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Gene Expression and Association Analyses of the Phosphodiesterase 4B (PDE4B) Gene in Major Depressive Disorder in the Japanese Population

Shusuke Numata,[1]* Jun-ichi Iga,[1] Masahito Nakataki,[1] Shin'Ya Tayoshi,[1] Kyoko Taniguchi,[1] Satsuki Sumitani,[1] Masahito Tomotake,[1] Toshihito Tanahashi,[2] Mitsuo Itakura,[2] Yoko Kamegaya,[3] Masahiko Tatsumi,[4] Akira Sano,[5] Takashi Asada,[6] Hiroshi Kunugi,[3] Shu-ichi Ueno,[7,8] and Tetsuro Ohmori[1]

[1]Department of Psychiatry, Course of Integrated Brain Sciences, Medical Informatics, Institute of Health Biosciences, The University of Tokushima Graduate School, Tokushima, Japan

[2]Division of Genetic Information, Institute for Genome Research, The University of Tokushima Graduate School, Tokushima, Japan

[3]Department of Mental Disorder Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Tokyo, Japan

[4]Yokohama Shinryo Clinic, Kanagawa, Japan

[5]Department of Psychiatry, Kagoshima University Graduate School of Medical and Dental Science, Kagoshima, Japan

[6]Department of Psychiatry, Institute of Clinical Medicine, University of Tsukuba, Tsukuba, Japan

[7]Department of Community and Psychiatric Nursing, School of Health Sciences, The University of Tokushima Graduate School, Tokushima, Japan

[8]Department of Neuropsychiatry, Neuroscience, Ehime University Graduate School of Medicine, Ehime, Japan

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The phosphodiesterase 4B (PDE4B) interacts with disrupted-in-schizophrenia 1 (DISC1), which is a known genetic risk factor for schizophrenia, bipolar disorder and major depressive disorder (MDD). PDE4B is also important in the regulation of cAMP signaling, a second messenger implicated in learning, memory, and mood. In this study, we determined mRNA expression levels of the PDE4B gene in the peripheral blood leukocytes of patients with MDD and control subjects (n = 33, each). Next we performed two-stage case-controlled association analyses (first set; case = 174, controls = 348; second set; case = 481, controls = 812) in the Japanese population to determine if the PDE4B gene is implicated in MDD. In the leukocytes, a significantly higher expression of the PDE4B mRNA was observed in the drug-naïve MDD patients compared with control subjects ($P < 0.0001$) and the expression of the MDD patients significantly decreased after antidepressant treatment ($P = 0.030$). In the association analysis, we observed significant allelic associations of four SNPs (the most significant, rs472952; $P = 0.002$) and a significant haplotypic association (permutation $P = 0.019$) between the PDE4B gene and MDD in the first-set samples. However, we could not confirm these significant associations in the following independent second-set of samples. Our results suggest that the PDE4B gene itself does not link to MDD but the elevated mRNA levels of PDE4B might be implicated in the pathophysiology of MDD.

Key words: PDE4B; DISC1; association analysis; expression analysis; major depressive disorder; peripheral blood leukocytes-

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*Correspondence to:
Shusuke Numata, M.D., Ph.D., Department of Psychiatry, Course of Integrated Brain Sciences, Medical Informatics, Institute of Health Biosciences, The University of Tokushima Graduate School, 3-18-15 Kuramoto-cho, Tokushima 770-8503, Japan.
E-mail: shu-numata@umin.ac.jp
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INTRODUCTION

The lifetime population prevalence for major depressive disorder (MDD) is 5–10%. Heritability based on twin studies of MDD is 40–50% and adoption studies provide some support for a role for genetic factors [Levinson, 2006]. Phosphodiesterases control intracellular concentrations of cyclic adenosine monophosphate (cAMP), a second messenger implicated in learning, memory, and mood, by catalyzing its hydrolysis [Davis et al., 1995; Lamprecht, 1999; Bauman et al., 2004]. The phosphodiesterase 4B (PDE4B) gene is located at chromosome 1p31 and is directly disrupted by a chromosomal translocation in a patient diagnosed with schizophrenia (SZ) and a cousin with chronic psychiatric illness in Scotland [Millar et al., 2005]. They reported that disrupted-in-schizophrenia 1 (DISC1), which is an important genetic risk factor for mental disorders such as SZ, bipolar disorder (BP) and MDD [Hennah et al., 2003; Hodgkinson et al., 2004; Hashimoto et al., 2006], interacts with the UCR2 domain of PDE4B and that elevation of cellular cAMP leads to dissociation of PDE4B from DISC1 and an increase in PDE4B activity [Millar et al., 2005]. Long PDE4 isoforms are activated upon phosphorylation of UCR1 by protein kinase A (PKA) and are transiently inhibited by phosphorylation of their catalytic domains by extra cellular signal regulated kinase (ERK) [Houslay and Adams, 2003; Houslay et al., 2005] and genetic variation of the DISC1 gene is associated with lower biological activity on ERK signaling [Hashimoto et al., 2006]. These results imply that the DISC1-PDE4B interaction is important in the regulation of cAMP signaling and previous studies have demonstrated adaptations of the cAMP signal transduction cascade in response to antidepressant treatment [Nestler et al., 1989; Nibuya et al., 1996; Duman et al., 1997].

Several studies provide evidences that PDE4B is an MDD susceptibility factor. PDE4B is localized in high levels within midbrain regions that are implicated in depression [Cherry and Davis, 1999]. PDE4B is involved in not only serotonin- but also noradrenalin-mediated neurotransmission signaling because repeated antidepressant administration of fluoxetine and desipramine decreases the expression of the Pde4b mRNA in mouse hippocampus [Dlaboga et al., 2006]. And chronic nicotine treatment, which may have an antidepressant effect, caused a down-regulation of Pde4b transcripts in rat hippocampus [Polesskaya et al., 2007]. Furthermore, Pde4b knockout mice showed a modest decrease in immobility time in the forced swim test (depression-like behavior) and altered hippocampal levels of serotonin [Siuciak et al., 2008]. Rolipram, a selective inhibitor of PDE4, is also reported to be efficacious in MDD patients [Laux et al., 1988; Fleischhacker et al., 1992].

Recently, Pickard et al. and our group reported significant associations between the PDE4B gene and SZ [Pickard et al., 2007; Numata et al., 2008]. Maier et al. [1993] reported that SZ probands had an increased familial risk for unipolar MDD and that there could be a familial relationship between the predispositions to SZ and to MDD. Families in which multiple cases of SZ, BP and MDD occur have been identified [Millar et al., 2000]. These reports support that SZ and MDD have at least a partially common genetic background.

Taken together, the findings mentioned above suggest that the PDE4B gene may be a susceptibility one to MDD. In this study, we determined mRNA expression levels of the PDE4B gene in the peripheral leukocytes of patients with MDD and control subjects, and performed case-controlled association analyses to determine if the PDE4B gene is implicated in MDD in the Japanese population.

MATERIALS AND METHODS

Gene Expression Analysis

Subjects for analysis. For the expression studies, we obtained blood samples from 33 drug-naïve MDD patients (8 male [mean age: 39.1 ± 12.7 years] and 25 female [mean age: 41.2 ± 14.0 years]) from Tokushima University Hospital in Japan. Twenty-eight patients were in the first depressive episode and other five were in the recurrent episode. The diagnosis of MDD was made by at least two experienced psychiatrists according to DSM-IV criteria [American Psychiatric Association, 1994]. Clinical symptoms were evaluated by the 17-item Hamilton Depressive Rating Scale [SIGH-D 17, Williams, 1988] when blood samples were taken. Mean HAM-D scores were 21.9 ± 6.9. Thirty-three sex- and age-matched controls were selected from volunteers (8 male [mean age: 38.0 ± 13.2 years] and 25 female [mean age: 39.6 ± 11.2]) who were in good physical health with a history of neither psychiatric nor serious somatic disease and were not taking any medication. We were able to follow up 18 patients treated with paroxetine. The mean paroxetine dose was 28.3 ± 9.2 mg/day and the mean duration of treatment was 54.5 ± 20.9 days. All subjects signed written informed consent to participate in the expression studies approved by the institutional ethics committees.

Quantitative reverse transcriptase polymerase chain reaction. Total RNA was extracted from the peripheral leukocytes of whole blood samples using the PAXgene Blood RNA kit [Qiagen, Tokyo, Japan] according to the protocol recommended by the manufacturer. Two micrograms of total RNA was used for cDNA synthesis by random (N6) primers and Quantiscript Reverse Transcriptase [Qiagen, Tokyo, Japan] after assessing RNA quality and quantity with NanoDrop (NanoDrop Technologies, Wilmington, DE). Real-time quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) analysis was performed with the ABI PRISM 7900 Sequence Detection System. The human PDE4B gene encodes four isoforms (the long PDE4B1 and PDE4B3, the short PDE4B2, and the super-short PDE4B5) [Cheung et al., 2007] and we measured the expression levels of total of these PDE4B isoforms because all these isoforms have been shown to interact with DISC1 [Millar et al., 2005; Cheung et al., 2007]. Taqman primer/probes for the PDE4B (Hs00963641_m1) gene were purchased from Applied Biosystems with the GAPDH and HPRT gene as endogenous references. All reactions were performed in triplicate. A comparative threshold cycle (C_t) method validation experiment was done. One sample was chosen as the calibrator and was amplified in each plate to correct for experimental differences among consecutive PCR runs. The amounts of PDE4B mRNA were normalized to the endogenous reference and expressed relative to the calibrator as $2^{-\Delta\Delta C_t}$ (comparative C_t method).

Statistical analysis. Statistical calculations were carried out using the SPSS Statistical Software Package 11.5 (SPSS, Tokyo, Japan). Expressional differences between patients and control subjects were calculated using the Mann–Whitney *U*-test. Regression analyses were used to examine the variability in the distribution of demographic variables (age, sexes, number of episodes, age of onset, hereditary load and HAM-D scores).

Association Study

Subjects for analysis. The first sample set was collected in the Tokushima University and the Ehime University Hospital in Japan. 174 MDD patients (74 male [mean age: 45.1 ± 12.4 years] and 100 female [mean age: 45.4 ± 15.5 years]) and 348 controls selected from volunteers (148 male [mean age: 45.1 ± 12.4 years] and 200 female [mean age: 44.9 ± 13.0]) were genotyped. The replication sample set was collected in Showa University School of Medicine and National Center of Neurology and Psychiatry in Japan (second sample set). Four hundred eighty-one MDD patients (187 male [mean age: 46.6 ± 14.6 years] and 294 female [mean age: 54.9 ± 16.5 years]) and 812 controls selected from volunteers (308 male [mean age: 44.1 ± 17.4 years] and 504 female [mean age: 44.3 ± 15.9]) were genotyped. The diagnosis of MDD was made by at least two experienced psychiatrists according to DSM-IV criteria [American Psychiatric Association, 1994]. Control subjects were healthy volunteers who had no current or past contact to psychiatric services. All subjects signed written informed consent to participate in the genetic association studies approved by the institutional ethics committees.

Genotyping. Genotyping was performed using commercially available TaqMan probes for the PDE4B gene with the ABI 7500 Fast Real Time PCR System and the ABI PRISM 7900 Sequence Detection System, according to the protocol recommended by the manufacturer (Applied Biosystems, Foster City, CA). We selected 19 single nucleotide polymorphic (SNP) markers at an average density of 21.4 kb in the PDE4B gene.

Statistical analysis. Allelic and genotypic frequencies of patients and control subjects were compared using Fisher's exact test. The SNPAlyze 3.2Pro software [DYNACOM, Japan] was used to estimate haplotype frequencies, LD, permutation p values (10,000 replications). Pair-wise LD indices (D′) were calculated for the control subjects. Power calculations for our sample size performed using the G*Power program [Erdfelder et al., 1996]. The criterion for significance was set at $P < 0.05$ for all tests.

RESULTS

Gene Expression Analysis

The PDE4B mRNA levels in the peripheral leukocytes before treatment. Drug-naïve MDD patients showed significantly higher expression levels of the PDE4B gene normalized by the GAPDH gene in the peripheral leukocytes (Patients; 1.59 ± 0.80, Controls; 0.91 ± 0.35: Mann–Whitney *U*-test, $P < 0.0001$, Fig. 1a). This difference was also confirmed using normalization by the HPRT gene (Mann–Whitney *U*-test, $P < 0.001$). With regard to demographic characteristics (age, sexes, number of episodes, age of onset, hereditary load, and HAM-D scores), there were not significant differences in mRNA expression levels of the PDE4B gene.

The PDE4B mRNA levels in the peripheral leukocytes after several week-paroxetine treatment. HAM-D scores were significantly improved after paroxetine treatment (N = 18, the mean HAM-D scores at baseline; 20.7 ± 6.5, at after treatment; 8.2 ± 7.1, Wilcoxon rank sum test, $P < 0.0001$) and the PDE4B mRNA levels of MDD patients significantly decreased after treatment compared with those before treatment (N = 18, the mean the PDE4B mRNA levels at baseline; 1.52 ± 0.86, at after treatment; 1.26 ± 0.55, Wilcoxon rank sum test, $P = 0.030$, Fig. 1b).

Genetic Association Study

In the first sample set, we genotyped 19 SNPs of the PDE4B gene in 174 MDD patients and 348 controls. Genotypic and allelic frequencies of these 19 SNPs in the PDE4B gene are shown in Table I.

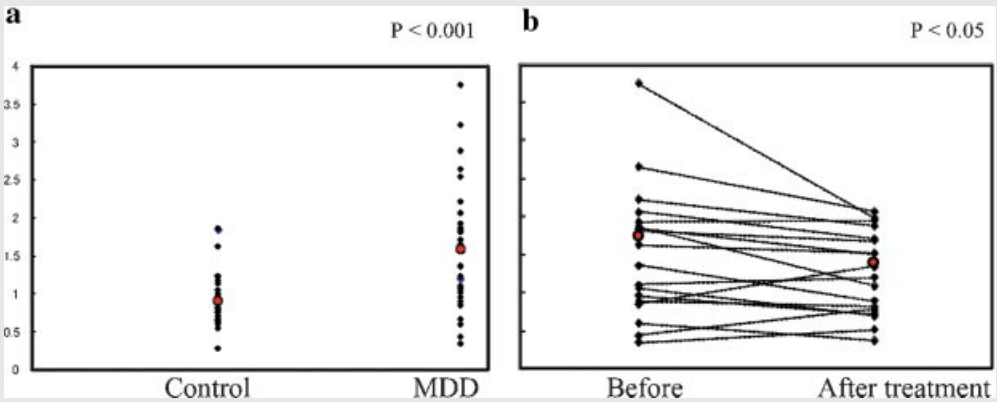

FIG. 1. mRNA expression of the PDE4B gene in the peripheral leukocytes. a: Patients with MDD showed significantly higher expression levels of PDE4B normalized by GAPDH in the peripheral leukocytes compared to control subjects (n = 33, each: patients; 1.59 ± 0.80, controls; 0.91 ± 0.35, Mann–Whitney *U*-test, $P < 0.0001$). b: After antidepressant treatment, the expression decreased significantly (n = 18, at baseline; 1.52 ± 0.86, at after treatment; 1.26 ± 0.55, Wilcoxon rank sum test, $P = 0.030$).

TABLE I. Association of SNPs in PDE4B with Major Depressive Disorder (First Sample Set)

						Allele			Genotype				
SNP	**Marker**	**Position**	**Diagnosis**	**HWE**	**n**	**C**	**G**	***P*-value**	**C/C**	**C/G**	**G/G**	***P*-value**	**Frequency**
SNP1	rs1317611	0	MDD	0.494	172	194	150	0.894	52	90	30	0.691	0.44
			CT	0.854	348	396	300		114	168	66		0.43

						Allele			Genotype				
SNP	**Marker**	**Position**	**Diagnosis**	**HWE**	**n**	**A**	**G**	***P*-value**	**A/A**	**A/G**	**G/G**	***P*-value**	**Frequency**
SNP2	rs12567612	8,736	MDD	0.556	172	212	132	1	63	86	23	0.966	0.38
			CT	0.597	348	430	266		130	170	48		0.38

						Allele			Genotype				
SNP	**Marker**	**Position**	**Diagnosis**	**HWE**	**n**	**C**	**G**	***P*-value**	**C/C**	**C/G**	**G/G**	***P*-value**	**Frequency**
SNP3	rs1937443	17866	MDD	0.55	173	185	161	0.742	47	91	35	0.283	0.47
			CT	0.116	348	364	332		103	158	87		0.48

						Allele			Genotype				
SNP	**Marker**	**Position**	**Diagnosis**	**HWE**	**n**	**A**	**G**	***P*-value**	**A/A**	**A/G**	**G/G**	***P*-value**	**Frequency**
SNP4	rs1354061	54924	MDD	0.06	172	132	212	0.495	19	94	59	0.364	0.38
			CT	0.521	348	251	445		42	167	139		0.36
SNP5	rs7539350	94599	MDD	0.495	171	202	140	0.946	57	88	26	0.95	0.41
			CT	0.606	345	410	280		119	172	54		0.41

						Allele			Genotype				
SNP	**Marker**	**Position**	**Diagnosis**	**HWE**	**n**	**G**	**T**	***P*-value**	**G/G**	**G/T**	**T/T**	***P*-value**	**Frequency**
SNP6	rs4004	125825	MDD	0.207	173	270	76	0.369	102	66	5	0.261	0.22
			CT	0.944	348	560	136		226	108	14		0.2

						Allele			Genotype				
SNP	**Marker**	**Position**	**Diagnosis**	**HWE**	**n**	**A**	**G**	***P*-value**	**A/A**	**A/G**	**G/G**	***P*-value**	**Frequency**
SNP7	rs2503174	157377	MDD	0.176	172	133	211	0.191	21	91	60	0.191	0.39
			CT	0.913	348	239	457		41	157	150		0.34

						Allele			Genotype				
SNP	**Marker**	**Position**	**Diagnosis**	**HWE**	**n**	**C**	**T**	***P*-value**	**C/C**	**C/T**	**T/T**	***P*-value**	**Frequency**
SNP8	rs1338719	197,757	MDD	0.281	172	129	215	0.839	28	73	71	0.118	0.38
			CT	0.096	348	266	430		43	180	125		0.38
SNP9	rs6700971	227,372	MDD	0.186	173	130	216	0.787	29	72	72	0.231	0.38
			CT	0.483	348	268	428		48	172	128		0.39
SNP10	rs6588190	245,591	MDD	0.013	173	245	101	0.667	94	57	22	0.016	0.29
			CT	0.291	347	481	213		162	157	28		0.31
SNP11	rs4320761	259,191	MDD	0.006	174	243	105	0.571	93	57	24	0.011	0.3
			CT	0.377	342	465	219		154	157	31		0.32

						Allele			Genotype				
SNP	**Marker**	**Position**	**Diagnosis**	**HWE**	**n**	**A**	**G**	***P*-value**	**A/A**	**A/G**	**G/G**	***P*-value**	**Frequency**
SNP12	rs599381	308,783	MDD	0.854	173	21	325	0.602	0	21	152	0.5	0.06
			CT	0.296	347	50	644		0	50	297		0.07
C	T	C/C	C/T	T/T									

(*Continued*)

TABLE I. (*Continued*)

						Allele			Genotype				
SNP	**Marker**	**Position**	**Diagnosis**	**HWE**	**n**	**A**	**G**	***P*-value**	**A/A**	**A/G**	**G/G**	***P*-value**	**Frequency**
SNP13	rs498448	315,003	MDD	0.048	174	194	154	0.895	61	72	41	0.16	0.44
			CT	0.896	345	388	302		108	172	65		0.44
						Allele			**Genotype**				
SNP	**Marker**	**Position**	**Diagnosis**	**HWE**	**n**	**A**	**T**	***P*-value**	**A/A**	**A/T**	**T/T**	***P*-value**	**Frequency**
SNP14	rsI040716	325,813	MDD	0.068	174	64	284	**0.012**	10	44	120	0.021	0.18
			CT	0.5	342	174	510		25	124	193		0.25
						Allele			**Genotype**				
SNP	**Marker**	**Position**	**Diagnosis**	**HWE**	**n**	**A**	**G**	***P*-value**	**A/A**	**A/G**	**G/G**	***P*-value**	**Frequency**
SNP15	rs2180335	334,153	MDD	0.42	174	59	289	**0.009**	7	45	122	0.029	0.17
			CT	0.526	347	168	526		23	122	202		0.24
						Allele			**Genotype**				
SNP	**Marker**	**Position**	**Diagnosis**	**HWE**	**n**	**C**	**T**	***P*-value**	**C/C**	**C/T**	**T/T**	***P*-value**	**Frequency**
SNP16	rs910694	344,449	MDD	0.42	174	59	289	**0.004**	7	45	122	0.013	0.17
			CT	0.956	347	172	522		22	128	197		0.25
						Allele			**Genotype**				
SNP	**Marker**	**Position**	**Diagnosis**	**HWE**	**n**	**A**	**G**	***P*-value**	**A/A**	**A/G**	**G/G**	***P*-value**	**Frequency**
SNP17	rs472952	348,987	MDD	0.46	173	54	292	**0.002**	6	42	125	0.007	0.16
			CT	0.515	348	168	528		23	122	203		0.24
SNP18	rs783036	379,593	MDD	0.456	173	48	298	0.154	5	38	130	0.192	0.14
			CT	0.944	348	122	574		10	102	236		0.18
SNP19	rs3767311	385,422	MDD	0.944	172	33	311	0.18	2	29	141	0.31	0.1
			CT	0.635	346	87	605		4	79	263		0.13

HWE mean *P* values for Hardy–Weinberg equilibrium. Statistical differences in genotypic and allelic distributions were evaluated using the Fisher's exact test. Values of $P < 0.05$ are shown in bold.

Genotypic distributions of these SNPs did not deviate from HW equilibrium in control group ($P > 0.05$). Significant differences in allelic frequencies were observed between MDD patients and controls for four SNPs (rs1040716, rs2180335, rs910694, and rs472952) ($P = 0.012$, 0.009, 0.004, and 0.002, respectively). After applying the Bonferroni correction test, one SNP (rs472952) still had significant allelic associations with MDD ($P = 0.032$). Next we performed haplotype analyses. The values of absolute D′ for the control subjects are presented in Figure 2. There were three LD blocks [Gabriel et al., 2002] in the PDE4B gene: rs1317611, rs12567613, and rs1937443 residing in block 1; rs6588190 and rs4320761 residing in block 2; and rs2180335 and rs910694 residing in block 3. The two marker haplotypes of block 3 were associated with MDD (permutation $P = 0.017$), while the three marker haplotypes of block 1 and the two marker haplotypes of block 2 were not associated with MDD (permutation $P = 0.944$ and 0.791, respectively). In power calculations using the G*Power program, the sample size in the first sample set had 0.58 power for detecting a significant association (alpha < 0.05) when an effect size index of 0.2 was used.

To examine whether or not this is a false-positive finding, we further genotyped an independent second sample set. We genotyped 4 SNPs, which were significantly associated with our first sample set, in 481 MDD patients and 812 controls of the second sample set. Genotypic and allelic frequencies of these four SNPs in the PDE4B gene are shown in Table II. Genotypic distributions of these SNPs did not deviate from HW equilibrium in control group ($P > 0.05$). Significant differences in allelic frequencies were not observed between MDD patients and controls for these four SNPs. There was no significant haplotypic association of block 3 ($P = 0.515$). In power calculations using the G*Power program, the sample size in the second sample set had 0.93 power for detecting a significant association (alpha < 0.05) when an effect size index of 0.2 was used.

DISCUSSION

In this study, we performed an mRNA expression analysis of the PDE4B gene in the peripheral blood leukocytes of MDD and control

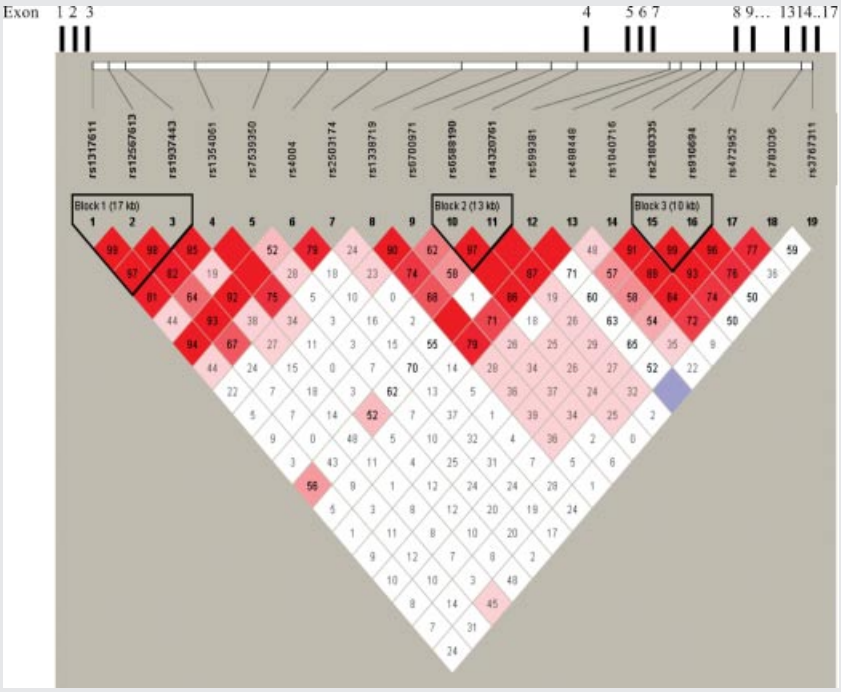

FIG. 2. Haplotype block structure of the PDE4B gene. Haplotype block structure was determined using the HAPLOVIEW program [Barrett et al., 2005]. Blocks were defined according to the criteria of Gabriel et al. [2002]. There were three LD blocks in the Japanese population (rs1317611, rs12567613, and rs1937443 reside in the block 1; rs6588190 and rs4320761 in the block 2; rs2180335 and rs910694 in the block 3). Each box represents the D′ corresponding to each pair-wise single nucleotide polymorphism. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE II. Association of SNPs in PDE4B With Major Depressive Disorder (Second Sample Set)

					Allele			Genotype				
SNP	**Marker**	**Diagnosis**	**HWE**	**n**	**A**	**T**	***P*-value**	**A/A**	**A/T**	**T/T**	***P*-value**	**Frequency**
SNP14	rs1040716	MDD	0.538	464	234	694	0.191	27	180	257	0.369	0.25
		CT	0.844	802	367	1,237		41	285	476		0.23
					Allele			**Genotype**				
SNP	**Marker**	**Diagnosis**	**HWE**	**n**	**A**	**G**	***P*-value**	**A/A**	**A/G**	**G/G**	***P*-value**	**Frequency**
SNP15	rs2180335	MDD	0.654	465	190	740	0.501	21	148	296	0.712	0.2
		CT	0.993	807	311	1,303		30	251	526		0.19
					Allele			**Genotype**				
SNP	**Marker**	**Diagnosis**	**HWE**	**n**	**C**	**T**	***P*-value**	**C/C**	**C/T**	**T/T**	***P*-value**	**Frequency**
SNP16	rs910694	MDD	0.557	461	187	735	0.641	21	145	295	0.701	0.2
		CT	0.729	807	314	1,300		29	256	522		0.19
					Allele			**Genotype**				
SNP	**Marker**	**Diagnosis**	**HWE**	**n**	**A**	**G**	***P*-value**	**A/A**	**A/G**	**G/G**	***P*-value**	**Frequency**
SNP17	rs472952	MDD	0.521	467	192	742	0.537	22	148	297	0.608	0.21
		CT	0.716	805	314	1,296		29	256	520		0.2

HWE means *P* values for Hardy–Weinberg equilibrium. Statistical differences in genotypic and allelic distributions were evaluated using the Fisher's exact test.

subjects and case-controlled association analyses of the PDE4B gene to clarify its implication in MDD.

First, we observed a significantly higher expression of the PDE4B mRNA in the peripheral blood leukocytes of drug-naïve patients with MDD when compared with control subjects. PDE4B shows high enrichment in human blood fractions and nervous system tissues [Cheung et al., 2007]. Increased mRNA expression of the PDE4B gene in the leukocytes may provide a clue for the pathophysiology in MDD because lymphocytes could reflect the metabolism of brain cells, and may be exploited as a neural and possible genetic probe in studies of psychiatric disorders [Gladkevich et al., 2004]. There have been several reports that the Pde4b expression is changed in depressive models. Jin and Conti reported that lipopolysaccharide (LPS), which may induce depressive-like behavior [Yirmiya, 1996; Frenois et al., 2007; O'Connor et al., 2008], induces the Pde4b2 in the blood leukocytes [Jin and Conti, 2002]. They concluded that this induction is consistent with the observation that LPS specifically increases PDE4B transcripts in human monocytes [Ma et al., 1999]. In our study, there was not significant relation between the mRNA expression levels of the PDE4B gene and HAM-D scores. Neither patients nor controls showed a significant difference of the mRNA expression levels of the PDE4B gene between genotypes of the SNP rs472952, which showed the most significant association with MDD in our first set samples. Our result of the gene expression analysis in the leukocytes may reflect altered PDE4B expression in the brain and dysfunctions of cAMP signaling of medication-free MDD patients.

Previous expression studies after antidepressant administration in rodents reported inconsistent results, depending on antidepressant drugs, species, and brain regions [Takahashi et al., 1999; Miró et al., 2002; Dlaboga et al., 2006]. Our result that the PDE4B mRNA levels of MDD patients decreased after paroxetine treatment is consistent with a study showing down-regulation of pde4b mRNA in hippocampus after SSRI administrations [Dlaboga et al., 2006]. The decrease of the PDE4B mRNA expression after treatment may be a consequence of pharmacological effects of paroxetine or clinical improvement.

During the preparation of this manuscript, Padmos et al. [2008] reported that the PDE4B mRNA expression in the monocytes of bipolar disorder patients is higher than those of controls. The PDE4B transcripts measured in their study were the same ones in our study. They and we measured the PDE4B expression levels of total of PDE4B isoforms without distinguishing four human isoforms (the long PDE4B1 and PDE4B3, the short PDE4B2 and the super-short PDE4B5). Both studies indicate that the elevated mRNA levels of PDE4B may play an important role in the pathophysiology of mood disorders. Future expression studies with separating each isoforms may further clarify the present results.

Second, we investigated the genetic association between the PDE4B gene and MDD. In the first sample set (174 MDD patients, 348 controls), we observed a significant allelic association of four SNPs in introns 7 and 8, however, we could not replicate these results in the second independent case–control samples (the second sample set; 481 MDD patients, 812 controls). Because the sample size of the second sample set was large enough, the positive result in the first sample set might be the result of type I error due to an inadequate sample size. Our negative result of the genetic association study is consistent with a previous study [Wong et al., 2006]. When we analyzed the pooled data from both first and second sample sets (a total of 655 patients and 1,160 controls), no significant association to MDD was found for any of the four SNPs typed at the allelic or genotypic level. There was no significant haplotypic association of block 3 in the pooled data, either (permutation $P = 0.295$). We did not analyze population stratification of our samples because there has been reported to be no population stratification in Japanese subjects. Because a previous study reported sex-dependent effect of PDE4B on association with SZ [Pickard et al., 2007], we subdivided this pooled data on the basis of sex. However, no significant association was observed in either male or female samples. In association studies, we selected 19 SNPs at an average density of 21.4 kb in the PDE4B gene. Further association studies with dense markers will be needed.

In conclusion, a significantly higher expression of the PDE4B mRNA in the leukocytes was observed in the MDD group compared with control subjects. However, none of SNPs analyzed in our study showed a significant association with MDD in the Japanese population. Our results indicate that the PDE4B gene itself does not link to MDD but that the elevated mRNA levels of PDE4B might be implicated in the pathophysiology of MDD.

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