

genotype effect of the Val66Met polymorphism on memory-related neuronal activity; i.e. abnormal intracellular trafficking and regulated secretion of BDNF in Met carriers may result in impaired hippocampal LTP or analogous synaptic events that may underlie encoding reflect decreased hippocampal activity, it seems to be plausible that effects of the polymorphism was found on encoding related hippocampal activity rather than retrieval related hippocampal activity. However, we cannot deny the possibility that inconsistencies between our study and the previous study may relate to sample differences, methodological differences, and also to possible genetic and allelic heterogeneity. These uncertainties cannot be addressed with the current data.

We did not detect the significant genotype effects on the memory performances during fMRI measurements, although previous studies reported the genotype effects. This could be explained by the difference of the difficulty between the two tasks and/or a ceiling effect of our task performance, as the mean hit rate of our retrieval task is above 95% and that of previous study was 88% (Val group, 91.6 ± 1.5 ; Met group, 84.5 ± 2.6). Thus, more difficult tasks would yield different findings. Our WMS-R results, although not reaching significance, do not always tend to be dose-dependent. Previous studies demonstrated genotype effect of the Val66Met polymorphism on human episodic memory on the behavioral level (Egan et al., 2003; Hariri et al., 2003), however, a recent study also demonstrated no association between episodic memory (estimated by logical memory) and BDNF genotype (Harris et al., 2006). Further studies should be conducted to clarify whether the Val66Met polymorphism have apparent effect on human episodic memory function.

Someone may argue that genotype effects of the Val66Met polymorphism on encoding related hippocampal activity observed in this study seem inconsistent with the result of behavioral measures; i.e. no significant BDNF genotype effects on memory-related behavioral measures. In general, it is considered that the difference of a missense polymorphism, i.e. the BDNF Val66Met polymorphism and the Val158Met polymorphism of the catechol-*O*-methyl transferase (COMT) gene, firstly causes the difference of function in the protein level, and then that the distinct protein function influences neuronal functions in the cellular level. Distinct cellular functions might affect brain activities in the network level and these differences of brain activities could result in the distinct performance in the behavioral level. Thus, genotype effects could be stronger in order of the protein level, the cellular level, the network level and the behavioral level. The difficulty to replicate the genotype effects in the behavioral level is well known particularly in the phenotype of psychiatric disorders (Preston and Weinberger, 2005). Thus, the brain activity might reflect genotype effects more directly than the behavior does in our study. A similar phenomenon – no significant effect of the COMT Val158Met polymorphism on working memory performance but significant effects on brain activities during a working memory task – was reported (Ho et al., 2005). Since there was no behavioral effect but effect observed in fMRI in hippocampus/parahippocampal gyrus (PHPG) region, this

raises another possibility that the fMRI effect of Val/Met is not relevant to memory but epiphenomenal, secondary to effects either in other, undetected brain regions projecting to PHPG or to PHPG activity unrelated to memory. The present study suggests that measurement of brain activity related to particular tasks should be useful to investigate relationships between several functional SNPs and human cognitive functions.

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No Association Between the Protein Tyrosine Phosphatase, Receptor-Type, Z Polypeptide 1 (*PTPRZ1*) Gene and Schizophrenia in the Japanese Population

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NRG1-ERBB signaling influences the risk for schizophrenia pathology. A recent study has reported that *MAG1*, *MAG2*, and protein tyrosine phosphatase, receptor-type, Z polypeptide 1 (*PTPRZ1*; located on 7q31.3) gene products regulate the NRG1-ERBB4 signaling pathway, and *PTPRZ1* is associated with schizophrenia in a Caucasian population. By applying a gene-based association concept, we analyzed any association between *PTPRZ1* tagging SNPs and schizophrenia in the Japanese population (576 schizophrenics and 768 controls). After linkage disequilibrium analysis, 29 single nucleotide polymorphisms (SNPs) were genotyped using a 5'-exonuclease allelic discrimination assay. We found a significant association of one tagging SNP in a genotype-wise analysis ($P = 0.007$); however, this might be resulted from type I error due to multiple testing ($P = 0.17$ after SNPSpD correction). No association was observed between schizophrenic patients and controls in either allelic, genotypic, or haplotypic analyses. Our results therefore suggest that *PTPRZ1* is unlikely to be related to the development of schizophrenia in the Japanese population.

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KEY WORDS: association study; *NRG1*; *ERBB4*; linkage disequilibrium; HapMap

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INTRODUCTION

Schizophrenia is a chronic and devastating psychiatric disorder with a global morbidity risk of approximately 1%. While schizophrenia is highly heritable (heritability score of approximately 0.8), the underlying genetics are complex, and the interpretation of genetics data has proven difficult (Freedman 2003; Ross et al. 2006). The hypothesis, which this disease is a developmental disorder of the nervous system with a late onset of characteristic symptoms, has been gaining acceptance over the past years, and several candidate predisposition genes such as neuregulin-1 (*NRG1*), disrupted-in-schizophrenia 1 (*DISC1*), dysbindin (*DTNBP1*), and glutamate decarboxylase 67 (*GAD1*) have been reported [Lewis and Levitt, 2002; Rapoport et al., 2005; Harrison, 2007].

Among these genes, *NRG1* is regarded as one of the most promising susceptibility genes for schizophrenia [Stefansson et al., 2002; Li et al., 2006; Munafò et al., 2006]. *NRG1*-ERBB signaling may contribute to the pathogenesis of schizophrenia by affecting neuronal migration, cortical connectivity, neurotransmitter receptor expression (NMDA and GABA_A), oligodendrocyte development, and myelination [Norton et al., 2006; Li et al., 2007; Woo et al., 2007]. Any associated alterations of *NRG1*-ERBB signaling would thus support a neurodevelopmental and a glutamate/GABA hypothesis of schizophrenia causation [Corfas et al., 2004].

Recently, *MAG1* proteins were identified as substrates for the *ERBB4* gene product by both a yeast two-hybrid analysis and a kinase assay [Montgomery et al., 2004]. Likewise, *MAG1* proteins are dephosphorylated by receptor protein tyrosine phosphatase beta (RPTPβ) [Fukada et al., 2005], and thus notably, RPTPβ may also regulate the *NRG1*-ERBB4 signaling [Buxbaum et al., 2007].

RPTPβ is the *PTPRZ1* (protein tyrosine phosphatase, receptor type, Z polypeptide 1) gene product, and RPTPβ protein is highly expressed during embryogenesis as a transmembrane protein, primarily in the central nervous system [Levy et al., 1993]. Tyrosine phosphorylation and dephosphorylation play a key role in the signaling of cell growth and differentiation, and the *PTPRZ1* gene product is believed to play a role in the recovery and survival of oligodendrocytes in demyelinating disease [Harroch et al., 2002].

In the aforementioned *NRG1*-ERBB4 study, a case-control association study between *PTPRZ1* and schizophrenia has been carried out, and *PTPRZ1* has been demonstrated to be associated with schizophrenia in a United Kingdom case-control cohort [Buxbaum et al., 2007]. However, while *PTPRZ1* is considered to be one of the plausible candidate genes for schizophrenia, replication of this positive association is required in order to demonstrate that *PTPRZ1* is a true susceptibility gene for this disease.

It is widely accepted that there are certain limitations in replicating a genetic association study using the same or a smaller number of single nucleotide polymorphisms (SNPs) as the original investigation. One major limitation is due to differences in allele frequency or variations of the linkage disequilibrium (LD) structure (population dependence) among each ethnicity. To overcome this limitation, a gene-based approach, rather than a SNP-based or haplotype-based approach, is currently recommended [Neale and Sham, 2004]. In such studies, it is important to include both the gene as well as the gene-flanking regions when testing for any associations, and it is also important to select genetic variants which adequately reflect the LD background by the standardized disequilibrium coefficient (D') and squared correlation coefficient (r^2) in the targeted population (e.g., tagging SNPs). By applying this gene-based association approach, we sought to determine the association, if any, between *PTPRZ1* tagging SNPs and schizophrenia in the Japanese population.

MATERIALS AND METHODS

Subjects

The cohorts used in this study consisted of 576 patients with schizophrenia (341 males and 235 females, mean age \pm SD = 50.1 \pm 15.1 years) and 768 healthy control subjects (365 males and 403 females, mean age \pm SD = 40.1 \pm 15.6 years). All subjects were unrelated to each other and were of Japanese ethnicity. The patients were all diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR) criteria for schizophrenia, with consensus reached among at least two experienced psychiatrists on the basis of unstructured interviews as well as a review of the subjects' medical records. All healthy control subjects were also psychiatrically screened with brief diagnostic unstructured interviews. Subjects who had current or past contact with psychiatric services were excluded. After describing the study to each subject, written informed consent was obtained. This study was approved by the Ethics Committee of the Nagoya University Graduate School of Medicine.

Tagging SNP Selection

To analyze genetic association, we implemented a gene-based approach. This method implies the inclusion of both the gene region and the gene-flanking regions in the association study [Neale and Sham, 2004]. The *PTPRZ1* gene contains 30 exons spanning approximately 188900 base pairs (bp), and three other splicing isoforms have been reported thus far [Garwood et al., 2003; Paul and Lombroso, 2003].

We first consulted the HapMap database (release#22; phase2, April 2007, population: Japanese in Tokyo, minor allele frequency (MAF): more than 0.05). All SNPs in the entire gene region covering all isoforms, as well as the 5,000 bp upstream 5' flanking region and the 5,000 bp downstream 3' flanking region, were listed.

Then we defined 28 tagging SNPs (Table I) with the criterion of an $r^2 > 0.8$ in 'pair-wise tagging only' mode using the 'Tagger' program, implemented by Haploview software version 4.0 (<http://www.broad.mit.edu/mpg/haploview/index.php>) [Barrett et al., 2005; de Bakker et al., 2005], considering two points in particular. First, we mandatorily included marginal and significant SNPs reported in previous study (Table I; rs6466808, rs10278079, rs1196471, rs2693657, rs1147502, rs1147497, rs1147489, and rs1206381; indicated by asterisk) as tagging SNPs, except for rs1196513 and rs13241278 [Buxbaum et al., 2007]. Because rs1196513 and rs13241278

were not listed in the HapMap database, we could not analyze the LD patterns of these SNPs. Second, due to unavailability of the reliable genotyping method for rs1206384, we genotyped rs1860721 (No.8) instead, whose r^2 value with rs1206384 was 1 (HapMap data). There were three validated exonic SNPs (MAF > 0.05) and some SNPs in the 3' or 5' flanking of the gene, and all these SNPs were covered by the selected tagging SNPs.

Of two other significant SNPs reported by Buxbaum et al. (rs1196513 and rs13241278), rs13241278 was included, but rs1196513 was not included in our analysis because this SNP was not validated and its frequency was not reported in the dbSNP database (NCBI). Overall, 29 SNPs were examined.

SNP Genotyping

Venous blood was drawn from each subject and genomic DNA was extracted from whole blood according to standard procedures. Genotyping of all tagging SNPs was carried out using a TaqMan 5'-exonuclease allelic discrimination assay (Applied Biosystems Japan Ltd., Tokyo, Japan). TaqMan probes and Universal PCR Master Mix were obtained from Applied Biosystems. Allelic-specific fluorescence was measured using the ABI PRISM 7900 Sequence Detector System (Applied Biosystems). Details regarding reagents or reaction conditions are available upon request.

Statistical Analysis

Genotype deviation from the Hardy-Weinberg equilibrium (HWE) and single-marker allelic association were evaluated using Haploview. Analysis of linkage disequilibrium between markers (r^2 and D') was also performed using Haploview. Genotypic association was tested by the chi-squared test or by Fisher's exact test. Genotypic association of SNPs that deviated from HWE was analyzed using the Cochran-Armitage trend test for a multiplicative model of inheritance [Balding, 2006]. For haplotype-wise analysis, LD blocks were initially defined in accordance with Gabriel's criteria using Haploview software. Haplotype analyses were performed with Unphased software version 2.403 [Dudbridge, 2003], which performs log-likelihood ratio tests under a log-linear model for global P values. Rare haplotypes found in less than 3% of both case and control subjects were excluded from the association analysis to provide greater sensitivity and accuracy when the effect was seen in common haplotypes, and the expectation-maximization algorithm was then employed. rs13241278 was excluded from haplotypic analysis because this SNP was not selected by Tagger. The significance level for all statistical tests was $P < 0.05$. Power calculations were performed using the genetic statistical package Genetic Power Calculator [Purcell et al., 2003] (<http://pngu.mgh.harvard.edu/~purcell/gpc/>). The number of effective independent SNPs assayed was estimated by the spectral decomposition method of Nyholt using SNPSpD software. This software is able to reflect the correlation of markers (LD) on corrected P values to control for inflation of the type I error rate [Nyholt, 2004].

RESULTS

The genotype and allele frequencies of each SNP from schizophrenic patients and healthy control subjects are summarized in Table I. The observed genotype frequencies of two tagging SNPs deviated from HWE (rs1206477 and rs13241278). The LD relationships between markers are provided in Table II. The LD patterns observed in our controls were nearly identical to those of the JPT HapMap samples, but obviously different from those of the CEU HapMap samples. Only rs1196511 showed a significant association with schizophrenia in a genotype-wise analysis ($P = 0.007$). However, this

TABLE I. Association Analysis of Twenty-Nine SNPs of PTPRZ1

No.	SNP ID	N			CON			SCZ			MAF			Allele P-value ^d	LD block ^d	GRR ^e	
		M/m	CON	SCZ	M/M	M/m	M/m	M/M	M/m	CON	SCZ	Genotype P-value	Corrected P-value				
1	rs13437930	Intron1	G/C	762	559	179	24	399	150	17	0.15	0.16	0.45 ^a	0.34	/	1.34	
2	rs24025893	Intron1	A/G	762	400	303	59	272	239	53	0.28	0.31	0.25 ^a	0.10	/	1.27	
3	rs1916885	Intron1	T/C	762	326	337	99	252	240	70	0.35	0.34	0.69 ^a	0.43	I	1.26	
4	rs3757548	Intron1	T/C	758	566	664	91	499	65	2	0.06	0.06	0.93 ^b	0.75	I	1.50	
5	rs1206477	Intron1	C/G	761	566	307	93	266	242	58	0.32	0.32	0.68 ^c	0.68	I	1.26	
6	rs6974265	Intron1	A/G	761	566	578	171	410	150	6	0.13	0.14	0.19 ^b	0.26	I	1.36	
7	rs1011692	Intron1	T/C	761	564	443	272	318	220	26	0.24	0.24	0.31 ^a	0.91	I	1.29	
8	rs1860721	Intron2	C/T	761	566	582	164	15	445	118	3	0.13	0.11	0.07 ^b	0.16	II	1.36
9	rs4731044	Intron2	C/G	762	563	323	112	238	256	69	0.36	0.35	0.39 ^a	0.54	II	1.26	
10	rs6466808*	Intron2	G/A	759	565	323	327	80	255	238	72	0.32	0.34	0.45 ^a	0.28	II	1.26
11	rs1196482	Intron2	C/T	761	566	495	237	29	356	193	17	0.19	0.20	0.42 ^a	0.67	II	1.31
12	rs10278079*	Intron2	A/G	762	566	392	295	75	268	235	63	0.29	0.32	0.32 ^a	0.14	II	1.27
13	rs1196511	Intron2	G/A	761	565	191	358	212	142	306	117	0.51	0.48	0.01 ^a	0.07	II	1.25
14	rs3817483	Intron6	T/C	762	566	592	158	12	443	111	12	0.12	0.12	0.69 ^a	0.99	II	1.37
15	rs10225212	Intron8	G/T	761	566	521	213	27	363	175	28	0.18	0.20	0.18 ^a	0.06	/	1.32
16	rs1196471*	Intron8	T/C	762	564	316	349	97	230	261	73	0.36	0.36	0.97 ^a	0.81	III	1.26
17	rs2693657*	Intron9	G/A	762	566	316	350	96	230	265	71	0.36	0.36	0.95 ^a	0.84	III	1.26
18	rs1196474	Intron10	G/A	761	566	379	304	78	273	236	57	0.30	0.31	0.81 ^a	0.70	III	1.37
19	rs17144005	Intron11	A/G	762	565	667	91	4	508	55	2	0.07	0.05	0.38 ^b	0.17	III	1.50
20	rs1147502*	Intron13	T/G	762	565	330	337	95	249	254	62	0.35	0.34	0.71 ^a	0.54	III	1.26
21	rs12670616	Intron13	T/G	762	565	697	63	2	524	42	0	0.04	0.04	0.53 ^b	0.32	III	1.61
22	rs1147497*	Intron14	T/G	761	565	313	357	91	232	265	68	0.35	0.36	1.00 ^a	0.97	III	1.26
23	rs1147492	Intron18	C/T	762	563	263	364	135	183	272	108	0.42	0.43	0.67 ^a	0.37	III	1.25
24	rs1147491	Intron18	A/G	762	565	457	257	48	341	203	21	0.23	0.22	0.10 ^a	0.37	III	1.29
25	rs1918031	Intron18	G/A	761	566	532	203	26	408	146	12	0.17	0.15	0.33 ^a	0.23	III	1.32
26	rs1147489*	Intron19	A/G	761	566	506	236	19	372	174	20	0.18	0.19	0.54 ^a	0.55	III	1.32
27	rs1147488	Intron22	A/G	762	566	571	174	17	436	122	8	0.14	0.12	0.46 ^b	0.27	III	1.35
28	rs1206381*	Intron27	A/C	761	566	322	348	91	249	253	64	0.35	0.34	0.82 ^a	0.53	III	1.26
	rs13241278*	Intron8	C/T	759	565	385	275	99	280	223	62	0.31	0.31	0.81 ^c	0.80	III	1.26

N, number; M, major allele; m, minor allele; CON, control; SCZ, schizophrenia; MAF, minor allele frequency; GRG, genotype relative risk ($\alpha = 0.05$, $1 - \beta = 0.8$). IDs with asterisk (*) represent significant or marginally significant SNPs in Buxbaum's report. Genotypic P-value was calculated by the chi-squared test (a), Fisher's exact test (b), and the Cochran-Armitage trend test (c). Allelic P-value was calculated and LD block (Gabriel's criteria) was defined using Haploview software (d). GRG was calculated using Genetic power calculator (e). Corrected P-value was calculated by Nyholt's method (f). Effective number of independent marker loci [Meff]: 24.8646. Experiment-wide significance threshold required to keep Type I error rate at 5%: 0.002.

significance may be the result of a type I error due to multiple testing. We performed *P*-value correction by using the SNPSpD program (the effective number of independent marker loci: 24.8646; the experiment-wide significance threshold required to keep type I error rate at a 0.05: 0.002; *P* = 0.17 after SNPSpD correction) [Nyholt, 2004]. No association was observed between the schizophrenic patients and their controls in allelic, genotypic, or haplotypic analyses (Tables I and III). Greater than 80% power in detecting any association with schizophrenia was obtained when the genotype relative risk (GRR) was set between 1.25 and 1.61 under a multiplicative model of inheritance assuming the disease prevalence to be 1% and the population susceptibility allele frequencies to be the values observed in control samples.

DISCUSSION

The ‘common disease-common variant’ hypothesis postulates that linkage disequilibrium should be detected by the haplotype association test if the risk haplotype is linked to causal variants for disease [Chakravarti, 1999]. Regarding the Japanese population, therefore, the data presented in this article do not provide sufficient evidence for the involvement of the *PTPRZ1* gene in conferring susceptibility for schizophrenia.

In this study, we could not replicate a previous report [Buxbaum et al., 2007], which revealed a significant association between *PTPRZ1* and schizophrenia in a Caucasian population. The discrepancy between Japanese and Caucasian populations may derive from ethnic differences in the etiology of schizophrenia. Although the sample size used here is smaller than the sample size of the original study, we obtained a statistical power expected to detect any possible association, and so the possibility of a type II error is unlikely. The GRR value calculated using power analysis was appropriate when compared to other promising candidate genes for schizophrenia [Shifman et al., 2002; Schwab et al., 2003]. In this regard, a prompt gene-based replication study has become feasible as the International HapMap project progresses. In this case, however, *PTPRZ1* is a complicated gene with a transcript that is spliced into four distinct isoforms, and the HapMap database focused only one isoform (NM_002851). With such a limitation, other SNPs involved in mRNA splicing, while having attracted great attention in the pathology of schizophrenia [Law et al., 2007], might nonetheless be overlooked. Thus, it might be useful to investigate not only exons but also splice junctions of this gene.

Two additional limitations need to be addressed in order to discuss the present results. First, age-unmatched and male-to-female ratio unmatched cohorts were examined in the present study. The mean age of the controls is younger than that of the patients. This means that a number of these younger controls, though likely not more than eight subjects given a lifetime morbidity risk of 1%, may later develop schizophrenia. This confounding factor might weaken the power of the present study. We also performed exploratory analysis to investigate the effect of age and gender on the disease status. Based on the results of the analysis using logistic regression model, these variables did not seem to be involved in the results of present association study (data not shown). Secondly, another limitation, which must be addressed, is that other candidates related to the *NRG1*-*ERBB* signaling would also be in the locus heterogeneity. In this case, causal variants with extremely rare MAFs and allelic heterogeneity should be also considered. Likewise, the combined effect between SNPs on *PTPRZ1* and SNPs on the other genes (*ERBB4*, *MAG1s*, etc) might actually prove to be a stronger predisposition factor.

Moreover, the definition of phenotypes is vital for a genetic association study. Therefore, endophenotypes (being more

TABLE II. Linkage Disequilibrium Analysis of *PTPRZ1*

No.	SNP ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	
1	r212437930																													
2	r22407593	0.45																												
3	r1915886	0.09	0.21																											
4	r3757548	0.01	0.03	0.04																										
5	r12004477	0.08	0.18	0.26	0.14																									
6	r1911692	0.03	0.05	0.09	0.07	0.05																								
7	r1911692	0.04	0.02	0.05	0.02	0.04	0.00																							
8	r1960721	0.04	0.12	0.16	0.02	0.14	0.00	0.05																						
9	r4731044	0.04	0.14	0.24	0.11	0.21	0.08	0.14	0.08	0.14																				
10	r6466808	0.11	0.40	0.94	0.03	0.20	0.23	0.01	0.07	0.27	0.11																			
11	r11948482	0.11	0.40	0.94	0.03	0.20	0.23	0.01	0.07	0.27	0.11	0.00																		
12	r10279079	0.14	0.31	0.62	0.02	0.18	0.11	0.00	0.06	0.23	0.88	0.10	1.00																	
13	r1196511	0.10	0.16	0.06	0.06	0.39	0.03	0.28	0.13	0.53	0.37	0.25	0.43	0.14																
14	r38417483	0.02	0.02	0.02	0.01	0.05	0.12	0.05	0.02	0.07	0.29	0.03	0.32	0.14	0.06															
15	r13244278	0.03	0.07	0.20	0.14	0.56	0.03	0.11	0.07	0.71	0.16	0.11	0.18	0.42	0.06	0.98														
16	r11964471	0.02	0.01	0.07	0.03	0.11	0.01	0.47	0.07	0.34	0.01	0.42	0.01	0.33	0.19	0.23	0.12	1.00												
17	r28989857	0.02	0.00	0.17	0.03	0.11	0.01	0.47	0.07	0.34	0.01	0.42	0.01	0.33	0.19	0.23	0.12	1.00	0.00											
18	r11964474	0.08	0.07	0.01	0.03	0.11	0.00	0.10	0.01	0.31	0.09	0.10	0.16	0.41	0.23	0.33	0.16	0.41	0.23	0.33	0.16	0.41	0.23	0.33	0.16	0.41	0.23	0.33	0.16	
19	r11746053	0.16	0.07	0.03	0.01	0.33	0.00	0.01	0.04	0.14	0.01	0.16	0.07	0.00	0.03	0.32	0.23	0.33	0.16	0.41	0.23	0.33	0.16	0.41	0.23	0.33	0.16	0.41	0.23	
20	r11964474	0.08	0.07	0.01	0.03	0.11	0.00	0.10	0.01	0.31	0.09	0.10	0.16	0.41	0.23	0.33	0.16	0.41	0.23	0.33	0.16	0.41	0.23	0.33	0.16	0.41	0.23	0.33	0.16	
21	r129570616	0.00	0.01	0.06	0.02	0.02	0.00	0.06	0.02	0.08	0.15	0.09	0.03	0.38	0.02	0.05	0.08	0.03	0.16	0.08	0.03	0.16	0.08	0.03	0.16	0.08	0.03	0.16	0.08	
22	r1147497	0.02	0.00	0.16	0.03	0.11	0.01	0.44	0.07	0.34	0.00	0.42	0.01	0.31	0.17	0.22	0.32	0.94	0.35	0.24	0.04	0.29	0.08	0.98	0.09	0.99	0.00	1.00	0.97	0.96
23	r1147462	0.00	0.00	0.31	0.09	0.33	0.00	0.18	0.10	0.45	0.10	0.06	0.06	0.24	0.09	0.51	0.00	0.35	0.35	0.07	0.05	0.71	0.03	0.27	0.63	0.37	0.10	1.00	0.96	
24	r1147491	0.03	0.01	0.94	0.02	0.08	0.01	0.05	0.43	0.13	0.02	0.06	0.03	0.00	0.01	0.10	0.32	0.15	0.15	0.67	0.22	0.15	0.61	0.16	0.22	0.36	0.14	0.06	0.59	0.99
25	r1918031	0.02	0.01	0.02	0.01	0.05	0.07	0.03	0.06	0.13	0.00	0.14	0.12	0.45	0.07	0.04	0.34	0.38	0.10	0.02	0.12	0.61	0.40	0.16	0.07	0.04	1.00	0.99	0.99	0.99
26	r1147468	0.00	0.01	0.13	0.02	0.04	0.03	0.30	0.03	0.06	0.08	0.59	0.07	0.13	0.03	0.09	0.05	0.38	0.38	0.10	0.02	0.12	0.61	0.40	0.16	0.07	0.04	1.00	0.99	0.99
27	r1147488	0.02	0.01	0.13	0.02	0.04	0.03	0.30	0.03	0.06	0.08	0.59	0.07	0.13	0.03	0.09	0.05	0.38	0.38	0.10	0.02	0.12	0.61	0.40	0.16	0.07	0.04	1.00	0.99	0.99
28	r1206381	0.01	0.00	0.15	0.03	0.11	0.01	0.41	0.07	0.34	0.01	0.40	0.01	0.32	0.17	0.19	0.10	0.58	0.88	0.21	0.03	0.25	0.08	0.36	0.15	0.37	0.04	0.09	0.64	0.09

Values shown above the diagonal are *r*² and values shown below are *r*⁻²

TABLE III. Haplotypic Analysis of *PTPRZ1*

No.	SNP ID	Haplotypic global <i>P</i> -value window size		
		2	3	4
1	rs13437930	0.22		
2	rs2402593	0.25	0.38	
3	rs1916885	0.64	0.46	0.57
4	rs3757548	0.84	0.66	0.60
5	rs1206477	0.54	0.74	0.77
6	rs6974265	0.64	0.77	0.90
7	rs1011692	0.15	0.37	0.50
8	rs1860721	0.21	0.31	0.45
9	rs4731044	0.58	0.34	0.26
10	rs6466808	0.41	0.44	0.40
11	rs1196482	0.21	0.28	0.35
12	rs10278079	0.18	0.19	0.24
13	rs1196511	0.16	0.26	0.26
14	rs3817483	0.13	0.21	0.17
15	rs10225212	0.21	0.29	0.41
16	rs1196471	0.21	0.23	0.34
17	rs2693657	0.84	0.23	0.44
18	rs1196474	0.92	0.96	0.44
19	rs17144005	0.26	0.43	0.41
20	rs1147502	0.27	0.33	0.41
21	rs12670616	0.51	0.27	0.26
22	rs1147497	0.58	0.73	0.43
23	rs1147492	0.62	0.58	0.07
24	rs1147491	0.62	0.62	0.64
25	rs1918031	0.57	0.62	0.39
26	rs1147489	0.22	0.32	0.48
27	rs1147488	0.47	0.45	0.54
28	rs1206381	0.50	0.39	0.36
		0.34	0.37	

Haplotypic global *P*-value was calculated using Unphased software.

specific than phenotypes) or symptoms are also thought to be important in this field [Gottesman and Gould, 2003; Craddock et al., 2006; Braff et al., 2007]. We did not take advantage of these analyses in order to test for a genetic association, but they may be useful in elucidating the potential role of *PTPRZ1* in schizophrenia. Because several studies have demonstrated that *Ptprz*-deficient mice suffer hippocampal dysfunction [Niisato et al., 2005; Tamura et al., 2006], additional endo-

phenotypic approaches such as cognitive function assessment, brain imaging and other phenotypes that reveal *PTPRZ1* traits would further contribute to our understanding of schizophrenia.

In summary, the findings of the present study suggest that *PTPRZ1* is unlikely to be related to the development of schizophrenia in the Japanese population. Further replication studies incorporating supplemental populations should be performed for conclusive results.

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Brief Research Communication

No Association Between Tagging SNPs of SNARE Complex Genes (STX1A, VAMP2 and SNAP25) and Schizophrenia in a Japanese Population

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Abnormalities in neural connections and the neurotransmitter system appear to be involved in the pathophysiology of schizophrenia. The soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex, which consists of Syntaxin1A, vesicle-associated membrane protein 2 (VAMP2) and synaptosomal-associated protein 25 kDa (SNAP25), plays an important role in the neurotransmitter system, and is therefore an attractive place to search for candidate genes for schizophrenia. We conducted a two-stage genetic association analysis of Syntaxin1A (*STX1A*), VAMP2 and SNAP25 genes with schizophrenia (first-set screening samples: 377 cases and 377 controls, second-set confirmation samples: 657 cases and 527 controls). Based on the linkage disequilibrium, 40 SNPs (*STX1A*, 8 SNPs; *VAMP2*, 3 SNPs; *SNAP25*, 29 SNPs) were selected as 'tagging SNPs'. Only nominally significant associations of an SNP (rs12626080) and haplotype (rs363014 and rs12626080) in *SNAP25* were detected in the first-set screening scan. To validate this significance, we carried out a replication analysis of these SNP and haplotype associations in second-set samples with a denser set of markers (including five additional SNPs). However, these associations could not be confirmed in the second-set analysis. These results suggest that the SNARE complex-related genes do not play a major role in susceptibility to schizophrenia in the Japanese population.

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This article contains supplementary material, which may be viewed at the American Journal of Medical Genetics website at <http://www.interscience.wiley.com/jpages/1552-4841/suppmat/index.html>.

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There is growing evidence that the presynapse is involved with the pathophysiology of schizophrenia. Within the presynaptic area, neurotransmitters are released by synaptic vesicle exocytosis, and the regulation of this release is critical for neural function. The machinery for this release consists of several groups of proteins that work together as a functional unit, the soluble *N*-ethylmaleimide sensitive factor attachment protein receptor (SNARE) complex [Montecucco et al., 2005].

The SNARE complex consists of Syntaxin1A, vesicle-associated membrane protein 2 (VAMP2) and synaptosomal-associated protein 25 kDa (SNAP25) [Marz and Hanson, 2002], and it has been reported that alterations in the components in the SNARE complex may underlie the pathophysiology of schizophrenia. First, postmortem studies measuring the level of SNARE complex protein or its mRNA revealed specific brain region alternations in schizophrenia [Gabriel et al., 1997; Thompson et al., 1998; Young et al., 1998; Karson et al., 1999; Sokolov et al., 2000; Hemby et al., 2002; Honer et al., 2002; Halim et al., 2003; Thompson et al., 2003]. Second, genetic association studies showed a significant association between SNPs in the Syntaxin1A gene (*STX1A*) and schizophrenic patients from Portugal and Toronto [Wong et al., 2004]. In addition, a very recent report showed that SNPs in *SNAP25* were associated with schizophrenia in Irish high-density families [Fanous et al., 2007].

In this study, we investigated whether genetic polymorphisms within *STX1A* (7p11.23: OMIM *186590), *VAMP2* (17p13.1: OMIM *185881) and *SNAP25* (20p12-p11.2: OMIM *600322) were associated with schizophrenia in a Japanese population.

A first-set screening analysis was conducted with 377 schizophrenic patients (196 males and 181 females; mean age ± standard deviation (SD) 42.4 ± 14.8 years) and 377 healthy controls (212 males and 172 females; 35.9 ± 14.7 years). In a

confirmation analysis a different panel of samples was used, consisting of 657 patients with schizophrenia (350 male and 307 female; 50.1 ± 14.4 years) and 527 controls (303 male and 224 female; 40.8 ± 15.3 years).

The 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. All healthy control subjects were also psychiatrically screened based on unstructured interviews. None of the subjects was known to be related to each other, and all were ethnically Japanese.

Written informed consent was obtained from each subject. This study was approved by the ethics committees at Fujita Health University, Nagoya University Graduate School of Medicine, Osaka University Graduate School of Medicine and Teikyo University School of Medicine.

After consulting the HapMap database (release#16.c.1, June 2005, www.hapmap.org, population: Japanese Tokyo: minor allele frequencies (MAFs) of more than 0.05 for *STX1A* and *VAMP2*, and 0.1 for *SNAP25*), 39 SNPs (*STX1A*, 7 SNPs; *VAMP2*, 3 SNPs; *SNAP25*, 29 SNPs) were selected as 'tagging SNPs' based on the criterion of an 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). For *STX1A*, since a previous report showed the positive association of an SNP in intron 7 [Wong et al., 2004], we included this SNP with the aforementioned 'tagging SNPs' for the association analysis. Overall, 40 SNPs were examined in this study (Supplementary Figures 1–3).

For denser mapping in the confirmation analysis, we added five SNPs around nominally significant SNPs or haplotypes detected in the first-set screening scan (rs610457, rs363013, rs363015, rs6039792 and rs363050).

For genotyping of these SNPs, a TaqMan assay (Applied Biosystems, CA), PCR-RFLP assay, and direct sequencing techniques were used. Detailed information is available in Supplementary Table 1. 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 was evaluated by a likelihood ratio test (allele-wise and haplotype-wise analyses) and χ^2 -test (genotype-wise analysis). For exhaustive screening, we tested all one-marker (by conventional allele-wise analysis), two-marker, and three-marker haplotypes (and seven-marker haplotypes for second-set confirmation analysis) using the COCAPHASE 2.403 program [Dudbridge, 2003].

The power and sample size calculations were performed with a statistical program (http://biostat.mc.vanderbilt.edu/twiki/bin/view/main/powersamplesize). This significance threshold for all statistical tests was 0.05.

All genotype frequencies of each group were in HWE (data not shown). The LD structures examined in our control samples were almost the same as the one shown in HapMap database (Supplementary Figure 1–3).

The SNP (rs12626080: *SNAP25*-M8: $P = 0.0236$, uncorrected) in *SNAP25* and the haplotype constructed by M7 (rs363014) and M8 in *SNAP25* showed a nominally significant association with schizophrenia in the first-set screening samples (global $P = 0.0215$, uncorrected), although no association was detected with any tagging SNP in *STX1A* and *VAMP2*, including the SNP reported to be associated with schizophrenia in Caucasian samples [Wong et al., 2003] (Tables I and II and Supplementary Table 2).

To validate this nominal significance, we carried out a replication analysis using an independent set of samples. In this analysis, five additional SNPs were further included for denser mapping around M7 and M8 (rs610457, rs363013, rs363015, rs6039792 and rs363050; Supplementary Figure 4). However, this second-set confirmation analysis showed no

TABLE I. First-Set Association Analysis of Tagging SNPs in *STX1A* and *VAMP2*

Genes	Marker IDs	Distance to next SNP (bp)	N ^a		MAF ^b			P-Values				
			SCZ	CON	SCZ	CON	Genotype	(1) Window ^c	(2) Window	(3) Window		
<i>STX1A</i> (minus strand)	SNP1	rs867500										
	SNP2	Intron7 SNP	0	375	0.204	0.217	0.754	0.527	0.947	0.946		
	SNP3	rs4363087	3592	373	0.260	0.261	0.767	0.978	0.859	0.896		
	SNP4	rs3793243	164	372	0.326	0.311	0.424	0.537	0.592	0.775		
	SNP5	rs875342	3151	377	0.419	0.386	0.304	0.196	0.553	0.698		
	SNP6	rs6951030	5751	370	0.228	0.238	0.211	0.653	0.781	0.924		
	SNP7	rs6954749	6143	373	0.0663	0.0665	0.826	0.594	0.593	0.872		
	SNP8	rs2030921	7145	376	0.483	0.469	0.460	0.602	0.869	0.869		
<i>VAMP2</i> (minus strand)	m1	rs2278637	0	372	0.430	0.436	0.348	0.724	0.732	0.802		
	m2	rs1061032	1981	377	0.399	0.403	0.750	0.975	0.694	0.694		
	m3	rs8067606	2800	375	0.425	0.431	0.490	0.765	0.765	0.765		

^aN, number; SCZ, schizophrenia; CON, control.

^bMAF, minor allele frequency.

^cIdentical as conventional allele-wise analysis.

TABLE II. First-Set Association Analysis of Tagging SNPs in SNAP25

Genes	Marker IDs	Distance to next SNP (bp)	N ^a			MAP ^b			P-Values		
			SCZ	CON	CON	SCZ	CON	Genotype	(1) Window ^c	(2) Window	(3) Window
SNAP25	M1	0	377	370	370	0.263	0.242	0.298	0.341	0.493	0.825
	M2	1,653	377	370	370	0.236	0.223	0.675	0.550	0.830	0.721
	M3	11,662	377	376	376	0.312	0.300	0.306	0.630	0.874	0.795
	M4	4,347	374	368	368	0.171	0.171	0.146	0.978	0.403	0.364
	M5	6,704	377	377	377	0.308	0.295	0.364	0.612	0.552	0.660
	M6	697	375	368	368	0.432	0.397	0.128	0.173	0.328	0.126
	M7	8,198	377	377	377	0.460	0.453	0.197	0.767	0.0215 ^d	0.0882
	M8	4,358	375	367	367	0.208	0.162	0.073	0.02366 ^d	0.0747	0.411
	M9	2,374	375	369	369	0.163	0.159	0.177	0.866	0.774	0.719
	M10	159	374	370	370	0.298	0.291	0.918	0.748	0.719	0.865
	M11	2,231	377	377	377	0.222	0.235	0.650	0.544	0.690	0.855
	M12	4,195	372	369	369	0.337	0.341	0.615	0.868	0.643	0.643
	M13	383	374	369	369	0.394	0.413	0.950	0.457	0.509	0.739
	M14	2,232	376	372	372	0.455	0.425	0.725	0.265	0.579	0.524
	M15	513	372	376	376	0.222	0.184	0.380	0.0712	0.254	0.375
	M16	952	377	376	376	0.144	0.129	0.259	0.379	0.0883	0.134
	M17	773	375	368	368	0.351	0.331	0.572	0.414	0.615	0.617
	M18	7,611	372	370	370	0.209	0.212	0.716	0.877	0.489	0.716
	M19	7,442	372	367	367	0.0970	0.0989	0.574	0.903	0.999	0.737
	M20	1,659	372	367	367	0.451	0.434	0.764	0.401	0.601	0.891
	M21	13,463	377	377	377	0.269	0.248	0.580	0.544	0.361	0.587
	M22	3,044	373	377	377	0.134	0.0082	0.873	1	0.208	0.510
	M23	422	374	368	368	0.182	0.192	0.893	0.647	0.732	0.689
	M24	865	374	370	370	0.379	0.401	0.325	0.391	0.679	0.788
	M25	657	377	376	376	0.400	0.373	0.253	0.286	0.526	0.807
	M26	1,923	377	376	376	0.401	0.405	0.892	0.889	0.612	0.548
	M27	468	376	376	376	0.460	0.465	0.614	0.850	0.942	0.862
	M28	2,666	372	367	367	0.260	0.249	0.746	0.634	0.642	0.676
	M29	3,876	377	377	377	0.237	0.206	0.176	0.156	0.437	0.676

^aN, number; SCZ, schizophrenia; CON, control.^bMAP, minor allele frequency.^cIdentical as conventional allele-wise analysis.^dBold numbers represent significant P-values.

evidence of the significance of these markers (*P*-values for M7-M8 combination: 0.541; Supplementary Table 3). To increase the power, the genotypes of these five new SNPs in the first-set samples were determined and we then combined the samples (first-set and second-set samples), but again we could not detect an association in this explorative analysis (*P*-values for M7-M8 combination: 0.280; Table III and Supplementary Table 4).

This genetic two-stage case-control association study revealed no association between SNARE complex-related genes (*STX1A*, *VAMP2* and *SNAP25*) and schizophrenia in the Japanese population. Because postmortem studies showed a change in expression of SNARE complex genes (see Introduction), the most interesting variants of these genes are SNPs located in the promoter regions that might affect gene expression. To cover such regions, particularly the 5' region of each gene, we applied the recently recommended 'gene-based' approach [Neale and Sham, 2004], in which it is important to include both the exon region and the flanking region. There is also emphasis on selecting genetic variants that adequately reflect the LD background in the targeted population (e.g., tagging SNPs). Our selection of tagging SNPs represented the all regions of these genes in the Japanese population, significantly reducing genotyping effort without much loss of power.

Moreover, we included confirmation analysis using an independent set of samples to check for Type I error, after significance was obtained in the screening samples. For *SNAP25*, an SNP and a two-marker haplotype were associated with schizophrenia in the first-set screening samples, but no significance could be seen in the larger second set, suggesting that the significance in the screening samples may have resulted from Type I error due to multiple testing or small sample size. We carried out power calculations and determined that our sample had sufficient power in the second-set analysis to detect association of 0.999 at *P* < 0.05, assuming an odds ratio of 1.69, which was shown in the first-set analysis of *SNAP25*-M8.

In addition, our sample size in the first-set screening analysis was large enough to deny Type II error in replicating the previous positive association of an SNP in *STX1A* intron 7 with schizophrenia in Caucasian samples [Wong et al., 2004]. The power was more than 0.997 at *P* < 0.05 when the odds ratio was set at 2.1, which is the estimated odds ratio of TDT in Wong's report [Wong et al., 2004]. One explanation for the different outcomes may be that *STX1A* susceptibility alleles were present in the Caucasian samples, but not in the Japanese population.

Although our sample size was large enough for replication of Wong's study, in general the odds ratios of common variants found to be associated with schizophrenia so far are less than 1.5. In this regard, a larger sample size might be required for conclusive results, since our sample size showed power surpassing 0.8 only when we set the odds ratio at more than 1.62.

With this statistical methodology, it is generally accepted that gene-gene interactions should be examined when a number of related genes are analyzed. We included explorative analysis to evaluate the interaction among these genes by multiple dimensionality reduction (MDR) [Hahn et al., 2003], but no interaction was detected (data not shown). In addition, we conducted MDR analysis for other genes related to SNARE complex genes, Complexin I and II (*CPLX1* and *CPLX2*), for which we previously found no association to schizophrenia [Kishi et al., 2006]. Again, no interaction could be detected in this analysis (data not shown).

There are numerous molecules related to the SNARE complex besides *CPLX* genes [Wang and Tang, 2006]. The most interesting molecule is dysbindin (*DTNBP1*: dystrobrevin-binding protein 1), for which there is evidence of an association

TABLE III. Confirmation Analysis Around the Nominally Significant SNPs Detected in First-Set Analysis

Marker ID ^a	Distance to next SNP (bp)	N ^b				MAF ^c							P-Values						
		SCZ	CON	SCZ	CON	SCZ	CON	SCZ	CON	Genotype	(1) Window ^d	(2) Window	(3) Window	(4) Window	(5) Window	(6) Window	(7) Window		
r#104571	0	1,031 (656)	892 (527)	0.216 (0.198)	0.213 (0.217)	0.970 (0.460)	0.804 (0.300)	0.092 (0.950)	0.146 (0.648)	0.183 (0.704)	0.136 (0.691)	0.107 (0.525)	0.107 (0.553)	0.107 (0.553)	0.107 (0.553)	0.107 (0.553)	0.076 (0.473)		
r#328013	1,661	1,031 (656)	892 (527)	0.011 (0.010)	0.011 (0.011)	0.902 (0.502)	0.872 (0.690)	0.057 (0.523)	0.759 (0.493)	0.194 (0.706)	0.142 (0.783)	0.142 (0.783)	0.142 (0.783)	0.142 (0.783)	0.142 (0.783)	0.142 (0.783)	0.142 (0.783)		
r#328014	2,283	1,031 (656)	892 (527)	0.449 (0.444)	0.455 (0.423)	0.724 (0.155)	0.710 (0.280)	0.289 (0.639)	0.195 (0.662)	0.144 (0.925)	0.136 (0.761)	0.107 (0.553)	0.107 (0.553)	0.107 (0.553)	0.107 (0.553)	0.107 (0.553)	0.076 (0.473)		
r#328015	5	1,031 (656)	892 (527)	0.060 (0.060)	0.062 (0.063)	0.340 (0.552)	0.733 (0.805)	0.272 (0.382)	0.144 (0.925)	0.136 (0.761)	0.107 (0.553)	0.107 (0.553)	0.107 (0.553)	0.107 (0.553)	0.107 (0.553)	0.107 (0.553)	0.076 (0.473)		
r#12526080	4,353	1,031 (657)	896 (523)	0.177 (0.191)	0.166 (0.168)	0.617 (0.461)	0.362 (0.204)	0.119 (0.884)	0.144 (0.925)	0.136 (0.761)	0.107 (0.553)	0.107 (0.553)	0.107 (0.553)	0.107 (0.553)	0.107 (0.553)	0.107 (0.553)	0.076 (0.473)		
r#6039792	1,040	1,031 (656)	892 (527)	0.212 (0.193)	0.209 (0.212)	0.953 (0.465)	0.768 (0.282)	0.050 (0.492)	0.146 (0.648)	0.183 (0.704)	0.136 (0.691)	0.107 (0.525)	0.107 (0.553)	0.107 (0.553)	0.107 (0.553)	0.076 (0.473)	0.076 (0.473)		
r#3363050	165	1,031 (656)	892 (527)	0.225 (0.207)	0.209 (0.205)	0.513 (0.991)	0.246 (0.923)	0.050 (0.492)	0.146 (0.648)	0.183 (0.704)	0.136 (0.691)	0.107 (0.525)	0.107 (0.553)	0.107 (0.553)	0.107 (0.553)	0.076 (0.473)	0.076 (0.473)		

^aN, number; SCZ, schizophrenia; CON, control.

^bMAF, minor allele frequency.

^cIdentical as conventional allele-wise analysis.

^dNumbers in parentheses indicate results from second-set samples.

with schizophrenia, since recent studies showed that dysbindin regulates the expression of *SNAP25* [Numakawa et al., 2004]. Therefore, it will be essential to evaluate the other candidate genes related to SNARE complex genes for conclusive results.

With regard to interpretation of the results from this study, several limitations should be mentioned. Firstly, we did not perform mutation screening of these genes. Secondly, our samples were un-matched for age and gender between cases and controls, and were not assessed with the use of a standard structured interview. Therefore, detailed association analysis with mutation search in well-phenotyped samples will be essential in future study.

To conclude, our results provide no evidence that SNARE complex genes play a major role in susceptibility for schizophrenia in the Japanese population. Our results also imply that caution is needed in drawing conclusions about positive associations from small-sample case-control studies. We strongly suggest that two-stage genetic association analysis be conducted when positive results are found in screening samples.

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Behavioral abnormalities and dopamine reductions in *sdj* mutant mice with a deletion in *Dtnbp1*, a susceptibility gene for schizophrenia

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ABSTRACT

Genetic susceptibility plays an important role in the pathogenesis of schizophrenia. Genetic evidence for an association between the dysbindin-1 gene (*DTNBP1*: dystrobrevin binding protein 1) and schizophrenia has been repeatedly reported in various populations worldwide. Thus, we performed behavioral analyses on homozygous *sandy* (*sdj*) mice, which lack dysbindin-1 owing to a deletion in the *Dtnbp1* gene. Our results showed that *sdj* mice were less active and spent less time in the center of an open field apparatus. Consistent with the latter observation, *sdj* mice also displayed evidence of heightened anxiety-like response and deficits in social interaction. Compared to wild-type mice, *sdj* mice displayed lower levels of dopamine, but not glutamate, in the cerebral cortex, hippocampus, and hypothalamus. These findings indicate that *sdj* mice display a number of behavioral abnormalities associated with schizophrenia and suggest that these abnormalities may be mediated by reductions in forebrain dopamine transmission.

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Schizophrenia is characterized by psychosis and profound disturbances of cognition, emotion, and social functioning. The dysbindin-1 gene (*DTNBP1*: dystrobrevin binding protein 1), have recently been identified as a susceptibility gene for schizophrenia [1,2]. In studies on postmortem brain tissue, decreased expression levels of dysbindin-1 protein [3] and mRNA [4] have been shown in patients with schizophrenia compared with controls. Chronic treatment of mice with antipsychotics did not affect the expression levels of dysbindin-1 protein and mRNA in their brains [3,5], suggesting that prior evidence of lower levels of dysbindin-1 protein and mRNA in the postmortem brains of schizophrenics is not likely to be a simple artifact of antemortem drug treatment. These data indicate that the dysbindin-1 gene may confer susceptibility to schizophrenia through reduced expression.

Dysbindin-1 is relatively ubiquitously expressed in neuronal cell bodies in most parts of the brain and in primary dendrites of

those neurons and is concentrated in axon terminals of some areas such as the hippocampal formation, striatum, substantia nigra, and cerebellum [3,6]. Previous studies reported that down-regulation of endogenous dysbindin-1 by small interfering RNA (siRNA) resulted in a reduction in the release of glutamate from primary cultured neurons [7] and an increase in the release of dopamine from PC12 cells [8]. These results suggest possible roles for the dysbindin-1 gene in glutamatergic and dopaminergic systems related to the pathophysiology of schizophrenia [9].

To investigate the functions of dysbindin-1 *in vivo*, we analyzed *sandy* (*sdj*) mutant mice, which express no dysbindin-1 protein owing to a deletion in the dysbindin-1 gene [10]. *Sdj* is a mutant mouse with diluted pigmentation that arose spontaneously in the DBA/2J inbred mouse strain and has simultaneous defects in melanosomes, lysosomes and platelet dense granules [11]. Thus, we first performed several behavioral analyses and measured dopamine and glutamate contents in several brain regions in *sdj* mice.

Materials and methods

Animals. We obtained *sdj* mice from the Jackson Laboratory (Bar Harbor, ME). *Sdj* mice have an autosomal recessive coat color

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mutation that arose spontaneously in the inbred DBA/2J strain. *Sdy* mice have a large deletion in the dysbindin-1 gene, from nucleotide 3701 of intron 5 to nucleotide 12377 of intron 7, and this deletion results in essentially total loss of dysbindin-1 [10]. Both *sd*y mice and wild-type mice derived from heterozygote crossings were used in all experiments. To minimize the influence of cage environment, they were bred under the same conditions after weaning at 3 weeks of age. The genotypes of mice were identified by their coat color and genomic PCR. Primers *i6_f* (5'-GCACTCAGGA GACCATGACA-3') and *i6_r* (5'-GGTGGACACTCTTGGGAAT-3') amplified a region in intron 6, and produced 305 bp PCR products from normal DNA. Primers *i5*, designed for intron 5 (5'-CCTAGCCCC TCAGGAATTGT-3'), and *i7*, designed for intron 7 (5'-GGGAATGGG GTCTTAATGGT-3'), amplified 733 bp PCR products from mutant DNA. The genomic sequences of these PCR products were confirmed by sequence analysis. The experimental protocols were approved by the Ethics Review Committee for Animal Experimentation of the National Institute of Neuroscience, Japan.

Experimental design for behavioral tests. All behavioral tests were carried out as described previously [12] using male mice that were 6–9 weeks old (*sd*y mice: $n = 119$; wild-type mice: $n = 120$). We used different batches of mice for each behavioral test. Mice were housed four per cage in a temperature-controlled room under a 12 h light–dark cycle (light on at 8:00 a.m.) with ad libitum access to food and water. All behavioral tests were performed between 10:00 a.m. and 7:00 p.m. After each trial, all apparatus were cleaned with water to prevent a bias based on olfactory cues.

Open field test. Locomotor activity was measured using an open field test. Activity was recorded during the first exposure to the open field apparatus (50 × 50 × 40 cm; O'Hara & Co., Tokyo, Japan). The illumination level was 40 lux at the floor of the open field. The field was divided by software (see below) into 16 equal-sized squares containing 4 central areas and 12 peripheral areas. Time spent in the central area defined as [stay time in center (%) = time spent in central areas/objective time for analysis (15 min or 30 min)] and the number of fecal boli were recorded. Data were collected for 30 min. Data acquisition and analysis were performed automatically, using Image OF software (see Behavioral data analysis).

Elevated plus maze test. The elevated plus maze consisted of two black plastic walkways (25 × 5 cm) 34 cm above the floor intersecting at right angles with one of the walkways having 15 cm high wall. To prevent animals from falling off the apparatus, 3-mm-high ledges were provided on the open arms (O'Hara & Co., Tokyo, Japan). A mouse was placed in the central square of the maze (5 × 5 cm), facing one of the enclosed arms. The behavior was recorded during a 20 min test session, because mice entered into the open arms a few times (*sd*y mice: 2.1 ± 0.7 , wild-type mice: 3.6 ± 0.8) for a 10 min test session. The illumination level was 40 lux at the central square of the maze. For data analysis, we used the following four measures: the number of entries into open arms, the total number of arm entries, the time spent on the open arms and the total distance traveled. Data acquisition and analysis were performed automatically, using Image EP software (see Behavioral data analysis).

Social interaction test. A pair of mice was placed simultaneously at opposing corners in the open field apparatus (50 × 50 × 40 cm; O'Hara & Co., Tokyo, Japan) and allowed to explore freely for 30 min. The pair of mice tested was composed of the same genotype (*sd*y–*sd*y pair or wild–wild pair) and had been housed in the same environmental conditions, but in different cages. The illumination level was 40 lux at the floor of the open field. Mice were familiar with the test arena by placing them singly in the apparatus, under the same light level (40 lux), for a 30 min period at least 2 days preceding the test. Total duration of contacts, the number of contacts and total distance traveled were measured. Analysis was

performed automatically using Image SI software (see Behavioral data analysis).

Behavioral data analysis. Behavioral data from the open field tests, elevated plus maze tests and social interaction tests were automatically analyzed as described previously [12]. Briefly, behaviors were monitored by a color charged-coupled device camera (Watec Co., Ltd., Yamagata, Japan) that was connected to a Macintosh computer. We used apparatuses with black-colored floors to detect behaviors of mice, because coat colors of mice were whitish (*sd*y) and dilute brown (wild-type). Images were captured at one or two frames per second. The applications used for the behavioral studies (Image OF, Image EP, Image SI, O'Hara & Co., Tokyo, Japan) were run using a Macintosh computer. These modified applications were based on the public domain NIH Image program developed at the U.S. National Institute of Mental Health.

Neurotransmitter measurements by HPLC-fluorometry. We measured the dopamine and glutamate levels in intact brain with a different batch of mice used for behavioral tests. Mice (male: 8–12 weeks old) were sacrificed by decapitation, and the decapitated heads were dropped directly into ice-cold water for 1 s to prevent degradation of neurotransmitters. Brains were removed from the calvarium and put on a chilled aluminum board. The brain was dissected into ten regions (olfactory bulb, OB; frontal pole cortex, FPC; non-frontal cerebral cortex, NF CX; cerebellum, CB; hippocampal formation, HF; striatum, ST; midbrain, MB; lower brainstem, LB; thalamus, TH; hypothalamus, HT) according to a previously reported method [13] with slight modification. Each block of brain tissue was put into a pre-weighed sampling tube. Brain tissues were homogenized in 9 volumes of 2% perchloric acid (PCA) solution (Katayama Chemical Industries Co., Ltd., Japan) including 1 mM EDTA- Na_2 and 1 mM $\text{Na}_2\text{S}_2\text{O}_5$ using a sonicator for 5–10 s. Homogenates were centrifuged at 10,000g for 30 min. The level of dopamine in the supernatant was determined by a fully automated HPLC system (Model HLC-725CA Catecholamine analyzer, Tosoh, Tokyo, Japan) using a diphenylethylenediamine condensation method [14]; glutamate levels were measured by a pre-label HPLC-fluorometric method [15].

Statistical analysis. Statistical analysis was conducted using SPSS 11.0J for Windows (SPSS Japan Inc., Tokyo, Japan). Data were analyzed by a two-tailed *t*-test unless otherwise noted. Fisher's exact test was used to compare *sd*y mice with wild-type mice for general health (physical characteristics, sensory/motor reflexes and the motor test). A repeated measures analysis of variance (ANOVA) was used to analyze differences in the time course of distance traveled in the open field test. For measurements of neurotransmitters, statistical significance was analyzed using the Student's *t*-test. All *p*-values reported are two-tailed. Statistical significance was defined as $p < 0.05$.

Results

General characteristics

There was no significant difference in body weight, physical characteristics (whiskers and fur), sensory-motor reflexes (eye blink, ear twitch, whisker response and righting reflex) or neuromuscular strength between *sd*y mice and wild-type mice (Supplementary Table S1). *Sdy* mice were more sensitive to 120dB auditory stimulation than wild-type mice, however, there was no significant difference in PPI between *sd*y and wild-type mice (data not shown).

Locomotor activity in a novel environment

Sdy mice showed a pronounced decrease in locomotor activity in the open field test compared with wild-type mice (Fig. 1A, geno-

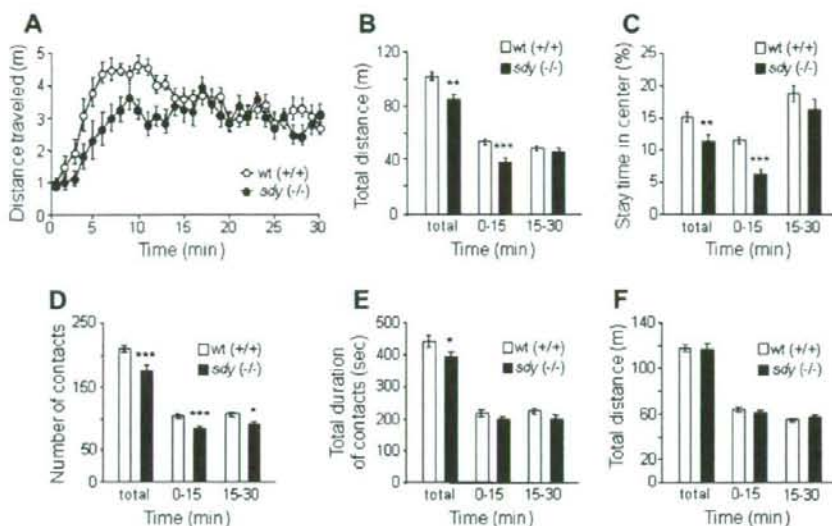


Fig. 1. Open field test with *sdymice* (A–C). Time course of distance traveled (A), total distance traveled (B) and time spent in the central area (C), are shown (*sdymice*: $n = 18$, wild-type mice: $n = 21$). Social interaction test in *sdymice* (D–F). The total number of contacts (D), total duration of contacts (E) and distance traveled (F), in the social interaction test, are shown (pairs of *sdymice*: $n = 22$, pairs of wild-type mice: $n = 21$). Data represent means \pm SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, compared with wild-type mice.

type effect, $F(1, 37) = 12.3$, $p = 0.001$). This hypoactivity phenotype was notable during the first half of the test period (Fig. 1B, total: $t(37) = 3.51$, $p = 0.001$, 0–15 min: $t(37) = 3.82$, $p < 0.001$, 15–30 min: $t(37) = 0.68$, $p = 0.50$). We then compared time spent in the center of the open field apparatus between *sdymice* and wild-type mice. As shown in Fig. 1C, *sdymice* spent significantly less time in the center (total: $t(37) = 2.99$, $p = 0.005$, 0–15 min: $t(37) = 5.26$, $p < 0.001$, 15–30 min: $t(37) = 1.19$, $p = 0.24$). There was no significant difference in the number of fecal boli during the open field test between *sdymice* and wild-type mice (*sdymice*: 8.0 ± 0.6 , wild-type mice: 9.3 ± 1.0 , $t(37) = 1.15$, $p = 0.26$).

Social interaction test

In the social interaction test, *sdymice* showed a significant decrease in the number of social contacts compared with wild-type mice (Fig. 1D, total: $t(41) = 3.57$, $p < 0.001$, 0–15 min: $t(41) = 3.87$, $p < 0.001$, 15–30 min: $t(41) = 2.65$, $p = 0.012$). The total duration of contacts was also decreased in *sdymice* during a 30 min test session (Fig. 1E, total: $t(41) = 2.05$, $p = 0.047$, 0–15 min: $t(41) = 1.49$, $p = 0.14$, 15–30 min: $t(41) = 1.71$, $p = 0.095$). The total distance traveled was not significantly different between the two genotypes (Fig. 1F, total: $t(41) = 0.24$, $p = 0.81$, 0–15 min: $t(41) = 1.01$, $p = 0.32$, 15–30 min: $t(41) = 0.62$, $p = 0.54$).

Elevated plus maze test

In the elevated plus maze test, *sdymice* exhibited a trend toward a reduced number of entries into the open arms during a 20 min test session compared with wild-type mice (Fig. 2A, total: $t(32) = 2.00$, $p = 0.054$). During the second half of the test period, the number of entries into the open arms was significantly lower in *sdymice* than wild-type mice (Fig. 2A, 0–10 min: $t(32) = 1.41$, $p = 0.17$, 10–20 min: $t(32) = 2.11$, $p = 0.042$). *Sdymice* also showed a significant decrease in the total number of arm entries during the second half of the test period and across the entire test period compared with wild-type mice (Fig. 2B, total: $t(32) = 2.35$, $p = 0.025$, 0–10 min: $t(32) = 2.00$, $p = 0.054$, 10–20 min: $t(32) = 2.19$, $p = 0.036$).

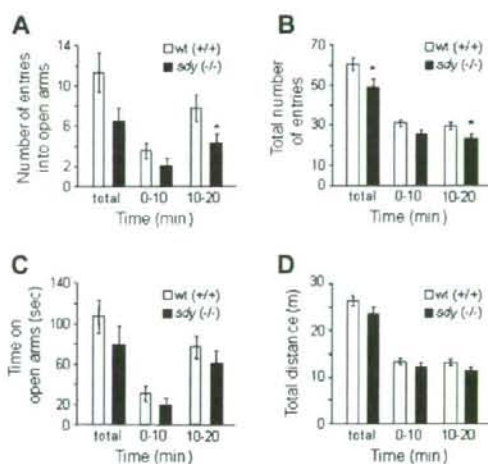


Fig. 2. Elevated plus maze test in *sdymice*. The number of open arm entries (A), total number of arm entries (B), time on open arms (C), and distance traveled (D), are shown (*sdymice*: $n = 16$, wild-type mice: $n = 18$). Data represent means \pm SEM. * $p < 0.05$, compared with wild-type mice.

There were no significant differences in the amount of time spent on the open arms (Fig. 2C, total: $t(32) = 1.13$, $p = 0.27$, 0–10 min: $t(32) = 1.18$, $p = 0.25$, 10–20 min: $t(32) = 0.94$, $p = 0.35$) or in the total distance traveled (Fig. 2D, total: $t(32) = 1.56$, $p = 0.13$, 0–10 min: $t(32) = 1.09$, $p = 0.28$, 10–20 min: $t(32) = 1.69$, $p = 0.10$) between *sdymice* and wild-type mice.

Dopamine and glutamate contents in the brain

Dopamine content was significantly reduced in three brain regions of *sdymice* compared with wild-type mice: non-frontal cerebral cortex (*sdymice*: 0.114 nmol/g, wild-type mice: 0.222 nmol/g,

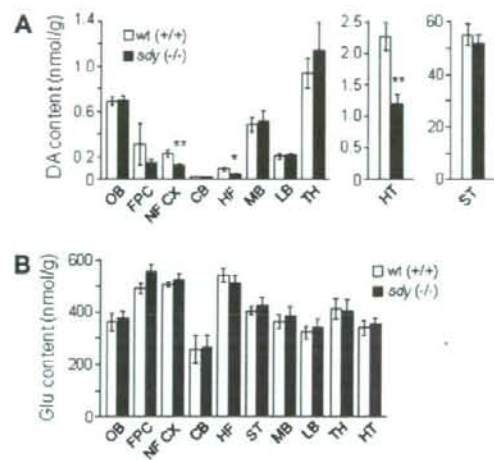


Fig. 3. Dopamine and glutamate content in the brains of *sdv* mice. Dopamine (DA) content (A) and glutamate (Glu) content (B) are shown (dopamine: $n = 4-8$, glutamate: $n = 11$). Olfactory bulb, OB; frontal pole cortex, FPC; non-frontal cerebral cortex, NF CX; cerebellum, CB; hippocampal formation, HF; striatum, ST; midbrain, MB; lower brainstem, LB; thalamus, TH; hypothalamus, HT. Data represent means \pm SEM. * $p < 0.05$, ** $p < 0.01$ compared with wild-type mice.

$p = 0.002$), hippocampal formation (*sdv* mice: 0.0392 nmol/g, wild-type mice: 0.0822 nmol/g, $p = 0.03$), and hypothalamus (*sdv* mice: 1.17 nmol/g, wild-type mice: 2.26 nmol/g, $p = 0.007$) (Fig. 3A). However, no significant difference in glutamate content was detected between *sdv* and wild-type mice in the brain areas examined (Fig. 3B).

Discussion

Several schizophrenia-related behaviors in rodents, such as hyperactivity, deficits in PPI, locomotor response to antipsychotics, disturbance in social interactions, and cognitive deficits, have commonly been observed in previous animal models for schizophrenia [16]. We report here that *sdv* mice, which express no dysbindin-1, show some, but not all, of these abnormalities.

In the open field test, *sdv* mice exhibited decreased locomotor activity during the first half of the test period compared with wild-type mice, and did not show habituation in a novel environment (Fig. 1A and B). It is unlikely that these abnormalities are due to a loss of motor function or general activity, which could be detected by the open field test, because of no differences in locomotor activity during the second half of the test or in neuromuscular strength between the two groups of animals. It could be caused by reduced adaptation, motivation to explore, and/or enhanced anxiety-like response in a novel environment. Indeed, *sdv* mice spent significantly less time in the center of the open field apparatus than wild-type mice (Fig. 1C), which is associated with anxiety-like response [17]. In addition, *sdv* mice showed a decrease in the number of entries into open arms and in the total number of arm entries in the elevated plus maze test (Fig. 2A and B), suggesting enhanced anxiety-related behavior in *sdv* mice. In the social interaction test, *sdv* mice showed reductions in the number of contacts and in the total duration of contacts without hypoactivity (Fig. 1D–F). The decreased social interactions may be caused largely by the proposed anxiogenic-like phenotype of the *sdv* mice and possibly by the reduced exploration. In rodents, reduced contacts with unfamiliar partners are indicative of social withdrawal, a clinical aspect of schizophrenia [16], suggesting a social with-

drawal phenotype in *sdv* mice. Previous study demonstrated that retinal melanosomes were deficient in *sdv* mice [10]. As we did not examine the visual acuity in *sdv* mice, there is a possibility that reduced visual acuity resulting from retinal defects affects multiple behavioral parameters in this study. Increased locomotor activity, which is observed in most animal models of schizophrenia, is considered to be a positive symptom of schizophrenia, like delusions and hallucinations. However, *sdv* mice showed less activity, which is unique in an animal model for schizophrenia. This phenotype could be due to a decreased motivation to explore, which might be related to the negative symptoms of schizophrenia (blunted affect, decreased motivation, and social withdrawal). Other phenotypes such as less time in the center of the open field apparatus, abnormal response in elevated plus maze, and decreased social interactions could also be related to reduced exploratory tendencies. Indeed, two recent studies show a significant association between risk haplotypes of the dysbindin-1 gene and negative symptoms in patients with schizophrenia [18,19]. These data imply that *sdv* mice, which exhibit reduced exploratory activity, heightened anxiety-like response and deficits in social interaction, could be a potential genetic model for negative symptom endophenotypes of schizophrenia.

Decreased dopamine content in three brain regions of *sdv* mice measured by HPLC-fluorometry in the present study (Fig. 3A) was consistent with dopamine reduction measured by HPLC with an electrochemical detection in the previous study [20]. Recent studies reported that reduced dysbindin-1 protein by *DTNBP1* siRNA transfection increased surface expression of dopamine D2 receptor (DRD2) and blocked dopamine-induced internalization of DRD2 in SH-SY5Y cells [21], and that dopamine release was increased by siRNA-mediated silencing of dysbindin-1 protein in PC12 cells [8]. These results suggest that the lack of dysbindin-1 causes an imbalance of the dopaminergic system. As DRD2 mutant mice show decreased activity [22,23], decreased locomotor activity in *sdv* mice could be due to the abnormal regulation of dopaminergic system by lack of dysbindin-1 protein. As glutamate content in *sdv* mouse brain was not altered, behavioral abnormalities in *sdv* mice could be related to the dopaminergic system rather than the glutamatergic system.

This new genetic mouse model could shed light on the etiology of schizophrenia and lead us to new hypotheses, novel diagnostic tools, and more effective therapies for the disorder.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2008.06.016.

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A Case of Schizophrenia with Chromosomal Microdeletion of 17p11.2 Containing a Myelin-Related Gene *PMP22*

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Abstract: We report a patient with schizophrenia who had a chromosomal deletion of 17p11.2 containing a myelin-related gene *PMP22* by using comparative genomic hybridization (CGH) array and quantitative PCR. Since genetic linkage to 17p11, reduced expression of *PMP22*, and alterations in myelination have previously been reported, this report further suggests an etiological role of *PMP22* in schizophrenia.

Keywords: Schizophrenia, *PMP22*, 17p11.2.

INTRODUCTION

Chromosomal aberrations found in patients with schizophrenia have provided possibly important insights into the molecular mechanism of the illness [1,2], although such aberrations are rare [3]. For example, 22q11 deletion syndrome (22q11DS) is a well known risk factor for schizophrenia [4]. *DISC1*, which is one of promising candidate genes of schizophrenia, was found as a disrupted gene by chromosomal translocation [5,6]. In this context, we have been screening chromosomal abnormalities by using the array comparative genomic hybridization (CGH) in 42 schizophrenia subjects and found a patient who had a chromosomal microdeletion of 17p11.2 containing the myelin-related gene, *PMP22*. *PMP22* is causal to Charcot-Marie-Tooth neuropathy type 1A (CMT1A) [7] and hereditary neuropathy with liability to pressure palsies (HNPP) [8]. Three copies of *PMP22* (duplication) result in CMT1A, while one copy (deletion) in HNPP. Patients with CMT1A have reduced nerve conduction velocities [9]. HNPP is characterized by diverse sensory or motor nerve palsies which are often precipitated by minor trauma.

CASE REPORT

Case History

The proband was 32-year-old man who was the first child of reportedly unrelated parents. He had a surgical operation

for inguinal herniation when he was 2 months old. According to his mother, the development of his verbal communication was delayed. When he was 11 years old, he lost consciousness for a few minutes with no apparent external event inducing such loss of consciousness; however, no abnormality was detected with electroencephalography or the computed tomography (CT) scan of the head immediately after the loss of consciousness. The CT film is not available now because the brain CT scan was carried out more than 20 years ago in another hospital. He entered ordinary elementary and junior high schools without receiving any special education. Although he went on to enter a high school, he dropped out at the age of 17 years. When he was 19 years old, auditory hallucination and delusion of persecution developed. Six months later, he started antipsychotic treatment at a psychiatric clinic. Then he was introduced to our hospital to control his psychotic symptoms and adverse effects (tremor) induced by the antipsychotics with a clinical diagnosis of schizophrenia and mental retardation. Finally, his psychiatric symptoms and tremor were controlled by 100mg floripamide, 2mg trihexyphenidyl, and 1mg biperiden. He had never showed motor paralysis or sensory disturbance. His intelligence quotient (IQ) was 42 by the Wechsler adult intelligence scale-revised (WAIS-R) [10] at the age of 29 years. Laboratory tests for blood and urine did not show any abnormality. His final diagnoses were schizophrenia and mental retardation, according to the structured interview of DSM-IV [11, 12].

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This study was approved by the ethics committee of the National Center of Neurology and Psychiatry, Tokyo, Japan. Written informed consent was obtained from the proband and his parents to perform chromosomal examination, and publish this case report.

Family History

His father was a professor of a university and his mother was a housewife with normal social function. Structural interview by Mini-International Neuropsychiatric Interview [13,14] did not indicate any present or past psychiatric diagnosis in his parents. The proband had a younger sister who had no current or past history of psychiatric illness according to the parents.

Array CGH Findings

The proband participated in our ongoing screening of chromosomal abnormalities in a series of patients with schizophrenia. Chromosomal abnormalities were assessed with the array CGH method developed by Miyake *et al.* [15] using a newly developed 4.2K microarray with 4,235 FISHed BAC clones. A typical 1.4-Mb microdeletion at 17p11.2, containing *PMP22*, was identified. The deletion was also confirmed by fluorescence *in situ* hybridization (FISH) analysis (Fig. 1).

Quantitative PCR for Copy Number Variation

We examined copy number of *PMP22* for the proband and his parents by the quantitative PCR of genomic DNA. TaqMan probes were chosen, and delta-delta Ct method was applied, according to a previous study [16]. Quantitative PCR was done with the ABI prism 7900 (Applied Biosystems, Foster city, CA, USA). A Delta-delta Ct value around 0.5 indicates that the copy number of the gene is one and the value around 1 means two copies. The delta-delta Ct values of the proband, his father, and his mother were 0.65, 0.50, and 1.06, respectively, suggesting that the microdeletion of the proband was transmitted from his father.

Neurological Assessment

A clinical neurologist assessed neurological symptoms of the proband. However, no symptom of HNPP was apparent including abnormality of deep tendon reflex. A nerve conduction study was carried out for the proband; however, the result was within normal limit except for the distal motor latency of right tibial nerve. He does not show *pes cavus* or hammer toes. These findings are incongruent with the phenotype of *PMP22* deletion described by Mouton *et al.* [17]. His father did not report any neurological symptoms, either, although a nerve conduction study was not carried out for him.

DISCUSSION

We report a case of schizophrenia who had a microdeletion of chromosome 17p11.2 containing a myelin-related gene, *PMP22*. To our knowledge, this is the first report of such a case. Chromosomal microdeletion was confirmed by FISH and RT-PCR in addition to the initial array CGH method. Thus it is unlikely that the detected deletion was an

artifact, although the case we report here was asymptomatic with respect to HNPP and had no clear family history of HNPP. Mouton *et al.* [17] investigated 99 individuals with the 17p11.2 deletion in 22 families and found that fourteen individuals showed no symptom. Thus asymptomatic individuals like our case seem to be not rare.

It is possible that coexistence of schizophrenia and the deletion of 17p11.2 in the proband may have occurred by simple coincidence. The absence of psychiatric history in the proband's father, who had the same deletion, further supports such a possibility. However, it is also possible that incomplete penetrance of the genetic effect of the deletion may explain the discrepancy in psychiatric condition between the proband and his father. Indeed, several lines of evidence in addition to our case have suggested that *PMP22* may play a role in the pathogenesis of schizophrenia. Dracheva *et al.* [18] reported that mRNA of *PMP22* was reduced in the hippocampus and anterior cingulate cortex in post mortem brains of schizophrenia. *PMP22* is highly expressed and plays a critical role in functions of oligodendrocytes, which accords with previous studies indicating the oligodendrocyte dysfunction in schizophrenia [19,20,21]. Decreased number of perineuronal oligodendrocytes was reported in the prefrontal cortex of patients with schizophrenia [22]. Transgenic mice which have oligodendrocyte dysfunction have increased levels of dopamine receptors and transporters [23]. Then defects in white matter can cause hyper-dopaminergic symptoms (e.g. delusion and hallucination). Reduced fraction anisotropy in white matter of patients with schizophrenia by a diffusion tensor imaging study [24] may be due to oligodendrocyte dysfunction. Oligodendrocytes produce trophic factors such as brain derived neurotrophic factor (BDNF) [25] and neuregulins (NRGs) [26]. BDNF and NRG1 are believed in playing an important role in the etiology of schizophrenia. *PMP22* dysfunction may decrease the function of BDNF [27] and NRG1 [28]. Finally, some genome wide linkage studies provide evidence for linkage to 17p11.2-q25.1 in schizophrenic pedigrees [29,30,31]. To further elucidate the possible role of *PMP22* in schizophrenia, molecular genetic studies and psychiatric examination on individuals with CMT1A and HNPP are warranted.

CONCLUSIONS

In conclusion, we found a patient with schizophrenia who had a chromosomal microdeletion of 17p11.2 containing *PMP22*, a gene critical to oligodendrocyte functions. Since reduced expression of *PMP22*, alterations in oligodendrocyte functions, and genetic linkage to 17p11 have previously been reported, our case further supports an etiological role of *PMP22* in schizophrenia.

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