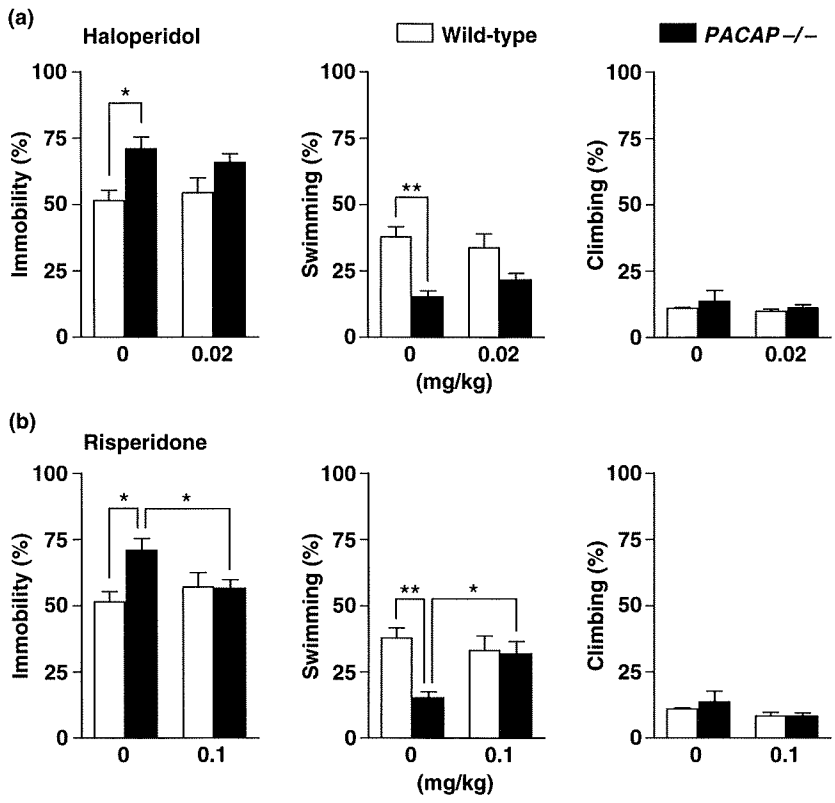

Fig. 2 Behavioral effects of the antipsychotic haloperidol and risperidone in FST in PACAP–/– mice. PACAP–/– (closed bars) and wild-type (open bars) mice (n = 4–9 per group) were treated intraperitoneally with haloperidol (a) and risperidone (b) at the indicated doses, or vehicle, and duration of immobility (left), swimming (center), and climbing (right) in FST was determined. Values for control are common between (a) and (b). $^{*}p < 0.05$, $^{**}p < 0.01$.

significantly between the mutant and wild-type mice (Fig. 1a).

Desipramine is an antidepressant known to decrease immobility time in FST (Petit-Demouliere *et al.* 2005), and indeed it decreased the immobility time and increased the climbing time in wild-type mice (Fig. 1b). In PACAP–/– mice, desipramine decreased the immobility and increased the climbing behavior to a similar extent as in wild-type mice (Fig. 1b).

Amelioration of depression-like behavior in FST by risperidone, ritanserin, and intracerebroventricular PACAP

We examined the effect of the antipsychotic drugs on FST behavior in PACAP–/– mice. When the typical antipsychotic haloperidol was administered, the immobility did not significantly change in either wild-type or PACAP–/– mice (Fig. 2a). Higher doses of haloperidol, however, induced catalepsy in both mutant and wild-type mice (data not shown). In contrast, the atypical antipsychotic risperidone decreased the immobility time and increased the swimming time in PACAP–/– mice, but had no effect on wild-type mice (Fig. 2b). The selective 5-HT_2 antagonist ritanserin also completely ameliorated the depression-like behavior in PACAP–/– mice (Fig. 3a). To further address the mechanism underpinning increased immobility behavior in PACAP–/– mice, animals were injected intracerebroventricularly with PACAP38 and subjected to the test. As shown in Fig. 3(b), PACAP38 injection resulted in a tendency to reduced immobility time and increased swimming time in PACAP–/– mice.

Increased head twitch and ear scratch responses induced by DOI in PACAP–/– mice

To examine the possible alteration in the 5-HT_2 receptor function in PACAP–/– mice, we measured the 5-HT_2 agonist DOI-induced head twitch response and ear scratch response that are mediated via activation of 5-HT_{2A} receptors (Darmani *et al.* 1996). As shown in Fig. 4(a and b), DOI increased head twitch and ear scratch responses in both wild-type and PACAP–/– mice. However, both responses were significantly higher in PACAP–/– mice compared with wild-type mice (head twitch, $p < 0.01$; ear scratch, $p < 0.01$; two-way ANOVA).

Impaired circadian corticosterone rhythm and body temperature rhythm in PACAP–/– mice, and reduced mRNA expression of glucocorticoid receptor in the PACAP–/– hippocampus

As altered activity of the hypothalamus–pituitary–adrenal (HPA) axis is one of the most commonly observed neuroendocrine abnormalities associated with depressive symptoms (Thomson and Craighead 2008), we examined the plasma corticosterone levels and its circadian rhythm in PACAP–/– mice (Fig. 4c). The typical circadian rhythm in corticosterone levels was observed in wild-type mice, in

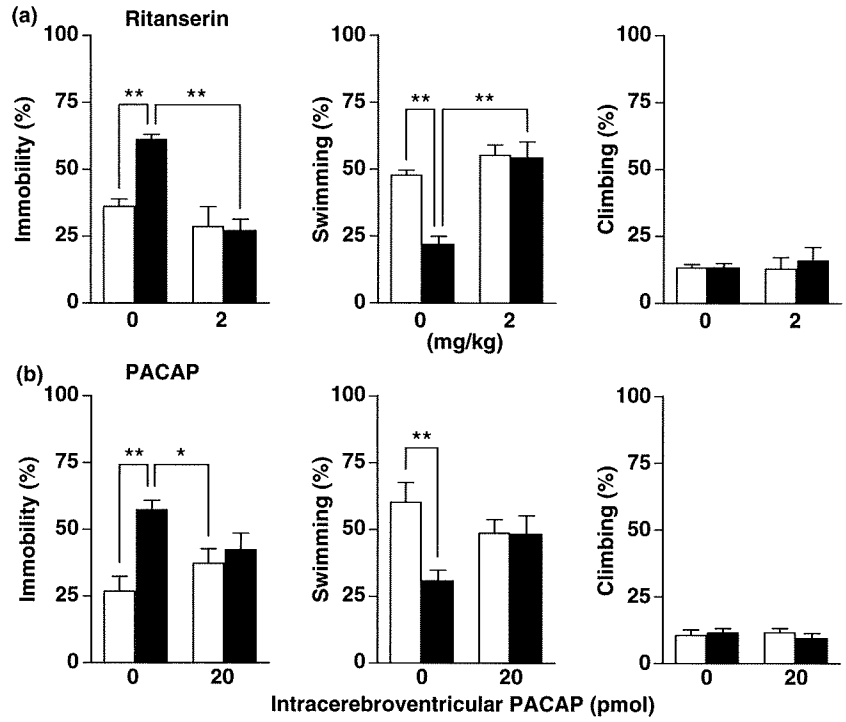

Fig. 3 Amelioration of depression-like behavior in FST by the 5-HT_2 antagonist ritanserin and intracerebroventricular PACAP. PACAP−/− (closed bars) and wild-type (open bars) mice were injected intraperitoneally with ritanserin (a; $n = 4–5$ per group) and intracerebroventricularly with PACAP38 (b; $n = 10–15$ per group) at the indicated doses, or vehicle, and duration of immobility (left), swimming (center), and climbing (right) in FST was determined. $^*p < 0.05$, $^{**}p < 0.01$.

which the levels peaked at zeitgeber time 12. In contrast, corticosterone levels in PACAP−/− mice showed no significant differences due to the time of day sampled. The dexamethasone-induced suppression in corticosterone levels was normally seen in mutant mice compared with wild-type mice (Fig. 4d). The hippocampal mRNA level of glucocorticoid receptor was significantly reduced in the mutants, while that of mineralocorticoid receptor was the same between the two groups (Fig. 4e). In addition, telemetry recording of core temperature revealed that circadian rhythm of body temperature seen in wild-type mice was virtually absent in PACAP−/− mice (Fig. 4f). In wild-type mice, body temperature was high during the dark phase, whereas, in PACAP−/− mice, this rise in body temperature was not observed.

Discussion

The aim of this study was to examine if PACAP deficiency in mice results in increased depression-like behavior, given that PACAP may share the same pathway with DISC1 (Hattori *et al.* 2007), one of the most probable risk factors for major psychiatric disorders, including both schizophrenia and depression. In the present study, PACAP−/− mice exhibited depression-like behavior in the FST, impaired circadian corticosterone rhythm, reduced expression of glucocorticoid receptor mRNA in the hippocampus, and flattened and low body temperature. All these phenotypes are considered to be related to clinical features of depression (Porsolt *et al.* 1977; van Londen *et al.* 2001; Petit-Demouliere *et al.* 2005; Ridder *et al.* 2005; Thomson and Craighead 2008). A limitation in the present study is that we could not provide data from other behavioral tests related to depression. Although we performed a tail suspension test in PACAP−/− mice, we could not obtain a determinate result because of some problem conducting this test in PACAP−/− mice.

Risperidone is a combined dopamine D_2 and 5-HT_2 receptor antagonist, while ritanserin, the predecessor of risperidone, is a selective 5-HT_2 antagonist. Pharmacological study with these drugs suggests that 5-HT_2 receptor signaling is relevant to the depression-like behavior in the FST in PACAP−/− mice. Exaggerated DOI-induced 5-HT syndrome in PACAP−/− mice suggests altered 5-HT_2 receptor signaling. It has been demonstrated that enhancement of 5-HT neurotransmission mediates swimming behavior in FST, whereas enhancement of norepinephrine neurotransmission increases climbing behavior (Page *et al.* 1999). Indeed, we observed that desipramine, a selective norepinephrine reuptake inhibitor, increased climbing behavior, but not swimming behavior, to a similar extent in wild-type and PACAP−/− mice. However, risperidone and ritanserin normalized immobility behavior solely via an increase in swimming behavior in PACAP−/− mice, without any effect in wild-type mice. These observations may suggest the 5-HT-dependent mechanisms for reduced swimming time in the FST in PACAP−/− mice. In line with this possibility, the 5-HT metabolite 5-hydroxyindoleacetic acid was slightly decreased in the cortex and striatum of the PACAP−/− mouse brain (Hashimoto *et al.* 2001) and the hypothermic response to 5-HT_{1A} agonists was significantly reduced in

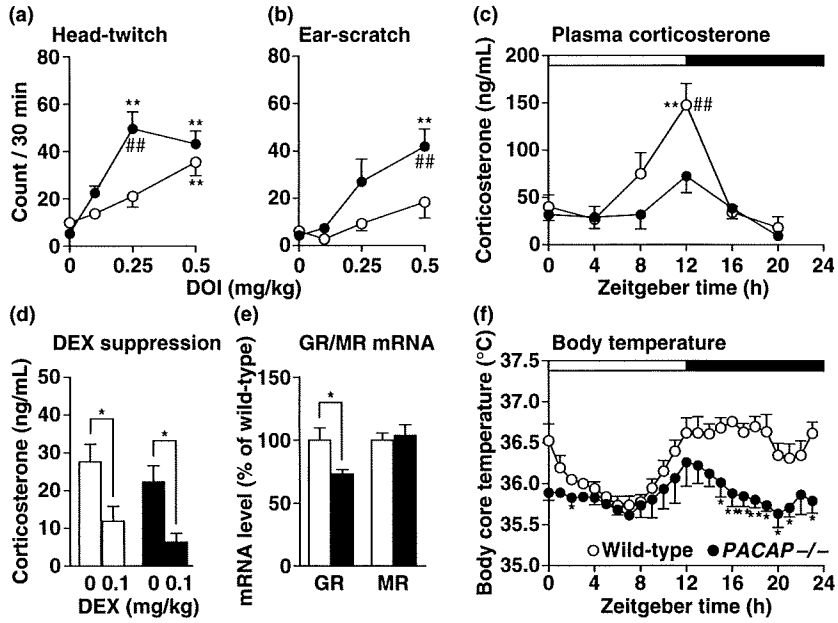

Fig. 4 The 5-HT_2 agonist DOI-induced head twitch and ear scratch responses, circadian and dexamethasone-suppressed corticosterone levels, glucocorticoid and mineralocorticoid receptor mRNA levels, as well as circadian body temperature rhythm in PACAP−/− mice. (a, b) PACAP−/− (closed circles) and wild-type (open circles) mice (n = 7–10 per group) were treated intraperitoneally with DOI at the indicated doses, and the head twitch (a) and ear scratch (b) responses for 30 min were counted. $^{**}p < 0.01$ compared with vehicle-treated mice of the same genotype, $^{\#\#}p < 0.01$ compared with the wild-type mice at the same dose. (c) PACAP−/− (closed circles) and wild-type (open circles) mice (n = 4 per group) were maintained in a 12-h light (100 lux)/12-h dark cycle, and plasma corticosterone levels were determined at the indicated times. Bar above the graph indicates the light/dark conditions (open, light; close, dark). $^{**}p < 0.01$ compared with zeitgeber time 0 of the same genotype; $^{\#\#}p < 0.01$ compared with the PACAP−/− mice at the same time. (d) PACAP−/− (closed bars) and wild-type (open bars) mice (n = 4–7 per group) were treated intraperitoneally with dexamethasone (DEX) at zeitgeber time 6 and the plasma corticosterone levels were determined at zeitgeber time 10, $^{*}p < 0.05$. (e) mRNA expression of glucocorticoid receptor (GR) and mineralocorticoid receptor (MR) was determined in hippocampus by quantitative real-time reverse transcriptase PCR in PACAP−/− (closed bars) and wild-type (open bars) mice (n = 5 per group), $^{*}p < 0.05$. (f) Body core temperature in PACAP−/− (closed circles) and wild-type (open circles) mice (n = 3–4 per group) were recorded using a telemetry-recording system. Bar above the graph indicates the light/dark conditions (open, light; close, dark). $^{*}p < 0.05$, $^{**}p < 0.01$ compared with the wild-type mice at the same time.

these mice (Tanaka *et al.* 2006), although we have not yet been able to find any change in 5-HT receptor expression in the PACAP−/− mouse brain. Taking these observations into consideration, it is suggested that PACAP deficiency leads to certain depressive conditions, but not a typical 'depression-like' profile, that might be due to perturbed 5-HT signaling, and therefore, is responsive to atypical antipsychotics and 5-HT_2 receptor antagonism.

As increasing evidence has suggested that PACAP plays diverse roles in mammalian neurogenesis and patterning (Waschek *et al.* 1998; Wu *et al.* 2006), we conducted a rescue experiment to determine whether the behavioral defect is due to an early developmental impairment, or a consequence of the absence of the PACAP pathway in otherwise normal behavior. The observation that intracerebroventricular injection of PACAP resulted in a tendency to reduced depression-like behavior in PACAP−/− mice implies a direct causal link between PACAP deficiency and depression-like behavior.

In PACAP−/− mice, the circadian plasma corticosterone level was flattened, although overall corticosterone secretion was lower, instead of higher, than wild-type mice. This observation is inconsistent with a previous study by Hamelink *et al.* (2002) that showed that the diurnal rhythm of plasma corticosterone was not altered in their PACAP−/− mouse line developed separately from our colony. Although the reason for this discrepancy remains unclear, it may in part be attributable to different genetic backgrounds (a mixed genetic background of C57/BL6 and 129 vs. CD1).

Altered activity of the HPA axis and cortisol secretion are commonly associated with depression (Thomson and Craighead 2008). In addition, altered glucocorticoid receptor signaling has been implicated in depression-like bahvaior (Boyle *et al.* 2005). Mice with forebrain-specific disruption of glucocorticoid receptors show increased depression-like behavior, such as increased immobility in the FST. In the present study, glucocorticoid receptor mRNA levels were reduced in the PACAP−/− hippocampi, however, the dexamethasone-induced suppression in corticosterone levels was normally seen. The flattened circadian corticosterone level seen in our mutant mice might be consistent with studies

demonstrating depressed individuals exhibit a relatively flat and unresponsive pattern of cortisol secretion (Young *et al.* 1994; Burke *et al.* 2005). As a large body of evidence indicates that the 5-HT system and the HPA axis have complex inter-relationships that may central to the pathophysiology of depression (Maes *et al.* 1995; Porter *et al.* 2004), further studies are needed to address the mechanisms through which altered PACAP-signaling influences the 5-HT–corticosteroid interaction relevant to depression-like behavior. Such scrutiny may lead to dissection of cause and effect between HPA axis activation, glucocorticoid receptor down-regulation, and altered 5-HT systems.

Circadian rhythms of body temperature are regulated by the biological clock in the hypothalamic suprachiasmatic nucleus and, in some depressed patients, a weak 24-h periodicity of body temperature has been shown (van Londen *et al.* 2001). PACAP is co-stored with glutamate, an essential modulator of light entrainment, in a subset of retinal ganglion cells and in the retinohypothalamic tract, and relays photic information from the eyes to the suprachiasmatic nucleus (Hannibal *et al.* 2000). Recently, we have shown that PACAP−/− mice exhibit circadian defects characterized by an attenuated-phase advanced response to light stimulation at the late night/predawn period (Kawaguchi *et al.* 2003). Taking these results together, PACAP involvement in adjustment of the biological clock might underlie the development of depressive symptoms.

It has been known that the non-competitive NMDA receptor antagonist phencyclidine (PCP) reproduces a schizophrenia-like psychosis including both positive and negative symptoms. In mice, PCP-induced enhancement of immobility in FST is attenuated by the atypical antipsychotics, but not by the typical antipsychotics (Mouri *et al.* 2007). Given that the deficiency of PACAP leads to a hypofunction of NMDA receptor-mediated neurotransmission (Mabuchi *et al.* 2004; Ohnishi *et al.* 2008), the responsiveness to the atypical antipsychotics in FST common to the PCP model and PACAP−/− mice might be reasonably explained.

We have previously demonstrated that the novelty-induced hyperactivity and pre-pulse inhibition deficits in PACAP−/− mice are ameliorated by amphetamine, a psychostimulant widely prescribed to treat attention-deficit hyperactivity disorder (Tanaka *et al.* 2006). This result and the findings that the PACAP gene might be associated with schizophrenia (Hashimoto *et al.* 2007) suggest that PACAP is involved in endophenotypes, such as impairment of neurophysiology of mental and cognitive processes, rather than being associated with specific psychiatric disorders. Therefore, PACAP is implicated as a pre-disposing risk factor for neuropsychiatric disorders. This situation is similar to DISC1, as the locus of which is implicated as a risk factor for neuropsychiatric disorders, including schizophrenia, depression and autistic spectrum disorders (Millar *et al.* 2000; Blackwood *et al.* 2001; Kilpinen *et al.* 2008).

The pathophysiology of mental disorders can be a combination of subtle alterations of major signaling pathways. Products of promising risk genes can influence multiple pathways, and consequently act as strong predisposing factors. PACAP is known to have pleiotropic actions, e.g. modulation of various signaling systems such as dopamine (Takei *et al.* 1998), 5-HT (Hashimoto *et al.* 2001), and NMDA receptor-mediated signaling (Mabuchi *et al.* 2004; Ohnishi *et al.* 2008), as well as neurotrophic and neuroendocrine actions (Vaudry *et al.* 2000). Therefore, it is plausible that PACAP is part of a common genetic etiology shared by multiple mental disorders, and that PACAP signaling may be a target candidate for new therapies.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Duration of immobility behavior in FST in PACAP−/− mice.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

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Regular Article

Association between the dysbindin gene (*DTNBP1*) and cognitive functions in Japanese subjects

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Aim: The dysbindin gene (dystrobrevin binding protein 1: *DTNBP1*) is a susceptibility gene for schizophrenia. Susceptibility genes for schizophrenia have been hypothesized to mediate liability for the disorder at least partly by influencing cognitive performance. This report investigated the relationship between cognitive function and the dysbindin gene.

Methods: The possible association between a single nucleotide polymorphism (SNP) of *DTNBP1* (rs2619539: P1655), which is a risk-independent SNP for schizophrenia in Japanese populations, and memory and IQ was investigated in 70 schizophrenia patients and 165 healthy volunteers in a Japanese population.

Results: This SNP was associated with two memory scales, verbal memory and general memory, on the Wechsler Memory Scale–Revised (WMS-R), and three subcategories of the Wechsler Adult Intelligence Scale–Revised (WAIS-R), vocabulary, similarities and picture completion in healthy subjects. The SNP, however, did not influence either the indices of WMS-R, IQ or subcategories of WAIS-R in schizophrenia patients.

Conclusion: A risk-independent SNP in *DTNBP1* may have an impact on cognitive functions such as memory and IQ in healthy subjects.

Key words: *DTNBP1*, dysbindin, IQ, memory, schizophrenia.

SCHIZOPHRENIA IS A complex genetic disorder characterized by profound disturbances of cognition, emotion and social functioning. It affects approximately 1% of the general population worldwide. A recent study implicated a gene on chromosome 6p, dystrobrevin binding protein 1 (*DTNBP1*; dysbindin, Online Mendelian Inheritance in Man [OMIM] 607145; National Center for Biotechnology Information [NCBI] gene ID 84062), as a susceptibility locus in Irish pedigrees.[1] Since then a significant association between schizophrenia and genetic variations in *DTNBP1* has been reported in various populations from Ireland, Wales, Germany/Hungary/Israel, Sweden, Bulgaria, USA, China, and Japan,[2–11] and only a few studies did not support this association.[12,13] Post-mortem brain studies have indicated reduced expression of the *DTNBP1* mRNA in hippocampus and prefrontal cortices and *DTNBP1* protein in the hippocampus of schizophrenia patients.[14–16] Long-term treatment of mice with typical or atypical antipsychotics did not alter the mRNA expression levels or protein levels of dysbindin in the frontal cortex and hippocampus,[16,17] suggesting that the prior

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evidence of decreased expression of *DTNBP1* in the post-mortem brains of schizophrenia patients is not likely to be a simple artifact of ante-mortem drug treatment.

Schizophrenia is a neuropsychiatric disorder characterized by cognitive dysfunction. The heritability study of a collection of endophenotypes for schizophrenia suggests that endophenotypes including cognitive function are important measures to consider in characterizing the genetic basis of schizophrenia.[18] Susceptibility genes for schizophrenia have been hypothesized to mediate liability for the disorder at least partly by influencing cognitive performance.[19] There have been few studies, however, on the relationship between cognitive function and the dysbindin gene. It is reported that a *DTNBP1* risk haplotype for schizophrenia was associated with general cognitive ability (g) in both schizophrenia patients and healthy controls.[20] The same group subsequently reported the association between the risk haplotype and cognitive decline in schizophrenia.[21] Another preliminary study assessed the association between another *DTNBP1* risk haplotype for schizophrenia and verbal and spatial memory, working memory, attentional control, and premorbid IQ in schizophrenia patients.[22] Patients carrying the dysbindin risk haplotype had significantly lower spatial working memory performance than patients who were non-risk carriers. Zinkstok *et al.* reported an association between genetic variations in *DTNBP1* and intelligence, IQ.[23] Recently, we reported the memory and learning impairment in sandy (sdy) mutant mice with a deletion in the dysbindin gene such as long-term memory retention and working memory,[24] supporting roles of dysbindin in cognitive function. In the present study we examined a possible association between a genetic variant of *DTNBP1*, which was not associated with schizophrenia in Japanese populations, and memory function and general cognitive ability.

METHODS

Subjects

Seventy schizophrenia patients and 165 healthy controls were used to study the association between neurocognitive functions and a single nucleotide polymorphism (SNP) in *DTNBP1*. Patients under treatment at the National Center Neurology and Psychiatry Musashi Hospital, Tokyo, Japan were recruited. Consensus diagnosis according to DSM-IV criteria was made by treating and research clinicians who were all senior psychiatrists, based on clinical interviews, observations and case notes. No patient was diagnosed on the basis of medical records alone. All patients were diagnosed as having chronic schizophrenia and were prescribed a stable dose of antipsychotic medication for at least 3 months prior to neuropsychological test sessions. Healthy controls with no history of contact with psychiatric services were recruited from the staff and their associates, through fliers and by word of mouth. Individuals who had a history of regular use of psychotropic agents were not enrolled. 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 alcohol/drug dependence or mental retardation. The Wechsler Memory Scale–Revised (WMS-R)[25,26] and full versions of the Wechsler Adult Intelligence Scale–Revised (WAIS-R)[27,28] were administered to the subjects, as described previously.[29] Five indices of the WMS-R and 11 subcategories as well as verbal, performance and total IQ measures of the WAIS-R were used for the analysis. After a description of the study, written informed consent was obtained from every subject. The study protocol was approved by the institutional ethics committee in National Center of Neurology and Psychiatry and this study has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

SNP genotyping

Venous blood was drawn from the subjects and genomic DNA was extracted from whole blood according to standard procedures. An SNP (P1655: rs2619539) adopted in the Straub *et al.* study[1] was genotyped using the TaqMan 5′-exonuclease allelic discrimination assay described in the previous study.[3] Although this SNP was not associated with schizophrenia in two independent Japanese studies,[3,11] association between an SNP and an intermediate phenotype may be more robust than that between an SNP and a disease. In order to produce high power of detection of the association in a Japanese sample, this SNP with the highest minor allele frequency in Japanese subjects (C allele: 0.31) was selected from SNP examined in our previous study.[3] The genotype distributions of the SNP were in Hardy–Weinberg

equilibrium for both schizophrenia patients and controls ($P = 0.3$, $P = 0.8$).

Statistical analysis

Statistical analyses were carried out using SPSS for Windows version 11.0 (SPSS Japan, Tokyo, Japan). Group comparisons of demographic data were performed using analysis of covariance (ANOVA) or χ^2, as appropriate. The effects of the P1655 SNP in *DTNBP1* on scales of the WMS-R or the WAIS-R were tested using ANOVA. Post-hoc comparisons were performed using Bonferroni correction. All P reported are two-tailed. Statistical significance was defined as $P < 0.05$.

RESULTS

We examined the associations between an SNP (P1655) in *DTNBP1* and two cognitive tests in 70 schizophrenia patients and 165 healthy controls. As expected, schizophrenia patients performed significantly worse than controls in all cognitive tests (all $P < 0.00001$). There were huge differences in cognitive performance between patients and controls (an average difference of the means is around two SD; e.g. verbal memory: patients, 79.1 ± 19.5; controls, 111.1 ± 13.4). Thus, we analyzed the effect of genotype in patients and controls, separately.

The characteristics of subjects are presented in Table 1. There was no significant difference between the genotype group in any of the variables, including illness features in schizophrenia patients. The genotype groups in healthy controls did not significantly differ in gender or education years, but there was a significant difference in age ($F = 4.23$, $P = 0.016$, post hoc G/G vs G/C, $P = 0.012$).

We assessed the effects of the SNP on the WMS-R and the WAIS-R scores in schizophrenia patients and control subjects (Table 2). Significant effects of the SNP were found in verbal memory ($F = 3.24$, $P = 0.042$), general memory ($F = 3.28$, $P = 0.040$), vocabulary ($F = 3.71$, $P = 0.027$), similarities ($F = 3.74$, $P = 0.026$) and picture completion ($F = 9.53$, $P = 0.00012$) in healthy controls. In contrast, no effect of the genotype on the results of cognitive tests was observed in schizophrenia patients.

We focused on the effects of the SNP on verbal memory in the WMS-R scores, because general memory is a component of verbal memory, and on picture completion in the WAIS-R scores, because the effect of the SNP was much stronger than that in vocabulary or similarities in controls (Fig. 1). On post-hoc analysis subjects carrying the G/G genotype had significantly higher verbal memory scores than those with the C/C genotype ($P = 0.025$; Fig. 1a). Conversely, subjects with the G/G genotype performed significantly worse in picture completion tasks than subjects with the G/C or C/C genotype ($P = 0.00015$, $P = 0.015$; Fig. 1b). Similar effects of the SNP were observed in general memory of the

Table 1. Subject details

	Schizophrenia				Control			
Variables	G/G ($n = 39$)	G/C ($n = 27$)	C/C ($n = 4$)	P	G/G ($n = 69$)	G/C ($n = 80$)	C/C ($n = 16$)	P
Age (years)	45.7 ± 12.2	43.6 ± 15.0	49.0 ± 7.3	0.68	34.1 ± 10.9	39.8 ± 12.8	38.4 ± 11.7	0.016
Gender (M/F)	24/15	17/10	2/2	0.88	19/50	30/50	5/11	0.43
Education years	13.5 ± 2.7	12.7 ± 3.6	14.3 ± 2.9	0.45	16.0 ± 2.6	16.3 ± 3.3	15.9 ± 3.0	0.79
Family history of psychiatric diseases (Yes/No)	15/24	7/20	2/2	0.53				
Age at onset (years)	24.5 ± 8.2	24.6 ± 10.0	29.5 ± 16.2	0.29				
Duration of illness (years)	21.6 ± 14.0	18.0 ± 13.8	16.8 ± 14.5	0.54				
CPZeq of total antipsychotic drugs (mg/day)	806 ± 658	793 ± 612	850 ± 918	0.99				

CPZeq, chlorpromazine equivalent; G/G, G/C, and C/C, genotypes of P1655.

Table 2. Cognitive test results and a genetic variation in *DTNBP1* in schizophrenia patients (mean ± SD)

		Schizophrenia				Control			
	Cognitive tests	G/G	G/C	C/C	*P*	G/G	G/C	C/C	*P*
WMS-R	Verbal Memory	77.4 ± 19.1	82.4 ± 20.8	74.0 ± 15.3	0.27	113.0 ± 12.3	110.9 ± 14.0	103.4 ± 13.0	*0.042*
	Visual memory	79.3 ± 21.9	81.7 ± 20.7	78.5 ± 26.5	0.68	109.8 ± 9.0	110.2 ± 8.8	107.7 ± 12.3	0.66
	General memory	75.5 ± 18.9	81.2 ± 20.6	70.0 ± 21.7	0.16	113.5 ± 11.2	112.6 ± 11.8	104.9 ± 12.7	*0.040*
	Attention/Concentration	89.0 ± 17.6	87.9 ± 17.3	89.5 ± 13.2	0.97	105.3 ± 13.8	104.7 ± 14.9	100.8 ± 10.4	0.45
	Delayed recall	75.4 ± 20.4	80.5 ± 22.2	81.3 ± 14.5	0.40	113.2 ± 11.3	112.0 ± 12.3	108.8 ± 11.8	0.52
WAIS-R	Information	8.7 ± 3.9	7.4 ± 3.5	10.0 ± 3.6	0.49	10.1 ± 2.9	10.9 ± 2.8	10.1 ± 3.3	0.27
	Digit Span	8.2 ± 2.7	8.2 ± 3.7	8.5 ± 2.1	0.75	11.1 ± 3.2	11.0 ± 2.6	10.5 ± 2.5	0.65
	Vocabulary	8.7 ± 3.3	7.2 ± 3.6	8.5 ± 2.9	0.36	10.4 ± 3.1	11.8 ± 2.8	11.6 ± 2.8	*0.027*
	Arithmetic	7.5 ± 3.1	7.2 ± 3.0	9.5 ± 3.8	0.54	10.7 ± 3.3	11.8 ± 2.9	10.9 ± 3.2	0.14
	Comprehension	7.2 ± 3.9	6.5 ± 3.4	7.3 ± 3.1	0.95	10.7 ± 3.0	10.9 ± 2.8	10.9 ± 2.0	0.94
	Similarities	9.4 ± 3.3	8.6 ± 3.6	11.5 ± 4.0	0.53	11.5 ± 2.5	12.5 ± 2.1	12.4 ± 1.6	*0.026*
	Picture Completion	8.1 ± 3.3	7.5 ± 3.6	9.8 ± 3.6	0.57	9.0 ± 2.3	10.6 ± 2.3	10.8 ± 1.7	*0.00012*
	Picture Arrangement	7.4 ± 3.2	6.9 ± 3.2	8.8 ± 5.5	0.82	11.3 ± 2.5	11.5 ± 2.2	10.9 ± 2.4	0.64
	Block Design	8.7 ± 4.2	8.1 ± 3.8	10.0 ± 2.3	0.86	12.4 ± 3.1	12.7 ± 2.1	13.0 ± 2.9	0.63
	Object Assembly	7.7 ± 3.6	7.6 ± 4.0	8.8 ± 2.2	0.92	11.7 ± 3.1	11.7 ± 2.7	11.8 ± 2.8	0.99
	Digit Symbol	6.9 ± 2.8	6.2 ± 3.3	8.5 ± 1.7	0.62	12.7 ± 2.8	12.8 ± 2.9	13.8 ± 2.5	0.40
	Verbal IQ	89.2 ± 17.4	84.4 ± 18.4	95.0 ± 17.8	0.81	104.9 ± 14.5	109.6 ± 12.3	107.1 ± 10.6	0.17
	Performance IQ	84.3 ± 18.3	80.1 ± 18.3	93.8 ± 18.6	0.58	109.2 ± 13.3	111.9 ± 10.0	113.1 ± 10.4	0.29
	Full scale IQ	86.0 ± 18.0	80.9 ± 19.2	94.3 ± 19.4	0.65	107.2 ± 12.9	111.6 ± 11.0	110.4 ± 8.3	0.12

G/G, G/C, and C/C, genotypes of P1655; *DTNBP1*, dystrobrevin binding protein 1; WAIS-R, Wechsler Adult Intelligence Scale-Revised; WMS-R, Wechsler Memory Scale-Revised.

WMS-R (higher performance in G/G genotype compared with C/C genotype, $P = 0.028$) and in vocabulary and similarities of the WAIS-R (lower performance in G/G genotype compared with G/C genotype, $P = 0.014$ and $P = 0.017$).

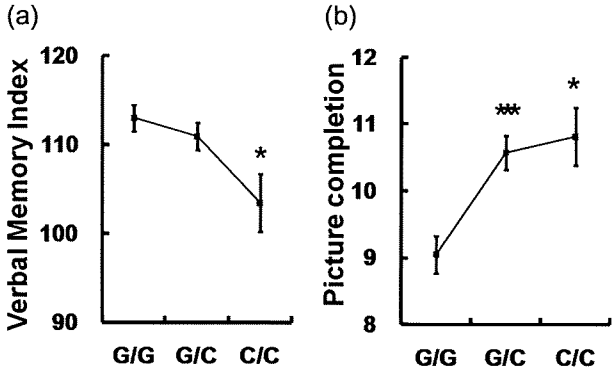

Figure 1. Association between cognitive functions and a single nucleotide polymorphism (SNP) in *dystrobrevin binding protein 1* (*DTNBP1*). An SNP in *DTNBP1* (P1655) was associated with (a) verbal memory and (b) picture completion in control subjects. G/G, G/C, and C/C represent genotypes of P1655. Data given as mean ± SE. $^{*}P < 0.05$; $^{***}P < 0.001$ compared with the G/G genotype.

DISCUSSION

In the present study we evaluated the relationship between an SNP in *DTNBP1* and several domains of memory performance measured on the WMS-R, IQ score, and its subscales measured on the WAIS-R in schizophrenia patients and healthy volunteers. Results indicated that this SNP was associated with two memory scales, verbal memory and general memory, and three subcategories of the WAIS-R, vocabulary, similarities and picture completion in control subjects. These results suggest that *DTNBP1* may be a candidate gene for human memory performance and cognitive ability. We have first reported the association between memory performance and the dysbindin gene in healthy subjects. Our data and a previous preliminary study did not find an association between the dysbindin gene and such memory performance, verbal memory, in schizophrenia patients.[22] Taken together, the effects of the dysbindin gene on memory performance could be affected by the disease and/or medication. Although one study reported an association between a risk haplotype of *DTNBP1* and general cognitive ability (g)[20] and another study found an association between several SNPs in *DTNBP1* and IQ in a Caucasian

sample,[23] we could not find an association between the SNP and IQ in the present Japanese sample. Three subcategories of the WAIS-R, however, were associated with the SNP in control subjects. This inconsistency could be due to several reasons, such as the use of differential genetic variations in the three studies; allelic heterogeneity; false-negatives in the present study; false-positives in the previous studies; and ethnic difference. For example, the G allele of P1635, which is significantly in excess in Japanese schizophrenia patients (3.0%),[3] was also overtransmitted in Irish samples (10.2%),[1] but undertransmitted in German samples (17.6%),[10] suggesting that this SNP might be a marker rather than a polymorphism responsible for susceptibility. Taken together, the genetic variation in *DTNBP1* might be a marker that is differentially associated with IQ among different populations. Thus, further examination such as association analysis using the same genetic variation studied in the previous study and the present study, and an independent study with a new cohort, are needed to draw any conclusions.

We observed that healthy subjects with the G/G genotype performed better in verbal memory tests and worse in several WAIS-R scores than those with the C/C genotype. These data suggest that this genetic variation in *DTNBP1* could contribute to the variation in human cognitive ability in both positive and negative ways. Although it apparently seems to be inconsistent, these results could explain the diversity of human cognitive domains. It is well known that each individual subject has strong points and weak points in specific cognitive functions. Subjects with the G/G genotype might have strengths in verbal memory and weaknesses in vocabulary, similarities and picture completion. And even though we used the Bonferroni correction for multiple testing, we could not exclude the possibility that these data were false-positive results.

The mechanisms underlying the effect of genetic variations in *DTNBP1* on cognitive function are unknown. No genetic variant in *DTNBP1* has produced direct evidence of functional effects. But *DTNBP1* is widely distributed in several brain regions, including the frontal cortex, temporal cortex, hippocampus, caudate, putamen, nucleus accumbens, amygdala, thalamus and midbrain.[15] A reduction in the expression of *DTNBP1* in the hippocampus and dorsolateral prefrontal cortex, known to be important areas for cognitive function, has been reported.[14–16] *DTNBP1* plays roles in neurotransmission,[3,30,31] cellular signaling[3,32] and neuronal survival.[3] The reduced expression of *DTNBP1* could be related to the reduced release of glutamate and the increased release of dopamine.[3,31] Reductions of dopamine content in sdy mice, which lack dysbindin-1 owing to a deletion in the *DTNBP1* gene, have been reported.[33,34] Reduced dysbindin-1 protein increased surface expression of dopamine D2 receptor and blocked dopamine-induced internalization of dopamine receptor D2 (DRD2) in SH-SY5Y cells.[35] Taken together, the reduced expression of *DTNBP1* could be related to impairment of glutamatergic and dopaminergic systems, which are implicated in the neuropathology in schizophrenia.[36] The association of the dysbindin gene with cognitive functions might be related to the effects of the dysbindin gene on glutamatergic and/or dopaminergic systems.

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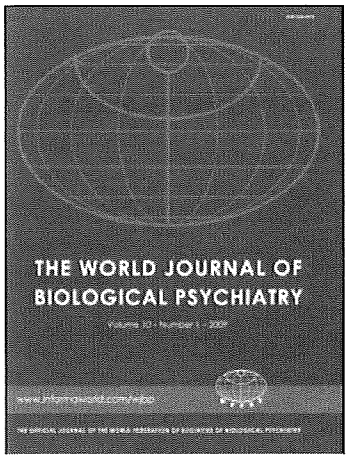

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A genetic variation in the dysbindin gene (DTNBP1) is associated with memory performance in healthy controls

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ORIGINAL INVESTIGATION

A genetic variation in the dysbindin gene *(DTNBP1)* is associated with memory performance in healthy controls

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Abstract
Schizophrenia is a common psychiatric disorder characterized by disturbances of cognition, emotion and social functioning. There are few studies investigating a possible genetic basis for the underlying mechanism of cognitive dysfunctions. A genetic variation in the dysbindin gene (DTNBP1: dystrobrevin binding protein 1), a susceptibility gene for schizophrenia, has been reported to be associated with general cognitive ability and cognitive decline in patients with schizophrenia. Although profound disturbances of memory performance are observed in schizophrenia, only one study has reported a relationship between this gene and spatial working memory in a Caucasian population. We examined a possible association between a protective haplotype of *DTNBP1* for developing schizophrenia and memory performance measured by the Wechsler Memory Scale-Revised (WMS-R) and the Wechsler Adult Intelligence Scale-Revised (WAIS-R) in 165 healthy volunteers and 70 patients with schizophrenia in a Japanese population. Healthy controls that carry the protective haplotype showed higher performance in several memory domains measured by the WMS-R than those who did not. Genotype effect on memory performance was not observed in patients with schizophrenia. This haplotype did not affect IQ and its sub-scores as measured by the Wechsler Adult Intelligence Scale-Revised in both groups. These data suggest that *DTNBP1* may have impact on parts of memory functions.

Key words: *Schizophrenia, dysbindin, DTNBP1, memory, polymorphism*

Introduction

Schizophrenia is a common psychiatric disorder characterized by profound disturbances of cognition, emotion and social functioning. It affects approximately 1% of the general population worldwide. A recent study implicated a gene on chromosome 6p, DTNBP1 (dystrobrevin binding protein 1; dysbindin, Online Mendelian Inheritance in Man [OMIM] 607145; National Center for Biotechnology Information [NCBI] Gene ID 84062), as a susceptibility locus in Irish pedigrees (Straub et al. 2002). Since then, a significant association between schizophrenia and genetic variations in *DTNBP1* has been reported in various populations from Ireland,

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Wales, Germany/Hungary/Israel, Sweden, Bulgaria, United States, China, and Japan (Schwab et al. 2003; Tang et al. 2003; Van Den Bogaert et al. 2003; van den Oord et al. 2003; Funke et al. 2004; Kirov et al. 2004; Numakawa et al. 2004; Williams et al. 2004; Li et al. 2005; Tochigi et al. 2006) and only a few studies did not support this association (Holliday et al. 2006; Joo et al. 2006). Two postmortem brain studies have indicated reduced expression of *DTNBP1* in the brain of patients with schizophrenia (Talbot et al. 2004; Weickert et al. 2004). Talbot et al. found that dysbindin-1 protein levels were reduced in the hippocampal formation of patients with schizophrenia (Talbot et al. 2004). This presynaptic reduction was observed especially in the inner molecular layer of the dentate gyrus. The expression levels of *DTNBP1* mRNA were also reduced in the prefrontal cortices of patients with schizophrenia (Weickert et al. 2004). Long-term treatment of mice with typical or atypical antipsychotics did not alter the mRNA expression levels or protein levels of dysbindin-1 in the frontal cortex and hippocampus (Talbot et al. 2004; Chiba et al. 2006), suggesting that the prior evidence of decreased expression of *DTNBP1* in the postmortem brains of schizophrenia is not likely to be a simple artifact of antemortem drug treatment.

DTNBP1 was originally found as a binding partner of alpha- and beta-dystrobrevins, which are causative genes of Duchenne muscular dystrophy (Benson et al. 2001). Dystrobrevins are parts of the dystrophin-associated protein complex, which plays important roles in the normal functions of muscle (Blake et al. 2002). Cognitive impairments are commonly found in patients with Duchenne muscular dystrophy, and these are thought to be due to an abnormality in the neuronal membrane caused by a lack of dystrophin (Blake and Kroger 2000). Recently, a genetic variation of *DTNBP1* was reported to influence general cognitive ability and to be associated with cognitive decline in schizophrenia (Burdick et al. 2006; Burdick et al. 2007). Moreover, some clinical features of schizophrenia, such as its negative symptoms, are associated with a risk haplotype of *DTNBP1* (Fanous et al. 2005; DeRosse et al. 2006). Memory function is one of the representative neurobiological traits related to the risk for developing schizophrenia (Toulopoulouand and Murray 2004; Boyer et al. 2007; Piskulic et al. 2007; Wobrock et al. 2008). However, there was only one report investigating the relationship between a genetic variation in *DTNBP1* and memory function, indicating the association with spatial working memory performance in a Caucasian population (Donohoe et al. 2007). Thus, we examined a possible association between a genetic variation of *DTNBP1* and memory functions assessed by the WMS-R in a Japanese population.

Materials and methods

Subjects

The subjects used to determine the haplotypes associated with schizophrenia, whose frequency is more than 10%, included 670 patients with schizophrenia and 588 healthy comparison subjects; these were the same subjects used in our previous study (Numakawa et al. 2004). Consensus diagnosis according to The Diagnostic and Statistical Manual of Mental Disorders-IV (DSM-IV) criteria was made by treating and research clinicians who were all senior psychiatrists, based on clinical interviews, observations and case notes. Healthy controls with no history of mental diseases and contact with psychiatric services were recruited from the hospital staff and their associates, through fliers and by word of mouth.

A subset of the subjects used in our previous study (Numakawa et al. 2004), seventy patients with schizophrenia and 165 healthy controls, was agreed to receive neurocognitive tests and completed the full versions of the Wechsler Adult Intelligence Scale-Revised (WAIS-R) (Wechsler 1981; Shinagawa et al. 1990) and the Wechsler Memory Scale-Revised (WMS-R) (Wechsler 1987; Sugishita 2001). They were used to examine the association between memory functions and a genetic variant of *DTNBP1*. Five indices of the WMS-R and total IQ (intelligence quotient) of the WAIS-R were used for the analysis. The subset of the patients for neurocognitive assessments were diagnosed as having chronic schizophrenia and were prescribed a stable dose of antipsychotic medication for at least 3 months prior to neuropsychological test sessions. Individuals who had a history of regular use of psychotropic agents were not enrolled in the control group. Participants were excluded from both patient and control groups if they had prior medical histories of central nervous system disease or severe head injury, or if they met the criteria for alcohol/drug dependence or mental retardation.

After a description of the study, written informed consent was obtained from every subject. This study has been approved by the local ethics committee and has therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

SNP genotyping

Venous blood was drawn from the subjects and genomic DNA was extracted from whole blood

according to standard procedures. Six single nucleotide polymorphisms (SNPs; P1655: rs2619539, P1635: rs3213207, P1325: rs1011313, P1320: rs760761, P1763: rs2619522, and SNPA: rs2619538) adopted in Straub's and Williams's work (Straub et al. 2002; Williams et al. 2004) were genotyped using the TaqMan 5'-exonuclease allelic discrimination assay described in the previous study (Numakawa et al. 2004).

Statistical analysis

Statistical analysis of an association study was performed using SNPAlyse (DYNACOM, Yokohama, Japan). Case–control haplotype analysis was performed by the permutation method to obtain the empirical significance (Good 2000). The global *P* values represent the overall significance using the χ^2-test when the observed versus expected frequencies of all of the haplotypes are considered together. The individual haplotypes were tested for association by grouping all others together and applying the χ^2-test with 1 df. *P* values were calculated based on 10,000 replications. Individual diplotypes were estimated by the maximum likelihood method based on the expectation-maximization algorithm using a haplotype inference function. Statistical analyses of the association between cognitive tests and a genotype were carried out using SPSS for Windows version 11.0 (SPSS Japan, Tokyo). Group comparisons of demographic data were performed by using analysis of covariance (ANOVA) or χ^2, as appropriate. The effects of a genotype in *DTNBP1* on scales of the WMS-R or the WAIS-R were assessed by multiple regression under the hypothesis that the number of the minor haplotype was parametrically related to the cognitive performance. Gender and education years were treated as covariates, as they were possible confounding factors. Age was also considered to be a possible confounding factor; however, it was not treated as a covariate, because the indices of the WMS-R and the WAIS-R were already corrected by age. Post hoc comparisons were performed using Tukey's HSD test. All *P* values reported are two tailed. Statistical significance was defined as $P<0.05$.

Results

Selection of a genetic variation of DTNBP1

To examine the association between a schizophrenia-associated genetic variation in *DTNBP1* and neurocognitive tests, it is necessary to find a genetic variation of *DTNBP1* that is associated with schizophrenia with high frequency. Our previous study showed that four out of six SNPs associated with schizophrenia (P1655: $P=0.748$, P1635: $P=0.0013$, P1325: $P=0.372$, P1320: $P=0.027$, P1763: $P=0.022$, SNPA: $P=0.040$) (Numakawa et al. 2004). The minor allele frequencies of SNPs associated with schizophrenia are less than 10% (P1635: 0.011 and 0.030 (control and schizophrenia), P1320: 0.071 and 0.095, P1763: 0.070 and 0.095, SNPA: 0.024 and 0.040) and *D'* values ranged between 0.5 and 1.0 between SNPs indicated strong to intermediate LD between the markers, as shown by our previous study (Numakawa et al. 2004). Thus, we performed haplotype analysis of a combination of six genotyped SNPs to find a haplotype associated with schizophrenia with a minor frequency of more than 10%.

We previously performed case–control haplotype analysis and found that a three-marker haplotype (rs3213207-rs1011313-rs760761) was associated with schizophrenia (permutation: global *P* value 0.007) (Numakawa et al. 2004). Examination of the contribution of individual haplotypes revealed that the 1-1-1 haplotype (P1635-P1325-P1320) was less frequent in patients with schizophrenia than controls (estimated frequencies: patients 72.7% vs. controls 77.9%, $P=0.038$), suggesting that this haplotype might be a protective haplotype (Table I). The 2-1-2 haplotype was enriched in patients with schizophrenia than in controls (estimated frequencies: patients 1.1% vs. controls 2.8%, $P=0.017$), suggesting that this haplotype might be a risk haplotype (Table I). Similar haplotype frequencies were observed in the other two haplotypes in patients with schizophrenia and controls (Table I). Only the 1-1-1 haplotype was fulfilled the criteria for the analysis, more than 10% haplotype frequency in the population and association with schizophrenia. Thus, the 1-1-1 haplotype was selected for further analysis and was named haplotype A, while other haplotypes were combined into haplotype O (others). The reason why we combined all others was that the estimated frequencies of the other haplotypes were too low to analyse independently.

Table I. A protective haplotype for developing schizophrenia.

	Frequency		Permutation
P1635–P1325–P1320	Control	Schizo	*P* value
1-1-1	0.779	0.727	0.038
1-2-1	0.152	0.165	0.508
1-1-2	0.051	0.072	0.153
2-1-2	0.011	0.028	0.017

Individual *P* values and estimated frequencies for the haplotypes in controls and patients are indicated. All haplotypes with a relative frequency not exceeding 1% were excluded from this table.

Association analysis between a protective haplotype in DTNBP1 and memory performances

We examined the associations between a protective haplotype in *DTNBP1* and memory performance in 165 healthy controls and 70 patients with schizophrenia in a Japanese population. As expected, patients with schizophrenia performed significantly worse than controls in all memory tests (all P values <0.00001). There were huge differences in memory performance between patients and controls (an average difference of the means is around two SD; for example, verbal memory: patients: 79.1 ± 19.5, controls: 111.1 ± 13.4). Thus, we analysed the effects of genotype in patients and controls, separately.

The characteristics of subjects are presented in Table II. The protective haplotype groups did not differ significantly in age, gender, education years or full-scale IQ among the controls. There was no significant difference between the protective haplotype groups in any of the variables, including illness features in patients with schizophrenia, except for education years of patients with the protective haplotype ($F=6.61$, $P=0.002$, post hoc A/A vs. O/O, $P=0.015$, A/O vs. O/O, $P=0.002$). The number of subjects in O/O genotype in patients with schizophrenia is only four and education years were significantly different with other two genotype groups. Furthermore, dosage of total antipsychotic drugs in four patients was apparently high compared with other two groups, although it did not reach statistical significance. As this would not be appropriate to examine the genotype effects on cognitive function in this O/O genotype group, we focused to analyse genotype effects on memory function between two genotypes (A/A vs. A/O). There was no significant difference between the two protective haplotype groups (A/A vs. A/O) in any of the variables, including illness features in patients with schizophrenia.

We firstly assessed the effects of the protective haplotype on the WMS-R scores and WAIS-R scores of control subjects (Table III). Significant effects of the haplotype were found in four indices of the WMS-R (verbal memory: $F=5.87$, $P=0.0035$, visual memory: $F=4.63$, $P=0.011$, general memory: $F=4.88$, $P=0.0087$ and delayed recall: $F=3.16$, $P=0.045$). There was no significant genotype effect on scores of 11 subscales of WAIS-R, verbal IQ, performance IQ or full-scale IQ in control subjects. No effect of the haplotype on the results of memory tests or IQ tests was observed in patients with schizophrenia (Table III). The genotype effects in verbal memory in control subjects were statistically significant after Bonferroni correction (corrected $P=0.035$).

Table II. Demographic information.

	Controls				Patients with schizophrenia				
Variables	A/A ($n=90$)	A/O ($n=62$)	O/O ($n=13$)	P value	A/A ($n=40$)	A/O ($n=26$)	O/O ($n=4$)	P value (A/A vs. A/O vs. O/O)	P value (A/A vs. A/O)
Age	37.6 (12.5)	36.4 (11.8)	39.2 (12.2)	0.70	43.7 (13.3)	46.3 (13.5)	50.5 (10.6)	0.52	0.45
Gender (M/F)	31/59	18/44	5/8	0.71	22/18	19/7	2/2	0.30	0.14
Education years	16.0 (2.9)	16.3 (3.0)	16.4 (3.4)	0.80	13.0 (2.9)	14.2 (2.4)	8.8 (4.0)	0.002	0.08
Full scale IQ	109.2 (12.1)	110.3 (11.5)	109.2 (11.6)	0.84	84.3 (16.8)	86.5 (20.4)	73.8 (24.7)	0.98	0.65
Family history of psychiatric diseases (Yes/No)					13/27	8/16	2/2	0.75	0.95
Age at onset (years)					24.4 (10.0)	25.2 (8.4)	29.5 (10.6)	0.59	0.71
Duration of illness (years)					18.7 (12.5)	21.6 (15.5)	21.0 (17.6)	0.37	0.42
CPZeq of total antipsychotic drugs (mg/day)					780 (620)	736 (639)	1480 (706)	0.09	0.79

Means (SD) are presented.

Table III. WMS-R and WAIS-R results and a protective haplotype in *DTNBP1*.

		Controls				Patients with schizophrenia			
		A/A	A/O	O/O	*P* value	A/A	A/O	O/O	*P* value
WMS-R	Verbal memory	111.1(13.7)	113.4(11.3)	100.2(15.6)	0.0035	76.9(19.4)	83.9(19.2)	70.0(21.6)	0.16
	Visual memory	109.4(9.2)	111.7(8.7)	103.2(9.5)	0.011	81.2(20.5)	79.3(23.2)	75.5(23.6)	0.74
	General memory	111.9(12.6)	114.5(10.2)	103.8(10.9)	0.0087	75.3(18.6)	82.0(20.5)	68.5(23.6)	0.19
	Attention/ Concentration	104.3(13.3)	105.3(15.2)	103.1(13.9)	0.71	88.6(15.5)	89.3(19.5)	84.5(19.3)	0.87
	Delayed recall	112.0(12.5)	113.9(9.5)	105.2(15.4)	0.045	76.3(20.2)	81.3(21.1)	68.8(25.9)	0.34
WAIS-R	Information	10.5(3.0)	10.6(2.7)	9.9(3.4)	0.69	8.1(3.3)	9.1(4.1)	6.0(5.0)	0.29
	Digit span	10.8(2.6)	11.3(3.2)	10.9(2.9)	0.49	8.0(2.7)	8.8(3.5)	7.0(2.9)	0.29
	Vocabulary	11.1(2.8)	11.3(3.2)	11.2(3.3)	0.94	8.2(3.1)	8.4(4.0)	6.0(4.1)	0.80
	Arithmetic	11.4(3.2)	11.1(2.9)	11.2(3.5)	0.88	7.0(2.6)	8.6(3.6)	5.8(2.2)	0.06
	Comprehension	10.7(2.7)	11.0(3.1)	10.9(2.7)	0.87	7.2(3.1)	6.8(4.2)	4.5(4.7)	0.67
	Similarities	11.9(2.3)	12.4(2.3)	12.0(1.9)	0.43	9.1(3.6)	9.8(3.2)	6.8(3.3)	0.41
	Picture completion	9.8(2.4)	10.2(2.3)	10.1(2.1)	0.59	8.2(3.2)	7.8(3.7)	7.5(4.8)	0.65
	Picture arrangement	11.2(2.6)	11.6(2.0)	10.8(2.4)	0.40	7.2(3.2)	7.7(3.6)	5.8(3.6)	0.54
	Block design	12.6(2.5)	12.6(2.8)	12.6(2.5)	0.98	8.8(3.6)	8.5(4.4)	6.5(4.2)	0.84
	Object assembly	11.7(2.9)	11.7(2.8)	11.5(2.5)	0.92	7.9(3.5)	7.4(3.8)	8.3(5.4)	0.60
	Digit symbol	13.0(2.9)	12.7(2.7)	12.9(2.8)	0.69	6.9(3.1)	6.7(2.8)	5.0(2.3)	0.79
	Verbal IQ	106.9(12.7)	108.3(13.8)	106.8(15.4)	0.73	86.7(15.4)	91.2(20.0)	74.5(23.2)	0.34
	Performance IQ	110.6(12.1)	111.5(11.1)	110.3(9.8)	0.92	84.2(17.4)	82.9(19.4)	76.5(24.6)	0.79
	Full scale IQ	109.2(12.1)	110.3(11.5)	109.2(11.6)	0.84	84.3(16.8)	86.5(20.4)	73.8(24.7)	0.65

Means (SD) are presented.

As the strongest effects of the genetic variation in *DTNBP1* on the WMS-R scores were observed in verbal memory, we focused on the association analysis between this score and the protective haplotype (Figure 1). Post hoc analysis of the verbal memory scores of control subjects revealed significantly poorer performance in subjects with the O/O haplotype, who do not carry the protective haplotype of *DTNBP1*, compared with A/A subjects ($P=0.016$) or A/O subjects ($P=0.0032$) (Figure 1). Similar effects of the haplotype on other indices of the WMS-R were seen in control subjects (data not shown). Similar performance has been observed in the verbal memory scores between A/A patients and A/O patients. The verbal memory scores in O/O patients with schizophrenia were also lower than those in A/A or A/O patients, however, we did not examine the statistical comparison due to the small number of the O/O patients. These data suggest that the genetic risk associated with *DTNBP1* could be related to memory performance, one of the neurobiological traits linked to the risk for developing schizophrenia.

Discussion

In the present study, we evaluated the relationship between a protective haplotype in *DTNBP1* and several domains of memory performance measured by the WMS-R in healthy volunteers and patients with schizophrenia. This protective haplotype was selected due to high estimated haplotype frequency. It is difficult to compare the present protective haplotype and those in most previous studies, because examined SNPs were different among studies and haplotype analyses were not routinely published for all analysed SNPs in each study. However, four previous studies reported haplotype analysis including our haplotype (P1635–P1325–P1320). Oord et al. showed P1635–P1325–P1765–P1757–P1320–P1763–P1578–P1792 haplotype was associated with schizophrenia and identified risk haplotype as 2-1-2-2-2-2-1-2 in an Irish population (Van Den Bogaert et al. 2003). P1635–P1325–P1765–P1320 haplotype was associated with schizophrenia in a German sample (Schwab et al. 2003). Bogaert et al. reported the association between P1635–P1325–P1757–P1320–P1578 haplotype and schizophrenia in a Swedish sample (Van Den Bogaert et al. 2003). On the other hand, P1635–P1635–P1325–P1320 haplotype was not associated with schizophrenia in a Japanese population (Tochigi et al. 2006). Three out of four studies showed positive association between haplotypes and schizophrenia. Only one study identified risk haplotype and this haplotype (2-1-2-2-2-2-1-2) was different from our protective haplotype (1-1-1) and the risk haplotype in our study (2-1-2) was matched to the previous report by Bogaert et al. (2003). Our results are consistent to those in previous studies.

We found that healthy subjects who carried the protective haplotype performed better on several

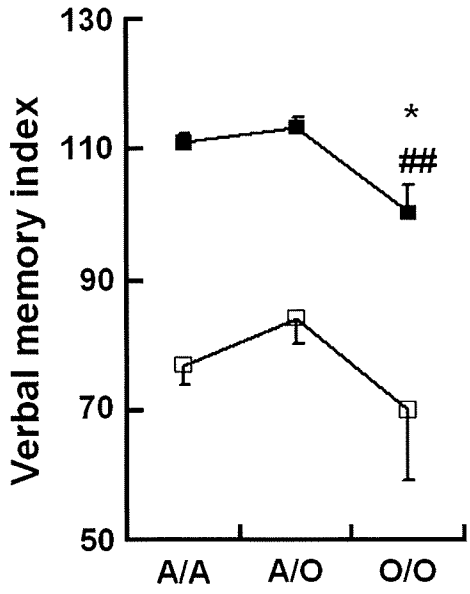

Figure 1. The association between verbal memory and the protective haplotype of *DTNBP1*. Control subjects without the protective haplotype had poorer performance on verbal memory tests. Haplotype A (protective haplotype) is defined as the 1-1-1 haplotype (P1635–P1325–P1320). A/A: protective/protective, A/O: protective/others, O/O: others/others; defined in the text. Filled squares: controls, open squares: patients with schizophrenia. Data represent the means ± SE. $*P<0.05$ compared with the A/A haplotype. $\#\#P<0.01$ compared with the A/O haplotype.

scales of the WMS-R, including verbal memory, visual memory, general memory and delayed recall, all of which are impaired in patients with schizophrenia compared with healthy subjects. These results suggest that *DTNBP1* may be a candidate gene for human memory performance. These results could be false-positive results due to small sample size of O/O control group and large sample size of the A/O group. We did not find a statistically significant effect for the protective haplotype on WMS-R scores in patients with schizophrenia. This might be due to several reasons; for example, the effects of the genetic variation might be masked by the illness, medication, the smaller number of patients with schizophrenia than controls in our study or a greater deviation in the performance of patients with schizophrenia. Further, all patients were under antipsychotic treatment which might severely affect cognitive performance. The number of patients is rather low and further, only four patients are of the O/O genotype, thus only 66 patients entered the calculation. This means that no conclusion can be made for patients.

Although one study reported an association between a risk haplotype of *DTNBP1* and IQ (Burdick et al. 2006), we could not replicate this association in our sample. This inconsistency could be due to several reasons, such as the use of differential haplotypes in the two studies, allelic heterogeneity, false-negative results of our study, ethnic difference, and small sample size for patient group. There are only four O/O carriers from a total of 70 patients with schizophrenia. Thus, further examination such as association analysis with the same haplotype studied in the previous study and our own, and an independent study with a new cohort, are needed to draw any conclusions.

Several intermediate phenotypes such as neurocognitive dysfunction, abnormal brain morphology, and deficits in pre-pulse inhibition of the startle response could contribute to the risk for developing schizophrenia (Preston and Weinberger 2005; Braff et al. 2007). Several susceptibility genes for schizophrenia, including *DTNBP1*, could contribute to the deficits of intermediate phenotype (Harrison and Weinberger 2005; Hashimoto et al. 2006). Our results support the notion that memory disturbance, an intermediate phenotype, could be related to the increased risk for developing schizophrenia possibly due to a genetic variation in *DTNBP1*. It is thought that there are other susceptibility genes for schizophrenia that are associated with memory performance.

The mechanisms underlying the effect of a genetic variation in *DTNBP1* on cognitive function are unknown. No genetic variant in *DTNBP1* provided direct evidence of functional effects. However, *DTNBP1* is widely distributed in several brain regions, including the frontal cortex, temporal cortex, hippocampus, caudate, putamen, nucleus accumbens, amygdala, thalamus and midbrain (Weickert et al. 2004). A reduction in the expression of *DTNBP1* in the hippocampus and dorsolateral prefrontal cortex, known to be important areas for cognitive function, has been reported (Talbot et al. 2004; Weickert et al. 2004). The reduced expression of *DTNBP1* could be related to the reduced release of glutamate and increased release of dopamine (Numakawa et al. 2004; Kumamoto et al. 2006). Recent studies reported that reduced dysbindin-1*1* protein by *DTNBP1* siRNA transfection increased surface expression of dopamine D2 receptor and blocked dopamine-induced internalization of DRD2 in SH-SY5Y cells (Iizuka et al. 2007). Reductions of dopamine content in sandy (sdy) mice, which lack dysbindin-1 owing to a deletion in the *DTNBP1* gene, have been reported (Murotani et al. 2007; Hattori et al. 2008). Furthermore, we recently reported deficits of long-term memory retention and working memory in sdy mice (Takao et al. 2008). Impairments of glutamatergic and dopaminergic systems in these critical brain regions are implicated in the neuropathology in schizophrenia. Further studies are needed to elucidate an underlying

genetic vulnerability to neurobiological traits in schizophrenia.

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Conflict of Interest

All authors declare that they have no conflict of interest.

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