Table 1 Pair-wise linkage disequilibrium in *Epsin 4*

		MS1 D5S1400	MS2 D5S1403	SNP1 rs1186922	MS3 AAAT11	SNP2 rs10046055	SNP3 rs1894962	SNP4 rs6556290	SNP5 rs7735412	SNP6 rs1145585	SNP7 rs1186930	SNP8 rs1145603	SNP9 rs1186934	SNP10 rs11744778	SNP11 rs1145602	SNP12 rs1186998	SNP13 rs17055032	SNP14 rs254664
MS1	D5S1400		0.071	0.123	0.399	0.347	0.216	*0.979*	0.207	0.026	0.030	0.031	0.038	0.337	0.046	0.087	0.336	0.344
MS2	D5S1403	0.001		0.309	0.250	0.235	0.310	*0.981*	0.246	0.102	0.240	0.243	0.237	0.293	0.114	0.304	0.090	0.294
SNP1	rs1186922	0.005	0.051		*0.879*	*0.999*	*1.000*	*0.999*	0.465	*0.861*	0.579	0.581	0.566	0.032	*0.813*	0.717	0.347	0.062
MS3	AAAT11	0.011	0.014	0.045		*0.897*	0.757	*1.000*	0.741	0.673	0.426	0.430	0.434	0.442	0.668	0.633	0.307	0.441
SNP2	rs10046055	0.008	0.013	0.063	0.775		*0.999*	*1.000*	0.589	*0.993*	0.487	0.490	0.508	0.502	0.581	0.707	0.400	0.516
SNP3	rs1894962	0.007	0.004	0.425	0.015	0.027		0.053	*0.989*	0.772	0.720	0.721	0.699	*0.859*	0.716	*0.855*	0.136	*0.927*
SNP4	rs6556290	0.002	0.004	0.008	0.001	0.001	0.000		*1.000*	0.257	*0.997*	*0.996*	*0.998*	*1.000*	0.259	*0.998*	*1.000*	1.000
SNP5	rs7735412	0.001	0.002	0.013	0.002	0.001	0.003	0.000		*0.923*	*1.000*	*0.999*	*1.000*	*0.955*	*0.835*	*0.999*	*0.987*	*1.000*
SNP6	rs1145585	0.000	0.002	0.415	0.015	0.035	0.455	0.001	0.004		*1.000*	*0.985*	*0.970*	*0.912*	*0.942*	*0.982*	*0.989*	*0.999*
SNP7	rs1186930	0.000	0.041	0.251	0.065	0.092	0.165	0.006	0.046	0.416		*1.000*	*0.997*	*0.984*	*0.931*	*0.992*	*0.996*	*1.000*
SNP8	rs1145603	0.000	0.043	0.254	0.067	0.094	0.166	0.006	0.046	0.406	*0.994*		*0.997*	*0.984*	*0.932*	*1.000*	*0.996*	*1.000*
SNP9	rs1186934	0.000	0.042	0.243	0.068	0.101	0.159	0.006	0.044	0.399	*0.980*	*0.987*		*0.984*	*0.939*	*0.996*	*0.995*	*0.992*
SNP10	rs11744778	0.009	0.035	0.000	0.185	0.249	0.020	0.000	0.110	0.029	0.372	0.375	0.375		*0.999*	*0.991*	0.509	*0.978*
SNP11	rs1145602	0.000	0.003	0.368	0.014	0.012	0.395	0.001	0.003	*0.883*	0.363	0.365	0.374	0.034		*0.937*	0.692	*0.919*
SNP12	rs1186998	0.003	0.050	0.295	0.111	0.150	0.179	0.005	0.036	0.309	0.759	0.766	0.755	0.292	0.284		*1.000*	*1.000*
SNP13	rs17055032	0.003	0.000	0.022	0.034	0.057	0.008	0.000	0.001	0.012	0.030	0.029	0.029	0.003	0.006	0.105		0.514
SNP14	rs254664	0.010	0.034	0.000	0.184	0.262	0.023	0.000	0.116	0.035	0.384	0.386	0.381	0.957	0.029	0.297	0.003	

Upper diagonal figures are D' and lower diagonal figures are r^2

Pairwise LD mesurement using the online software SHEsis

Pairs in LD ($D' > 0.8$ or $r^2 > 0.8$) are shown in italics

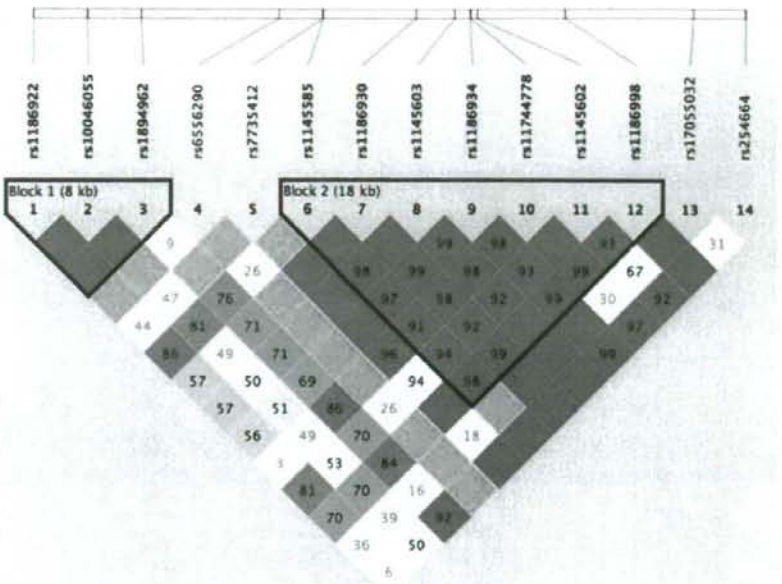

Fig. 2 Linkage disequilibrium (*LD*) block structure estimated from 14 SNPs by using Haploview

Discussion

The purpose of this investigation was to replicate Pimm et al.'s (2005) study of *Epsin 4* and its association with schizophrenia in a Japanese population. Pimm et al. (2005) originally reported that two SNPs, rs254664 (intron 1) and rs10046055 (5′ upstream region), in addition to two microsatellite markers D5S1403 (5′ flanking region) and AAAT11 (5′ upstream region), may be involved in the susceptibility to schizophrenia in the English, Irish, Welsh, and Scottish populations. In the present study, we examined a total of 17 polymorphisms and obtained weak evidence for an association at microsatellite 2 (D5S1403). However, when multiple testing was taken into consideration, the finding no longer reached significance. In addition, the risk-conferring allele was inconsistent between the two studies; we found an enrichment of the 203rd allele of microsatellite 2 in schizophrenics, whereas Pimm et al. (2005) found an enrichment of the same allele in controls. Furthermore, in the haplotype-based analysis, we did not find any evidence for an association.

Thus, we failed to replicate the widespread and highly significant associations with schizophrenia across 200,000 bp of *Epsin 4* reported by Pimm et al. (2005). Though we examined this region thoroughly by using 17 markers, we obtained no evidence for an association. The discrepancy found between Pimm et al.'s (2005) study and ours could result from a combination of factors. The first possibility may be due to the putative difference in allele frequencies between the English, Irish, Welsh, and Scottish population versus the Japanese population. It has been suggested that variations between ethnicities and populations in allele frequencies can dramatically affect the power to detect marginal differences (Marchini et al. 2005). According to the data bank of the International HapMap project (http://www.hapmap.org/index.html.en/), the allele frequency variation for rs10046055 and rs254664 is noticeable. Therefore, ethnic diversity of allele frequency of the SNPs may be a reason for negative findings in our present study. It is possible that there is a potential risk locus that is in strong LD with the investigated genetic variants in Pimm et al.'s (2005) population that is in weak or no LD with the variants in our population. However, to address this issue, we analyzed ten additional SNPs to thoroughly cover the entire genomic region of interest according to the HapMap database for the Japanese population; therefore, such a possibility is unlikely. Another possibility is that our negative results may be a type II error due to the potentially inadequate sample size. Our sample size (354 patients and 365 controls) had a power of 90% to detect an odds ratio of 1.9 or more if the T/A haplotype of HapB was assumed to be a risk (see Table 3). Incidentally, this haplotype gave rise to the most significant result ($P = 0.0005$) in the study of Pimm et al. (2005). This relatively weak power is due to the low frequency (0.06) of the T/A haplotype in our Japanese controls. If the A allele

Table 2 Allelic and haplotypic association analyses between *Epsin 4* and schizophrenia

dbSNP ID	Position[a]	Inter-SNP distance (bp)	Allele frequency			χ^2	P^c	P^d	Haplotype P (global)[e]			
			Allele[b]	Control	Schizophrenia				2 Locus	3 Locus	4 Locus	5 Locus
MS1 D5S1400	157439237	–	368	104 (0.16)	115 (0.18)	1.03	0.31					
			371	304 (0.47)	292 (0.46)	0.07	0.80					
			377	124 (0.19)	101 (0.16)	2.20	0.14					
			380	55 (0.08)	70 (0.11)	2.43	0.12	(T3)				
			383	45 (0.07)	45 (0.07)	0.01	0.90	0.12				
MS2 D5S1403	157346617	92,620	203	417 (0.66)	444 (0.71)	4.26	0.04					
			207	114 (0.18)	99 (0.16)	1.00	0.32					
			211	18 (0.03)	15 (0.02)	0.23	0.63	(T3)				
			215	67 (0.11)	56 (0.09)	0.91	0.34	0.04	0.64			
SNP1	157302083	44,534	A	179 (0.25)	189 (0.27)							
rs1186922			T	547 (0.75)	511 (0.73)	1.02	0.31		0.23	0.43		
MS3 AAAT11	157301697	386	248	567 (0.84)	562 (0.87)	1.48	0.22	(T1)				
			252	100 (0.15)	86 (0.13)	0.71	0.40	0.13	0.47	0.58	0.34	
SNP2	157297912	3,785	A	120 (0.17)	99 (0.14)							
rs10046055			T	606 (0.83)	607 (0.86)	1.74	0.19		0.23	0.43	0.77	0.31
SNP3	UTR	4,191	C	86 (0.12)	99 (0.14)							
rs1894962	157293721		T	642 (0.88)	605 (0.86)	1.61	0.21		0.23	0.26	0.47	0.77
SNP4	UTR	10,737	C	2 (0.00)	2 (0.00)							
rs6556290	157282984		T	726 (1.00)	706 (1.00)	0.00	0.98		0.22	0.23	0.27	0.47
SNP5	UTR	3,395	C	14 (0.02)	16 (0.02)							
rs7735412	157279589		T	712 (0.98)	690 (0.98)	0.20	0.66		1.00	0.21	0.23	0.26
SNP6	UTR	42	C	608 (0.84)	589 (0.83)							
rs1145585	157279547		T	116 (0.16)	117 (0.17)	0.08	0.78		0.76	0.84	0.43	0.38
SNP7	UTR	7,239	T	494 (0.68)	482 (0.68)							
rs1186930	157272308		C	232 (0.32)	222 (0.32)	0.03	0.86		0.78	0.67	0.67	0.52
SNP8	UTR	2,984	C	496 (0.68)	484 (0.69)							
rs1145603	157269324		T	230 (0.32)	222 (0.31)	0.01	0.92		0.92	0.74	0.64	0.63
SNP9	UTR	1,215	A	229 (0.32)	217 (0.31)							
rs1186934	157268109		G	491 (0.68)	479 (0.69)	0.06	0.80		0.89	0.89	0.68	0.57
SNP10	UTR	42	A	114 (0.16)	102 (0.14)							
rs11744778	157268067		G	608 (0.84)	604 (0.86)	0.50	0.48		0.78	0.75	0.75	0.72
SNP11	UTR	517	A	117 (0.16)	115 (0.16)							
rs1145602	157267550		G	609 (0.84)	587 (0.84)	0.02	0.89		0.78	0.58	0.56	0.56
SNP12	Intron 1	6,895	C	279 (0.38)	258 (0.37)							
rs1186998	157260655		G	447 (0.62)	448 (0.63)	0.54	0.46		0.23	0.30	0.42	0.54
SNP13	Intron 1	10,039	A	49 (0.07)	37 (0.05)							
rs17055032	157250616		C	679 (0.93)	671 (0.95)	1.44	0.23		0.39	0.36	0.54	0.51
SNP14	Intron 1	4,036	C	611 (0.84)	603 (0.85)							
rs254664	157246580		T	113 (0.16)	103 (0.15)	0.29	0.59		0.47	0.51	0.48	0.51

[a] Chromosome position was obtained from the HapMap database

[b] Microsatellites alleles presented by PCR fragment size (bp) with frequencies >0.03 in controls in either study

[c] P from 2 × 2 χ^2, with one *df*

[d] P from CLUMP Monte Carlo

[e] P from COCAPHASE-EM, -droprare 0.03 options

Table 3 Replication study of haplotype-based analysis between *Epsin 4* and schizophrenia

Groupe	Markers and haplotype			Case freq.	Cont freq.	*P* value[a]	
						Individual	Global
HapA	rs254664	rs10046055					
	C	A		0.05	0.08	0.098	
	T	A		0.09	0.09	0.743	
	C	T		0.80	0.77	0.151	
	T	T		0.06	0.07	0.664	0.341
HapB	rs1186930	rs10046055					
	C	A		0.10	0.10	0.563	
	T	A		0.04	0.06	0.133	
	C	T		0.22	0.22	0.835	
	T	T		0.64	0.62	0.418	0.471
HapC	rs10046055	AAAT11					
	T	248		0.85	0.82	0.189	
	A	252		0.12	0.14	0.249	0.234
HapD	rs254664	rs10046055	AAAT11				
	G	T	248	0.80	0.76	0.10	
	G	T	248	0.06	0.07	0.42	
	T	A	252	0.05	0.06	0.25	
	T	A	252	0.07	0.08	0.58	0.429
HapE	rs1145603	rs10046055	AAAT11				
	C	T	248	0.64	0.61	0.292	
	T	T	248	0.21	0.22	0.969	
	C	A	252	0.04	0.06	0.131	
	T	A	252	0.08	0.08	0.698	0.440
HapF	rs1145603	rs254664					
	C	C		0.69	0.68	0.940	
	T	C		0.17	0.16	0.671	
	T	T		0.15	0.16	0.591	0.819

[a] *P* from COCAPHASE-EM, -droprare 0.03 options

of rs10046055, which gave rise to the second significant result ($P = 0.002$) by Pimm et al. (2005), was assumed to be a risk, our sample size had a power of 90% to detect an odds ratio of 1.6 or more. Thus our sample size may have been limited by the inadequacy to detect a potentially weak effect of *Epsin 4* in Japanese. It is also possible that there may be a differential effect of *Epsin 4* on the development of schizophrenia between ethnic groups due to unknown factors. Alternatively, the positive association between schizophrenia and *Epsin 4* reported by Pimm et al. (2005) may have arisen by chance.

Subsequent to Pimm et al.'s (2005) investigation, two replication studies have been reported. Tang et al. (2006) reported a significant association of schizophrenia with some haplotypes of *Epsin 4* (252/T composed of AAAT11 and rs10046055, global $P = 0.0021$; T/T of rs1145603 and rs254664, $P = 0.0033$) in Han Chinese family trios, providing evidence for an association between schizophrenia and *Epsin 4*. The other replication study of Liou et al. (2006) found some evidence for an association, discovering a significant difference in allele frequency at rs1186922 ($P = 0.038$) and a significant difference in the frequency of a haplotype composed of two markers (rs1186922 and rs10046055). Liou et al. (2006) conservatively concluded that these findings occurred by multiple testing and chance and that their data did not support an association between schizophrenia and *Epsin 4*. However, Gurling et al. (2007) argued that the data of Liou et al. (2006) should be interpreted as supportive evidence for association. Since these two studies and ours were performed in Asian populations, if the association really exists, the obtained results should be similar. However, we obtained no evidence supporting the positive findings of Tang et al. (2006) or Liou et al. (2006).

In conclusion, we failed to identify a significant association between genetic variations of *Epsin 4* and schizophrenia in a Japanese population, suggesting that the examined region (5′ side) of *Epsin 4* does not have a major influence on susceptibility to schizophrenia in Japanese. Given the inconsistent findings across studies, more studies

should be conducted within different ethnic populations to draw more concrete conclusions. A meta-analysis of such studies and a multicenter study with a very large sample size will eventually be required. Since we examined only the 5′ side of the gene, the possibility remains that other unknown polymorphisms linked to the 3′ region of the *Epsin 4* gene may be associated with schizophrenia.

Acknowledgments We thank all patients for their participation in this study. This study was supported by the Japan Society for Promotion of Science (M. R.), Health and Labor Sciences Research Grants (Research on psychiatric and neurological diseases and mental health) (H. K.), Japan Health Sciences Foundation (Research on health sciences focusing on drug innovation) (H. K.), Grant-in-Aid for Scientific Research on Priority Areas of Applied Genomics from the Ministry of Education, Culture, Sports, Science and Technology of Japan (H. K.).

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J Hum Genet (2008) 53:914–919
DOI 10.1007/s10038-008-0327-6

ORIGINAL ARTICLE

Microarray comparative genomic hybridization analysis of 59 patients with schizophrenia

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Received: 18 April 2008 / Accepted: 8 July 2008 / Published online: 7 August 2008

Abstract Schizophrenia is a common psychiatric disorder with a strong genetic contribution. Disease-associated chromosomal abnormalities in this condition may provide important clues, such as *DISC1*. In this study, 59 schizophrenia patients were analyzed by microarray comparative genomic hybridization (CGH) using custom bacterial artificial chromosome (BAC) microarray (4,219 BACs with 0.7-Mb resolution). Chromosomal abnormalities were found in six patients (10%): 46,XY,der(13)t(12;13)(p12.1; p11).ish del(5)(p11p12); 46,XY, ish del(17)(p12p12); 46,XX.ish dup(11)(p13p13); and 46,X,idic(Y)(q11.2); and in two cases, mos 45,X/46XX. Autosomal abnormalities in three cases are likely to be pathogenic, and sex chromosome abnormalities in three follow previous findings. It is noteworthy that 10% of patients with schizophrenia have (sub)microscopic chromosomal abnormalities, indicating that genome-wide copy number survey should be considered in genetic studies of schizophrenia.

Keywords Schizophrenia · Chromosomal abnormality · Array comparative genomic hybridization · Copy number variation

Introduction

Schizophrenia is a common psychiatric disorder involving approximately 1% of the population worldwide. Family, twin, and adoption studies suggest genetic factors contribute to this illness (Lang et al. 2007; McGuffin et al. 1995). Meta-analysis including 18 genome scans revealed strong evidence at chromosomal regions 22q, 8p, and 13q

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as the susceptibility loci (Badner and Gershon 2002), and another meta-analysis of 20 genome-wide scans suggested regions of chromosomes 2q, 5q, 3p, 11q, 6p, 1q, 22q, 8p, 20q, and 14p as the significant loci (Lewis et al. 2003). Chromosomal abnormalities in patients with schizophrenia may provide useful information regarding the susceptible loci (Bassett et al. 2000). Disrupted in schizophrenia 1 (*DISC1*) gene isolated from a large Scottish family with t(1;11)(q42.1;q14.3) and high risk of schizophrenia in velo-cardio-facial syndrome (VCFS) with a 22q11 deletion are good examples (Arinami 2006; Millar et al. 2000; Murphy 2002). Some linkage and association studies support that schizophrenia could be associated with *DISC1* and genes at 22q11 (Chubb et al. 2008; Liu et al. 2002; O'Donovan et al. 2003; Shifman et al. 2002).

Microarray technologies have now become practical tools for detection of submicroscopic copy number changes. Using custom bacterial artificial chromosome (BAC) microarray (4,219 BACs at 0.7-Mb resolution), we analyzed 59 patients with schizophrenia. Chromosomal abnormalities found in this study are presented.

Materials and methods

Subjects

A total of 59 subjects (31 men and 28 women) with schizophrenia were recruited in this study. Forty-one had family history. Diagnosis was made for each patient according to the *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition (DSM-IV) criteria on the basis of unstructured interviews and information from medical records. Participants were excluded if they had organic brain diseases, including head injury and infection, or if they met criteria for alcohol/drug dependence. After written informed consent, genomic deoxyribonucleic acid (DNA) from lymphoblastoid cell line (LCL) of all patients was isolated using DNA isolation systems [Quick Gene-800 (Fujifilm, Tokyo, Japan) and/or NA-3000 (Kurabo, Osaka, Japan)]. Micorarray comparative genomic hybridization (CGH) and fluorescence in situ hybridization (FISH) analysis were performed using materials from LCL. Peripheral blood lymphocytes were reevaluated in ID394, MZ102, and MZ127, but could not be obtained for reexamination in ID67, ID345, or ID391. Only parents of ID345 subjects were available for familial analysis. Other parents or sibs could not be evaluated. Experimental protocols were approved by the Committee for Ethical Issues at Yokohama City University School of Medicine.

Microarray CGH analysis

Comparative genomic hybridization analysis was performed using our custom BAC microarray containing 4,219 BAC clones, as previously described (Saitsu et al. 2008). In brief, after complete digestion using *Dpn*II, subject's DNA was labeled with Cy-5 dCTP (Amersham Biosciences, Piscataway, NJ), and reference DNA was labeled with Cy-3 deoxycytidine triphosphate (dCTP) (Amersham Biosciences) using the DNA random primer Kit (Invitrogen). Prehybridization, probe hybridization, washing, and drying steps for arrays were preformed on a Tecan hybridization station HS400 (Tecan Japan, Kawasaki, Japan). Arrays were scanned by GenePix 4000B (Axon Instruments, Union City, CA, USA) and analyzed using GenePix Pro 6.0 (Axon Instruments). The signal intensity ratio between patient and control DNA was calculated from the data of the single-slide experiment using the ratio of means formula (F635 mean − B635 median/F532 mean − B532 median) according to GenePix Pro. 6.0. The standard deviation was calculated from the data of all clones. We regarded the signal ratio as abnormal if it ranged out of ±3 standard deviations (SD). Clones showing abnormal copy number were checked to see whether they were in the position of previously registered copy number variations using the Human Genome Variation Database (http://www.hgvbase.org/) (Iafrate et al. 2004). Unregistered changes were considered for further confirmation. Genome position was based on the UCSC genome browser Human Mar. 2006 (hg18) assembly.

Fluorescence in situ hybridization

To confirm status of clones with a possibly abnormal copy number, FISH was performed, as previously described (Shimokawa et al. 2005). BAC DNA was labeled with SpectrumGreen™-11-deoxyuridine triphosphate (dUTP) or SpectrumOrange™-11-dUTP (Vysis, Downers Grove, IL, USA) by nick translation and denatured at 70°C for 10 min. Probe-hybridization mixtures (15 μl) were applied on chromosomes, incubated at 37°C for 16–72 h, then washed and mounted in antifade solution (Vector, Burlingame, CA, USA) containing 4'-6'-diamidino-2-phenylindole (DAPI). Photographs were taken on an AxioCam MR CCD fitted to Axioplan2 fluorescence microscope (Carl Zeiss, Oberkochen, Germany). In ID394 and MZ102, we counted 100 interphase nuclei to validate the number of cells with X aneuploidy, as well as 30 metaphases.

Results and discussion

Six patients showed chromosomal abnormalities (10%, 6/59) (Table 1). As we could not obtain materials from most

Table 1 Summary of six patients with (sub)microscopic chromosomal rearrangements

Patient	Gender	FH	Karyotype	Size of imbalance
ID67	M	No	46,XY,der(13)t(12;13)(p12.1; p11).ish del(5)(p11p12)	1.7 Mb deletion (chr.5) 23.1 Mb gain (chr.12)
MZ127	F	Yes	46,XX.ish dup(11)(p13p13)	430 bp (?) gain (chr.11)
ID345	M	No	46,XY, ish del(17)(p12p12)	1.3 Mb deletion (chr.17)
MZ102	F	Yes	Mos45,X/46,XX	Whole X loss (mosaic)
ID394	F	Yes	Mos45,X/46,XX	Whole X loss (mosaic)
ID391	M	Yes	46,X,idic(Y)(q11.2)	Yq12-qter deletion Yq11.23-Yq12 gain

FH family history of schizophrenia and/or other psychiatric disorders

of their parents and sibs, heritability of the abnormalities could not fully be investigated. According to our experiences of microarray CGH analysis of more than 200 Japanese patients associated with mental-retardation-related disorders, all chromosomal abnormalities described here were never detected. Thus, it is less likely that the changes are polymorphisms.

In ID67, arr cgh 5p12p12(RP11-1037A10 → RP11-929P16) × 1, 12pterp12.1(GS-124K20 → RP11-12D15) × 3 was found. A 23.2-Mb copy number gain from 12pter to 12p12.1 (chr12: 0–23,176,547 bp) was detected (Fig. 1a). G-banded chromosomal analysis revealed that 12pter-12p12.1 was translocated to 13p11 (Fig. 1a). The 12p12.1 translocation breakpoint was localized between two BAC clones, RP11-35A22 and RP11-349E13, by FISH (chr12: 23,176,547–23,861,227 bp) (data not shown). Additionally, a 1.7-Mb submicroscopic deletion at 5p12 from RP11-1037A10 to centromeric sequence gap (chr5: 44,778 009–46,437 323 bp) was also found in this patient (Fig. 1a). The 12p trisomy is recognized as multiple congenital anomalies/mental retardation (MCA/MR) syndrome characterized by dysmorphic face, heavy birth weight, foot deformities, hypotonia, and mental retardation (Allen et al. 1996). A previous study suggested that partial duplication of 12pter-p13.2 is sufficient for recognizable phenotype of 12p trisomy (Rauch et al. 1996). The 23.1-Mb duplicated region contained at least 229 genes. Dysmorphic facial features of 12p trisomy (Rauch et al. 1996) were not recognized in this patient. It is interesting that ID67 also had a 1.7-Mb deletion at 5p12, containing two genes, *MRPS30* (the mitochondrial ribosomal protein S30 gene) and *HCN1* (the hyperpolarization-activated cyclic nucleotide-gated potassium channel 1 gene). It is worth noting linkage findings within the vicinity of this region in Costa Rican schizophrenia samples (Cooper-Casey et al. 2005). *HCN1* is an intriguing candidate gene. The general *Hcn*1 loss in mice led to a defect in the learning of motor tasks, and specific deletion of the gene in forebrain neurons resulted in an unexpected enhancement of spatial learning and memory (Herrmann et al. 2007; Nolan et al. 2003). ID67 (a 72-year-old male) developed psychotic symptoms (delusions, hallucinations, and psychomotor excitement) at age 20 years. He had received electroconvulsive therapy many times and continuous sleep therapy until antipsychotic medication (chlorpromazine) was introduced at age 23 years. Since the onset of the illness, he has spent most of his life in psychiatric hospitals because of exacerbations of psychotic episodes and marked deterioration of social functions. Intelligent quotient (IQ) at 72 years was 72. He had no family history of major psychosis within the first-degree relatives.

In MZ127, arr cgh 11p13p13(RP11-51J14) × 3 was recognized. Duplication of RP11-51J14 at 11p13 (chr11: 33,302,231–33,302,660 bp) was confirmed by FISH using LCL and peripheral blood lymphocytes (Fig. 1b). According to the genome browser, the size of RP11-51J14 is 430 bp, indicating that the reference sequence is somehow odd and may contain a deletion overlapping with RP11-51J14 as FISH signals of RP11-51J14 are strong enough to detect on a microscope, suggesting that its size is at least >10 kb. *HIPK3* (the homeodomain interactive protein kinase 3 gene) was corresponding to this clone. HIPK3 is a Fas-associated death-domain (FADD)-interacting kinase involved in apotosis (Curtin and Cotter 2003), remaining unknown in relation to schizophrenia. MZ127 (42-year-old woman) presented with epilepsy at age 12 years and has had recurrent depression and slight mania since age 29 years. She began to exhibit auditory hallucination, not synchronizing with mood swing, and was diagnosed as schizophrenia at 40 years. Her mother and sister suffered from major depression and schizophrenia, respectively. Her father committed suicide induced by depression.

In ID345, arr cgh 17p12p12(RP11-78J16 → RP11-103P10) × 1 was found, as previously described (Ozeki et al. 2008). The deletion from RP11-246F16 to RP11-103P10 (chr17: 14,061,460–15,374,745 bp) is 1.4 Mb, compatible with the common deletion found in approximately 85% of hereditary neuropathy with liability to pressure palsies (HNPP; OMIM #162500) (Stogbauer et al.

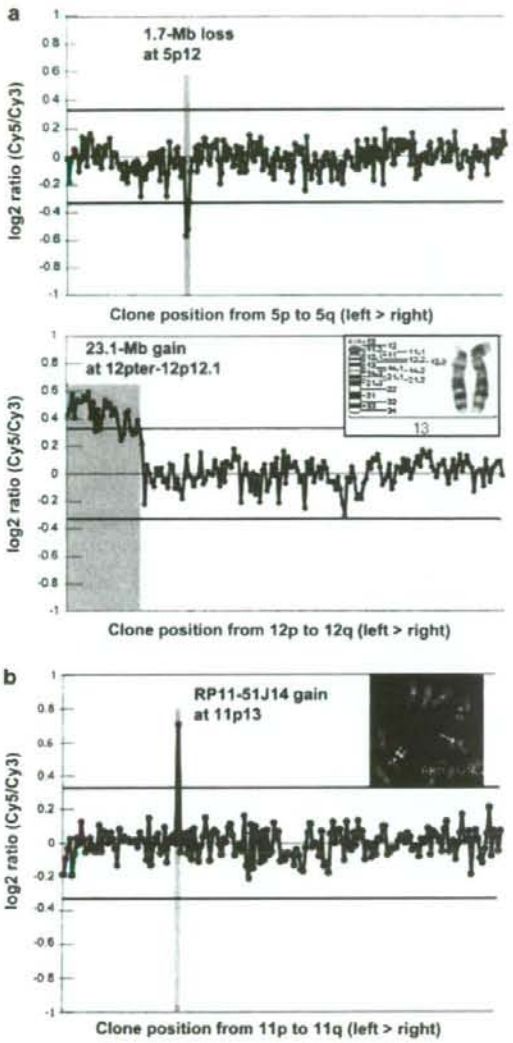

Fig. 1 Results of microarray comparative genomic hybridization (*CGH*) in ID67 (**a**) and MZ127 (**b**). Chromosomes 5 (*upper*) and 12 (*lower*) are displayed (**a**). The karyotype is arr cgh 5p12p12(RP11-1037A10 → RP11-929P16) × 1, 12pterp12.1(GS-124K20 → RP11-12D15) × 3. Partial karyotype clearly shows a 12pter-p12.1 segment is translocated to 13p11. Chromosome 11 is presented (**b**) . The karyotype is arr cgh 11p13p13(RP11-51J14) × 3. RP11-51J14 at 11p13 is duplicated

2000). The deletion was also identified in his father's chromosomes from peripheral blood lymphocytes. He suffered from auditory hallucination and delusion of persecution and received antipsychotic treatment at age 19. Neurological examination did not reveal any manifestations of HNPP (Ozeki et al. 2008). Pareyson et al. (1996) reported that about 25% of individuals with HNPP deletion are asymptomatic. The peripheral myelin protein 22 gene (*PMP22*) may be a candidate that is not only expressed in the peripheral nervous system but also in the central nervous system (Ohsawa et al. 2006), this being supported by linkage studies of psychotic bipolar disorder (Park et al. 2004) and schizophrenia (Owen et al. 2004). No family history regarding psychiatric disorders was observed in ID345.

Entire X chromosome copy number aberration was suspected in two patients, ID394 and MZ102 (data not shown). FISH analysis using RP11-65B15 at Xq23 revealed mosaic monosomy of chromosome X: mos45,X[41]/46,XX[59] in ID394 and mos45,X[84]/46,XX[16] in MZ102. X aneuploidy is well known to be seen in elderly normal females (Stone and Sandberg 1995). ID394 and MZ102 were 67 and 38 years old, respectively. The fraction of cells with X monosomy was very high (84% and 41%) in lymphoblastoid cell lines of these patients. Reevaluation of peripheral blood lymphocytes showed mos 45,X[7]/46,XX[98] in ID394 and mos 45,X[4]/46,XX[96] in MZ102. These findings may support involvement of X-chromosomal abnormalities in schizophrenia (Kumra et al. 1998; Kunugi et al. 1999), but mosaic X monosomy is also found in age-matched normal controls (Toyota et al. 2001). ID394 (a 67-year-old woman) developed psychotic symptoms (paranoid delusion and hallucinations) at age 31 years when she delivered her second child. Since then, she had been admitted to a psychiatric hospital three times (each for a few months). She quit her job as a pharmacist after the onset of the illness and has lived as a housewife. She has been managed by antipsychotic medications without major exacerbation for the past decade. The second child developed schizophrenia-like symptoms, including social withdrawal and lack of volition. MZ102 (a 38-year-old woman) exhibited psychomotor excitement and was diagnosed as having schizophrenia at age 23 years. Her father showed psychotic disorder, and her uncle had schizophrenia. In ID391, arr cgh Ypterq11.23(GS-98C4 → RP11-214M24) × 3, Yq11.23qter(RP11-263C17 → RP11-80F8) × 1 was identified. FISH analysis using BACs, RP11-74L17 at PAR1, RP11-375P13 at Yp11.2, RP11-655E20 at Yq11.2, and RP11-80F8 at Yq12 revealed the isodicentric Y chromosome [46,X,idic(Y)(q11.2)] (data not shown). Previously, two cases of idic(Yp) were reported in schizophrenia, although idic(Yp) is one of the most common rearrangements in the Y chromosome (Nanko et al. 1993; Yoshitsugu et al. 2003). ID391 (a 29-year-old man) developed hallucinations and abnormal sense of self at age 21 years, when he was admitted to a psychiatric hospital for 3 months. Since then, his illness has been well controlled by antipsychotic medication. He quit university after the onset

of illness and has not obtained a job, suggesting deterioration of functioning. His younger sister (apparently without the Y chromosome) has schizophrenia. Thus, contribution of sex chromosomal abnormalities found in this study is less likely.

Four microarray CGH studies of schizophrenia were reported: 1,440 BAC microarray for 30 patients, 2,460 BAC microarray for 35 patients, a tiling-path microarray consisting of ~36,000 BACs for 93 patients, and high-resolution microarrays (85,000–2,100,000 oligos) for 150 patients (Kirov et al. 2008; Moon et al. 2006; Walsh et al. 2008; Wilson et al. 2006). We could not replicate any similar abnormalities, though microarray platforms were all different in terms of clones and genome coverage. In this study, (sub)microscopic rearrangements were detected in 10% of patients. Similarly, 15% of patients analyzed by high-resolution microarrays were found to possess submicroscopic chromosomal changes (Walsh et al. 2008). Various kinds of recurrent and unique submicroscopic changes were found in 10–17% of idiopathic mental retardation and 7% of autism by microarray CGH analysis (Miyake et al. 2006; Sebat et al. 2007; Zahir and Friedman 2007). Importantly, a 22q13 deletion (in autism) involving Sh3 and multiple ankyrin repeat domains 3 (*SHANK3*), whose point mutation was related to autism (Durand et al. 2007), strongly supports this approach as one of the most powerful and straightforward strategies in neuropsychiatric disorders.

In conclusion, microarray technologies could provide good opportunity to identify chromosomal copy number changes in relation to mental and psychiatric disorders, and genome-wide copy number survey should be considered in genetic studies of these disorders.

Acknowledgments We thank all patients for their participation in this study. This work was supported by Research Grants from the Ministry of Health, Labour and Welfare (HK and NM), and Grant-in-Aid for Scientific Research on Priority Areas from the Ministry of Education, Culture, Sports, Science and Technology of Japan (RH and NM).

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Neuroscience Research 61 (2008) 360–367

Neuroscience Research

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Dose-dependent effect of the Val66Met polymorphism of the brain-derived neurotrophic factor gene on memory-related hippocampal activity

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Received 3 July 2007; accepted 11 April 2008
Available online 20 April 2008

Abstract

Brain-derived neurotrophic factor (BDNF) plays a critical role in activity-dependent neuroplasticity underlying learning and memory in the hippocampus. Recent human studies have indicated that a common single nucleotide polymorphism of the BDNF gene, the Val66Met polymorphism, has impact on episodic memory, hippocampal morphology and memory-related hippocampal activity measured by functional magnetic resonance imaging (fMRI). However, two issues remain to be clarified: (1) whether the genotype effect of this polymorphism on memory-related brain activity is allele dose dependent and (2) whether the effect of this polymorphism in Asian population is the same as effects observed in Caucasian sample. To clarify these issues, we studied the relationship of the Val66Met polymorphism genotype and hippocampal activity during episodic memory task using fMRI in healthy 58 biologically unrelated Japanese. Although there was no genotype effect on episodic memory function obtained by behavioral assessments, fMRI measurements revealed a significantly negative correlation between the dose of Met-BDNF allele and encoding related brain activity in the bilateral hippocampi and right parahippocampal gyrus. There was no genotype effect on retrieval related brain activity. These data indicated a genetic mechanism for normal variation in human memory and suggest effects of BDNF signaling on hippocampal function in humans.

Keywords: Brain-derived neurotrophic factor (BDNF); Hippocampus; Human memory; Gene; Polymorphism; fMRI

1. Introduction

Brain-derived neurotrophic factor (BDNF), a member of neurotrophin family, has important roles in hippocampal plasticity and hippocampal-related learning and memory through long-term potentiation (LTP) (Poo, 2001). It is also important for long-term developmental phenomena, such as neuronal survival, differentiation, and activity-dependent refinement of synaptic architecture (Huang and Reichardt, 2001; Malcangio and Lessmann, 2003). A common single nucleotide polymorphism (SNP) of the BDNF gene producing an amino acid substitution of valine to methionine (Val66Met) affects intracellular packaging and activity-dependent secretion of BDNF (Egan et al., 2003; Chen et al., 2004). A previous study revealed that normal individuals with the Met-BDNF allele had poorer episodic memory accompanying with lesser engagement of the hippocampi during memory task observed in a functional magnetic resonance imaging (fMRI) when compared to individuals homozygous for the Val-BDNF allele (Hariri et al., 2003). They also demonstrated that Met-BDNF

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0168-0102/$ – see front matter
doi:10.1016/j.neures.2008.04.003

carriers had smaller hippocampus when compared to individuals homozygous for the Val-BDNF allele (Pezawas et al., 2004; Bueller et al., 2006).

Although these results strongly suggest that the Val66Met polymorphism of the BDNF gene should contribute inter-individual differences of human episodic memory function and memory-related brain structures and brain activity, at least two issues remain to be clarified. First, because of relatively small sample size, particularly homozygous for Met-BDNF allele, the previous study might demonstrate different brain activity related to memory task between homozygous for Val-BDNF allele and Met-BDNF carriers (Hariri et al., 2003). It has been unclear whether the genotype effect of the Val66Met polymorphism on memory-related brain activity is dose dependent. Indeed, their behavioral study with larger sample demonstrated dose-dependent effect of the Val66Met polymorphism on episodic memory performance in normal individuals (Egan et al., 2003). Second, some studies suggested racial differences of effects of the Val66Met polymorphism, such as discrepancy in associations between BDNF polymorphism and the prevalence of neuropsychiatric diseases (Sklar et al., 2002; Kunugi et al., 2004). In terms of brain morphology, our previous structural MR study demonstrated that effects of the Val66Met polymorphism on brain morphology in Japanese were different from those in Caucasians; i.e. the effect of the Val66Met polymorphism was noted in not hippocampal volume but volumes of the parahippocampal gyrus and caudate nucleus (Nemoto et al., 2006). Taken together, it would be possible that genotype effects of the Val66Met polymorphism on brain activity related to episodic memory task in Asian population may differ from those in Caucasians.

To clarify these two issues, we performed fMRI measurements with memory task in larger samples consisted of normal Japanese individuals.

2. Methods

2.1. Subjects

Fifty-eight healthy subjects participated in the study. Written informed consent was obtained from all the subjects in accordance with ethical guidelines in place at local ethical committee. All the subjects were recruited from local advertisements and underwent a Wechsler Adult Intelligence Scale-Revised (WAIS-R), Wechsler Memory Scale-Revised (WMS-R) and MRI scanning. They were also screened by a questionnaire on medical history and excluded if they had neurological, psychiatric or medical conditions that could potentially affect the central nervous system, such as substance abuse or dependence, atypical headache, head trauma with loss of consciousness, asymptomatic or symptomatic cerebral infarction detected by T2 weighted MRI, hypertension, chronic lung disease, kidney disease, chronic hepatic disease, cancer, or diabetes mellitus. All the subjects were biologically unrelated Japanese. Table 1 shows characteristics of subjects. The age range of the sample was 22–63 years (mean 36.4, standard deviation [S.D.] 10.6). According to genotypes, subjects were categorized into three groups; the homozygous Val-BDNF group ($n = 17$, male:female = 5:12, one was left-handed), the Val/Met-BDNF group ($n = 29$, male:female = 5:24, two were left-handed), and the remaining, homozygous Met-BDNF group ($n = 12$, male:female = 2:10, all were right-handed). The genotype distribution of this SNP was not deviated with Hardy–Weinberg equilibrium (chi-square = 0.003, $p = 0.95$). The one way analysis of variance revealed that there was no significant difference of age, education years, full scale IQ, or scores of WMS-R between groups (all $p > 0.05$). The chi-square test also revealed that there was no significant difference of distribution of gender ratio (d.f. = 2, chi-square = 1.1, $p = 0.57$), handedness (d.f. = 2, chi-square = 0.85, $p = 0.65$) or the ApoE genotype (d.f. = 2, chi-square = 2.9, $p = 0.24$).

2.2. Genotyping

Venous blood was drawn from the subjects and genomic DNA was extracted from whole blood according to the standard procedures. Genotyping the Val66Met SNP (dbSNP accession: rs6265) was performed with the TaqMan 5′-exonuclease allelic discrimination assay, described previously (Nemoto et al., 2006). Primers and probes for detection of the SNP (TaqMan SNP Genotyping assays on demand) were purchased from Applied Biosystems (ABI, Foster City, CA, USA). PCR cycling conditions were: at 95 °C for 10 min, 45 cycles of 92 °C for 15 s and 60 °C for 1 min. ApoE genotypes were determined by the polymerase chain reaction (PCR) restriction fragment length polymorphism (RFLP) method. We used the primers designed by Hixson and Vernier (Hixson and Vernier, 1990). The procedures have been previously described in detail (Asada et al., 1996). Hha I—digested PCR fragments were subjected to gel electrophoresis and visualized by staining with ethidium bromide.

2.3. fMRI study

Cerebral activation was measured with fMRI using blood oxygen level-dependent contrast. After automatic shimming, a time course series of 145 volumes was obtained using single-shot gradient-refocused echo-planar imaging (TR = 4000 ms, TE = 60 ms, flip angle = 90°, in-plane resolution

Table 1
Characteristics of subjects

	Val/Val-BDNF	Val/Met-BDNF	Met/Met-BDNF	Genotype F (p)	Chi square (p)
Number of subjects	17	29	12		
Age	37.6 (2.9)	35.1 (10.1)	38.0 (10.6)	0.47 (0.63)	
Gender (M/F)	5/12	5/24	2/10		1.1 (0.57)
Handedness (R/L)	16/1	27/2	12/0		0.85 (0.65)
Education	15.4 (2.7)	16.4 (2.4)	15.9 (2.7)	0.97 (0.36)	
ApoE epsilon4 allele (non-carrier/carrier)	15/2	21/8	11/1		2.9 (0.24)
Full scale IQ (WAIS-R)	108.9 (13.7)	108.3 (12.2)	110. 8 (13.2)	0.12 (0.89)	
WMS-R					
Verbal memory	117 (12.4)	108.3 (12.8)	113.08 (11.8)	2.51 (0.09)	
Visual memory	109.5 (11.2)	108.9 (9.1)	106.3 (9.1)	0.41 (0.67)	
General memory	116.8 (11.7)	109.4 (12.0)	112.7 (10.7)	2.05 (0.14)	
Attention/concentration	106.6 (16.3)	104.1 (14.2)	105.9 (15.9)	0.14 (0.97)	
Delayed recall	116.9 (13.5)	110.4 (11.9)	108.3 (16.7)	1.81 (0.17)	

WMS-R (Wechsler memory scale-revised); mean value (standard deviation).

3.44 mm × 3.44 mm, FOV = 22 cm, contiguous 4-mm slices to cover the entire brain) with a 1.5 T MAGNETOM Vision plus MR scanner (Siemens, Erlangen, Germany) using the standard head coil. The first five volumes of each fMRI scan were discarded because of non-steady magnetization, with the remaining 140 volumes used for the analysis. Head motion was minimized by placing tight but comfortable foam padding around the subject's head. Before the collection of fMRI data for each subject, we acquired a reference EPI scan and visually inspected it for artifacts (i.e. ghosting) as well as for good signal across the entire volume of acquisition, including the medial temporal lobes.

The fMRI paradigm consisted of the encoding and subsequent retrieval of novel, complex scenes. Stimuli were presented in a blocked paradigm; seven encoding blocks were followed by seven retrieval blocks in an interleaved design with a passive viewing of scrambled picture condition (control condition). During encoding blocks, subjects viewed five images, presented serially for 4 s each, and were asked to remember them. All scenes were of neutral emotional valence and were derived from the International Affective Picture System (Lang et al., 1997). During subsequent retrieval blocks, subjects again viewed five images, presented serially for 3 s each, and determined whether each scene was "new" (presented during the encoding blocks) or "old" (not presented during the encoding blocks). In each retrieval block, half of the scenes were "old" and remaining half were "new". During the interleaved rest blocks, subjects were instructed to fixate on a centrally presented cross-hair. During scanning, all subjects responded by button presses with their dominant hand, allowing for the determination of accuracy and reaction time.

Analysis of the fMRI data were completed using statistical parametric mapping 2 (SPM2). Images for each subject were realigned to the first volume in the time series to correct for head motion, spatially normalized into a standard stereotactic space (Montreal Neurological Institute template) using a 12 parameter affine model and smoothed with a Gaussian filter, set at 8 mm full-width at half-maximum. Voxel-wise signal intensities were ratio normalized to the whole-brain global mean. Task-related activations were calculated using a t-statistic, producing a statistical image for the contrasts of encoding versus control and retrieval versus control for each subject. These individual contrast images were then used in second-level random effects models, which account for both scan-to-scan and subject-to-subject variability, to determine task-specific regional responses at the group level for the entire sample (main effects of tasks). We used $p < 0.001$, corrected for multiple comparisons with false discovery rate (FDR) < 0.05 as a statistical threshold for these whole-brain search. To test dose-dependent genotype effects of the BDNF gene, we performed correlational analysis. In this analysis, we treated Met-BDNF gene dose (i.e. the number of Met-BDNF allele in a person's BDNF genotype) as a covariate of interest and treated the genotype of the ApoE gene, age, and gender as nuisance variables. Because

Table 2
Brain activity related to encoding and retrieval

Brain regions	Brodmann area	Cluster size	Tarailach coordinates			Corrected p value	T value
			x	y	z		
Activity related to encoding (Fig. 1)							
Rt. Middle frontal gyrus	46	36	44	17	21	$<5.0 \times 10^{-4}$	4.5
Lt. Middle frontal gyrus	46	38	−44	17	25	5.0×10^{-3}	3.8
Rt. Parahippocampal gyrus, hippocampus	30/36/19	2617	36	−32	−22	$<5.0 \times 10^{-4}$	11.8
Lt. Parahippocampal gyrus, hippocampus	30/36/19	2617	−24	−39	−11	$<5.0 \times 10^{-4}$	8.9
Rt. Precuneus	19	2617	32	−72	33	$<5.0 \times 10^{-4}$	8.3
Lt. Precuneus	19	2617	−28	−76	37	$<5.0 \times 10^{-4}$	7.5
Lt. Superior parietal lobule	7	2617	−24	−72	44	$<5.0 \times 10^{-4}$	6.9
Rt. Fusiform gyrus	19/37	2617	28	−39	−11	$<5.0 \times 10^{-4}$	10.5
Rt. Middle temporal gyrus	19	2617	40	−81	19	$<5.0 \times 10^{-4}$	13.1
Rt. Angular gyrus	39	2617	36	−76	30	$<5.0 \times 10^{-4}$	9.7
Lt. Fusiform gyrus	37	2617	−48	−59	−17	$<5.0 \times 10^{-4}$	13.7
Lt. Middle temporal gyrus	19	2617	−40	−81	19	$<5.0 \times 10^{-4}$	9.7
Rt. Lingual gyrus	19	2617	32	−58	−4	$<5.0 \times 10^{-4}$	7.7
Rt. Middle occipital gyrus	37	2617	44	−70	7	$<5.0 \times 10^{-4}$	11.6
Lt. Superior occipital gyrus	39	2617	−32	−76	30	$<5.0 \times 10^{-4}$	8.2
Lt. Middle occipital gyrus	18/19	2617	−36	−86	−2	$<5.0 \times 10^{-4}$	17.5
Activity related to retrieval (Fig. 2)							
Lt. Middle frontal gyrus	9	74	−40	13	25	$<5.0 \times 10^{-4}$	6.4
Rt. Middle frontal gyrus	9/46	56	44	13	25	$<5.0 \times 10^{-4}$	5.4
Rt. Parahippocampal gyrus, hippocampus	19/30/36/37	2393	16	−50	3	$<5.0 \times 10^{-4}$	9.4
Rt. Posterior cingulate	29	2393	4	−42	6	$<5.0 \times 10^{-4}$	9.2
Lt. Parahippocampal gyrus, hippocampus	30/35/36/37	2393	−24	−47	−8	$<5.0 \times 10^{-4}$	6.2
Lt. Posterior cingulate	29	2393	−4	−42	6	$<5.0 \times 10^{-4}$	10.5
Rt. Precuneus	7	2393	4	−71	51	$<5.0 \times 10^{-4}$	7.3
Lt. Precuneus	7	2393	−4	−75	52	$<5.0 \times 10^{-4}$	6.4
Rt. Fusiform gyrus	20/37/39	2393	36	−55	−14	$<5.0 \times 10^{-4}$	14.8
Lt. Fusiform gyrus	37	2393	−44	−59	−17	$<5.0 \times 10^{-4}$	13.0
Rt. Middle occipital gyrus	18/19	2393	40	−85	4	$<5.0 \times 10^{-4}$	18.1
Lt. Lingual gyrus	18	2393	−8	−82	−13	$<5.0 \times 10^{-4}$	9.9
Lt. Middle occipital gyrus	19	2393	−28	−89	15	$<5.0 \times 10^{-4}$	15.6
Lt. Inferior occipital gyrus	18	2393	−40	−82	−6	$<5.0 \times 10^{-4}$	15.0
Genotype effects on brain activity related to encoding							
R Parahippocampal gyrus, hippocampus		44	27	−35	−5	$<5.0 \times 10^{-4}$	4.8
L Hippocampus		52	−36	−20	−9	2.0×10^{-3}	4.0
Genotype effects on brain activity related to retrieval							
Not significant							

of our *a priori* hypothesis regarding the differential response of the hippocampus and parahippocampal gyrus, a statistical threshold of $p < 0.05$, a small volume correction for multiple comparisons within the *a priori* volume of interest, was used to identify dose-dependent genotype effects on brain activity. For this hypothesis-driven analysis, we used the Wake Forest University PickAtlas (Maldjian et al., 2003). Whole-brain correlational analyses were also calculated using second-level random effect models. Because we had no *a priori* hypotheses regarding the activity of brain regions outside of the memory-related areas, we used a statistical threshold of $p < 0.05$, corrected for multiple comparisons across all supra-threshold voxels, for these whole-brain correlational analyses.

3. Results

3.1. Task performance during fMRI measurements

The percentage of correct answers (S.D.) for the retrieval task in each genotype was as follows; 98.9% (2.13) for Val/Val-BDNF group, 97.2% (6.06) for Val/Met-BDNF group, and 96.7% (3.89) for Met/Met-BDNF group, respectively. There was no significant genotype effect on the percentage of correct answers of the retrieval task ($F = 0.8$, $p = 0.45$). The mean reaction time (S.D.) of the retrieval task in each genotype was as follows; 1239 (232) ms for Val/Val-BDNF group, 1227 (175) ms for Val/Met-BDNF group, and 1312 (219) ms for Met/Met-BDNF group, respectively. There was no significant genotype effect on reaction time for the retrieval task ($F = 0.76$, $p = 0.48$).

3.2. Results of brain activity

When all subjects were treated one group, we found a significant activation in the bilateral hippocampus, parahippocampal gyri, visual association areas, parietal association areas including precunei and the dorsolateral prefrontal cortices during encoding period (Table 2 and Fig. 1). During retrieval period, a significant activation was also found in the bilateral hippocampus, parahippocampal gyri, posterior cingulate cortex (PCC), visual association areas, parietal association areas and the dorsolateral prefrontal cortices (Table 2 and Fig. 2).

Regarding brain activity related to encoding, a significantly negative correlation between the dose of Met-BDNF gene and brain activity was noted in the bilateral hippocampi and right parahippocampal gyrus (Table 2 and Figs. 3 and 4). On the other hand, a weak negative correlation between dose of Met-BDNF gene and brain activity related retrieval in the bilateral parahippocampal gyri at a lenient statistical threshold (uncorrected $p < 0.05$), however, no voxels could survive after the correction for multiple comparisons within the ROI (corrected $p > 0.05$). Since the previous study demonstrated genotype effect on retrieval related hippocampal activity in the group comparison (Val/Val BDNF subjects versus Met-BDNF carriers), we additionally performed the same analysis to estimate genotype effect on retrieval related activity. With a lenient statistical threshold (uncorrected $p < 0.05$), Val/Val-BDNF group showed stronger activation during retrieval in the

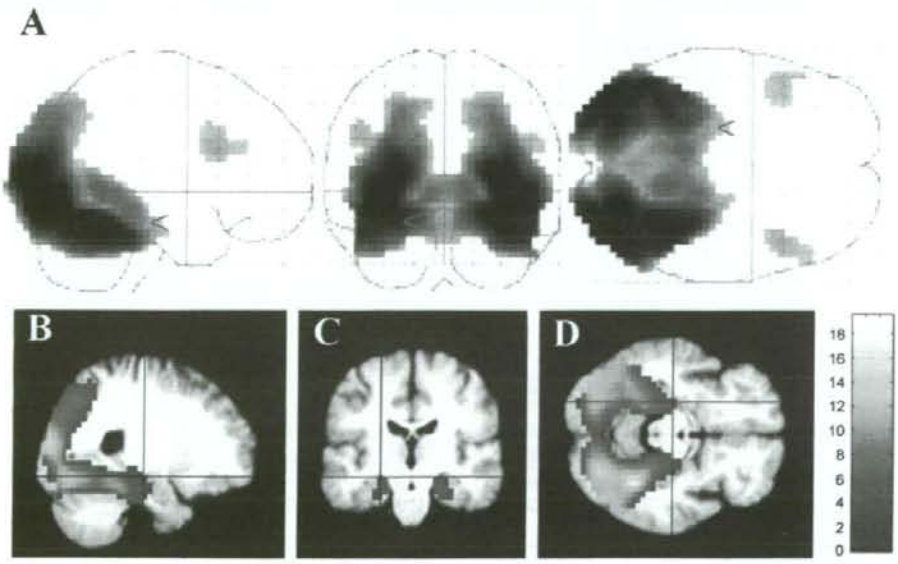

Fig. 1. Brain activity associates with encoding in the whole group. (A) The SPM {*t*} is displayed in a standard format as a maximum-intensity projection (MIP) viewed from the right, the back and the top of the brain as SPM2 "glass brain" representation. The anatomical space corresponds to the atlas of Talairach and Tournoux. Representation in stereotaxic space of brain areas with significant activation was demonstrated. Task-related activations were calculated using a *t*-statistic of SPM2. A significant activation associated with encoding is noted in the bilateral hippocampus, parahippocampal gyri, visual association areas, parietal association areas including precunei and the dorsolateral prefrontal cortices ($p < 0.05$, corrected for multiple comparisons) and anatomical localization, as projected on (B) sagittal, (C) coronal, and (D) axial sections of a normal MRI, spatially normalized into the Montreal Neurological Institute template. The scale of *T* value is indicated on the bottom right.

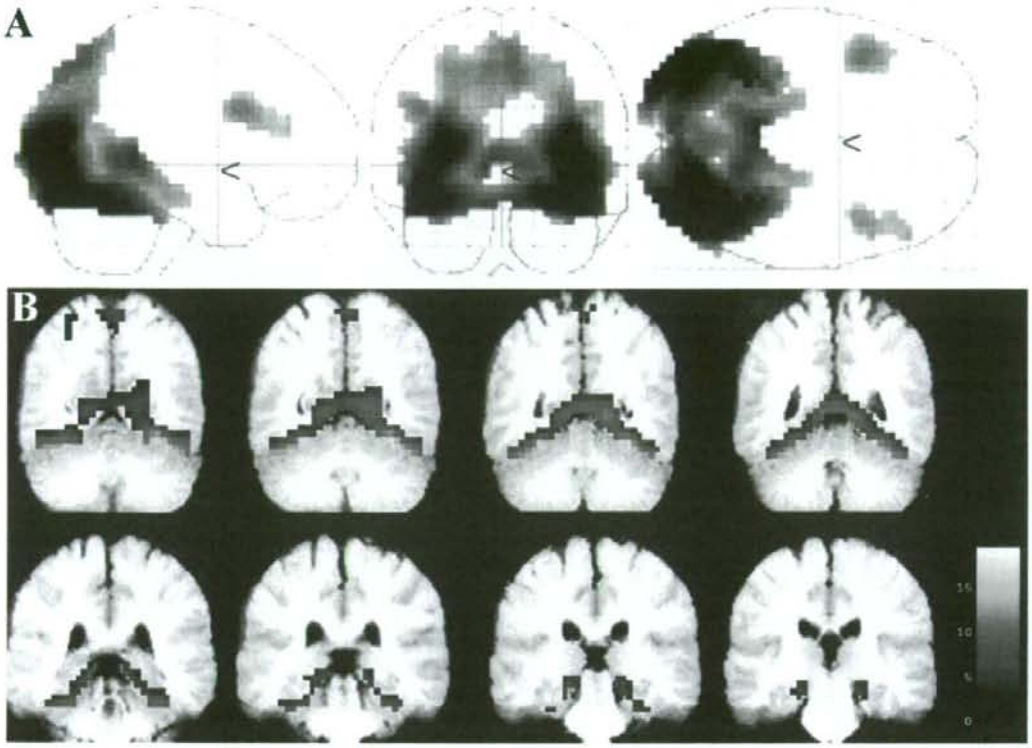

Fig. 2. Brain activity associates with retrieval in the whole group. (A) The SPM {*t*} is displayed in a standard format as a MIP viewed from the right, the back and the top of the brain as SPM2 "glass brain" representation. The anatomical space corresponds to the atlas of Talairach and Tournoux. Representation in stereotaxic space of brain areas with significant activation was demonstrated. Task-related activations were calculated using a *t*-statistic of SPM2. A significant activation was also found in the bilateral hippocampus, parahippocampal gyri, posterior cingulate cortex, visual association areas, parietal association areas and the dorsolateral prefrontal cortices ($p < 0.05$, corrected for multiple comparisons) and anatomical localization, as projected on (B) coronal sections of a normal MRI, spatially normalized into the Montreal Neurological Institute template. The scale of *T* value is indicated on the bottom right.

bilateral parahippocampal gyri and hippocampi. However, no voxels could survive after the correction for multiple comparisons within the ROI (corrected $p > 0.05$). Whole brain correlational analyses demonstrated that the Val66Met polymorphism had no impact on activity within the distributed cortical network, such as visual association areas, parietal association areas and the dorsolateral prefrontal cortex (corrected $p > 0.05$).

As age range of our subjects is relatively broad, age might be a possible confounding factor. Thus, we analyzed the data including age as a covariate. We further re-analyzed the data using subjects leaving out the subjects older than 55 years (Val/Val = 3, Val/Met = 1 and Met/Met = 0) and obtained essentially same results in all analysis (data not shown).

4. Discussion

In the whole subjects' analysis, we found significant bilateral activation in the hippocampus and parahippocampal gyrus during both encoding and retrieval. The data was well concordant with previous fMRI studies of episodic memory. We also replicated significant bilateral activations in the inferotemporal, parietal, and frontal cortices, a distributed network critical for visuospatial information processing (Ungerleider and Haxby, 1994) during both encoding and retrieval. In addition, we found significant activation in the bilateral PCC during retrieval. Several studies have indicated that the PCC have prominent response properties associated with episodic memory, particularly, successful retrieval (Wagner et al., 2005). A significant activation in the PCC associated with retrieval in the present study is well consistent with previous studies.

In terms of effects of the Val66Met polymorphism of the BDNF gene on memory-related brain activity, we obtained two main findings: (1) the Val66Met polymorphism of the BDNF gene affects encoding related neuronal activity in the hippocampal regions with a dose-dependent manner; i.e. a negative correlation between the number of Met allele and the degree of activation in the bilateral hippocampi and parahippocampal gyri during encoding and (2) the Val66Met polymorphism of the BDNF gene did not affects retrieval related neural activity in the hippocampal regions. Further, this

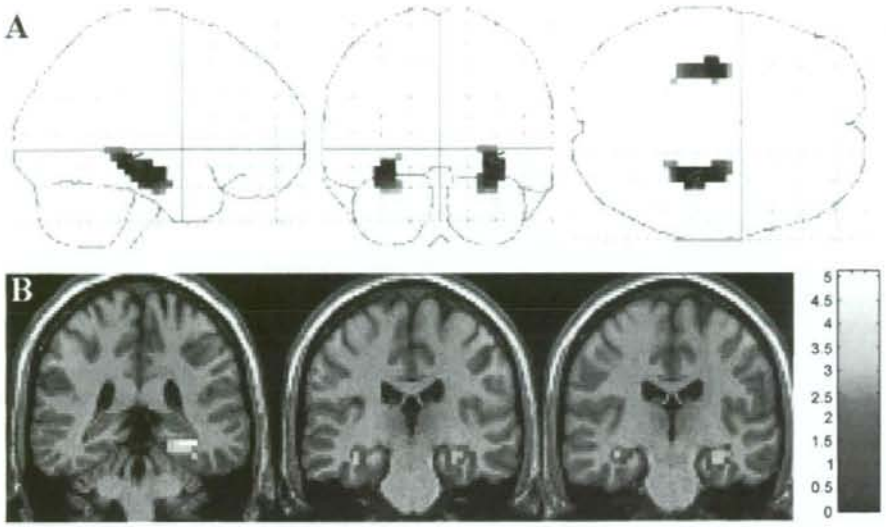

Fig. 3. Genotype effect of the Val66Met polymorphism of the BDNF gene on encoding related hippocampal activity. (A) The SPM {*t*} is displayed in a standard format as a MIP viewed from the right, the back and the top of the brain as SPM2 "glass brain" representation. The anatomical space corresponds to the atlas of Talairach and Tournoux. Representation in stereotaxic space of brain areas with significant activation was demonstrated. A significant negative correlation between numbers of Met allele and degree of activation is noted in the bilateral hippocampal regions calculated by correlational analysis of SPM2 ($p < 0.05$, corrected for multiple comparisons) and anatomical localization, as projected on (B) coronal sections of a normal MRI, spatially normalized into the Montreal Neurological Institute template.

polymorphism had no effects on activity within the other distributed cortical network, such as visual association areas, parietal association areas and the dorsolateral prefrontal cortex involved with general visuo-spatial information processing. Consistent with the previous fMRI study of the Val66Met polymorphism (Hariri et al., 2003), our data also suggest that this polymorphism could have impact on memory-related neuronal activity of the hippocampal region in an Asian sample. In addition, our data indicate Met-BDNF dose-dependent

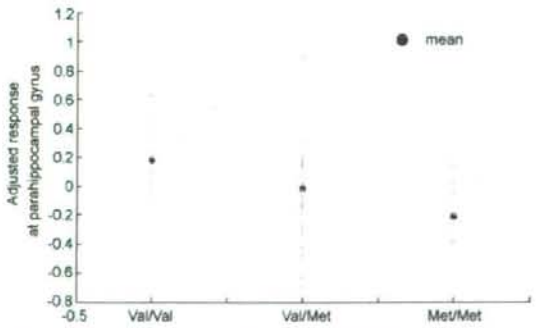

Fig. 4. Dose-dependent effect of Met allele on hippocampal activation. Activation in parahippocampal gyrus during encoding task was calculated in each genotype individual (Val/Val = 17, Val/Met = 29 and Met/Met = 12) by the SPM2. *X*-axis: adjusted response at parahippocampal gyrus; *Y*-axis: genotype group. A gray dot indicates each individual response and a black dot indicates a mean response in the genotype groups. Negative correlation between activation associated with encoding and genotype was calculated by correlational analysis of SPM2 ($p < 0.05$, corrected for multiple comparisons).

effects of the Val66Met polymorphism on hippocampal activity during encoding. The specific effect of BDNF on hippocampal activity is well concordant with the expression pattern of BDNF in the brain, which is highest in the hippocampus (Murer et al., 2001). This effect of the Val66Met polymorphism on BOLD response in the hippocampal region may be mediated through alterations in activity-dependent hippocampal processes requiring BDNF-regulated secretion, particularly the abnormal intracellular trafficking and regulated secretion of BDNF in Met carriers, which may result in impaired hippocampal LTP that could underlie encoding (Egan et al., 2003; Chen et al., 2004). Most neuroimaging studies also have demonstrated genotype effects on hippocampal region, i.e. Met allele is associated with reduced hippocampal volume and impaired hippocampal function (Hariri et al., 2003; Pezawas et al., 2004; Bueller et al., 2006). However, owing to the relatively small sample size and the low frequency of the Met allele, these studies, including the previous memory fMRI study, used combined Met/Met and Val/Met subjects (Hariri et al., 2003; Pezawas et al., 2004; Bueller et al., 2006). On the other hand, our study with a relatively larger sample, particularly individuals with Met-allele, revealed dose-dependent genotype effects of the Val66Met polymorphism on encoding-related hippocampal activity. Our data support and strengthen the results of the previous fMRI study.

The previous study demonstrated that the Val66Met polymorphism affected both on encoding and retrieval related hippocampal activity (Hariri et al., 2003), however, we could not found genotype effects on retrieval related hippocampal activity. Considering the possible underlying mechanism of

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

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

Someone may argue that genotype effects of the Val66Met polymorphism on encoding related hippocampal activity observed in this study seem inconsistent with the result of behavioral measures; i.e. no significant BDNF genotype effects on memory-related behavioral measures. In general, it is considered that the difference of a missense polymorphism, i.e. the BDNF Val66Met polymorphism and the Val158Met polymorphism of the catechol-*O*-methyl transferase (COMT) gene, firstly causes the difference of function in the protein level, and then that the distinct protein function influences neuronal functions in the cellular level. Distinct cellular functions might affect brain activities in the network level and these differences of brain activities could result in the distinct performance in the behavioral level. Thus, genotype effects could be stronger in order of the protein level, the cellular level, the network level and the behavioral level. The difficulty to replicate the genotype effects in the behavioral level is well known particularly in the phenotype of psychiatric disorders (Preston and Weinberger, 2005). Thus, the brain activity might reflect genotype effects more directly than the behavior does in our study. A similar phenomenon – no significant effect of the COMT Val158Met polymorphism on working memory performance but significant effects on brain activities during a working memory task – was reported (Ho et al., 2005). Since there was no behavioral effect but effect observed in fMRI in hippocampus/parahippocampal gyrus (HPHG) region, this raises another possibility that the fMRI effect of Val/Met is not relevant to memory but epiphenomenal, secondary to effects either in other, undetected brain regions projecting to HPHG or to HPHG activity unrelated to memory. The present study suggests that measurement of brain activity related to particular tasks should be useful to investigate relationships between several functional SNPs and human cognitive functions.

Acknowledgments

The authors thank Ms. Tomoko Shizuno for technical assistance. This work was supported in part by Grants-in-Aid from the Japanese Ministry of Health, Labor and Welfare (H17-kokoro-007); the Japanese Ministry of Education, Culture, Sports, Science and Technology; Japan Society for the Promotion of Science; CREST (Core research for Evolutional Science and Technology) of JST (Japan Science and Technology Agency); Japan Foundation for Neuroscience and Mental Health.

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American Journal of Medical Genetics Part B (Neuropsychiatric Genetics) 147B:1013–1018 (2007)

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

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NRG1-ERBB signaling influences the risk for schizophrenia pathology. A recent study has reported that *MAGI1*, *MAGI2*, and protein tyrosine phosphatase, receptor-type, Z polypeptide 1 (*PTPRZ1*; located on 7q31.3) gene products regulate the NRG1-ERBB4 signaling pathway, and *PTPRZ1* is associated with schizophrenia in a Caucasian population. By applying a gene-based association concept, we analyzed any association between *PTPRZ1* tagging SNPs and schizophrenia in the Japanese population (576 schizophrenics and 768 controls). After linkage disequilibrium analysis, 29 single nucleotide polymorphisms (SNPs) were genotyped using a 5′-exonuclease allelic discrimination assay. We found a significant association of one tagging SNP in a genotype-wise analysis ($P = 0.007$); however, this might be resulted from type I error due to multiple testing ($P = 0.17$ after SNPSpD correction). No association was observed between schizophrenic patients and controls in either allelic, genotypic, or haplotypic analyses. Our results therefore suggest that *PTPRZ1* is unlikely to be related to the development of schizophrenia in the Japanese population.

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

How to cite this article: Ito Y, Yamada S, Takahashi N, Saito S, Yoshimi A, Inada T, Noda Y, Ozaki N. 2008. No Association Between the Protein Tyrosine Phosphatase, Receptor-Type, Z Polypeptide 1 (*PTPRZ1*) Gene and Schizophrenia in the Japanese Population. Am J Med Genet Part B 147B:1013–1018.

Grant sponsor: Ministry of Education, Culture, Sports, Science and Technology of Japan; Grant sponsor: Ministry of Health, Labour and Welfare of Japan; Grant sponsor: Japanese Health Sciences Foundation (Research on Health Sciences Focusing on Drug Innovation).

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Received 29 August 2007; Accepted 13 November 2007

DOI 10.1002/ajmg.b.30692

Published online 9 January 2008 in Wiley InterScience (www.interscience.wiley.com)

INTRODUCTION

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

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

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

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

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