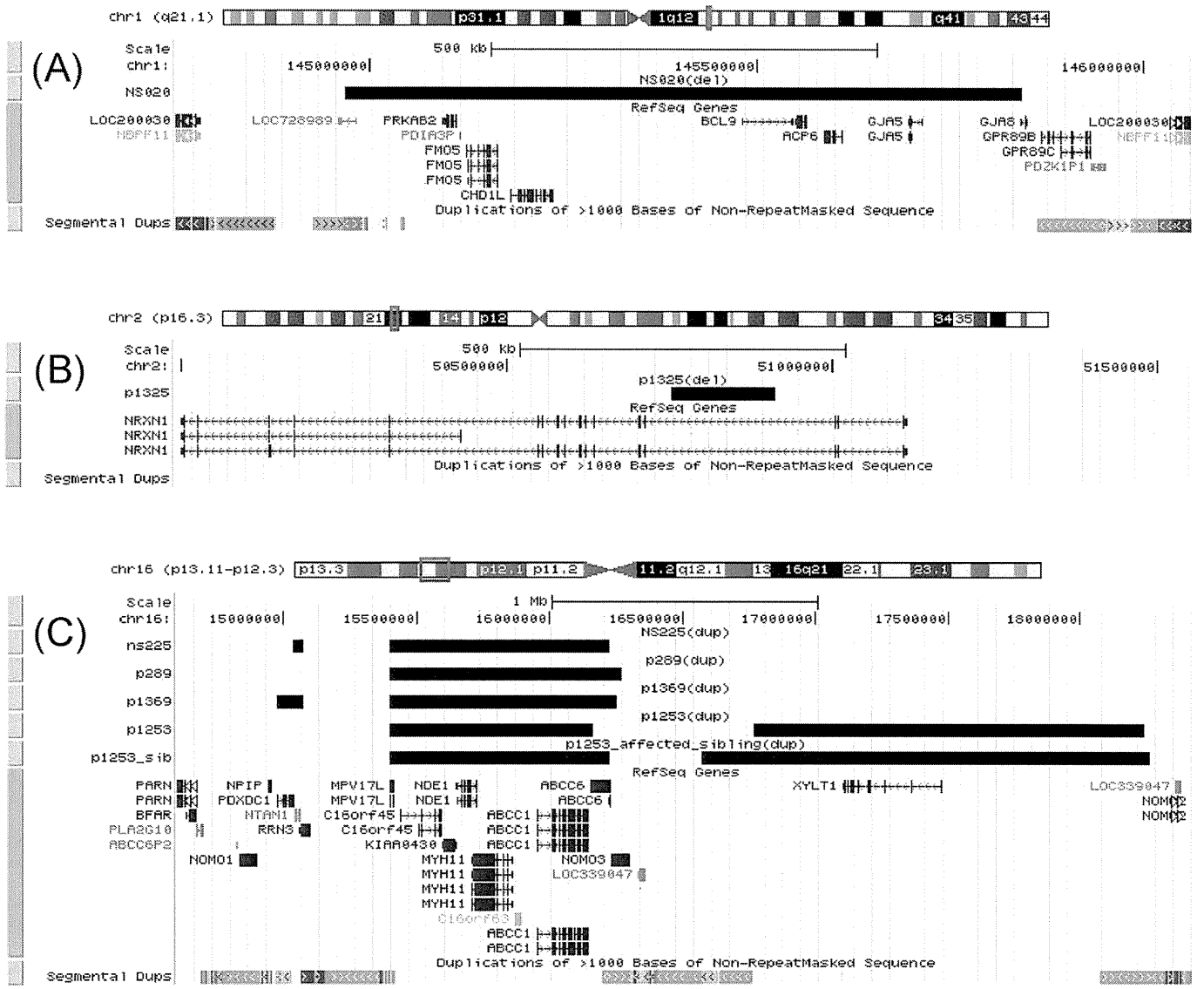

Figure 1. Positions of CNVs according to the validation experiments. CNV validation was undertaken using Illumina HumanHap 660W quad bead arrays (for CNVs at 1q21.1 and *NRXN1*) or 610-quad bead arrays (for CNVs at 16p13.1). Figures are produced on the UCSC Genome Browser according to NCBI Build 36.1, March 2006, hg18 (http://www.genome.ucsc.edu/) and indicate the positions of the CNVs: **(A)** 1q21.1; **(B)** *NRXN1*; and **(C)** 16p13.1: the last trace is that of the affected sibling of "p1253." CNV, copy number variation; NCBI, National Center for Biotechnology Information; UCSC, University of California, Santa Cruz.

implicated CNV risk factors for schizophrenia (4,8). Among large duplications, the most notable is that on 16p13.1, which was found in four cases and one control subject, while one more control subject had the reciprocal deletion (Fisher exact test $p = .19$, one-tailed). These CNVs in cases were confirmed using Illumina arrays (Figure S1 and Tables S1 and S2 in Supplement 2). One of the patients with 16p13.1 duplication had an affected sibling and unaffected mother who had also provided DNA. The duplication was found in the affected sibling but not the unaffected mother (DNA from the father was not available and there is no indication that he suffers with mental illness). The duplication in this family extends further on the centromeric side compared with the region usually included in CNVs of this region (Figure 1).

Of the remaining susceptibility loci reported in the recent studies (4,7,8), we found no deletions at 22q11.2 or 15q13.3. We also find no support for the 15q11.2 locus, where three deletions were found in control subjects and only one in a case (Fisher exact test $p = .37$, two-tailed, a trend in the opposite direction).

We also searched for CNVs that intersected genes and were present only in cases, reasoning as have others (2,3) that such CNVs are good candidates (Tables S3 and S4 in Supplement 2). One of the singleton deletions was in *NRXN1*, a gene implicated in previous studies (2,6,7,9,10) (Figure S1 and Table S2 in Supplement 2). Several more contain intriguing candidate genes (e.g., deletions in *PARK2, GRIK2, MAGEL2,* and *ATXN2L* and duplications in *CHRNA7* and *NRG4*), which have been implicated in neurodegenerative disorders or have possible functional relevance for neurodevelopment.

Discussion

In this study, we do not find a significant increase in the burden of CNVs in schizophrenia, either overall or for any

specific size range of CNVs, as proposed in previous studies (2–4,7). We did, however, find several trends in the same direction and of a similar magnitude as the largest global CNV survey of schizophrenia (4). Not all research has found such an increased burden, e.g., no evidence was obtained from a study in the Chinese population (5). It is possible that genuine population differences might drive this discrepancy between Caucasian and Asian samples, as might our exclusion of subjects with mental retardation or epilepsy. Sample size could also have played a role. Our sample had a modest power of ~.65 to detect a single CNV in a case for the following very strong candidate loci: 1q21.1, 15q13.3, and 22q11.2 and *NRXN1*, where approximately .2% of affected persons have deletions. In fact, we did find one deletion each in two of these loci (1q21.1 and *NRXN1*).

We found stronger support for association with duplications at 16p13.1, which contain the candidate gene *NDE1*. It is within the interval duplicated in all patients (Figure 1). Deletions and duplications of this region were implicated in autism (13) and schizophrenia (7), while deletions have been implicated in mental retardation (14). The most recent study surveying children with unexplained intellectual disability also reported significant association for both deletions and duplications at this locus ($p = 4.7 \times 10^{-5}$) (15), suggesting that this duplication is also pathogenic for a broad range of neuropsychiatric disorders. Our result for an excess of duplications in schizophrenic probands does not reach statistical significance; however, the frequency of the duplication is fourfold higher in cases than in control subjects (.8% vs. .2%), which is very similar to the rate found in our previous study from the United Kingdom (.6% vs. .2%) (7) and in the ISC study (.4% vs. .2%) (4). We found an identical duplication in an affected sibling. Larger CNVs in this locus, as in one of our probands, were also found in three cases and two control subjects in the ISC (4). The four probands in our study who carry 16p13.1 duplications do not appear to share any specific clinical features (Table S2 in Supplement 2).

We also found one deletion in a case at 1q21.1 and *NRXN1* and none in control subjects, which is close to the reported frequency of .2% in cases. Unlike those deletions of *NRXN1* that were associated with schizophrenia in a previous study (10), the CNV reported here does not intersect exons (10). However, it is large compared with most exon-sparing deletions reported in control subjects (10), and a new reanalysis of all *NRXN1* deletions shows that large (>100 kb) deletions in this gene might be almost as relevant as those affecting exons (16). The relevance to schizophrenia of the other CNVs found only in cases can only be assessed in future meta-analyses of such studies, but we note here that the three deletions we found in *PARK2* are of particular interest, as they have been implicated as a susceptibility factor for autism (17).

In summary, we provide support for the role of CNVs at 16p13.1, 1q21.1, and *NRXN1* in the etiology of schizophrenia. Although we find similar, but not significant, trends for an increased overall burden of CNVs, as well as for the involvement of duplications in the 100 kb to 200 kb range as proposed in the ICS study (4), in this population we could not find an increased burden of very large CNVs (>500 kb) in schizophrenia, which has been the main finding in recent studies (4,7). The discrepancy with previous studies could be due to our exclusion of patients with neurodevelopmental disorders, epilepsy, or known mental retardation, as such features are found in many of the carriers of large CNVs, e.g., 15q13.3 (15). Given the rarity of the CNVs that have been implicated so far in schizophrenia, there is a need for more large studies, studies in non-European populations, and meta-analyses.

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Supplementary material cited in this article is available online.

1. Cook EH Jr, Scherer SW (2008): Copy-number variations associated with neuropsychiatric conditions. *Nature* 455:919–923.
2. Walsh T, McClellan JM, McCarthy SE, Addington AM, Pierce SB, Cooper GM, *et al.* (2008): Rare structural variants disrupt multiple genes in neurodevelopmental pathways in schizophrenia. *Science* 320:539–543.
3. Xu B, Roos JL, Levy S, van Rensburg EJ, Gogos JA, Karayiorgou M (2008): Strong association of de novo copy number mutations with sporadic schizophrenia. *Nat Genet* 40:880–885.
4. The International Schizophrenia Consortium (2008): Rare chromosomal deletions and duplications increase risk of schizophrenia. *Nature* 455: 237–241.
5. Shi YY, He G, Zhang Z, Tang W, Zhang J Jr, Zhao Q, *et al.* (2008): A study of rare structural variants in schizophrenia patients and normal controls from Chinese Han population. *Mol Psychiatry* 13:911–913.
6. Need AC, Ge D, Weale ME, Maia J, Feng S, Heinzen EL, *et al.* (2009): A genome-wide investigation of SNPs and CNVs in schizophrenia. *PLoS Genet* 5:e1000373.
7. Kirov G, Grozeva D, Norton N, Ivanov D, Mantripragada KK, Holmans P, *et al.* (2009): Support for the involvement of large CNVs in the pathogenesis of schizophrenia. *Hum Mol Genet* 18:1497–1503.
8. Stefansson H, Rujescu D, Cichon S, Pietilainen OP, Ingason A, Steinberg S, *et al.* (2008): Large recurrent microdeletions associated with schizophrenia. *Nature* 455:232–236.
9. Kirov G, Gumus D, Chen W, Norton N, Georgieva L, Sari M, *et al.* (2008): Comparative genome hybridization suggests a role for NRXN1 and APBA2 in schizophrenia. *Hum Mol Genet* 17:458–465.
10. Rujescu D, Ingason A, Cichon S, Pietilainen OP, Barnes MR, Toulopoulou T, *et al.* (2009): Disruption of the neurexin 1 gene is associated with schizophrenia. *Hum Mol Genet* 18:988–996.
11. Korn JM, Kuruvilla FG, McCarroll SA, Wysoker A, Nemesh J, Cawley S, *et al.* (2008): Integrated genotype calling and association analysis of SNPs, common copy number polymorphisms and rare CNVs. *Nat Genet* 40: 1253–1260.
12. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, *et al.* (2007): PLINK: A tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 81:559–575.
13. Ullmann R, Turner G, Kirchhoff M, Chen W, Tonge B, Rosenberg C, *et al.* (2007): Array CGH identifies reciprocal 16p13.1 duplications and deletions that predispose to autism and/or mental retardation. *Hum Mutat* 28:674–682.
14. Hannes FD, Sharp AJ, Mefford HC, de Ravel T, Ruivenkamp CA, Breuning MH, *et al.* (2008): Recurrent reciprocal deletions and duplications of 16p13.11: The deletion is a risk factor for MR/MCA while the duplication may be a rare benign variant. *J Med Genet* 46:223–232.
15. Mefford HC, Cooper GM, Zerr T, Smith JD, Baker C, Shafer N, *et al.* (2009): A method for rapid, targeted CNV genotyping identifies rare variants associated with neurocognitive disease. *Genome Res* 19:1579–1585.
16. Kirov G, Rujescu D, Ingason A, Collier DA, O'Donovan MC, Owen MJ (2009): Neurexin 1 (NRXN1) deletions in schizophrenia. *Schizophr Bull* 35:851–854.
17. Glessner JT, Wang K, Cai G, Korvatska O, Kim CE, Wood S, *et al.* (2009): Autism genome-wide copy number variation reveals ubiquitin and neuronal genes. *Nature* 459:569–573.

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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 2-1-2-2-2-1-2 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 (2-1-2-2-2-1-2) 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

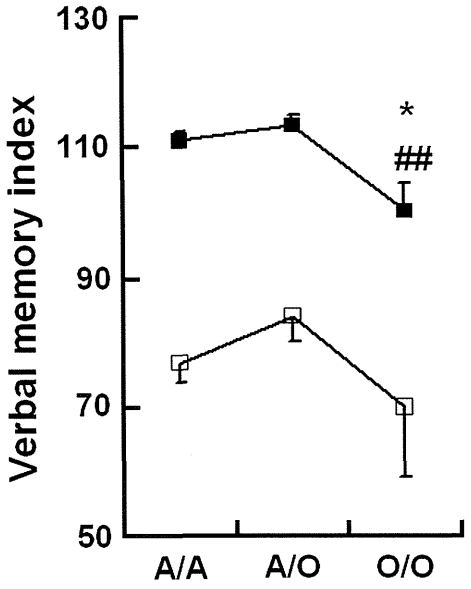

Figure 1. The association between verbal memory and the protective haplotype of *DTNBP1*. Control subjects without the protective haplotype had poorer performance on verbal memory tests. Haplotype A (protective haplotype) is defined as the 1-1-1 haplotype (P1635–P1325–P1320). A/A: protective/protective, 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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Statement of interest

All authors declare that they have no conflict of interest.

References

Benson MA, Newey SE, Martin-Rendon E, Hawkes R, Blake DJ. 2001. Dysbindin, a novel coiled-coil-containing protein that interacts with the dystrobrevins in muscle and brain. J Biol Chem 276(26):24232–24241.

Blake DJ, Kroger S. 2000. The neurobiology of duchenne muscular dystrophy: learning lessons from muscle? Trends Neurosci 23(3):92–99.

Blake DJ, Weir A, Newey SE, Davies KE. 2002. Function and genetics of dystrophin and dystrophin-related proteins in muscle. Physiol Rev 82(2):291–329.

Boyer P, Phillips JL, Rousseau FL, Ilivitsky S. 2007. Hippocampal abnormalities and memory deficits: new evidence of a strong pathophysiological link in schizophrenia. Brain Res Rev 54(1):92–112.

Braff DL, Freedman R, Schork NJ, Gottesman II. 2007. Deconstructing schizophrenia: an overview of the use of endophenotypes in order to understand a complex disorder. Schizophr Bull 33(1):21–32.

Burdick KE, Lencz T, Funke B, Finn CT, Szeszko PR, Kane JM, et al. 2006. Genetic variation in DTNBP1 influences general cognitive ability. Hum Mol Genet 15(10):1563–1568.

Burdick KE, Goldberg TE, Funke B, Bates JA, Lencz T, Kucherlapati R, et al. 2007. DTNBP1 genotype influences cognitive decline in schizophrenia. Schizophr Res 89(1–3): 169–172.

Chiba S, Hashimoto R, Hattori S, Yohda M, Lipska B, Weinberger DR, et al. 2006. Effect of antipsychotic drugs on DISC1 and dysbindin expression in mouse frontal cortex and hippocampus. J Neural Transm 113(9):1337–1346.

DeRosse P, Funke B, Burdick KE, Lencz T, Ekholm JM, Kane JM, et al. 2006. Dysbindin genotype and negative symptoms in schizophrenia. Am J Psychiatry 163(3):532–534.

Donohoe G, Morris DW, Clarke S, McGhee KA, Schwaiger S, Nangle JM, et al. 2007. Variance in neurocognitive performance is associated with dysbindin-1 in schizophrenia: A preliminary study. Neuropsychologia 45(2):454–8.

Fanous AH, van den Oord EJ, Riley BP, Aggen SH, Neale MC, O'Neill FA, et al. 2005. Relationship between a high-risk haplotype in the DTNBP1 (dysbindin) gene and clinical features of schizophrenia. Am J Psychiatry 162(10):1824–1832.

Funke B, Finn CT, Plocik AM, Lake S, DeRosse P, Kane JM, et al. 2004. Association of the DTNBP1 locus with schizophrenia in a U.S. population. Am J Hum Genet 75(5):891–898.

Good P. (2000). Permutation tests. A practical guide to resampling methods for testing hypothesis. New York: Springer-Verlag.

Harrison PJ, Weinberger DR. 2005. Schizophrenia genes, gene expression, and neuropathology: on the matter of their convergence. Mol Psychiatry 10(1):40–68.

Hashimoto R, Hattori S, Chiba S, Yagasaki Y, Okada T, Kumamaru E, et al. 2006. Susceptibility genes for schizophrenia. Psychiatry Clin Neurosci 60 (Suppl 1):S4–10.

Hattori S, Murotani T, Matsuzaki S, Ishizuka T, Kumamoto N, Takeda M, et al. 2008. Behavioral abnormalities and dopamine reductions in sdy mutant mice with a deletion in Dtnbp1, a susceptibility gene for schizophrenia. Biochem Biophys Res Commun 373(2):298–302.

Holliday EG, Handoko HY, James MR, McGrath JJ, Nertney DA, Tirupati S, et al. 2006. Association study of the dystrobrevin-binding gene with schizophrenia in Australian and Indian samples. Twin Res Hum Genet 9(4):531–539.

Iizuka Y, Sei Y, Weinberger DR, Straub RE. 2007. Evidence that the BLOC-1 protein dysbindin modulates dopamine D2 receptor internalization and signaling but not D1 internalization. J Neurosci 27(45):12390–12395.

Joo EJ, Lee KY, Jeong SH, Ahn YM, Koo YJ, Kim YS. 2006. The dysbindin gene (DTNBP1) and schizophrenia: no support for an association in the Korean population. Neurosci Lett 407(2):101–106.

Kirov G, Ivanov D, Williams NM, Preece A, Nikolov I, Milev R, et al. 2004. Strong evidence for association between the dystrobrevin binding protein 1 gene (DTNBP1) and schizophrenia in 488 parent-offspring trios from Bulgaria. Biol Psychiatry 55(10):971–975.

Kumamoto N, Matsuzaki S, Inoue K, Hattori T, Shimizu S, Hashimoto R, et al. 2006. Hyperactivation of midbrain dopaminergic system in schizophrenia could be attributed to the down-regulation of dysbindin. Biochem Biophys Res Commun 345(2):904–909.

Li T, Zhang F, Liu X, Sun X, Sham PC, Crombie C, et al. 2005. Identifying potential risk haplotypes for schizophrenia at the DTNBP1 locus in Han Chinese and Scottish populations. Mol Psychiatry 10(11):1037–1044.

Murotani T, Ishizuka T, Hattori S, Hashimoto R, Matsuzaki S, Yamatodani A. 2007. High dopamine turnover in the brains of Sandy mice. Neurosci Lett 421(1):47–51.

Numakawa T, Yagasaki Y, Ishimoto T, Okada T, Suzuki T, Iwata N, et al. 2004. Evidence of novel neuronal functions of dysbindin, a susceptibility gene for schizophrenia. Hum Mol Genet 13(21):2699–2708.

Piskulic D, Olver JS, Norman TR, Maruff P. 2007. Behavioural studies of spatial working memory dysfunction in schizophrenia: a quantitative literature review. Psychiatry Res 150(2): 111–121.

Preston GA, Weinberger DR. 2005. Intermediate phenotypes in schizophrenia: a selective review. Dialogues Clin Neurosci 7(2):165–179.

Schwab SG, Knapp M, Mondabon S, Hallmayer J, Borrmann-Hassenbach M, Albus M, et al. 2003. Support for association of schizophrenia with genetic variation in the 6p22.3 gene, dysbindin, in sib-pair families with linkage and in an additional sample of triad families. Am J Hum Genet 72(1):185–190.

Shinagawa F, Kobayashi S, Fujita K, Maekawa H. (1990). Japanese Wechsler Adult Intelligence Scale-Revised. Tokyo: Nihonbunkakagakusha.

Straub RE, Jiang Y, MacLean CJ, Ma Y, Webb BT, Myakishev MV, et al. 2002. Genetic variation in the 6p22.3 gene DTNBP1, the human ortholog of the mouse dysbindin gene, is associated with schizophrenia. Am J Hum Genet 71(2): 337–348.

Sugishita M. (2001). Japanese Wechsler Memory Scale-Revised. Tokyo: Nihonbunkakagakusha.

Takao K, Toyama K, Nakanishi K, Hattori S, Takamura H, Takeda M, et al. 2008. Impaired long-term memory retention and working memory in sdy mutant mice with a deletion in Dtnbp1, a susceptibility gene for schizophrenia. Mol Brain 1(1):11.

Talbot K, Eidem WL, Tinsley CL, Benson MA, Thompson EW, Smith RJ, et al. 2004. Dysbindin-1 is reduced in intrinsic, glutamatergic terminals of the hippocampal formation in schizophrenia. J Clin Invest 113(9):1353–1363.

Tang JX, Zhou J, Fan JB, Li XW, Shi YY, Gu NF, et al. 2003. Family-based association study of DTNBP1 in 6p22.3 and schizophrenia. Mol Psychiatry 8(8):717–718.

Tochigi M, Zhang X, Ohashi J, Hibino H, Otowa T, Rogers M, et al. 2006. Association study of the dysbindin (DTNBP1) gene in schizophrenia from the Japanese population. Neurosci Res 56(2):154–158.

Toulopoulouand T, Murray RM. 2004. Verbal memory deficit in patients with schizophrenia: an important future target for treatment. Expert Rev Neurother 4(1):43–52.

Van Den Bogaert A, Schumacher J, Schulze TG, Otte AC, Ohlraun S, Kovalenko S, et al. 2003. The DTNBP1 (dysbindin) gene contributes to schizophrenia, depending on family history of the disease. Am J Hum Genet 73(6):1438–1443.

van den Oord EJ, Sullivan PF, Jiang Y, Walsh D, O'Neill FA, Kendler KS, et al. 2003. Identification of a high-risk haplotype for the dystrobrevin binding protein 1 (DTNBP1) gene in the Irish study of high-density schizophrenia families. Mol Psychiatry 8(5):499–510.

Wechsler D. (1981). Wechsler Adult Intelligence Scale, Revised. New York: Psychological Corporation.

Wechsler D. (1987). Wechsler Memory Scale Manual, Revised. San Antonio, TX: Psychological Corporation.

Weickert CS, Straub RE, McClintock BW, Matsumoto M, Hashimoto R, Hyde TM, et al. 2004. Human dysbindin (DTNBP1) gene expression in normal brain and in schizophrenic prefrontal cortex and midbrain. Arch Gen Psychiatry 61(6):544–555.

Williams NM, Preece A, Morris DW, Spurlock G, Bray NJ, Stephens M, et al. 2004. Identification in 2 independent samples of a novel schizophrenia risk haplotype of the dystrobrevin binding protein gene (DTNBP1). Arch Gen Psychiatry 61(4):336–344.

Wobrock T, Ecker UK, Scherk H, Schneider-Axmann T, Falkai P, Gruber O. 2008. Cognitive impairment of executive function as a core symptom of schizophrenia. World J Biol Psychiatry:1–10.

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Possible association between the pituitary adenylate cyclase-activating polypeptide (PACAP) gene and major depressive disorder

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ABSTRACT

Pituitary adenylate cyclase-activating polypeptide (PACAP, ADCYAP1: adenylate cyclase-activating polypeptide 1) is a neuropeptide with neurotransmission modulating activity. The associations of the PACAP gene with schizophrenia and hippocampal volume have been reported. We recently reported depression-like behavior in the forced swimming test in PACAP deficient mice. Here we examined a possible association between the PACAP gene and major depressive disorder (MDD) in 637 patients and 967 controls and found that a genetic variant in the gene was associated with MDD. The present results suggest that PACAP signaling might contribute to the pathogenesis of MDD.

The adenylate cyclase-activating polypeptide 1 (ADCYAP1) gene encodes pituitary adenylate cyclase-activating polypeptide (PACAP), a neuropeptide originally isolated from ovine hypothalamus based on its ability to stimulate adenylate cyclase in rat anterior pituitary cell cultures. PACAP is a member of the vasoactive intestinal peptide (VIP)/secretin/glucagon family. PACAP has been recognized as a pleiotropic neuropeptide that acts as a neurotransmitter, neuromodulator or neurotrophic factor through the activation of G-protein-linked receptors [5]. Mice lacking the PACAP gene exhibited marked behavioral abnormalities such as novelty-induced hyperactivity [6] and deficits in prepulse inhibition [16]. Further, we recently observed depression-like behavior in the forced swimming test in PACAP deficient mice [4], implying that PACAP is involved in fundamental mental processes.

Major depressive disorder (MDD) is a common and highly prevalent mental disorder with symptoms that include deficits in a range of cognitive, psychomotor and emotional processes. MDD is caused by a complex interaction of a large number of genetic (estimated heritability: 31–42%) and non-genetic factors, each with a relatively small contribution to the disorder [3]. The PACAP gene is located on 18p11, in which linkage studies have suggested as a locus for schizophrenia and bipolar disorder [13]. We recently reported the evidence for a possible association between PACAP signaling and schizophrenia [7]. In considering the emerging picture that major psychiatric disorders might share, at least in part, common genetic etiologies, it is plausible to assume that PACAP may be a risk factor for major mental illnesses, beyond schizophrenia. In the present study, we therefore pursued the possible association of the PACAP gene with MDD.

There were 637 patients with MDD [40.9% male, with a mean age of 51.3 years (SD 16.2) and mean age of onset of 46.2 years (SD 15.3)], and 967 healthy controls [47.7% male, with a mean age of 40.4 years (SD 16.1)]. All the subjects were biologically unrelated

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Table 1
Allele frequencies of seven SNPs in the PACAP gene (ADCYAP1) in patients with major depressive disorder and controls.

SNP-ID	dbSNP	Distance from SNP1	Major/minor polymorphism	Location	Number of subjects		Minor allele frequency		*P* value	Odds ratio (95% CI)
					Controls	Patients	Controls	Patients		
SNP1	rs2846584	–	C/T	5′-Region	967	637	0.362	0.362	0.972	
SNP2	rs2231181	712	G/C	5′-UTR	960	626	0.336	0.334	0.904	
SNP3	rs1893154	1071	G/A	Intron 1	951	633	*0.126*	*0.101*	*0.031*	*0.78(0.62–0.97)*
SNP4	rs1893153	1149	T/A	Intron 1	953	621	0.174	0.159	0.279	
SNP5	rs2856966	3656	A/G	Exon 3 (D54G)	953	630	0.047	0.063	0.058	1.35 (0.98–1.82)
SNP6	rs928978	4481	C/A	Intron 4	958	624	0.475	0.496	0.259	
SNP7	rs1610037	6581	A/G	3′-UTR	962	626	0.216	0.224	0.597	

Minor allele frequencies in controls are shown. CI: confidence interval. Significant results ($P < 0.05$) are italised.

Japanese. Patients were recruited at the National Center Hospital of Neurology and Psychiatry, Department of Psychiatry, Nagoya University Graduate School of Medicine, Department of Psychiatry, Fujita Health University School of Medicine, or Department of Psychiatry, Showa University School of Medicine. Healthy controls were recruited from local advertisements in Tokyo and Aichi, including hospital and institutional staffs. Consensus diagnosis was made for each patient by at least two trained psychiatrists, according to the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV), based on unstructured clinical interviews and other available information including medical records and other research assessments. No patient was diagnosed by medical records alone. All patients were receiving treatment at the time that blood drawings were performed. Subjects with comorbid schizophrenia, bipolar disorder, or patients who had a history of substance abuse were excluded from the study. Controls were healthy volunteers who had no current or past contact with psychiatric services. After a description of the study, written informed consent was obtained from every subject. The study protocol was approved by institutional ethics committees and was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Venous blood was drawn from subjects and genomic DNA was extracted from whole blood according to standard procedures. Seven single nucleotide polymorphisms (SNPs) in the PACAP gene were genotyped using the TaqMan 5′-exonuclease allelic discrimination assay, as described previously [7,9,10]. Primers and probes for detection of the SNPs are available upon request. Statistical analysis of genetic association studies was performed using SNPAlyse (DYNACOM, Yokohama, Japan). The presence of Hardy–Weinberg equilibrium was examined by using the χ^2-test for goodness of fit. Allele distributions between patients and controls were analyzed by the χ^2-test for independence. Statistical significance was defined as $P < 0.05$.

We have recently demonstrated an association between schizophrenia and the SNP3 (rs1893154) of the PACAP gene [7]. Therefore, in this study, we examined the possible association between this genetic variant and MDD. In total, 967 healthy controls and 637 patients with MDD were studied. The major allele of the SNP3 of the PACAP gene was in excess in patients with MDD ($\chi^2 = 4.7$, $P = 0.031$, odds ratio = 0.78, 95% confidence interval 0.62–0.97; Table 1). We also tested six other SNPs in the PACAP gene and found that SNP5 (rs2856966) tended to be associated with MDD, with a marginal *P* value of 0.058 (Table 1). The genotype distributions of all examined SNPs in the PACAP gene were in Hardy–Weinberg equilibrium for both the controls and patients with MDD ($P > 0.4$).

An association between MDD and the PACAP gene has been demonstrated for the first time in our sample of 637 patients and 967 healthy controls. We selected SNPs according to our previous association study between the PACAP gene and schizophrenia [7]. Our method of SNP selection was not a gene based method such as selection of Tagging SNPs. Five SNPs in the region of the PACAP gene (approximately 7.3 kb) were selected as Tag SNPs using SNPs consulted the HapMap database (www.hapmap.org, population: Japanese Tokyo) and Tagger program of the HAPLOVIEW software. Three out of five SNPs (SNP3, SNP5 and SNP7) was examined in our study. However, there is a possibility of genotyping more tagSNPs to fully understand the role of this gene in the etiology of MDD. The G/A SNP (SNP3) of the PACAP gene associated with the disorder is intronic, and so far, we could not detect whether this SNP influences the expression and/or function of the PACAP gene. However, the SNP3 has also been shown to have a possible impact on brain morphology. Homozygous carriers of the G allele showed smaller bilateral hippocampal volumes compared with A-carriers [7]. This is in line with a previous imaging study that has demonstrated that patients with a history of MDD have reduced hippocampal volumes [14]. The evidence for genetic associations with MDD (SNP3, odds ratio = 0.78; SNP5, odds ratio = 1.35) in the present study and with schizophrenia (SNP3, odds ratio = 0.74; SNP5, odds ratio = 1.38) in the previous study [7] suggests that the effect size of the contribution of the PACAP gene might be similar to both MDD and schizophrenia.

There are several limitations in our results. Lack of structured interview or standardized tests (e.g., Beck Depression Inventory) in patients could influence the results. As the huge heterogeneity was found in MDD, more detailed information for clinical variables in MDD was required such as severity of symptoms, with or without psychotic symptoms, pharmacoresistance, duration of illness, duration of treatment, family history of MDD and/or other psychiatric disorders, inpatient or outpatient, times of hospitalization, etc. However, we do not have the detail clinical information in our sample. These specific variables such as depressive symptom severity could be associated with the PACAP gene. As the PACAP SNP was also associated with schizophrenia, it may be more strongly associated with a subset of patients, for example, those with psychotic symptoms and/or reactivity to atypical antipsychotic medications [15]. Further studies differentiating MDD into various clinical subgroups are warranted in the future.

Altered HPA axis activity and cortisol secretion are commonly associated with MDD [17]. In addition, altered glucocorticoid receptor signaling has been implicated in this disorder [1]. Mice with forebrain-specific disruption of glucocorticoid receptors show increased depression-like behavior, such as increased immobility in the forced swim test. In mice lacking the PACAP gene, the circadian plasma corticosterone level was flattened, although overall corticosterone secretion was lower, instead of higher, than wild-type mice [4]. Glucocorticoid receptor mRNA levels were reduced in the hippocampi of PACAP deficient mice, however, the dexamethasone-induced suppression in corticosterone levels was normally seen [4]. The flattened circadian corticosterone level in these mutant mice might be consistent with studies demonstrating that depressed individuals exhibit a relatively flat and unresponsive pattern of cortisol secretion [2].

The finding that the PACAP gene might be associated with hippocampal volume [7] suggests that PACAP is involved in endophenotypes, such as impairments of neurophysiology of mental and cognitive processes, rather than being associated with specific psychiatric disorders. The pathophysiology of mental disorders can be a combination of subtle alterations of major signaling pathways, which are influenced by products of risk genes. A good example might be that the interaction between Disrupted-in-schizophrenia 1 (DISC1) and its binding protein, DISC1-Binding Zinc-finger protein, is involved in the PACAP signaling pathway [11]. DISC1 is a gene disrupted by a translocation that segregates with major psychiatric disorders including schizophrenia, bipolar disorder and MDD [12]. Subsequent study revealed that the DISC1 gene was associated with MDD [8]. Therefore, PACAP could be a part of a common genetic etiology shared by multiple mental disorders including MDD.

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References

[1] M.P. Boyle, J.A. Brewer, M. Funatsu, D.F. Wozniak, J.Z. Tsien, Y. Izumi, L.J. Muglia, Acquired deficit of forebrain glucocorticoid receptor produces depression-like changes in adrenal axis regulation and behavior, Proc. Natl. Acad. Sci. U.S.A. 102 (2005) 473–478.
[2] H.M. Burke, M.C. Davis, C. Otte, D.C. Mohr, Depression and cortisol responses to psychological stress: a meta-analysis, Psychoneuroendocrinology 30 (2005) 846–856.
[3] K.P. Ebmeier, C. Donaghey, J.D. Steele, Recent developments and current controversies in depression, Lancet 367 (2006) 153–167.
[4] H. Hashimoto, R. Hashimoto, N. Shintani, K. Tanaka, A. Yamamoto, M. Hatanaka, X. Guo, Y. Morita, M. Tanida, K. Nagai, M. Takeda, A. Baba, Depression-like behavior in the forced swimming test in PACAP-deficient mice: amelioration by the atypical antipsychotic risperidone, J. Neurochem. 110 (2009) 595–602.
[5] H. Hashimoto, N. Shintani, A. Baba, New insights into the central PACAPergic system from the phenotypes in PACAP- and PACAP receptor-knockout mice, Ann. NY Acad. Sci. 1070 (2006) 75–89.
[6] H. Hashimoto, N. Shintani, K. Tanaka, W. Mori, M. Hirose, T. Matsuda, M. Sakaue, J. Miyazaki, H. Niwa, F. Tashiro, K. Yamamoto, K. Koga, S. Tomimoto, A. Kunugi, S. Suetake, A. Baba, Altered psychomotor behaviors in mice lacking pituitary adenylate cyclase-activating polypeptide (PACAP), Proc. Natl. Acad. Sci. U.S.A. 98 (2001) 13355–13360.
[7] R. Hashimoto, H. Hashimoto, N. Shintani, S. Chiba, S. Hattori, T. Okada, M. Nakajima, K. Tanaka, N. Kawagishi, K. Nemoto, T. Mori, T. Ohnishi, H. Noguchi, H. Hori, T. Suzuki, N. Iwata, N. Ozaki, T. Nakabayashi, O. Saitoh, A. Kosuga, M. Tatsumi, K. Kamijima, D.R. Weinberger, H. Kunugi, A. Baba, Pituitary adenylate cyclase-activating polypeptide is associated with schizophrenia, Mol. Psychiatry 12 (2007) 1026–1032.
[8] R. Hashimoto, T. Numakawa, T. Ohnishi, E. Kumamaru, Y. Yagasaki, T. Ishimoto, T. Mori, K. Nemoto, N. Adachi, A. Izumi, S. Chiba, H. Noguchi, T. Suzuki, N. Iwata, N. Ozaki, T. Taguchi, A. Kamiya, A. Kosuga, M. Tatsumi, K. Kamijima, D.R. Weinberger, A. Sawa, H. Kunugi, Impact of the DISC1 Ser704Cys polymorphism on risk for major depression, brain morphology and ERK signaling, Hum. Mol. Genet. 15 (2006) 3024–3033.
[9] R Hashimoto, T. Okada, T. Kato, A. Kosuga, M. Tatsumi, K. Kamijima, H. Kunugi, The breakpoint cluster region gene on chromosome 22q11 is associated with bipolar disorder, Biol. Psychiatry 57 (2005) 1097–1102.
[10] R. Hashimoto, T. Suzuki, N. Iwata, Y. Yamanouchi, T. Kitajima, A. Kosuga, M. Tatsumi, N. Ozaki, K. Kamijima, H. Kunugi, Association study of the frizzled-3 (FZD3) gene with schizophrenia and mood disorders, J. Neural. Transm. 112 (2005) 303–307.
[11] T. Hattori, K. Baba, S. Matsuzaki, A. Honda, K. Miyoshi, K. Inoue, M. Taniguchi, H. Hashimoto, N. Shintani, A. Baba, S. Shimizu, F. Yukioka, N. Kumamoto, A. Yamaguchi, M. Tohyama, T. Katayama, A novel DISC1-interacting partner DISC1-binding zinc-finger protein: implication in the modulation of DISC1-dependent neurite outgrowth, Mol. Psychiatry 12 (2007) 398–407.
[12] J.K. Millar, J.C. Wilson-Annan, S. Anderson, S. Christie, M.S. Taylor, C.A. Semple, R.S. Devon, D.M. Clair, W.J. Muir, D.H. Blackwood, D.J. Porteous, Disruption of two novel genes by a translocation co-segregating with schizophrenia, Hum. Mol. Genet. 9 (2000) 1415–1423.
[13] J.I. Nurnberger Jr., T. Foroud, Genetics of bipolar affective disorder, Curr. Psychiatry Rep. 2 (2000) 147–157.
[14] Y.I. Sheline, P.W. Wang, M.H. Gado, J.G. Csernansky, M.W. Vannier, Hippocampal atrophy in recurrent major depression, Proc. Natl. Acad. Sci. U.S.A. 93 (1996) 3908–3913.
[15] R.C. Shelton, G.D. Tollefson, M. Tohen, S. Stahl, K.S. Gannon, T.G. Jacobs, W.R. Buras, F.P. Bymaster, W. Zhang, K.A. Spencer, P.D. Feldman, H.Y. Meltzer, A novel augmentation strategy for treating resistant major depression, Am. J. Psychiatry 158 (2001) 131–134.
[16] K. Tanaka, N. Shintani, H. Hashimoto, N. Kawagishi, Y. Ago, T. Matsuda, R. Hashimoto, H. Kunugi, A. Yamamoto, C. Kawaguchi, T. Shimada, A. Baba, Psychostimulant-induced attenuation of hyperactivity and prepulse inhibition deficits in Adcyap1-deficient mice, J. Neurosci. 26 (2006) 5091–5097.
[17] F. Thomson, M. Craighead, Innovative approaches for the treatment of depression: targeting the HPA axis, Neurochem. Res. 33 (2008) 691–707.

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ORIGINAL PAPER

Lack of Association Between *MAGEL2* and Schizophrenia and Mood Disorders in the Japanese Population

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Abstract Several investigations have reported that abnormalities in circadian rhythms might be related with the pathophysiology of psychiatric disorders, since many psychiatric patients have insomnia and sleep-awake disturbance. A recent animal study reported that *Magel2*, which encodes a member of the MAGE/necdin family of proteins, might be associated in the pathophysiology of psychiatric disorders. Magel2 gene knockout mice showed altered concentrations of both dopamine and serotonin in several parts of the brain compared with controls. In addition, the authors of that study detected a bilateral reduction in cortical volume in distinct regions of the Magel2 gene knockout mice brain, including focused regions in the parieto-temporal lobe of the cerebral cortex, the amygdala, the hippocampus, and the nucleus accumbens. These mice were also found to have hypoactivity and abnormalities in circadian rhythms. From this evidence, we considered Magel2 gene (*MAGEL2*) to be a good candidate gene for the pathophysiology of schizophrenia and mood disorder, and we conducted a case–control study among Japanese (731 schizophrenia patients, 465 MDD patients, 156 BP patients and 758 controls) using three tagging SNPs in *MAGEL2* (rs850815, rs8920 and rs4480754), selected using the HapMap database. We did not find any association between *MAGEL2* and schizophrenia, BP or MDD in allele/genotype-wise analysis or haplotype-wise analysis. Our results suggest that *MAGEL2* may not play a role in the pathophysiology of schizophrenia and mood disorders in the Japanese population. A replication study using larger samples may be required for conclusive results, since our sample size was small and our study analyzed only three SNPs in *MAGEL2*.

Keywords Major depressive disorder · Bipolar disorder · Schizophrenia · *MAGEL2* · Linkage disequilibrium · Tagging SNP

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Introduction

Several investigations have reported that abnormalities in circadian rhythms might be related with the pathophysiology of psychiatric disorders (Barbini et al. 1998; McClung 2007a, c, d), since many psychiatric patients have insomnia and sleep-awake disturbance. Also because all psychotropic drugs, such as dopamine and serotonin, act on neurotransmitter systems in the brain, altered neural transmission is hypothesized to be a susceptibility factor for several psychiatric disorders (Maier et al. 2005; Muller and Schwarz 2008). Recently, these neurotransmitter systems have been reported to have reciprocal interactions with circadian

rhythms (Barnard and Nolan 2008; Voderholzer et al. 2007). Clock genes were also suggested to regulate not only circadian rhythms but also dopamine neural transmission (McClung 2007b). Abnormalities in dopamine neural transmission are known to be involved in the pathophysiology of schizophrenia (Lang et al. 2007), bipolar disorder (BP) (Berk et al. 2007) and major depressive disorder (MDD) (Nestler and Carlezon 2006). In addition, some genetic studies have shown significant associations between schizophrenia/schizo-affective disorder and timeless homolog gene (*TIMELESS*) (Mansour et al. 2006), neuronal PAS domain protein 2 gene (*NPAS2*) (Mansour et al. 2009) and period homolog 3 gene (*PER3*) (Mansour et al. 2006); and between BP and clock gene (*CLOCK*) (Soria et al. 2010), *NPAS2* (Mansour et al. 2009), early growth response 3 (*EGR3*) (Mansour et al. 2009), RAR-related orphan receptor alpha gene (*RORB*) (Mansour et al. 2009), vasoactive intestinal peptide gene (*VIP*) (Soria et al. 2010), rev-erb alpha gene (*NR1D1*) (Severino et al. 2009), prokineticin 2 receptor gene (*PROKR2*) (Kishi et al. 2009g), Bmal1 gene (*ARNTL*) (Nievergelt et al. 2006), *TIMELESS* (Mansour et al. 2006) and *PER3* (Mansour et al. 2006; Nievergelt et al. 2006). In addition, *PROKR2* (Kishi et al. 2009g), cryptochrome 1 gene (*CRY1*) (Soria et al. 2010), and *NPAS2* (Soria et al. 2010) were reported to have an association with MDD. Genetic studies also showed that *NPAS2* was associated with schizophrenia (Mansour et al. 2009), BP (Mansour et al. 2009) and MDD (Soria et al. 2010). These results suggest the possibility of only a partial overlap in genetic predisposition between BP, schizophrenia and MDD. Evidence supporting this hypothesis includes that the neurotrophic tyrosine kinase receptor 3 (*NTRK3*), which is located on 15q, was shown to be associated with schizophrenia (Otnaess et al. 2009), BP (Feng et al. 2008) and MDD (Feng et al. 2008; Verma et al. 2008). In a previous study, we also reported that the clock gene (*CLOCK*) might predict fluvoxamine response in Japanese MDD patients (Kishi et al. 2009d). These facts suggest a crucial relationship between circadian rhythms and psychiatric disorders, and so genes associated with the molecular clock mechanism are good candidates for the etiology of psychiatric disorders. We thought these psychiatric disorders may have some shared mechanisms related to circadian rhythms and considered it reasonable to assess all these disorders.

A recent animal study reported that *Magel2*, which encodes a member of the MAGE/necdin family of proteins, might be associated in the pathophysiology of psychiatric disorders. *Magel2* null mice were found to have circadian rhythm abnormalities (Kozlov et al. 2007; Mercer et al. 2009). Abnormalities in circadian rhythms have been reported to be involved in the pathophysiology of major mental illness, such as schizophrenia and mood disorders. *Magel2* null mice showed altered concentrations of both dopamine and serotonin compared with controls in several parts of brain, including the hypothalamus (Mercer et al. 2009). The authors of the above study detected bilateral reduction in cortical volume in distinct regions of the *Magel2*-null adult mouse brain, including focused regions in the parieto-temporal lobe of the cerebral cortex, the amygdala, the hippocampus, and the nucleus accumbens (Mercer et al. 2009). Several investigations reported that psychiatric disorder patients showed reduced volumes in several parts of the brain compared with healthy controls. Yang and colleagues reported reduced gray matter volume in the hippocampus and parahippocampal gyrus in schizophrenia compared with normal controls (Yang et al. 2010). Savitz and colleagues reported that BP patients showed smaller left and right amygdala volumes than healthy controls (Savitz et al. 2010). Abe and colleagues reported significant gray matter volume reduction in MDD patients compared with controls in several brain regions, such as hippocampus (Abe et al. 2010). More detailed evidence can be seen in the following reviews (Bora et al. 2009; Brambilla et al. 2008; Brunet-Gouet and Decety 2006; Konarski et al. 2008). Several studies have suggested that Magel2 gene (*MAGEL2*) might be a susceptibility gene for Pradar-Willie syndrome (Bischof et al. 2007; Kanber et al. 2009). Pradar-Willie syndrome is known to be comorbid with psychiatric symptoms including mood symptoms, insomnia and psychosis (Soni et al. 2007, 2008). Prader-Willi syndrome is a hypothalamic disorder in which psychotic symptoms may occur that resemble schizophrenia (Soni et al. 2007, 2008).

The *MAGEL2* of humans (OMIM * 605283, 1 exon in this genomic region spanning 3,280 bp) is located at 15q11-q12 (Mercer et al. 2009). The copy number variations (CNVs) on chromosome 15q11.2 were associated with schizophrenia in a multisite European sample (Stefansson et al. 2008). Several linkage studies also reported that 15q11 was significantly associated with autism (Depienne et al. 2009; Hogart et al. 2008; Kim et al. 2008; van der Zwaag et al. 2009).

Based on the above evidence, we selected *MAGEL2* as a candidate gene for the pathophysiology of psychiatric disorders, and conducted a case–control study among Japanese (731 schizophrenia, 465 major depressive disorder (MDD), 156 BP and 758 healthy controls) using tagging SNPs in *MAGEL2*, selected using the HapMap database.

Materials and Methods

Subjects

The subjects in the association analysis were 731 schizophrenia patients (393 males and 338 females; mean age ± standard deviation 36.3 ± 18.4 years), 465 MDD

patients (217 males and 248 females; mean age ± standard deviation 48.7 ± 16.0 years), 156 BP patients (80 males and 76 females; 99 patients with bipolar I disorder and 57 patients with bipolar II disorder; 47.2 ± 13.9 years) and 758 healthy controls (326 males and 432 females; 37.2 ± 15.9 years). A total of 589 schizophrenia patients and 145 BP patients were diagnosed according to DSM-IV criteria with the consensus of at least two experienced psychiatrists on the basis of unstructured interviews and a review of medical records, of which 142 schizophrenia patients and 11 BP patients underwent the Structured Clinical Interview for DSM-IV disorders (SCID-1). A total of 256 MDD patients were diagnosed according to DSM-IV criteria with the consensus of at least two experienced psychiatrists on the basis of unstructured interviews and a review of medical records, of which 209 of the patients underwent the Mini-International Neuropsychiatric Interview (MINI). Of the 465 MDD patients, 267 were treated with fluvoxamine, sertraline or paroxetine. Although 189 of 267 patients treated with SSRIs were assessed with the MINI and the Structured Interview Guide for Hamilton Rating Scale for Depression (SIGH-D) (Williams 1988), the other 78 of 267 patients were assessed with only the SIGH-D. None had severe medical complications such as liver cirrhosis, renal failure, heart failure or other Axis-I disorders according to DSM-IV. No structured methods were used to assess psychiatric symptoms in the controls, which included hospital staff and medical students. Yamaguchi-Kabata and colleagues reported that different proportions of individuals from different regions of Japan in case and control groups can lead to statistical error (Yamaguchi-Kabata et al. 2008); however, another recent study confirmed that there is no population stratification in our control samples (Ikeda et al. 2009). The study was described to subjects and written informed consent was obtained from each. This study was approved by the Ethics Committees at Fujita Health University, Nagoya University School of Medicine, and the University of Occupational and Environmental Health.

SNP Selection and LD Evaluation

We first consulted the HapMap database (release#24/phase II, Nov 2008, www.hapmap.org, population: Japanese Tokyo, minor allele frequencies (MAFs) of more than 0.05) and selected 6 SNPs (rs850815, rs850817, rs850818, rs8920, rs850823 and rs4480754) covering *MAGLE2* (5′-flanking regions including about 2 kb from the exon 1 and about 2 kb bp downstream (3′) from the exon 1: HapMap database contig number chr 15q: 21438034. 21445838). Three 'tagging SNPs' (rs850815, rs8920 and rs4480754) in *MAGLE2* were then selected with the criteria of r^2 threshold greater than 0.8 in 'pair-wise tagging only' mode using the 'Tagger' program (Paul de Bakker, http://www/broad.mit.edu/mpg/tagger) in Haploview for the following association analysis (Barrett et al. 2005).

SNP Genotyping

We used TaqMan assays (ABI: Applied Biosystems, Inc., Foster City, CA,) for all SNPs. One allelic probe was labeled with FAM dye and the other with fluorescent VIC dye. The plates were heated for 2 min at 50°C and 95°C for 10 min, followed by 45 cycles of 95°C for 15 s and 58°C for 1 min. Please refer to ABI for the primer sequence. Detailed information is available on request.

Statistical Analysis

Genotype deviation from the Hardy–Weinberg equilibrium (HWE) was evaluated by chi-square test (SAS/Genetics, release 8.2, SAS Japan Inc, Tokyo, Japan). Marker-trait association analysis was used to evaluate allele and genotype association with the chi-square test (SAS/Genetics, release 8.2, SAS Japan Inc, Tokyo, Japan), and haplotype association analysis was evaluated with a likelihood ratio test using the COCAPHASE2.403 program (Dudbridge 2003). In the haplotype analysis, we determined that the cutoff for testing haplotype frequency was 0.05. We used the permutation test option as provided in the haplotype analysis to avoid spurious results and correct for multiple testing. Permutation test correction was performed using 1000 iterations (random permutations). Power calculation was performed using a genetic power calculator (Purcell et al. 2003). We also performed a multiple regression analysis of the possible correlations between total SIGH-D score at the baseline and each examined SNP genotype. In these analyses, total SIGH-D score at the baseline was set as the dependent variable, and age, gender and each examined SNP genotype were set as the independent variables. Polymorphisms were categorized into three genotypes: homozygous wild-type, heterozygous, and homozygous variant. Homozygosity for the more common allele was treated as the reference category. Tests for linear trend of odds ratio (additive model) were calculated using an ordered categorical variable by assigning scores to the genotypes: 0 (no variant allele), 1 (carrying one variant allele) and 2 (carrying two variant alleles). All statistical analyses were performed using JMP (JMP 5.0. 1 J, SAS Japan Inc, Tokyo, Japan). The significance level for statistical tests was 0.05.

Results

Schizophrenia, BP and MDD group and healthy control group were analyzed using a t-test or a chi-square test.

There was some significant differences in gender distribution among these groups (Age: $P_{schizophrenia} < 0.557$, $P_{BP} < 0.00100$ and $P_{MDD} < 0.00100$, Gender: $P_{schizophrenia} < 0.00200$, $P_{BP} < 0.126$ and $P_{MDD} < 0.336$).

The LD structure as determined from our control samples can be seen in Fig. 1. The LD from all tagging SNPs was not tight, according to the HapMap database samples (Fig. 1). Genotype frequencies of all SNPs were in HWE (Table 1). As a measure of genotyping quality control, we added twenty-five randomly selected samples that were genotyped again, and the genotype consistency rates for all four SNPs were 100%. The MAFs in our healthy control samples were similar to those in the HapMap database.

We did not detect any associations between *MAGEL2* and schizophrenia or mood disorders in the allele/genotype or haplotype analyses (schizophrenia: $P_{haplotype} = 0.255$, MDD: $P_{haplotype} = 0.555$ and BP: $P_{haplotype} = 0.840$) (Table 1).

In the logistic regression analysis, we detected a significant correlation between SIGH-D total score at the baseline and age at recruitment (P value: 0.0134).

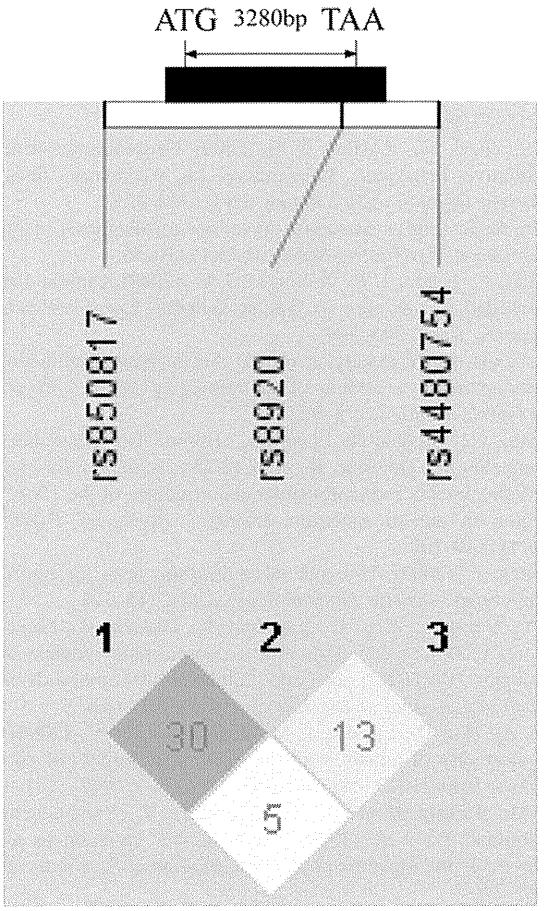

Fig. 1 LD evaluation and tagging SNPs in *MAGEL2*. ATG is the start codon and TAA is the stop codon. Vertical bar represents exon. Color scheme is based on r^2 value. Other information can be seen at the Haploview website

However, SIGH-D total score at the baseline was not associated with three tagging SNP genotypes.

In the power analysis, we obtained power of more than 80% for the detection of association when we set the genotype relative risk at 1.24–1.27, 1.35–1.41 and 2.65–3.31 in schizophrenia, MDD and BP, respectively, for *MAGEL2* under a multiplicative model of inheritance.

Discussion

We first performed a gene-based association analysis between *MAGEL2* and schizophrenia and mood disorders including BP and MDD in the Japanese population. However, we did not find an association between three tagging SNPs in *MAGEL2* and schizophrenia or mood disorders in the Japanese population.

Several limitations in the present study are worth noting. First, the lack of association between *MAGEL2* and schizophrenia and mood disorder patients may have been due to biased samples, such as small sample sizes or unmatched age. In the power analysis, we obtained enough power, of more than 80%, for the detection of association in schizophrenia and MDD. However, for a small sample of BP, it is difficult to conclude that there is a negative association. On average, the controls were much younger than the patients. This means that a number of young controls may go onto develop one of these disorders, most likely MDD, since the incidence of major depression is as high as 5% or more. Most of our subjects did not undergo structured interviews, and it is reported that MDD patients who are not diagnosed by structured interview may develop bipolar disorder in the future (Bowden 2001; Stensland et al. 2008). However, in this study patients were carefully diagnosed according to DSM-IV criteria with consensus of at least two experienced psychiatrists on the basis of a review of medical records. In addition, when we found a patient who had been misdiagnosed, we promptly excluded the misdiagnosed case to maintain the precision of our sample (Kishi et al. 2009a, b, c, e, f, g, h, i; Kishi et al. 2008, 2010). Second, we did not perform a mutation scan of *MAGEL2*. The rare copy number variations (CNVs) on chromosome 15q11.2 were associated with schizophrenia in a multisite European sample (Stefansson et al. 2008). However, because we consider it to be difficult to evaluate the association of extremely rare variants from the viewpoint of statistical power, a replication study using a larger sample will be required for conclusive results. No analysis of CNVs, acetylation and methylation rates in *MAGEL2* was performed in our study.

In conclusion, our results suggest that *MAGEL2* may not play a role in the pathophysiology of schizophrenia and mood disorders in the Japanese population. A replication

Table 1 Tagging SNPs and association analysis of *MAGEL2*

SNP ID[a]	Phenotype	MAF[c]	*N*	Genotype distribution[d]			*P*-value		
				M/M	M/m	m/m	HWE[e]	Genotype	Allele
rs850815	Controls	0.252	758	426	282	50	0.718		
5′ near gene	Schizophrenia	0.280	731	373	306	52	0.314	0.132	0.0789
A > G	MDD	0.251	465	254	189	22	0.0759	0.257	0.936
	BP	0.266	156	88	53	15	0.104	0.363	0.604
rs8920	Controls	0.220	758	470	243	45	0.0741		
Exon 1 3′UTR	Schizophrenia	0.219	731	446	250	35	0.997	0.475	0.959
A > T	MDD	0.199	465	295	155	15	0.322	0.102	0.223
	BP	0.189	156	102	49	5	0.763	0.365	0.231
rs4480754	Controls	0.467	758	215	378	165	0.962		
3′ near gene	Schizophrenia	0.478	731	203	357	171	0.563	0.755	0.544
G > T	MDD	0.451	465	137	237	91	0.526	0.652	0.427
	BP	0.471	156	43	79	34	0.840	0.978	0.894

[a] Major allele > minor allele

[b] *MDD* major depressive disorder, *BP* bipolar disorder

[c] *MAF* minor allele frequency

[d] *M* major allele, *m* minor allele

[e] Hardy–Weinberg equilibrium

study using larger samples may be required for conclusive results, since our sample size was small.

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References

Abe, O., Yamasue, H., Kasai, K., Yamada, H., Aoki, S., Inoue, H., et al. (2010). Voxel-based analyses of gray/white matter volume and diffusion tensor data in major depression. *Psychiatry Research, 181*(1), 64–70.

Barbini, B., Benedetti, F., Colombo, C., Guglielmo, E., Campori, E., & Smeraldi, E. (1998). Perceived mood and skin body temperature rhythm in depression. *European Archives of Psychiatry and Clinical Neuroscience, 248*(3), 157–160.

Barnard, A. R., & Nolan, P. M. (2008). When clocks go bad: neurobehavioural consequences of disrupted circadian timing. *PLoS Genet, 4*(5), e1000040.

Barrett, J. C., Fry, B., Maller, J., & Daly, M. J. (2005). Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics, 21*(2), 263–265.

Berk, M., Dodd, S., Kauer-Sant'anna, M., Malhi, G. S., Bourin, M., Kapczinski, F., et al. (2007). Dopamine dysregulation syndrome: implications for a dopamine hypothesis of bipolar disorder. *Acta Psychiatrica Scandinavica. Supplementum, 434*, 41–49.

Bischof, J. M., Stewart, C. L., & Wevrick, R. (2007). Inactivation of the mouse Magel2 gene results in growth abnormalities similar to Prader-Willi syndrome. *Human Molecular Genetics, 16*(22), 2713–2719.

Bora, E., Yucel, M., & Allen, N. B. (2009). Neurobiology of human affiliative behaviour: Implications for psychiatric disorders. *Current Opinion in Psychiatry, 22*(3), 320–325.

Bowden, C. L. (2001). Strategies to reduce misdiagnosis of bipolar depression. *Psychiatric Services, 52*(1), 51–55.

Brambilla, P., Hatch, J. P., & Soares, J. C. (2008). Limbic changes identified by imaging in bipolar patients. *Current Psychiatry Reports, 10*(6), 505–509.

Brunet-Gouet, E., & Decety, J. (2006). Social brain dysfunctions in schizophrenia: a review of neuroimaging studies. *Psychiatry Research, 148*(2–3), 75–92.

Depienne, C., Moreno-De-Luca, D., Heron, D., Bouteiller, D., Gennetier, A., Delorme, R., et al. (2009). Screening for genomic rearrangements and methylation abnormalities of the 15q11–q13 region in autism spectrum disorders. *Biological Psychiatry, 66*(4), 349–359.

Dudbridge, F. (2003). Pedigree disequilibrium tests for multilocus haplotypes. *Genetic Epidemiology, 25*(2), 115–121.

Feng, Y., Vetro, A., Kiss, E., Kapornai, K., Daroczi, G., Mayer, L., et al. (2008). Association of the neurotrophic tyrosine kinase receptor 3 (NTRK3) gene and childhood-onset mood disorders. *American Journal of Psychiatry, 165*(5), 610–616.

Hogart, A., Wu, D., Lasalle, J. M., & Schanen, N. C. (2008). The comorbidity of autism with the genomic disorders of chromosome 15q11.2–q13. *Neurobiology of Disease, 38*(2), 181–191.

Ikeda, M., Aleksic, B., Kirov, G., Kinoshita, Y., Yamanouchi, Y., Kitajima, T., et al. (2009). Copy number variation in schizophrenia in the Japanese population. *Biological Psychiatry, 67*(3), 283–286.

Kanber, D., Giltay, J., Wieczorek, D., Zogel, C., Hochstenbach, R., Caliebe, A., et al. (2009). A paternal deletion of MKRN3, MAGEL2 and NDN does not result in Prader-Willi syndrome. *European Journal of Human Genetics, 17*(5), 582–590.

Kim, S. J., Brune, C. W., Kistner, E. O., Christian, S. L., Courchesne, E. H., Cox, N. J., et al. (2008). Transmission disequilibrium testing of the chromosome 15q11–q13 region in autism. *American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics, 147B*(7), 1116–1125.

Kishi, T., Kitajima, T., Ikeda, M., Yamanouchi, Y., Kinoshita, Y., Kawashima, K., et al. (2008). Association analysis of nuclear receptor Rev-erb alpha gene (NR1D1) with mood disorders in the Japanese population. *Neuroscience Research, 62*(4), 211–215.

Kishi, T., Ikeda, M., Kitajima, T., Yamanouchi, Y., Kinoshita, Y., Kawashima, K., et al. (2009a). Association analysis of functional polymorphism in estrogen receptor alpha gene with schizophrenia and mood disorders in the Japanese population. *Psychiatric Genetics, 19*(4), 217–218.

Kishi, T., Ikeda, M., Kitajima, T., Yamanouchi, Y., Kinoshita, Y., Kawashima, K., et al. (2009b). A functional polymorphism in estrogen receptor alpha gene is associated with Japanese methamphetamine induced psychosis. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 33*(5), 895–898.

Kishi, T., Kitajima, T., Ikeda, M., Yamanouchi, Y., Kinoshita, Y., Kawashima, K., et al. (2009c). Association study of clock gene (CLOCK) and schizophrenia and mood disorders in the Japanese population. *European Archives of Psychiatry and Clinical Neuroscience, 259*(5), 293–297.

Kishi, T., Kitajima, T., Ikeda, M., Yamanouchi, Y., Kinoshita, Y., Kawashima, K., et al. (2009d). CLOCK may predict the response to fluvoxamine treatment in Japanese major depressive disorder patients. *Neuromolecular Medicine, 11*(2), 53–57.

Kishi, T., Kitajima, T., Ikeda, M., Yamanouchi, Y., Kinoshita, Y., Kawashima, K., et al. (2009e). Orphan nuclear receptor Rev-erb alpha gene (NR1D1) and fluvoxamine response in major depressive disorder in the Japanese population. *Neuropsychobiology, 59*(4), 234–238.

Kishi, T., Kitajima, T., Tsunoka, T., Ikeda, M., Yamanouchi, Y., Kinoshita, Y., et al. (2009f). Genetic association analysis of serotonin 2A receptor gene (HTR2A) with bipolar disorder and major depressive disorder in the Japanese population. *Neuroscience Research, 64*(2), 231–234.

Kishi, T., Kitajima, T., Tsunoka, T., Okumura, T., Ikeda, M., Okochi, T., et al. (2009g). Possible association of prokineticin 2 receptor gene (PROKR2) with mood disorders in the Japanese population. *Neuromolecular Medicine, 11*(2), 114–122.

Kishi, T., Tsunoka, T., Ikeda, M., Kawashima, K., Okochi, T., Kitajima, T., et al. (2009h). Serotonin 1A receptor gene and major depressive disorder: an association study and meta-analysis. *Journal of Human Genetics, 54*(11), 629–633.

Kishi, T., Yoshimura, R., Kitajima, T., Okochi, T., Okumura, T., Tsunoka, T., et al. (2009i). HTR2A is associated with SSRI response in major depressive disorder in a Japanese cohort. *Neuromolecular Medicine*, in press.

Kishi, T., Tsunoka, T., Ikeda, M., Kitajima, T., Kawashima, K., Okochi, T., et al. (2010). Serotonin 1A receptor gene is associated with Japanese methamphetamine-induced psychosis patients. *Neuropharmacology, 58*(2), 452–456.

Konarski, J. Z., McIntyre, R. S., Kennedy, S. H., Rafi-Tari, S., Soczynska, J. K., & Ketter, T. A. (2008). Volumetric neuroimaging investigations in mood disorders: bipolar disorder versus major depressive disorder. *Bipolar Disorders, 10*(1), 1–37.

Kozlov, S. V., Bogenpohl, J. W., Howell, M. P., Wevrick, R., Panda, S., Hogenesch, J. B., et al. (2007). The imprinted gene Magel2 regulates normal circadian output. *Nature Genetics, 39*(10), 1266–1272.

Lang, U. E., Puls, I., Muller, D. J., Strutz-Seebohm, N., & Gallinat, J. (2007). Molecular mechanisms of schizophrenia. *Cellular Physiology and Biochemistry, 20*(6), 687–702.

Maier, W., Hofgen, B., Zobel, A., & Rietschel, M. (2005). Genetic models of schizophrenia and bipolar disorder: overlapping inheritance or discrete genotypes? *European Archives of Psychiatry and Clinical Neuroscience, 255*(3), 159–166.

Mansour, H. A., Talkowski, M. E., Wood, J., Chowdari, K. V., McClain, L., Prasad, K., et al. (2009). Association study of 21 circadian genes with bipolar I disorder, schizoaffective disorder, and schizophrenia. *Bipolar Disorders, 11*(7), 701–710.

Mansour, H. A., Wood, J., Logue, T., Chowdari, K. V., Dayal, M., Kupfer, D. J., et al. (2006). Association study of eight circadian genes with bipolar I disorder, schizoaffective disorder and schizophrenia. *Genes, Brain, and Behavior, 5*(2), 150–157.

McClung, C. A. (2007a). Circadian genes, rhythms and the biology of mood disorders. *Pharmacology and Therapeutics, 114*(2), 222–232.

McClung, C. A. (2007b). Circadian rhythms, the mesolimbic dopaminergic circuit, and drug addiction. *Scientific World Journal, 7*, 194–202.

McClung, C. A. (2007c). Clock genes and bipolar disorder: Implications for therapy. *Pharmacogenomics, 8*(9), 1097–1100.

McClung, C. A. (2007d). Role for the Clock gene in bipolar disorder. *Cold Spring Harbor Symposia on Quantitative Biology, 72*, 637–644.

Mercer, R. E., Kwolek, E. M., Bischof, J. M., van Eede, M., Henkelman, R. M., & Wevrick, R. (2009). Regionally reduced brain volume, altered serotonin neurochemistry, and abnormal behavior in mice null for the circadian rhythm output gene Magel2. *American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics, 150B*(8), 1085–1099.

Muller, N., & Schwarz, M. J. (2008). A psychoneuroimmunological perspective to Emil Kraepelins dichotomy: Schizophrenia and major depression as inflammatory CNS disorders. *European Archives of Psychiatry and Clinical Neuroscience, 258*(Suppl 2), 97–106.

Nestler, E. J., & Carlezon, W. A., Jr. (2006). The mesolimbic dopamine reward circuit in depression. *Biological Psychiatry, 59*(12), 1151–1159.

Nievergelt, C. M., Kripke, D. F., Barrett, T. B., Burg, E., Remick, R. A., Sadovnick, A. D., et al. (2006). Suggestive evidence for association of the circadian genes PERIOD3 and ARNTL with bipolar disorder. *American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics, 141B*(3), 234–241.

Otnaess, M. K., Djurovic, S., Rimol, L. M., Kulle, B., Kahler, A. K., Jonsson, E. G., et al. (2009). Evidence for a possible association of neurotrophin receptor (NTRK-3) gene polymorphisms with hippocampal function and schizophrenia. *Neurobiology of Diseases, 34*(3), 518–524.

Purcell, S., Cherny, S. S., & Sham, P. C. (2003). Genetic power calculator: Design of linkage and association genetic mapping studies of complex traits. *Bioinformatics, 19*(1), 149–150.

Savitz, J., Nugent, A. C., Bogers, W., Liu, A., Sills, R., Luckenbaugh, D. A., et al. (2010). Amygdala volume in depressed patients with bipolar disorder assessed using high resolution 3T MRI: the impact of medication. *Neuroimage, 49*(4), 2966–2976.

Severino, G., Manchia, M., Contu, P., Squassina, A., Lampus, S., Ardau, R., et al. (2009). Association study in a Sardinian sample between bipolar disorder and the nuclear receptor REV-ERBalpha gene, a critical component of the circadian clock system. *Bipolar Disorders, 11*(2), 215–220.

Soni, S., Whittington, J., Holland, A. J., Webb, T., Maina, E., Boer, H., et al. (2007). The course and outcome of psychiatric illness in people with Prader-Willi syndrome: implications for management and treatment. *Journal of Intellectual Disability Research, 51*(Pt 1), 32–42.

Soni, S., Whittington, J., Holland, A. J., Webb, T., Maina, E. N., Boer, H., et al. (2008). The phenomenology and diagnosis of

psychiatric illness in people with Prader-Willi syndrome. *Psychological Medicine, 38*(10), 1505–1514.

Soria, V., Martinez-Amoros, E., Escaramis, G., Valero, J., Perez-Egea, R., Garcia, C., et al. (2010). Differential association of circadian genes with mood disorders: CRY1 and NPAS2 are associated with unipolar major depression and CLOCK and VIP with bipolar disorder. *Neuropsychopharmacology, 35*(6), 1279–1289.

Stefansson, H., Rujescu, D., Cichon, S., Pietilainen, O. P., Ingason, A., Steinberg, S., et al. (2008). Large recurrent microdeletions associated with schizophrenia. *Nature, 455*(7210), 232–236.

Stensland, M. D., Schultz, J. F., & Frytak, J. R. (2008). Diagnosis of unipolar depression following initial identification of bipolar disorder: a common and costly misdiagnosis. *Journal of Clinical Psychiatry, 69*(5), 749–758.

van der Zwaag, B., Staal, W. G., Hochstenbach, R., Poot, M., Spierenburg, H. A., de Jonge, M. V., et al. (2009). A co-segregating microduplication of chromosome 15q11.2 pinpoints two risk genes for autism spectrum disorder. *American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics*, in press.

Verma, R., Holmans, P., Knowles, J. A., Grover, D., Evgrafov, O. V., Crowe, R. R., et al. (2008). Linkage disequilibrium mapping of a chromosome 15q25–26 major depression linkage region and sequencing of NTRK3. *Biological Psychiatry, 63*(12), 1185–1189.

Voderholzer, U., Riemann, D., Huwig-Poppe, C., Kuelz, A. K., Kordon, A., Bruestle, K., et al. (2007). Sleep in obsessive compulsive disorder: polysomnographic studies under baseline conditions and after experimentally induced serotonin deficiency. *European Archives of Psychiatry and Clinical Neuroscience, 257*(3), 173–182.

Williams, J. B. (1988). A structured interview guide for the Hamilton Depression Rating Scale. *Archives of General Psychiatry, 45*(8), 742–747.

Yamaguchi-Kabata, Y., Nakazono, K., Takahashi, A., Saito, S., Hosono, N., Kubo, M., et al. (2008). Japanese population structure, based on SNP genotypes from 7003 individuals compared to other ethnic groups: effects on population-based association studies. *American Journal of Human Genetics, 83*(4), 445–456.

Yang, Y., Raine, A., Han, C. B., Schug, R. A., Toga, A. W., & Narr, K. L. (2010). Reduced hippocampal and parahippocampal volumes in murderers with schizophrenia. *Psychiatry Research, 182*(1), 9–13.