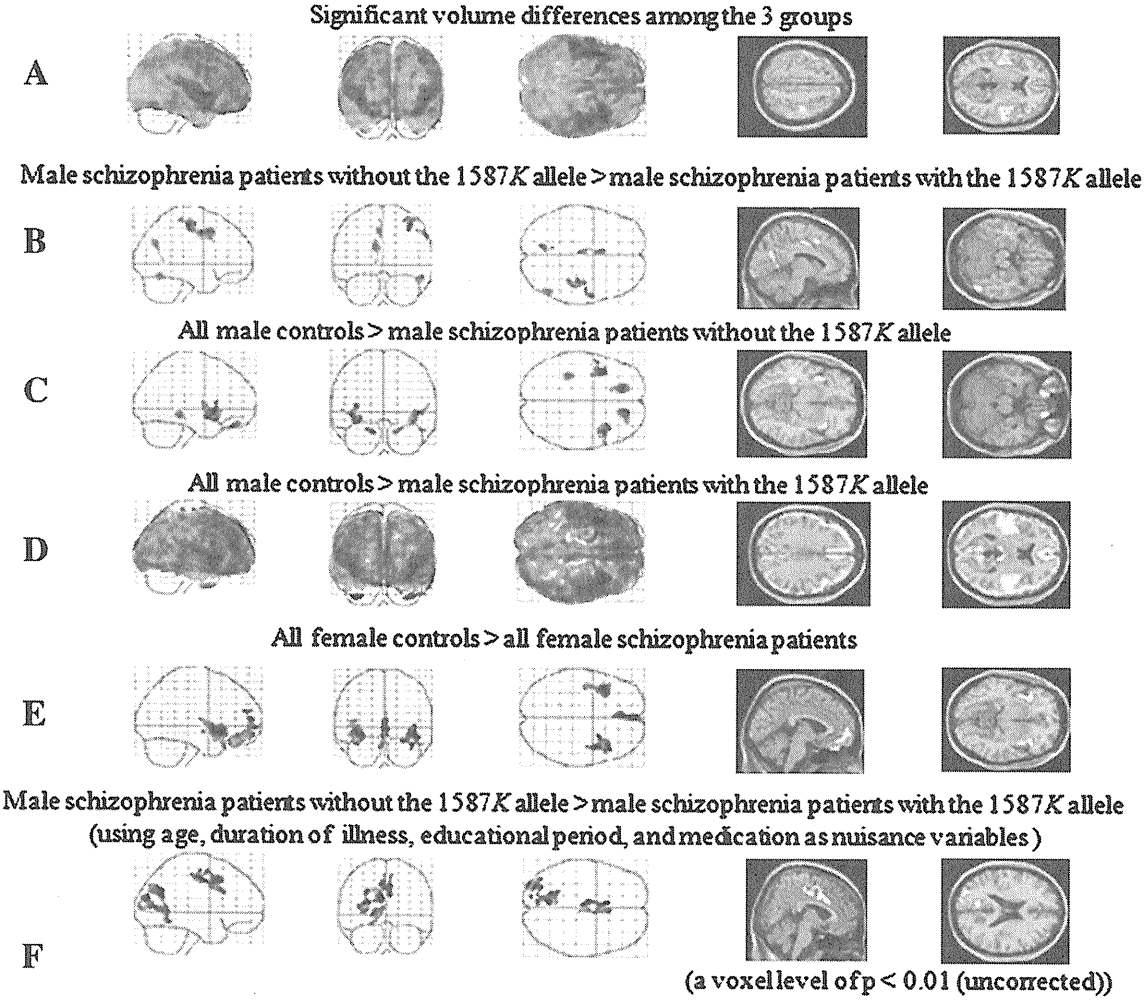

Fig. 1. Group effect was assessed using analysis of covariance (ANCOVA) (SPM2). Age was used as a nuisance variable. (A); There were statistically significant volume differences among the 3 groups of men, i.e., the male schizophrenia patients with and without the 1587*K* allele and the entire male control group. (B); Male schizophrenia patients carrying the 1587*K* allele showed gray matter volume reduction in the bilateral occipital regions and posterior cingulate cortices compared with those who did not carry this allele. (C); There were volume decreases in the bilateral insulae and orbitofrontal regions, and the left parahippocampal region in male patients with schizophrenia without the 1587*K* allele compared with all male controls. (D); Male patients with schizophrenia carrying the 1587*K* allele showed volume reduction in almost all the gray matter areas, compared with all male controls. (E); When all female schizophrenia patients were analyzed collectively, they showed gray matter volume reduction in the bilateral insulae, anterior cingulate cortex, and orbitofrontal cortex, compared with all female controls. (F); We also evaluated the difference in gray matter volume between the schizophrenic groups with and without the 1587*K* allele for each sex using ANCOVA, controlling for age, duration of illness, educational period, and medication. Male patients with schizophrenia carrying the 1587*K* allele showed small gray matter volume in the left occipital region and bilateral posterior cingulate cortices, compared with those who did not carry the 1587*K* allele controlling for age, duration of illness, educational period, and medication, at nominal trend level ($P<0.01$ uncorrected).

2007). Two broad theories have been proposed to describe the pattern of cerebral changes: the global and macro-circuit theories (Buchsbaum et al., 2006). According to the global theory, white matter reductions occur uniformly throughout the brain, possibly as a result of genetic abnormalities in the protein pathways controlling myelination (Konrad and Winterer, 2008). The alternative macro-circuit theory proposes that specific white matter tracts are disrupted in schizophrenia either as a cause or a consequence of a disorder in the gray matter regions they connect (Konrad and Winterer, 2008). The present results may accord with the global theory by showing smaller volume in almost the entire gray matter in male schizophrenia patients carrying the 1587*K* allele of *ABCA1*, because ABCA1 was regarded as the key regulator of brain cholesterol homeostasis and associated with structure and function in neurons such as myelination (Karasinska et al., 2009). Both male and female schizophrenia patients who did not carry the 1587*K* allele showed smaller volume in the medial temporal region, insulae, and anterior cingulate cortex, which have been referred to as predominantly impaired brain regions in schizophrenia, than control subjects (Glahn et al., 2008; Ellison-Wright et al., 2008). On the other hand, the male patients with schizophrenia carrying the 1587*K* allele showed the smaller volume in the occipital regions and posterior cingulate cortices, where it is known to remain unchanged from illness, than male patients not carrying 1587*K* allele. Intricate analysis controlling for age, duration of illness, educational period, and medication, male patients carrying 1587*K* allele showed the smaller volume in occipital and posterior cingulate cortices compared with male patients not carrying 1587*K* allele, only at the trend level, but these tendencies could not be detected in females even at the trend level. From these points, we suggest a male-specific association of the 1587*K* allele of *ABCA1* with susceptibility to schizophrenia and smaller gray matter volume in schizophrenia. In this study, we evaluated only a gray matter volume change, and no consideration was paid to the white matter. Further work with the diffusion tensor imaging data will be necessary to confirm our results.

Schizophrenia is a multifactorial disorder caused by a complex interaction of genetic and environmental factors (Bassett et al., 2001).

In this study, we found no significant difference in gray matter volume related to the *R1587K* polymorphism in healthy subjects. This may be accounted for by the possibility that *ABCA1* polymorphism interacts with other risk factors for schizophrenia and that these collectively influence brain vulnerability.

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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, 4th 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