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 ± SD = 50.1 ± 15.1 years) and 768 healthy control subjects (365 males and 403 females, mean age ± SD = 40.1 ± 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. Haplotypic 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 schizophrenics in a genotype-wise analysis ($P = 0.007$). However, this

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

No.	SNP ID		M/m	N		CON			SCZ			MAF		Genotype *P*-value	Corrected *P*-value	Allele *P*-value[d]	LD block[d]	GRR[e]
				CON	SCZ	M/M	M/m	m/m	M/M	M/m	m/m	CON	SCZ					
1	rs13437930	Intron1	G/C	762	566	559	179	24	399	150	17	0.15	0.16	0.45[a]		0.34	/	1.34
2	rs2402593	Intron1	A/G	762	564	400	303	59	272	239	53	0.28	0.31	0.25[a]		0.10	I	1.27
3	rs1916885	Intron1	T/C	762	565	326	337	99	255	240	70	0.35	0.34	0.69[a]		0.43	I	1.26
4	rs3757548	Intron1	T/C	758	566	664	91	3	499	65	2	0.06	0.06	0.93[b]		0.75	I	1.50
5	rs1206477	Intron1	C/G	761	566	361	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	12	410	150	6	0.13	0.14	0.19[b]		0.26	I	1.36
7	rs1011692	Intron1	T/C	761	564	443	272	46	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	327	112	238	256	69	0.36	0.35	0.39[a]		0.54	II	1.26
10	rs6466808*	Intron2	G/A	759	565	356	323	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]	0.17[f]	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		1.26

N, number; M, major allele; m, minor allele; CON, control; SCZ, schizophrenia; MAF, minor allele frequency; GRR, 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).
GRR 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 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*, MAGIs, 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	rs13437930		0.99	0.97	1.00	0.98	1.00	0.89	1.00	0.65	0.55	0.07	0.57	0.72	0.86	0.58	0.61	0.43	0.43	0.44	0.63	0.49	0.73	0.41	0.17	0.23	0.83	0.02	0.90	0.34
2	rs2402593	0.45		1.00	1.00	0.99	1.00	0.98	0.64	0.80	0.70	0.48	0.58	0.62	0.25	0.63	0.70	0.10	0.10	0.28	0.62	0.52	0.76	0.08	0.11	0.10	0.11	0.41	0.69	0.04
3	rs1916885	0.09	0.21		1.00	1.00	1.00	0.93	0.76	0.89	0.38	0.73	0.32	0.33	0.26	0.90	0.83	0.41	0.41	0.09	0.93	0.90	0.81	0.40	0.89	0.26	0.21	0.57	0.72	0.38
4	rs3757548	0.01	0.03	0.04		1.00	1.00	1.00	1.00	0.95	1.00	1.00	1.00	0.93	1.00	0.96	1.00	0.89	0.89	1.00	1.00	0.96	1.00	0.88	0.95	1.00	1.00	1.00	0.97	0.88
5	rs1206477	0.08	0.18	0.26	0.14		1.00	0.94	0.77	0.92	0.95	0.94	0.95	0.93	0.90	0.77	0.85	0.64	0.65	0.73	1.00	0.70	0.89	0.65	0.70	0.75	0.73	0.61	0.72	0.65
6	rs6974265	0.03	0.39	0.08	0.01	0.07		1.00	0.18	0.97	0.85	1.00	0.55	0.42	0.35	0.65	0.10	0.17	0.17	0.10	0.58	0.51	0.73	0.17	0.03	0.49	0.30	1.00	0.34	0.18
7	rs1011692	0.04	0.12	0.50	0.02	0.13	0.05		1.00	0.89	0.19	0.85	0.12	0.92	0.33	0.90	0.78	0.91	0.91	0.85	0.78	0.89	0.83	0.88	0.90	0.75	0.33	0.65	0.71	0.84
8	rs1860721	0.03	0.02	0.16	0.01	0.04	0.00	0.05		1.00	1.00	1.00	0.95	0.98	1.00	1.00	0.88	0.94	0.94	0.96	1.00	1.00	0.90	0.94	1.00	0.95	0.92	1.00	0.95	0.94
9	rs4731044	0.04	0.14	0.24	0.11	0.71	0.08	0.14	0.08		1.00	1.00	1.00	1.00	0.97	0.94	0.89	0.68	0.68	0.84	1.00	0.83	0.91	0.68	0.75	0.87	0.72	0.67	0.92	0.69
10	rs6466808	0.11	0.40	0.04	0.03	0.20	0.23	0.01	0.07	0.27		1.00	1.00	0.86	0.99	0.87	0.94	0.08	0.08	0.32	0.96	0.73	0.94	0.07	0.34	0.17	0.54	0.90	0.78	0.10
11	rs1196482	0.00	0.02	0.24	0.02	0.10	0.04	0.55	0.04	0.14	0.11		1.00	1.00	1.00	0.98	0.94	0.99	0.99	0.96	0.77	0.98	1.00	0.98	0.96	0.90	0.02	0.80	0.94	0.95
12	rs10278079	0.14	0.31	0.02	0.03	0.18	0.11	0.00	0.06	0.23	0.88	0.10		1.00	0.98	0.97	0.95	0.10	0.11	0.37	0.97	0.88	0.88	0.10	0.44	0.20	0.53	0.89	0.75	0.13
13	rs1196511	0.10	0.16	0.06	0.06	0.39	0.03	0.28	0.13	0.53	0.37	0.25	0.43		0.98	0.98	0.94	0.75	0.75	0.14	1.00	0.90	0.80	0.74	0.60	0.05	0.76	0.73	0.84	0.75
14	rs3817483	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		0.97	0.66	0.89	0.89	0.66	0.40	0.94	0.91	0.83	0.96	0.49	0.82	1.00	0.48	0.83
	rs13241278	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		1.00	0.95	0.96	0.89	1.00	0.92	1.00	0.94	0.89	0.86	0.86	0.95	0.93	0.88
15	rs10225212	0.31	0.27	0.08	0.01	0.07	0.01	0.04	0.02	0.09	0.40	0.05	0.47	0.20	0.01	0.10		1.00	1.00	0.91	0.99	0.87	1.00	1.00	0.05	0.41	0.93	1.00	0.58	0.91
16	rs1196471	0.02	0.00	0.17	0.03	0.11	0.01	0.47	0.07	0.14	0.01	0.42	0.01	0.32	0.19	0.23	0.12		1.00	0.97	0.93	0.95	0.97	0.98	0.94	0.95	0.96	0.97	0.98	0.96
17	rs2693657	0.02	0.00	0.17	0.03	0.11	0.01	0.47	0.07	0.15	0.01	0.42	0.01	0.33	0.19	0.23	0.12	1.00		0.97	0.93	0.96	0.97	0.98	0.95	0.95	0.96	0.97	0.98	0.95
18	rs1196474	0.08	0.07	0.01	0.03	0.11	0.00	0.10	0.31	0.17	0.09	0.10	0.13	0.01	0.03	0.16	0.41	0.23	0.23		1.00	1.00	1.00	1.00	0.46	0.98	0.97	1.00	0.98	0.95
19	rs17144005	0.16	0.07	0.03	0.01	0.03	0.00	0.01	0.01	0.04	0.14	0.01	0.16	0.07	0.00	0.03	0.32	0.03	0.03	0.16		1.00	1.00	1.00	0.96	0.98	0.88	1.00	1.00	0.86
20	rs1147502	0.02	0.06	0.23	0.12	0.45	0.02	0.13	0.08	0.64	0.13	0.12	0.17	0.41	0.06	0.73	0.09	0.27	0.27	0.23	0.04		1.00	1.00	0.98	0.97	0.98	1.00	0.97	0.94
21	rs12670616	0.00	0.01	0.06	0.00	0.02	0.00	0.10	0.01	0.02	0.09	0.01	0.09	0.03	0.28	0.02	0.01	0.08	0.08	0.02	0.00	0.02		1.00	1.00	1.00	0.98	0.71	1.00	0.97
22	rs1147497	0.02	0.00	0.16	0.03	0.11	0.01	0.44	0.07	0.14	0.00	0.42	0.01	0.31	0.17	0.22	0.12	0.94	0.95	0.24	0.04	0.29	0.08		0.98	0.99	0.99	1.00	0.97	0.96
23	rs1147492	0.00	0.00	0.31	0.09	0.33	0.00	0.18	0.10	0.45	0.04	0.16	0.06	0.24	0.09	0.51	0.00	0.35	0.35	0.07	0.05	0.71	0.03	0.37		1.00	1.00	1.00	1.00	0.98
24	rs1147491	0.03	0.01	0.04	0.02	0.08	0.01	0.05	0.43	0.13	0.02	0.06	0.03	0.00	0.01	0.10	0.12	0.15	0.15	0.67	0.22	0.15	0.01	0.16	0.22		1.00	1.00	1.00	0.96
25	rs1918031	0.02	0.01	0.02	0.01	0.05	0.07	0.07	0.03	0.06	0.13	0.00	0.14	0.12	0.45	0.07	0.04	0.34	0.34	0.08	0.01	0.10	0.22	0.36	0.14	0.06		0.94	1.00	0.99
26	rs1147489	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	0.01	0.40	0.16	0.07	0.04		1.00	0.99
27	rs1147488	0.02	0.03	0.15	0.01	0.04	0.00	0.03	0.83	0.08	0.05	0.03	0.04	0.10	0.01	0.06	0.01	0.08	0.08	0.35	0.01	0.08	0.01	0.08	0.11	0.52	0.03	0.04		1.00
28	rs1206381	0.01	0.00	0.15	0.03	0.11	0.01	0.41	0.07	0.14	0.01	0.40	0.01	0.32	0.17	0.19	0.10	0.88	0.88	0.21	0.03	0.25	0.08	0.91	0.36	0.15	0.37	0.40	0.09	

Values shown above the diagonal are D' and values shown below are r^2.

TABLE III. Haplotypic Analysis of *PTPRZ1*

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

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 endophenotypic 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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REFERENCES

Balding DJ. 2006. A tutorial on statistical methods for population association studies. Nat Rev Genet 7(10):781–791.

Barrett JC, Fry B, Maller J, Daly MJ. 2005. Haploview: Analysis and visualization of LD and haplotype maps. Bioinformatics 21(2):263–265.

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.

Buxbaum JD, Georgieva L, Young JJ, Plescia C, Kajiwara Y, Jiang Y, Moskvina V, Norton N, Peirce T, Williams H, Craddock NJ, Carroll L, Corfas G, Davis KL, Owen MJ, Harroch S, Sakurai T, O'Donovan MC. 2007. Molecular dissection of NRG1-ERBB4 signaling implicates PTPRZ1 as a potential schizophrenia susceptibility gene. Mol Psychiatry. (advance online publication, 17 April 2007) DOI: 10.1038/sj.mp.4001991.

Chakravarti A. 1999. Population genetics—Making sense out of sequence. Nat Genet 21(1 Suppl):56–60.

Corfas G, Roy K, Buxbaum JD. 2004. Neuregulin 1-erbB signaling and the molecular/cellular basis of schizophrenia. Nat Neurosci 7(6):575–580.

Craddock N, O'Donovan MC, Owen MJ. 2006. Genes for schizophrenia and bipolar disorder? Implications for psychiatric nosology. Schizophr Bull 32(1):9–16.

de Bakker PI, Yelensky R, Pe'er I, Gabriel SB, Daly MJ, Altshuler D. 2005. Efficiency and power in genetic association studies. Nat Genet 37(11):1217–1223.

Dudbridge F. 2003. Pedigree disequilibrium tests for multilocus haplotypes. Genet Epidemiol 25(2):115–121.

Freedman R. 2003. Schizophrenia. N Engl J Med 349(18):1738–1749.

Fukada M, Kawachi H, Fujikawa A, Noda M. 2005. Yeast substrate-trapping system for isolating substrates of protein tyrosine phosphatases: Isolation of substrates for protein tyrosine phosphatase receptor type z. Methods 35(1):54–63.

Garwood J, Heck N, Reichardt F, Faissner A. 2003. Phosphacan short isoform, a novel non-proteoglycan variant of phosphacan/receptor

protein tyrosine phosphatase-beta, interacts with neuronal receptors and promotes neurite outgrowth. J Biol Chem 278(26):24164–24173.

Gottesman II, Gould TD. 2003. The endophenotype concept in psychiatry: Etymology and strategic intentions. Am J Psychiatry 160(4):636–645.

Harrison PJ. 2007. Schizophrenia susceptibility genes and neurodevelopment. Biol Psychiatry 61(10):1119–1120.

Harroch S, Furtado GC, Brueck W, Rosenbluth J, Lafaille J, Chao M, Buxbaum JD, Schlessinger J. 2002. A critical role for the protein tyrosine phosphatase receptor type Z in functional recovery from demyelinating lesions. Nat Genet 32(3):411–414.

Law AJ, Kleinman JE, Weinberger DR, Weickert CS. 2007. Disease-associated intronic variants in the ErbB4 gene are related to altered ErbB4 splice-variant expression in the brain in schizophrenia. Hum Mol Genet 16(2):129–141.

Levy JB, Canoll PD, Silvennoinen O, Barnea G, Morse B, Honegger AM, Huang JT, Cannizzaro LA, Park SH, Druck T, Huebner K, Sap J, Ehrlich M, Musacchio JM, Schlessinger J. 1993. The cloning of a receptor-type protein tyrosine phosphatase expressed in the central nervous system. J Biol Chem 268(14):10573–10581.

Lewis DA, Levitt P. 2002. Schizophrenia as a disorder of neurodevelopment. Annu Rev Neurosci 25:409–432.

Li D, Collier DA, He L. 2006. Meta-analysis shows strong positive association of the neuregulin 1 (NRG1) gene with schizophrenia. Hum Mol Genet 15(12):1995–2002.

Li B, Woo RS, Mei L, Malinow R. 2007. The neuregulin-1 receptor erbB4 controls glutamatergic synapse maturation and plasticity. Neuron 54(4):583–597.

Montgomery JM, Zamorano PL, Garner CC. 2004. MAGUKs in synapse assembly and function: An emerging view. Cell Mol Life Sci 61 (7–8):911–929.

Munafo MR, Thiselton DL, Clark TG, Flint J. 2006. Association of the NRG1 gene and schizophrenia: A meta-analysis. Mol Psychiatry 11(6):539–546.

Neale BM, Sham PC. 2004. The future of association studies: Gene-based analysis and replication. Am J Hum Genet 75(3):353–362.

Niisato K, Fujikawa A, Komai S, Shintani T, Watanabe E, Sakaguchi G, Katsuura G, Manabe T, Noda M. 2005. Age-dependent enhancement of hippocampal long-term potentiation and impairment of spatial learning through the Rho-associated kinase pathway in protein tyrosine phosphatase receptor type Z-deficient mice. J Neurosci 25(5):1081–1088.

Norton N, Moskvina V, Morris DW, Bray NJ, Zammit S, Williams NM, Williams HJ, Preece AC, Dwyer S, Wilkinson JC, Spurlock G, Kirov G, Buckland P, Waddington JL, Gill M, Corvin AP, Owen MJ, O'Donovan MC. 2006. Evidence that interaction between neuregulin 1 and its receptor erbB4 increases susceptibility to schizophrenia. Am J Med Genet Part B 141B(1):96–101.

Nyholt DR. 2004. A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. Am J Hum Genet 74(4):765–769.

Paul S, Lombroso PJ. 2003. Receptor and nonreceptor protein tyrosine phosphatases in the nervous system. Cell Mol Life Sci 60(11):2465–2482.

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

Rapoport JL, Addington AM, Frangou S, Psych MR. 2005. The neurodevelopmental model of schizophrenia: Update 2005. Mol Psychiatry 10(5):434–449.

Ross CA, Margolis RL, Reading SA, Pletnikov M, Coyle JT. 2006. Neurobiology of schizophrenia. Neuron 52(1):139–153.

Schwab SG, Knapp M, Mondabon S, Hallmayer J, Borrmann-Hassenbach M, Albus M, Lerer B, Rietschel M, Trixler M, Maier W, Wildenauer DB. 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.

Shifman S, Bronstein M, Sternfeld M, Pisante-Shalom A, Lev-Lehman E, Weizman A, Reznik I, Spivak B, Grisaru N, Karp L, Schiffer R, Kotler M, Strous RD, Swartz-Vanetik M, Knobler HY, Shinar E, Beckmann JS, Yakir B, Risch N, Zak NB, Darvasi A. 2002. A highly significant association between a COMT haplotype and schizophrenia. Am J Hum Genet 71(6):1296–1302.

Stefansson H, Sigurdsson E, Steinthorsdottir V, Bjornsdottir S, Sigmundsson T, Ghosh S, Brynjolfsson J, Gunnarsdottir S, Ivarsson O, Chou TT, Hjaltason O, Birgisdottir B, Jonsson H, Gudnadottir VG, Gudmundsdottir E, Bjornsson A, Ingvarsson B, Ingason A, Sigfusson S, Hardardottir H, Harvey RP, Lai D, Zhou M, Brunner D, Mutel V, Gonzalo A, Lemke G, Sainz J, Johannesson G, Andresson T, Gudbjartsson D, Manolescu A, Frigge ML, Gurney ME, Kong A, Gulcher JR, Petursson H, Stefansson K. 2002. Neuregulin 1 and susceptibility to schizophrenia. Am J Hum Genet 71(4):877–892.

Tamura H, Fukada M, Fujikawa A, Noda M. 2006. Protein tyrosine phosphatase receptor type Z is involved in hippocampus-dependent memory formation through dephosphorylation at Y1105 on p190 RhoGAP. Neurosci Lett 399(1–2):33–38.

Woo RS, Li XM, Tao Y, Carpenter-Hyland E, Huang YZ, Weber J, Neiswender H, Dong XP, Wu J, Gassmann M, Lai C, Xiong WC, Gao TM, Mei L. 2007. Neuregulin-1 enhances depolarization-induced GABA release. Neuron 54(4):599–610.

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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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KEY WORDS: Schizophrenia; SNARE complex; Syntaxin; VAMP; SNAP25

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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 attachments 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

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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
STX1A (minus strand)	SNP1	rs867500	0	375	375	0.204	0.217	0.754	0.527	0.947	0.946
	SNP2	Intron7 SNP	3592	373	372	0.260	0.261	0.767	0.978	0.859	0.896
	SNP3	rs4363087	164	373	370	0.326	0.311	0.424	0.537	0.592	0.775
	SNP4	rs3793243	3151	375	377	0.419	0.386	0.304	0.196	0.553	0.698
	SNP5	rs875342	5751	375	370	0.228	0.238	0.211	0.653	0.781	0.924
	SNP6	rs6951030	6143	373	372	0.0563	0.0565	0.826	0.594	0.593	0.872
	SNP7	rs9654749	7145	376	374	0.483	0.469	0.460	0.602	0.869	
	SNP8	rs2030921	785	376	377	0.249	0.240	0.728	0.696		
VAMP2 (minus strand)	m1	rs2278637	0	372	369	0.430	0.436	0.348	0.724	0.732	0.802
	m2	rs1061032	1981	377	377	0.399	0.403	0.750	0.975	0.694	
	m3	rs8067606	2800	375	371	0.425	0.431	0.490	0.765		

[a]N, number; SCZ, schizophrenia; CON, control.
[b]MAF, minor allele frequency.
[c]Identical 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]		MAF[b]		P-Values			
				SCZ	CON	SCZ	CON	Genotype	(1) Window[c]	(2) Window	(3) Window
SNAP25	M1	rs6104567	0	377	370	0.263	0.242	0.298	0.341	0.493	
	M2	rs1889189	1,653	377	370	0.236	0.223	0.675	0.550	0.830	0.825
	M3	rs3787303	11,662	377	376	0.312	0.300	0.306	0.630	0.874	0.721
	M4	rs2423487	4,347	374	368	0.171	0.171	0.146	0.978	0.403	0.795
	M5	rs363012	6,704	377	377	0.308	0.295	0.364	0.612	0.552	0.364
	M6	rs363039	697	375	368	0.432	0.397	0.128	0.173	0.328	0.660
	M7	rs363014	8,198	377	377	0.460	0.453	0.197	0.767	**0.0215**[d]	0.126
	M8	rs12626080	4,358	375	367	0.208	0.162	0.073	**0.0236**[d]	0.0747	0.0882
	M9	rs363052	2,374	375	369	0.163	0.159	0.177	0.866	0.774	0.411
	M10	rs363053	159	374	370	0.298	0.291	0.918	0.748	0.719	0.865
	M11	rs4813024	2,231	377	377	0.222	0.235	0.650	0.544	0.690	0.855
	M12	rs6074113	4,195	372	369	0.337	0.341	0.615	0.868	0.509	0.643
	M13	rs363022	383	374	369	0.394	0.413	0.950	0.457	0.579	0.739
	M14	rs362564	2,232	376	372	0.455	0.425	0.725	0.265	0.254	0.524
	M15	rs362547	513	372	376	0.222	0.184	0.380	0.0712	0.0883	0.375
	M16	rs362567	952	377	376	0.144	0.129	0.259	0.379	0.615	0.134
	M17	rs362570	773	375	368	0.351	0.331	0.572	0.414	0.489	0.617
	M18	rs362584	7,611	372	370	0.209	0.212	0.716	0.877	0.999	0.716
	M19	rs16991334	7,442	372	367	0.0970	0.0989	0.574	0.903	0.401	0.737
	M20	rs6039807	1,659	372	367	0.451	0.434	0.580	0.544	0.601	0.891
	M21	rs362995	13,463	377	377	0.269	0.248	0.764	0.361	0.208	0.587
	M22	rs363006	3,044	373	370	0.0134	0.0082	0.873	1	0.732	0.510
	M23	rs6108463	422	374	368	0.182	0.192	0.893	0.647	0.679	0.689
	M24	rs362988	865	374	370	0.379	0.401	0.325	0.391	0.526	0.788
	M25	rs6039820	657	377	376	0.400	0.373	0.253	0.286	0.612	0.807
	M26	rs6108464	1,923	377	376	0.401	0.405	0.892	889	0.942	0.548
	M27	rs3787283	468	376	375	0.460	0.465	0.614	0.850	0.642	0.862
	M28	rs3746544	2,666	372	367	0.260	0.249	0.746	0.634	0.437	0.676
	M29	rs6133852	3,876	377	377	0.237	0.206	0.176	0.156		

[a]N, number; SCZ, schizophrenia; CON, control.
[b]MAF, minor allele frequency.
[c]Identical as conventional allele-wise analysis.
[d]Bold 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 (*CPLX I* and *CPLXII*), 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*: dustrobrevin-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 IDs		Distance to next SNP (bp)	N[a]		MAF[b]		*P*-Values								
			SCZ	CON	SCZ	CON	Genotype	(1) Window[c]	(2) Window	(3) Window	(4) Window	(5) Window	(6) Window	(7) Window
	rs6104571	0	1,031 (656)	892 (527)	0.216 (0.196)	0.213 (0.217)	0.970 (0.469)	0.804 (0.200)	00.992 (0.950)	0.146 (0.648)	0.183 (0.704)	0.136 (0.691)	0.107 (0.525)	0.076 (0.473)
	rs363013	1,661	1,031 (656)	892 (527)	0.011 (0.010)	0.011 (0.011)	0.902 (0.502)	0.872 (0.690)	0.657 (0.523)	0.759 (0.493)	0.194 (0.706)	0.142 (0.608)	0.107 (0.553)	
M7	rs363014	2,283	1,033 (656)	914 (537)	0.449 (0.444)	0.455 (0.422)	0.724 (0.155)	0.710 (0.280)	0.789 (0.659)	0.195 (0.662)	0.142 (0.783)	0.107 (0.500)		
	rs363015	5	1,031 (656)	892 (527)	0.060 (0.060)	0.062 (0.063)	0.340 (0.552)	0.733 (0.808)	0.192 (0.962)	0.144 (0.925)	0.136 (0.761)			
M8	rs12626080	4,353	1,032 (657)	890 (523)	0.177 (0.160)	0.166 (0.168)	0.617 (0.861)	0.362 (0.582)	0.119 (0.884)	0.136 (0.761)				
	rs6039792	1,040	1,031 (656)	892 (527)	0.212 (0.191)	0.209 (0.212)	0.953 (0.468)	0.768 (0.204)	0.056 (0.492)					
	rs363050	165	1,031 (656)	892 (527)	0.225 (0.207)	0.209 (0.205)	0.511 (0.991)	0.246 (0.923)						

[a]N, number; SCZ, schizophrenia; CON, control.
[b]MAF, minor allele frequency.
[c]Identical as conventional allele-wise analysis.
[d]Numbers 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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REFERENCES

Dudbridge F. 2003. Pedigree disequilibrium tests for multilocus haplotypes. Genet Epidemiol 25(2):115–121.

Fanous A, van den Oord E, Zhao Z, Wormley B, Amdur R, O'Neill FA, Walsh D, Kendler K, Riley B. 2007. SNAP-25 is Associated with Schizophrenia in 270 Irish High Density Families. Biol Psychiatry 61(8S):191S.

Gabriel SM, Haroutunian V, Powchik P, Honer WG, Davidson M, Davies P, Davis KL. 1997. Increased concentrations of presynaptic proteins in the cingulate cortex of subjects with schizophrenia. Arch Gen Psychiatry 54(6):559–566.

Hahn LW, Ritchie MD, Moore JH. 2003. Multifactor dimensionality reduction software for detecting gene–gene and gene–environment interactions. Bioinformatics 19(3):376–382.

Halim ND, Weickert CS, McClintock BW, Hyde TM, Weinberger DR, Kleinman JE, Lipska BK. 2003. Presynaptic proteins in the prefrontal cortex of patients with schizophrenia and rats with abnormal prefrontal development. Mol Psychiatry 8(9):797–810.

Hemby SE, Ginsberg SD, Brunk B, Arnold SE, Trojanowski JQ, Eberwine JH. 2002. Gene expression profile for schizophrenia: Discrete neuron transcription patterns in the entorhinal cortex. Arch Gen Psychiatry 59(7):631–640.

Honer WG, Falkai P, Bayer TA, Xie J, Hu L, Li HY, Arango V, Mann JJ, Dwork AJ, Trimble WS. 2002. Abnormalities of SNARE mechanism proteins in anterior frontal cortex in severe mental illness. Cereb Cortex 12(4):349–356.

Karson CN, Mrak RE, Schluterman KO, Sturner WQ, Sheng JG, Griffin WS. 1999. Alterations in synaptic proteins and their encoding mRNAs in prefrontal cortex in schizophrenia: A possible neurochemical basis for 'hypofrontality'. Mol Psychiatry 4(1):39–45.

Kishi T, Ikeda M, Suzuki T, Kitajima T, Yamanouchi Y, Kinoshita Y, Ozaki N, Iwata N. 2006. No association of complexin1 and complexin2 genes with schizophrenia in a Japanese population. Schizophr Res 82(2–3):185–189.

Marz KE, Hanson PI. 2002. Sealed with a twist: Complexin and the synaptic SNARE complex. Trends Neurosci 25(8):381–383.

Montecucco C, Schiavo G, Pantano S. 2005. SNARE complexes and neuroexocytosis: How many, how close? Trends Biochem Sci 30(7):367–372.

Neale BM, Sham PC. 2004. The future of association studies: Gene-based analysis and replication. Am J Hum Genet 75(3):353–362.

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

Sokolov BP, Tcherepanov AA, Haroutunian V, Davis KL. 2000. Levels of mRNAs encoding synaptic vesicle and synaptic plasma membrane proteins in the temporal cortex of elderly schizophrenic patients. Biol Psychiatry 48(3):184–196.

Thompson PM, Egbufoama S, Vawter MP. 2003. SNAP-25 reduction in the hippocampus of patients with schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry 27(3):411–417.

Thompson PM, Sower AC, Perrone-Bizzozero NI. 1998. Altered levels of the synaptosomal associated protein SNAP-25 in schizophrenia. Biol Psychiatry 43(4):239–243.

Wang Y, Tang BL. 2006. SNAREs in neurons-beyond synaptic vesicle exocytosis (Review). Mol Membr Biol 23(5):377–384.

Wong AH, Macciardi F, Klempan T, Kawczynski W, Barr CL, Lakatoo S, Wong M, Buckle C, Trakalo J, Boffa E. et al. 2003. Identification of candidate genes for psychosis in rat models, and possible association between schizophrenia and the 14-3-3eta gene. Mol Psychiatry 8(2):156–166.

Wong AH, Trakalo J, Likhodi O, Yusuf M, Macedo A, Azevedo MH, Klempan T, Pato MT, Honer WG, Pato CN. et al. 2004. Association between schizophrenia and the syntaxin 1A gene. Biol Psychiatry 56(1):24–29.

Young CE, Arima K, Xie J, Hu L, Beach TG, Falkai P, Honer WG. 1998. SNAP-25 deficit and hippocampal connectivity in schizophrenia. Cereb Cortex 8(3):261–268.

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Behavioral abnormalities and dopamine reductions in *sdy* 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* (*sdy*) mice, which lack dysbindin-1 owing to a deletion in the *Dtnbp1* gene. Our results showed that *sdy* mice were less active and spent less time in the center of an open field apparatus. Consistent with the latter observation, *sdy* mice also displayed evidence of heightened anxiety-like response and deficits in social interaction. Compared to wild-type mice, *sdy* mice displayed lower levels of dopamine, but not glutamate, in the cerebral cortex, hippocampus, and hypothalamus. These findings indicate that *sdy* 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* (*sdy*) mutant mice, which express no dysbindin-1 protein owing to a deletion in the dysbindin-1 gene [10]. *Sdy* 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 *sdy* mice.

Materials and methods

Animals. We obtained *sdy* mice from the Jackson Laboratory (Bar Harbor, ME). *Sdy* 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 *sdy* 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′-GGTTGACACTCTTGCGGAAT-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 (*sdy* 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 (*sdy* 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 (*sdy–sdy* 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 (*sdy*) 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 $Na_2S_2O_5$ using a sonicator for 5–10 s. Homogenates were centrifuged at 10,000*g* 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 *sdy* 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 *sdy* 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 *sdy* 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-

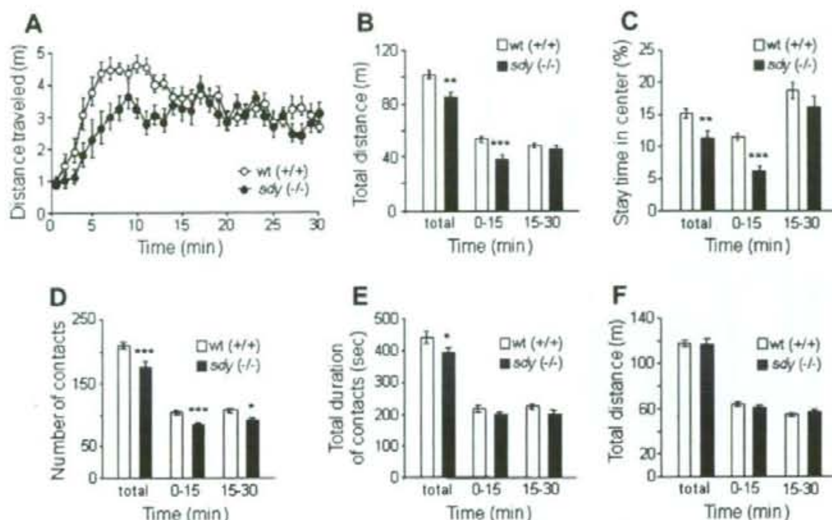

Fig. 1. Open field test with *sdy* mice (A–C). Time course of distance traveled (A), total distance traveled (B) and time spent in the central area (C), are shown (*sdy* mice: $n = 18$, wild-type mice: $n = 21$). Social interaction test in *sdy* mice (D–F). The total number of contacts (D), total duration of contacts (E) and distance traveled (F), in the social interaction test, are shown (pairs of *sdy* mice: $n = 22$, pairs of wild-type mice: $n = 21$). Data represent means ± 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 *sdy* and wild-type mice. As shown in Fig. 1C, *sdy* mice 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 *sdy* and wild-type mice (*sdy* mice: 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, *sdy* mice 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 *sdy* mice 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, *sdy* mice 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 *sdy* mice than wild-type mice (Fig. 2A), 0–10 min: $t(32) = 1.41$, $p = 0.17$, 10–20 min: $t(32) = 2.11$, $p = 0.042$). *Sdy* mice 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: $t(32) = 2.19$, $p = 0.036$).

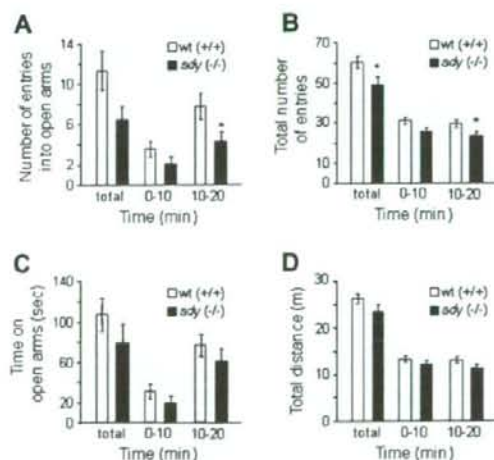

Fig. 2. Elevated plus maze test in *sdy* mice. The number of open arm entries (A), total number of arm entries (B), time on open arms (C), and distance traveled (D), are shown (*sdy* mice: $n = 16$, wild-type mice: $n = 18$). Data represent means ± SEM. $^{*}p < 0.05$, compared with wild-type mice.

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 *sdy* mice and wild-type mice.

Dopamine and glutamate contents in the brain

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

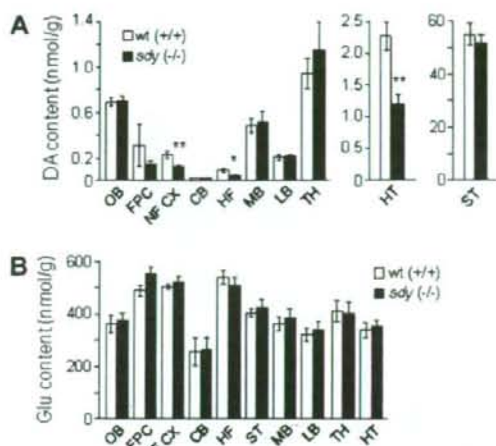

Fig. 3. Dopamine and glutamate content in the brains of *sdy* mice. Dopamine (DA) content (A) and glutamate (Glu) content (B) are shown (dopamine: $n = 4–8$, glutamate: $n = 11$). Olfactory bulb, OB; frontal pole cortex, FPC; non-frontal cerebral cortex, NF CX; cerebellum, CB; hippocampal formation,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

$p = 0.002$), hippocampal formation (*sdy* mice: 0.0392 nmol/g, wild-type mice: 0.0822 nmol/g, $p = 0.03$), and hypothalamus (*sdy* 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 *sdy* 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 *sdy* mice, which express no dysbindin-1, show some, but not all, of these abnormalities.

In the open field test, *sdy* 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, *sdy* 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, *sdy* 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 *sdy* mice. In the social interaction test, *sdy* 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 *sdy* 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 withdrawal phenotype in *sdy* mice. Previous study demonstrated that retinal melanosomes were deficient in *sdy* mice [10]. As we did not examine the visual acuity in *sdy* 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, *sdy* 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 *sdy* 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 *sdy* 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 *sdy* mice could be due to the abnormal regulation of dopaminergic system by lack of dysbindin-1 protein. As glutamate content in *sdy* mouse brain was not altered, behavioral abnormalities in *sdy* 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.

References

[1] P.J. Harrison, D.R. Weinberger, Schizophrenia genes, gene expression, and neuropathology: on the matter of their convergence, Mol. Psychiatry 10 (2005) 40–68.
[2] R. Hashimoto, S. Hattori, S. Chiba, Y. Yagasaki, T. Okada, E. Kumamaru, T. Mori, K. Nemoto, H. Tanii, H. Hori, H. Noguchi, T. Numakawa, T. Ohnishi, H. Kunugi, Susceptibility genes for schizophrenia, Psychiatry Clin. Neurosci. 60 (Suppl 1) (2006) S4–S10.
[3] K. Talbot, W.L. Eidem, C.L. Tinsley, M.A. Benson, E.W. Thompson, R.J. Smith, C.G. Hahn, S.J. Siegel, J.Q. Trojanowski, R.E. Gur, D.J. Blake, S.E. Arnold, Dysbindin-1

is reduced in intrinsic, glutamatergic terminals of the hippocampal formation in schizophrenia, J. Clin. Invest. 113 (2004) 1353–1363.

[4] C.S. Weickert, R.E. Straub, B.W. McClintock, M. Matsumoto, R. Hashimoto, T.M. Hyde, M.M. Herman, D.R. Weinberger, J.E. Kleinman, Human dysbindin (DTNBP1) gene expression in normal brain and in schizophrenic prefrontal cortex and midbrain, Arch. Gen. Psychiatry 61 (2004) 544–555.

[5] S. Chiba, R. Hashimoto, S. Hattori, M. Yohda, B. Lipska, D.R. Weinberger, H. Kunugi, Effect of antipsychotic drugs on DISC1 and dysbindin expression in mouse frontal cortex and hippocampus, J. Neural. Transm. 113 (2006) 1337–1346.

[6] K. Talbot, D.S. Cho, W.Y. Ong, M.A. Benson, L.Y. Han, H.A. Kazi, J. Kamins, C.G. Hahn, D.J. Blake, S.E. Arnold, Dysbindin-1 is a synaptic and microtubular protein that binds brain snapin, Hum. Mol. Genet. 15 (2006) 3041–3054.

[7] T. Numakawa, Y. Yagasaki, T. Ishimoto, T. Okada, T. Suzuki, N. Iwata, N. Ozaki, T. Taguchi, M. Tatsumi, K. Kamijima, R.E. Straub, D.R. Weinberger, H. Kunugi, R. Hashimoto, Evidence of novel neuronal functions of dysbindin, a susceptibility gene for schizophrenia, Hum. Mol. Genet. 13 (2004) 2699–2708.

[8] N. Kumamoto, S. Matsuzaki, K. Inoue, T. Hattori, S. Shimizu, R. Hashimoto, A. Yamatodani, T. Katayama, M. Tohyama, Hyperactivation of midbrain dopaminergic system in schizophrenia could be attributed to the down-regulation of dysbindin, Biochem. Biophys. Res. Commun. 345 (2006) 904–909.

[9] J.M. Stone, P. Morrison, L.S. Pilowsky, Glutamate and dopamine dysregulation in schizophrenia - a synthesis and selective review, J. Psychopharmacol. 21 (2007) 440–452.

[10] W. Li, Q. Zhang, N. Oiso, E.K. Novak, R. Gautam, E.P. O'Brien, C.L. Tinsley, D.J. Blake, R.A. Spritz, N.G. Copeland, N.A. Jenkins, D. Amato, B.A. Roe, M. Starcevic, E.C. Dell'Angelica, R.W. Elliott, V. Mishra, S.F. Kingsmore, R.E. Paylor, R.T. Swank, Hermansky-Pudlak syndrome type 7 (HPS-7) results from mutant dysbindin, a member of the biogenesis of lysosome-related organelles complex 1 (BLOC-1), Nat. Genet. 35 (2003) 84–89.

[11] R.T. Swank, H.O. Sweet, M.T. Davisson, M. Reddington, E.K. Novak, Sandy: a new mouse model for platelet storage pool deficiency, Genet. Res. 58 (1991) 51–62.

[12] S. Hattori, R. Hashimoto, T. Miyakawa, H. Yamanaka, H. Maeno, K. Wada, H. Kunugi, Enriched environments influence depression-related behavior in adult mice and the survival of newborn cells in their hippocampi, Behav. Brain Res. 180 (2007) 69–76.

[13] J. Glowinski, L.L. Iversen, Regional studies of catecholamines in the rat brain. I. The disposition of [3H]norepinephrine, [3H]dopamine and [3H]dopa in various regions of the brain, J. Neurochem. 13 (1966) 655–669.

[14] H. Nohta, A. Mitsui, Y. Ohkura, Spectrofluorometric determination of catecholamines with 1,2-diphenylethylenediamine, Anal. Chim. Acta 165 (1984) 171–176.

[15] H. Tamura, T.P. Hicks, Y. Hata, T. Tsumoto, A. Yamatodani, Release of glutamate and aspartate from the visual cortex of the cat following activation of afferent pathways, Exp. Brain Res. 80 (1990) 447–455.

[16] B.K. Lipska, D.R. Weinberger, To model a psychiatric disorder in animals: schizophrenia as a reality test, Neuropsychopharmacology 23 (2000) 223–239.

[17] J.N. Crawley, What's Wrong with My Mouse? Wiley-Liss, New York, 2000.

[18] A.H. Fanous, E.J. van den Oord, B.P. Riley, S.H. Aggen, M.C. Neale, F.A. O'Neill, D. Walsh, K.S. Kendler, Relationship between a high-risk haplotype in the DTNBP1 (dysbindin) gene and clinical features of schizophrenia, Am. J. Psychiatry 162 (2005) 1824–1832.

[19] P. DeRosse, B. Funke, K.E. Burdick, T. Lencz, J.M. Ekholm, J.M. Kane, R. Kucherlapati, A.K. Malhotra, Dysbindin genotype and negative symptoms in schizophrenia, Am. J. Psychiatry 163 (2006) 532–534.

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

[21] Y. Iizuka, Y. Sei, D.R. Weinberger, R.E. Straub, Evidence that the BLOC-1 protein dysbindin modulates dopamine D2 receptor internalization and signaling but not D1 internalization, J. Neurosci. 27 (2007) 12390–12395.

[22] J.H. Baik, R. Picetti, A. Saiardi, G. Thiriet, A. Dierich, A. Depaulis, M. Le Meur, E. Borrelli, Parkinsonian-like locomotor impairment in mice lacking dopamine D2 receptors, Nature 377 (1995) 424–428.

[23] M.A. Kelly, M. Rubinstein, T.J. Phillips, C.N. Lessov, S. Burkhart-Kasch, G. Zhang, J.R. Bunzow, Y. Fang, G.A. Gerhardt, D.K. Grandy, M.J. Low, Locomotor activity in D2 dopamine receptor-deficient mice is determined by gene dosage, genetic background, and developmental adaptations, J. Neurosci. 18 (1998) 3470–3479.

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 1 A (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 floropipamide, 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 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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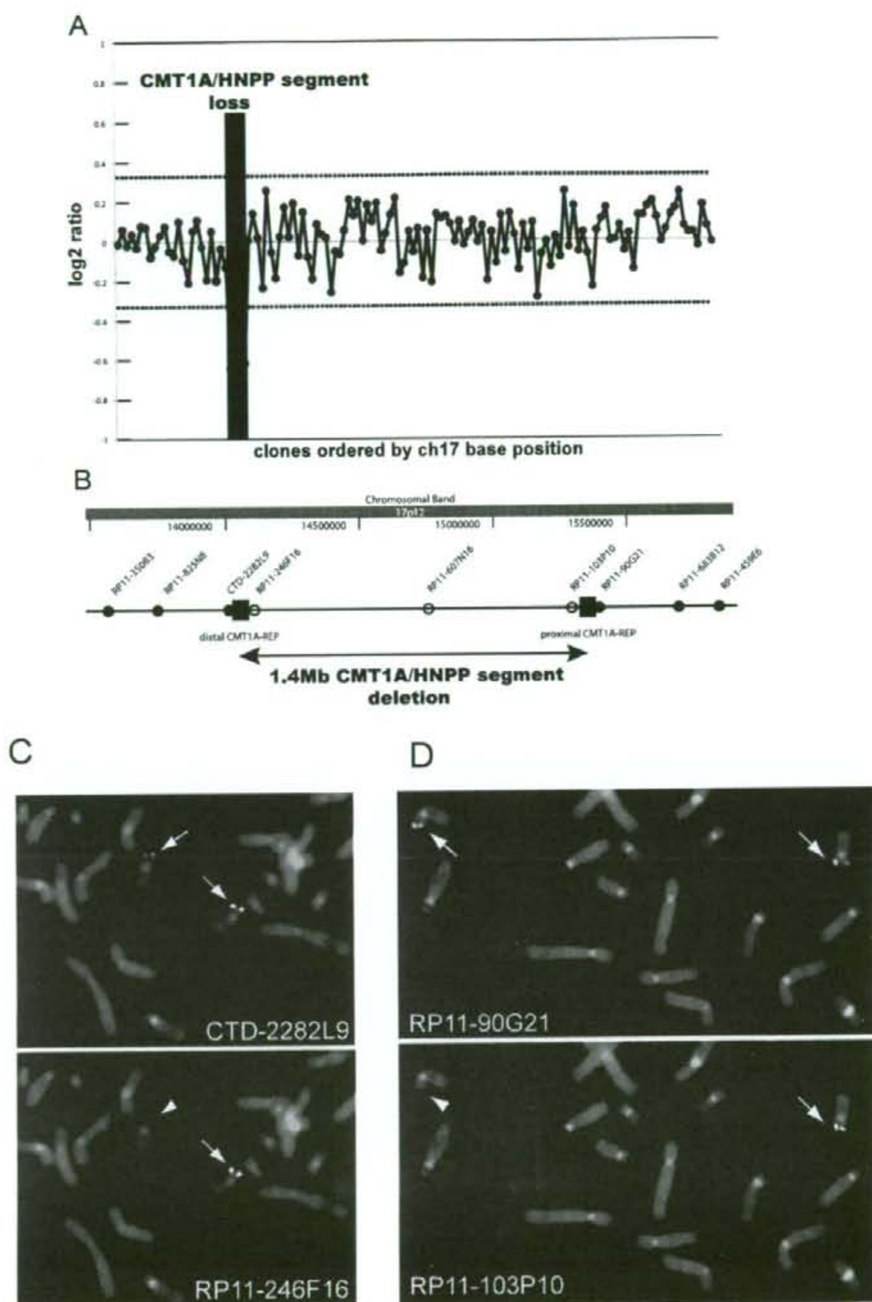

Fig. (1). (**A**) Microarray CGH analysis showing deletions at CMT1A/HNPP locus. (**B**) Schematic presentation of BAC clones delineating the CMT1A/HNPP deletion in the patient. Open circle: BAC clone deleted in the patient, closed circle: BAC clone not deleted in the patient. Closed square: low copy repeat which may have mediated genomic rearrangements. A common 1.4-Mb deletion of HNPP occurs between proximal CMT1A-REP and distal CMT1A-REP. (**C**,**D**) BAC FISH analysis in the proband. Arrows show intact signals in 17p11.2 region. Arrow heads show the loss of signal in 17p11.2 region. Clone positions are indicated in Fig. (**1B**).

REFERENCES

[1] MacIntyre DJ, Blackwood DH, Porteous DJ, Pickard BS, Muir WJ. Chromosomal abnormalities and mental illness. Mol Psychiatry 2003; 8: 275-87.

[2] Bassett AS, Chow EW, Weksberg R. Chromosomal abnormalities and schizophrenia. Am J Med Genet 2000; 97: 45-51.

[3] Kunugi H, Lee KB, Nanko S. Cytogenetic findings in 250 schizophrenics: evidence confirming an excess of the X chromosome aneuploidies and pericentric inversion of chromosome 9. Schizophr Res 1999; 40: 43-7.

[4] Millar JK, Wilson-Annan JC, Anderson S, *et al.* Disruption of two novel genes by a translocation co-segregating with schizophrenia. Hum Mol Genet 2000; 9: 1415-23.

[5] Murphy KC, Own MJ. The behavioural phenotype in velo-cardio-facial-syndrome. Am J Hum Genet 1997; 61: A5.

[6] Blackwood DH, Fordyce A, Walker MT, St Clair DM, Porteous DJ, Muir WJ. Schizophrenia and affective disorders--cosegregation with a translocation at chromosome 1q42 that directly disrupts brain-expressed genes: clinical and P300 findings in a family. Am J Hum Genet 2001; 69: 428-33.

[7] Lupski JR, de Oca-Luna RM, Slaugenhaupt S, *et al.* DNA duplication associated with Charcot-Marie-Tooth disease type 1A. Cell 1991; 66: 219-232.

[8] Chance PF, Alderson MK, Leppig KA, *et al.* DNA deletion associated with hereditary neuropathy with liability to pressure palsies. Cell 1993; 72: 143-51.

[9] Choi BO, Kim J, Lee KL, Yu JS, Hwang JH, Chung KW. Rapid diagnosis of CMT1A duplications and HNPP deletions by multiplex microsatellite PCR. Mol Cells 2007; 23: 39-48.

[10] Wechsler D, translated into Japanese by Shinagawa F, Kobayashi S, Fujita K, Maegawa H. Wechsler Adult Intelligence Scale-Revised Japanese version.; SACCESS BELL Co. Ltd., 1990.

[11] American Psychiatric Association. translated into Japanese by Takahashi S, Ohno Y, Someya T. In Diagnostic and Statistical Manual of Mental Disorders 4th Ed. Japanese version.; Tokyo: Igaku-Shoin Ltd., 1996.

[12] First MB, Spitzer RL, Gibbon M, Williams JBW, translated into Japanese by Kitamura T, Okano T. Structured Clinical Interview for DSM-IV Axis I Disorders (SCID). Japanese version.; Tokyo: NIPPON HYORONSHA Co. Ltd., 2003.

[13] Otsubo T, Tanaka K, Koda R, *et al.* Reliability and validity of Japanese version of the Mini-International Neuropsychiatric Interview. Psychiatry Clin Neurosci 2005; 59: 517-526.

[14] Sheehan DV, Lecrubier Y, Sheehan KH, *et al.* The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J Clin Psychiatry 1998; 59(Suppl 20): 22-57.

[15] Miyake N, Shimokawa O, Harada N, *et al.* BAC array CGH reveals genomic aberrations in idiopathic mental retardation. Am J Med Genet A 2006; 140: 205-11.

[16] Aarskog NK, Vedeler CA. Real-time quantitative polymerase chain reaction. A new method that detects both the peripheral myelin protein 22 duplication in Charcot-Marie-Tooth type 1A disease and the peripheral myelin protein 22 deletion in hereditary neuropathy with liability to pressure palsies. Hum Genet 2000; 107: 494-8.

[17] Mouton P, Tardieu S, Gouider R, *et al.* Spectrum of clinical and electrophysiologic features in HNPP patients with the 17p11.2 deletion. Neurology 1999; 52: 1440-46.

[18] Dracheva S, Davis KL, Chin B, Woo DA, Schmeidler JH, Haroutunian V. Myelin-associated mRNA and protein expression deficits in the anterior cingulate cortex and hippocampus in elderly schizophrenia patients. Neurobiol Dis 2006; 21: 531-40.

[19] Haroutunian V, Katsel P, Dracheva S, Stewart DG, Davis KL. Variations in oligodendrocyte-related gene expression across multiple cortical regions: implications for the pathophysiology of schizophrenia. Int J Neuropsychopharmacol 2007; 10: 565-73.

[20] Davis KL, Stewart DG, Friedman JI, *et al.* White matter changes in schizophrenia: evidence for myelin-related dysfunction. Arch Gen Psychiatry 2003; 60: 443-56.

[21] Hakak Y, Walker JR, Li C, *et al.* Genome-wide expression analysis reveals dysregulation of myelination-related genes in chronic schizophrenia. Proc Natl Acad Sci USA 2001; 98: 4746-51.

[22] Vostrikov VM, Uranova NA, Orlovskaya DD. Deficit of perineuronal oligodendrocytes in the prefrontal cortex in schizophrenia and mood disorders. Schizophr Res 2007; 94: 273-80.

[23] Roy K, Murtie JC, El-Khodor BF, *et al.* Loss of erbB signaling in oligodendrocytes alters myelin and dopaminergic function, a potential mechanism for neuropsychiatric disorders. Proc Natl Acad Sci USA 2007; 104: 8131-6.

[24] Shergill SS, Kanaan RA, Chitnis XA, *et al.* A diffusion tensor imaging study of fasciculi in schizophrenia. Am J Psychiatry 2007; 164: 467-73.

[25] Dougherty KD, Dreyfus CF, Black IB. Brain-derived neurotrophic factor in astrocytes, oligodendrocytes, and microglia/macrophages after spinal cord injury. Neurobiol Dis 2000; 7: 574-85.

[26] Deadwyler GD, Pouly S, Antel JP, Devries GH. Neuregulins and erbB receptor expression in adult human oligodendrocytes. Glia 2000; 32: 304-12.

[27] Hashimoto T, Lewis DA. BDNF Val66Met polymorphism and GAD67 mRNA expression in the prefrontal cortex of subjects with schizophrenia. Am J Psychiatry 2006; 163: 534-7.

[28] Stefansson H, Sigurdsson E, Steinthorsdottir V, *et al.* Neuregulin 1 and susceptibility to schizophrenia. Am J Hum Genet 2002; 71: 877-92.

[29] Bulayeva KB, Glatt SJ, Bulayev OA, Pavlova TA, Tsuang MT. Genome-wide linkage scan of schizophrenia: a cross-isolate study. Genomics 2007; 89: 167-77.

[30] Bulayeva KB, Leal SM, Pavlova TA, *et al.* Mapping genes of complex psychiatric diseases in Daghestan genetic isolates. Am J Med Genet B Neuropsychiatr Genet 2005; 132: 76-84.

[31] Williams NM, Norton N, Williams H, *et al.* A systematic genomewide linkage study in 353 sib pairs with schizophrenia. Am J Hum Genet 2003; 73: 1355-67.

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An association study of tachykinin receptor 3 gene with schizophrenia in the Japanese population

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The tachykinin receptor 3 (*TACR3*) gene encodes the neurokinin3 (NK3) receptor. Animal studies showed that agonist-induced stimulation of the NK3 receptor leads to the excessive release of dopamine in the ventral and dorsal striatal and prefrontal cortical regions. Data from clinical trials of selective NK3 receptor antagonists in schizophrenia have shown significant improvement in positive symptoms. We performed an association study of the *TACR3* gene in the Japanese population of 384 schizophrenic patients and 384 controls. Nine single nucleotide polymorphisms were genotyped using TaqMan assays and polymerase chain reaction-restriction fragment length polymorphism method. No significant association between schizophrenia and these single nucleotide polymorphisms was observed in single-marker and haplotype analyses. Our results suggest that *TACR3* is unlikely to be related to the development of schizophrenia in the Japanese population. *NeuroReport* 19:471–473 © 2008 Wolters Kluwer Health | Lippincott Williams & Wilkins.

Keywords: association study, dopamine, neurokinin3 receptor, schizophrenia, tachykinin receptor 3

Introduction

Schizophrenia is a severe, disabling and lifelong mental disorder with a global prevalence of 1%. Although it is generally accepted that genetic factors contribute to the development of this disease, its etiology has not yet been clarified.

The tachykinin receptor 3 (*TACR3*) gene located on chromosome 4q25 encodes the neurokinin 3 receptor (NK3). Its endogenous ligand is neurokinin B, a member of the tachykinin peptide family. It is expressed in the central nervous system and spinal cord [1]. Several animal studies have been performed investigating the interaction between NK3 receptors and dopamine (DA) and serotonin (5-HT) pathways. First, stimulation of NK3 receptors in the ventral mesencephalon increases DA release in the ventral and dorsal striatum and prefrontal cortex [2,3]. Second, infusion of NK3 receptor agonists into the ventral tegmental area evokes DA-mediated behaviors, such as yawning and chewing, which are potently inhibited by DA2 receptor antagonists such as haloperidol [4,5]. Third, injection of NK3 receptor agonists into the raphe area elicits 5-HT-mediated behaviors, such as head twitches, which are inhibited by 5-HT2A/5-HT2C receptor antagonists [6,7]. Additionally, a recent clinical study showed NK3 receptor antagonists improved the positive symptoms in schizophrenia [8]. These results suggest that altered form and function of the NK3 receptor might be related to the abnormalities of DA and 5-HT signaling, one of the major hypotheses explaining the pathophysiology of schizophrenia.

For all of these reasons, *TACR3* was hypothesized to be involved in the pathogenesis of schizophrenia. In this study, we performed linkage disequilibrium (LD) analysis of the *TACR3* gene and carried out case–control association studies between TACR3 polymorphisms and schizophrenia using single-marker association analysis and haplotype analysis in the Japanese population.

Methods

Participants

A total of 384 patients with schizophrenia [231 men, mean age $\pm$ standard deviation (SD) 48.8 ± 14.4; 153 women, 53.0 ± 15.9] and 384 controls (161 men, 40.8 ± 15.6; 223 women, 39.5 ± 13.8) were genotyped. All participants were ethnically Japanese and unrelated to each other. The schizophrenic patients, who were diagnosed according to the DSM-IV criteria with the consensus of at least two experienced psychiatrists on the basis of empirical diagnostic interviews and review of medical records, were recruited from several psychiatric hospitals around the Tokyo and Nagoya areas (within a 350 km radius). All healthy control participants with no current or past contact with psychiatric services were also screened on the basis of brief diagnostic interviews. This study was approved by the Ethics Committee of the Nagoya University Graduate School of Medicine, and written informed consent was obtained from each participant.

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