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Positive association of the PDE4B (phosphodiesterase 4B) gene with schizophrenia in the Japanese population

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Abstract

The phosphodiesterase 4B (PDE4B) gene is located at 1p31, a susceptibility region for schizophrenia (SZ). Moreover, PDE4B interacts with DISC1, which is a known genetic risk factor for SZ. Recently, it was reported that the PDE4B gene is associated with SZ in Caucasian and African American populations. In this study, case-controlled association analyses were performed in the Japanese population to determine if the PDE4B gene is implicated in SZ. Thirteen single nucleotide polymorphisms (SNPs) were analyzed in 444 schizophrenic patients and 452 control subjects. Three SNPs (rs2180335, rs910694 and rs472952) were significantly associated with SZ after applying multiple test correction ($p = 0.039$, 0.004 and 0.028). In addition, a significant association was found between specific haplotypes (rs2180335 and rs910694) and SZ (permutation $p = 0.001$). Our result suggests that variations at the PDE4B locus may play a significant role in the etiology of SZ in the Japanese population.
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1. Introduction

Schizophrenia (SZ) is a complex psychiatric disorder that afflicts approximately 1% of the population throughout the world and has high heritability (Craddock et al., 2005). The phosphodiesterase 4B (PDE4B) gene is located at 1p31, a susceptibility region for SZ (Faraone et al., 2006). PDE4B belongs to the PDE4 family of phosphodiesterases, which are orthologous to the Drosophila learning and memory gene dunce (Davis et al., 1995). The PDE4B gene has been found to be disrupted by a translocation breakpoint in two related individuals with psychosis in Scotland (Millar et al., 2005). Moreover, disrupted-in-schizophrenia 1 (DISC1), which is an important genetic risk factor for mental disorders such as SZ (Hennah et al., 2006; Ishizuka et al., 2006), has been shown to interact dynamically in a cyclic adenosine monophosphate (cAMP) dependent manner with PDE4B. DISC1 interacts with the UCR2 domain of PDE4B and elevation of cellular cAMP caused by protein kinase A (PKA) leads to dissociation of PDE4B from DISC1 and an increase in PDE4B

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activity (Millar et al., 2005). Long PDE4 isoforms are activated upon phosphorylation of UCR1 by 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). Moreover, Hashimoto et al. showed that genetic variation of the DISC1 gene is associated with lower biological activity on ERK signaling (Hashimoto et al., 2006). This implies that the DISC1–PDE4B interaction is important in the regulation of cAMP signaling. It was reported that patients with schizophrenia have decreased levels of intracellular cAMP (Muly, 2002) and that antipsychotic medications raise intracellular cAMP levels in the brain after blocking D2 receptors (Kelly et al., 2007). Recently, King et al. reported that variation in the PDE4B gene is associated with SZ in Caucasian and African American populations (King et al., 2006).

Taken together, the findings mentioned above suggest that the PDE4B gene may be a susceptibility one to SZ. In this study, we attempted to confirm the association of the PDE4B gene with SZ in Japanese subjects.

2. Materials and methods

2.1. Subjects for analysis

All patients and control subjects were biologically unrelated and Japanese. The diagnosis of SZ was made by at least two experienced psychiatrists according to DSM-IV criteria (American Psychiatric Association, 1994). For the genetic studies, we used genomic DNA samples from 444 SZ patients (265 male [mean age: 48.4 ± 13.9 years] and 179 female [mean age: 48.4 ± 15.0 years]) from thirteen psychiatric hospitals in the neighboring area of Tokushima Prefecture and the Ehime University Hospital in Japan. Controls (452) were selected from volunteers (271 male [mean age: 48.7 ± 12.1 years] and 181 female [mean age: 47.5 ± 12.7]) who were genetically unrelated residents living in Japan without either mental past histories or family histories of at least first degree relatives. All subjects signed written informed consent to participate in the genetic association studies approved by the institutional ethics committees.

2.2. Genotyping

We genotyped thirteen SNPs of the PDE4B gene. Genotyping was performed using commercially available TaqMan probes for the PDE4B gene with the Applied Biosystems 7500 Fast Real Time PCR System, according to the protocol recommended by the manufacturer (Applied Biosystems, California, USA.). We selected thirteen single nucleotide polymorphic (SNP) markers (rs1317611 (C/G), rs1354061 (A/G), rs4004 (G/T), rs6700971 (C/T), rs6588190 (C/T) and rs4320761 (C/T), rs599381 (A/G), rs498448 (C/T), rs1040716 (A/T), rs2180335 (A/G), rs910694 C/T), rs472952 (A/G), rs3767311 (A/G)) for genotyping from the public databases (dbSNP Home page) as reference for International Hap Map Project and the Applied Biosystems software SNPbrowser 3.5. Considering King's report that showed highly significant association between SNPs in introns 7 and 8 and SZ (King et al., 2006), we selected six SNPs that locate at the region from intron 7 to intron 8 and show high linkage disequilibrium (LD) between each pair of these SNPs. Haplotype block structure was determined using the HAPLOVIEW program (Barrett et al., 2005). Blocks were defined according to the criteria of Gabriel et al. (Gabriel et al., 2002).

2.3. Statistical analysis

Allelic and genotypic frequencies of patients and control subjects were compared using Fisher's exact test. Deviation from Hardy–Weinberg (HW) distribution of alleles was determined using the Haploview program. The SNPAlyze 3.2Pro software (DYNACOM, Japan) was used to estimate haplotype frequencies, LD, and permutation p values (10,000 replications). Pair-wise LD indices, D′ and r^2, 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.

3. Results

We genotyped thirteen SNPs in the PDE4B gene in 444 SZ patients and 452 controls. Genotypic and allelic frequencies of thirteen SNPs on PDE4B are shown in Table 1. There were two LD blocks (Gabriel et al., 2002) in PDE4B (Fig. 1) with rs6588190 and rs4320761 residing in block 1 and rs2180335 and rs910694 residing in block 2. Significant differences in allelic frequencies were observed between SZ patients and controls for four SNPs in introns 7 and 8, but not for the remaining nine SNPs. The T allele of rs1040716, the G allele of rs2180335, the T allele of rs910694 and the G allele of rs472952 occurred more frequently in the SZ patient' group than in control subjects ($p = 0.013$, 0.003, 0.0003, 0.002, respectively). After applying the Bonferroni correction test, these three SNPs (rs2180335, rs910694 and rs472952) still had significant allelic associations with schizophrenia ($p = 0.039$, 0.004, 0.028, respectively). Genotypic distributions of all SNPs were in Hardy–Weinberg equilibrium in control subjects, however, rs1040716 and rs2180335 showed deviations from Hardy–Weinberg equilibrium in SZ subjects ($p < 0.01$). In addition, we performed haplotype analyses for block 1 and block 2. The two marker haplotypes of block 2, containing SNPs (rs2180335 and rs910694), were associated with SZ (permutation $p = 0.001$, Table 2), while the two marker haplotypes of block 1 were not associated with SZ (permutation $p = 0.283$).

In power calculations using the G*Power program, we found that the sample size had >0.84 power for detecting

Table 1
Allele frequencies of thirteen SNPs in the PDE4B gene in patients with schizophrenia and control

SNP	Diagnosis	HWE	*n*	Allele		*p*-Value	Genotype			*p*-Value	Frequency
				C	G		C/C	C/G	G/G		
rs1317611	SZ	0.934	444	490	398	0.601	136	218	90	0.837	0.448
	CT	0.597	452	510	394		147	216	89		0.436
				A	G		A/A	A/G	G/G		
rs1354061	SZ	1	443	310	576	0.302	54	202	187	0.5	0.35
	CT	0.49	452	338	566		67	204	181		0.374
				G	T		G/G	G/T	T/T		
rs4004	SZ	0.64	444	709	179	1	285	139	20	0.32	0.202
	CT	0.116	451	721	181		283	155	13		0.201
				C	T		C/C	C/T	T/T		
rs6700971	SZ	0.47	444	332	556	1	66	200	178	0.912	0.374
	CT	0.93	452	338	566		64	210	178		0.374
				C	T		C/C	C/T	T/T		
rs6588190	SZ	0.799	443	638	248	0.232	228	182	33	0.462	0.28
	CT	0.858	452	627	277		216	195	41		0.306
				C	T		C/C	C/T	T/T		
rs4320761	SZ	1	443	637	249	0.178	229	179	35	0.398	0.281
	CT	1	452	623	281		215	193	44		0.311
				A	G		A/A	A/G	G/G		
rs599381	SZ	1	444	81	807	0.74	4	73	367	0.922	0.091
	CT	0.865	451	78	824		4	70	377		0.086
				C	T		C/C	C/T	T/T		
rs498448	SZ	0.159	444	522	366	0.316	161	200	83	0.58	0.412
	CT	0.362	452	510	394		149	212	91		0.436
				A	T		A/A	A/T	T/T		
rs1040716	SZ	0.001	444	183	705	0.013	31	121	292	0.012	0.206
	CT	0.305	449	230	668		34	162	253		0.256
				A	G		A/A	A/G	G/G		
rs2180335	SZ	0.005	444	160	728	0.003	24	112	308	0.0014	0.18
	CT	0.986	452	215	689		26	163	263		0.238
				C	T		C/C	C/T	T/T		
rs910694	SZ	0.018	444	158	730	0.0003	22	114	308	0.00013	0.178
	CT	0.623	451	223	679		25	173	253		0.247
				A	G		A/A	A/G	G/G		
rs472952	SZ	0.03	444	161	727	0.002	22	117	305	0.0037	0.181
	CT	0.729	452	218	686		28	162	262		0.241
				A	G		A/A	A/G	G/G		
rs3767311	SZ	0.906	444	98	790	0.354	6	86	352	0.528	0.11
	CT	0.693	452	89	815		3	83	366		0.098

a significant association (alpha < 0.05) when an effect size index of 0.2 was used.

4. Discussion

In this study, we performed a genetic and haplotypic-based association of the PDE4B gene with SZ in the Japanese population. We observed significant differences in allele frequency for rs2180335 and rs910694 of intron 7, and rs472952 of intron 8 between SZ-cases and controls after applying the Bonferroni correction test ($p = 0.039$, 0.004, 0.028, respectively). Furthermore two marker haplotypes covering rs2180335 and rs910694 in the same block were significantly associated with SZ (permutation $p = 0.001$). The most common haplotype (GT) was present in 82% of SZ -cases and 75% of controls. Therefore, this haplotype might be a risk factor for SZ. The second most common haplotype (AC) was present in 18% of SZ – cases and 24% of controls, suggesting that this haplotype might be protective against SZ. King et al. also reported that several SNPs, in particular two SNPs in introns 7 and 8, and three marker haplotypes showed highly significant association with SZ in Caucasian and African American populations (King et al., 2006). During the preparation of this manuscript, another study, demonstrating that three – SNP haplotypes in intron 3 are significantly associated with SZ in a female Scottish population (110 subjects), was published (Pickard et al.,

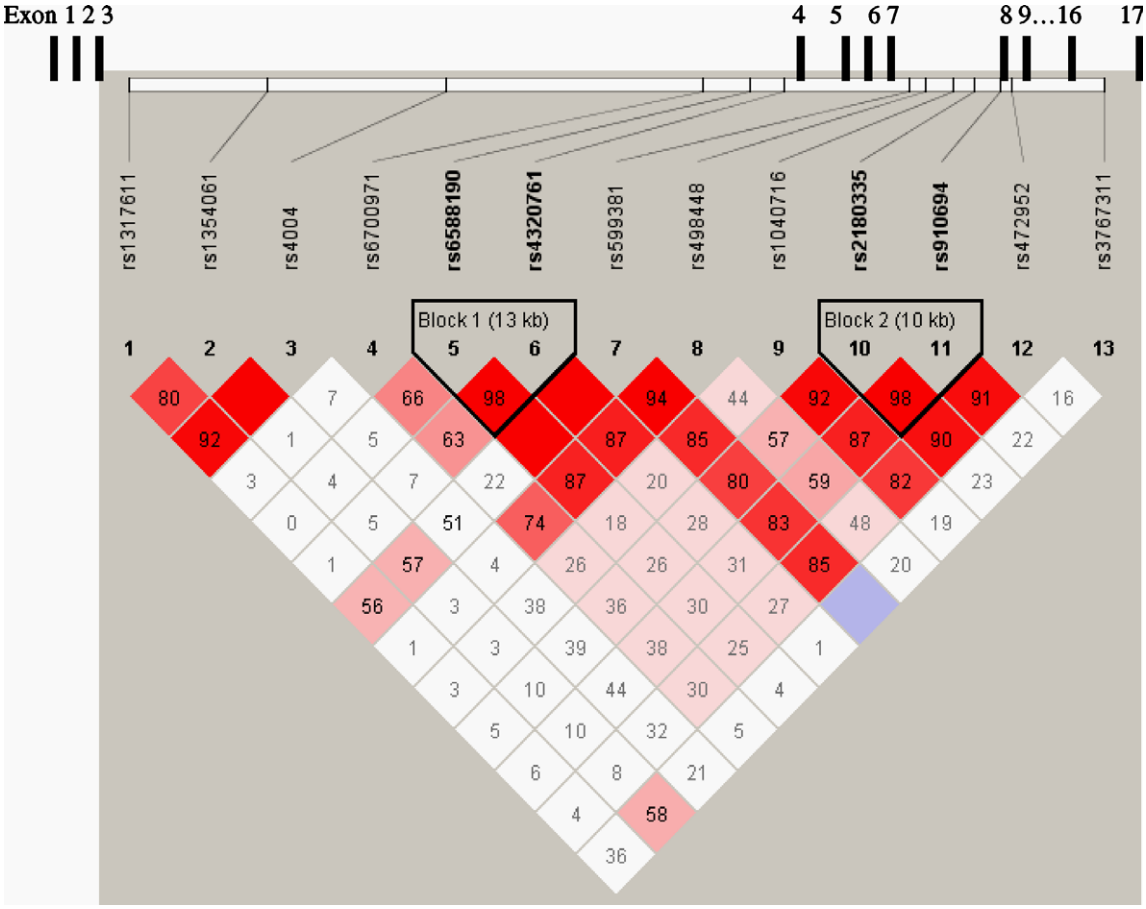

Fig. 1. Haplotype block structure of the PDE4B gene Haplotype block structure was determined using the HAPLOVIEW program (Barrett et al., 2005). Blocks were defined according to the criteria of Gabriel et al. (2002). There were two LD blocks in PDE4B. rs6588190 and rs4320761 reside in the block 1and rs2180335 and rs910694 reside in the block 2.

Table 2
Haplotype analysis among SZ and controls

Haplotype (rs2180335-rs910694)	Overall (%)	Schizophrenia	Control	Chi-Square	*p*-Value	Permutation *p*-value
(a) Schizophrenia						
GT	78.4	81.8	75.1	11.9	0.0006	0.0012
AC	20.6	17.6	23.6	9.99	0.0016	0.0021

Select locus	Chi-Square	*p*-Value	Permutation *p*-value	Replications
rs2180335/ rs910694	16.4	0.0009	0.0011	10000

Haplotypes were omitted from analysis if the estimated haplotype probabilities were less than 5%. The two marker haplotypes of the block 2 containing SNPs (rs2180335, rs910694) were associated with SZ (permutation $p = 0.0003$).

2007). In our study, when the data were subdivided on the basis of sex, no significant association was observed in single SNPs after the Bonferroni correction either in male and female samples. The two marker haplotypes of block 2, containing SNPs (rs2180335 and rs910694), were associated with SZ in male (permutation $p = 0.006$), while this two marker haplotypes were not associated with SZ in female (permutation $p = 0.208$). Three marker haplotypes (rs2180335-rs910694-rs472952, $D' > 0.9$ and $r^2 > 0.8$ in this region) were associated with SZ both in male and female (permutation $p = 0.009$, 0.039, respectively). Different results of gender effect and positive association regions between our study and Pickard's study may be caused by sample size, different SNPs examined and ethnic difference. However it is very interesting that all three reports including ours show positive association of the PDE4B gene with Schizophrenia. In our study, rs1040716 and rs2180335 showed deviations from HWE in SZ ($p < 0.01$), while all genotype frequencies in the PDE4B gene SNPs of control subjects were in HWE. This result

may reflect a SZ- specific mutation such as microdeletion of the region around rs1040716 and rs2180335, which causes the PDE4B expressional changes in our Japanese SZ samples.

Several recent studies provide evidence that PDE4 is a SZ susceptibility factor. Rolipram, a selective inhibitor of PDE4, reversed amphetamine (indirect dopamine agonist) – disrupted auditory sensory gating (Maxwell et al., 2004) and blocked the disruption of pre-pulse inhibition (PPI) caused by amphetamine in mice (Kanes et al., 2007). In rodents, rolipram suppressed conditioned avoidance responding (CAR), which is a commonly used test to screen for antipsychotic activity, at doses that did not produce response failures (Wadenberg and Hicks, 1999). Moreover, the dose-related effects of rolipram in CAR were similar to those seen with antipsychotics (Siuciak et al., 2006) and the same authors recently showed that PDE4B knockout mice exhibit a blunted response to rolipram in CAR (Siuciak et al., 2007). PDE4B is involved not only in the dopaminergic system, but also in the glutamatergic system. Rolipram attenuates MK-801 (NMDA receptor antagonist)-induced deficits in latent inhibition (Davis and Gould, 2005) and improves working -and reference-memory deficits induced by an NMDA receptor antagonist (O' Donnell and Zhang, 2004; Zhang et al., 2004). Furthermore, it has been reported that rolipram is efficacious in SZ patients (Pietzcker et al., 1979).

In conclusion, we here provide evidence that PDE4B is a genetic susceptibility factor for SZ. Larger studies are needed to confirm these associations by genotyping more PDE4B polymorphisms and haplotypes.

Conflict of interest

There are none.

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