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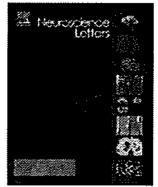

No association between the *Bcl2-interacting killer* (*BIK*) gene and schizophrenia

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ABSTRACT

The *Bcl2-interacting killer* (*BIK*) gene interacts with cellular and viral survival-promoting proteins, such as Bcl-2, to enhance apoptosis. The BIK protein promotes cell death in a manner analogous to Bcl-2-related death-promoting proteins, Bax and Bak. There have been lower Bcl-2 levels and increased Bax/Bcl-2 ratio in the temporal cortex of patients with schizophrenia compared with those in controls. Because the death-promoting activity of BIK was suppressed in the presence of the cellular and viral survival-promoting proteins, the BIK protein is suggested as a likely target for antiapoptotic proteins. The purpose of this study is to investigate the association between genetic variants in the *BIK* gene and schizophrenia in a large Japanese population (1181 patients with schizophrenia and 1243 healthy controls). We found nominal evidence for association of alleles, rs926328 ($\chi^2 = 4.44$, $p = 0.035$, odds ratio = 1.13) and rs2235316 ($\chi^2 = 4.41$, $p = 0.036$, odds ratio = 1.13), with schizophrenia. However, these associations were no longer positive after correction for multiple testing (rs926328: corrected $p = 0.105$, rs2235316: corrected $p = 0.108$). We conclude that *BIK* might not play a major role in the susceptibility of schizophrenia in Japanese population.

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Schizophrenia (MIM 181500) is a common complex psychiatric disease and is generally considered as a neurodevelopmental disorder. The lifetime morbidity rate is 0.5–1.0% across distinct populations. Family, twin, and adoption studies of schizophrenia have indicated that there are strong genetic factors with an estimated heritability of approximately 80% [5,20]. Regions on a number of chromosomes (e.g., 1, 6, 8, 10, 13, and 22) have been implicated as sites of potential vulnerability genes [17]. For example, 22q11–13 has been shown as a suggestive region in a number of linkage studies.

Apoptosis, a form of programmed cell death, is regulated by a complex cascade of pro- and anti-apoptotic members of the Bcl-2 family proteins. The ratio of pro-apoptotic (e.g. Bax, Bad) to anti-apoptotic (e.g. Bcl-2, Bcl-X_L) protein levels is a key determinant in regulating cytochrome *c* release and subsequent caspase activation, leading to rapid neuronal death [16,21]. Postmortem brain studies showed that markers of apoptosis, levels of apoptotic regulatory proteins and DNA fragmentation patterns, were altered in schizophrenia [1,2,12,13]. The Bcl-2 levels are 30% lower in temporal cortex in schizophrenia compared with controls [12]. There is a 50% increase in the Bax/Bcl-2 ratio in the temporal cortex of patients with schizophrenia compared to matched controls [13]. The cortical neuropathology in schizophrenia such as synaptic deficits and reduced neuropil without overall neuronal loss, and limited and often layer-specific reductions of neurons appears to be characterized by non-lethal and localized apoptotic activity at the level of synapses and terminal neuritis in schizophrenia [1,2,8,13]. Neuroimaging studies suggest that a progressive loss of cortical gray matter occurs in the early stage of the clinical course of schizophrenia [3,7,19]. Although the mechanisms underlying these data are

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Table 1
Genotype and allele distributions for SNPs in the *BIK* gene between patients with schizophrenia and controls.

Marker				SCZ			CON			Genotypic *p*-value (*df* = 2)	SCZ	CON	Allelic *p*-value (*df* = 1)	OR
dbSNP IDs	Position[a]	M/m	Gene	M/M	M/m	m/m	M/M	M/m	m/m		MAF			
rs926328	22896778	T/A	5′	0.29	0.50	0.21	0.26	0.51	0.24	0.10	0.46	0.49	0.035	0.89
rs4988372	22897148	C/T	5′	0.89	0.10	0.005	0.91	0.09	0.003	0.50	0.06	0.05	0.25	1.16
rs4988374	22897266	C/A	5′	0.89	0.10	0.005	0.91	0.09	0.003	0.45	0.06	0.05	0.21	1.18
rs2235316	22914504	A/C	Intron 3	0.26	0.50	0.24	0.29	0.49	0.22	0.10	0.49	0.46	0.036	1.13

SCZ, patients with schizophrenia; CON, healthy controls; m, minor allele; M, major allele; MAF, minor allele frequency; OR, odds ratio.
[a] db SNP build 129.

unknown, evidence for progressive clinical deterioration and subtle neurostructural changes following the onset of psychosis has led to the hypothesis that the increased apoptotic vulnerability may contribute to the pathophysiology of schizophrenia.

BIK (*Bcl2-interacting killer*) gene (MIM 603392) mapped on chromosome 22q13, which has a modest linkage to schizophrenia [6]. The gene contains 5 exons and spans approximately 19 kb [22]. *BCL-2* gene has been shown to enhance the survival of a variety of cell types exposed to diverse cell death-inducing stimuli. Bcl-2-related proteins either promote cell survival or accelerate cell death. The protein encoded by the *BIK* gene is known to interact with the cellular survival-promoting proteins Bcl-2 and Bcl-X_L, as well as with the viral survival-promoting proteins Epstein–Barr virus (EBV) BHRF1 and adenovirus E1B 19-kD to enhance apoptosis [4,9]. The BIK promotes cell death similar to the Bcl-2-related death-promoting proteins, Bax and Bak. Because the death-promoting activity of BIK was suppressed by coexpression of Bcl-2, Bcl-X_L, EBV BHRF1, and E1B 19-kD, the BIK protein might be associated with anti-apoptotic proteins. In this study, we investigated whether the *BIK* gene is associated with schizophrenia in a large Japanese population.

The subjects consisted of 1181 patients with schizophrenia [51.1% males (604/577), mean age ± SD; 46.1 ± 14.9 years] and 1243 healthy controls [46.5% male (578/665), mean age ± SD; 38.6 ± 15.7 years]. The sex ratio and the mean age differed significantly between groups (sex ratio; $\chi^2 = 5.2$, $p = 0.022$, mean age; $z = -13.0$, $p < 0.001$). All the subjects were biologically unrelated Japanese. Patients were recruited at the National Center Hospital of Neurology and Psychiatry, Showa University School of Medicine, Fujita Health University School of Medicine and Osaka University Graduate School of Medicine. Cases were recruited from both outpatients and inpatients at the hospitals. Each schizophrenic research subject had been diagnosed by at least two trained psychiatrists according to the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) criteria based on unstructured clinical interviews. Controls, including the hospital and institutional staffs, were recruited through local advertisements. Psychiatrically healthy controls were evaluated using unstructured interviews to exclude individuals who had current or past contact with psychiatric services. We did not assess the controls for the family history of psychiatric disorders, such as schizophrenia, bipolar disorder, or major depressive disorder. All experiments on human subjects were conducted in accordance with the Declaration of Helsinki and all procedures were carried out with the adequate understanding and written consent of the subjects.

Venous blood was drawn from the subjects and genomic DNA was extracted from whole blood according to standard procedures. Four single nucleotide polymorphisms (SNPs) in the *BIK* gene were selected from the public database (HAPMAP: http://www.hapmap.org/index.html.ja) in order to cover the *BIK* gene (4.75 kb per SNP). The four SNPs in the *BIK* gene (rs926328, rs4988372, rs4988374 and rs2235316) were genotyped using the TaqMan 5′-exonuclease allelic discrimination assay (Applied Biosystems, Foster City, California, USA), as described previously [10,11,15]. Primers and probes for detection of the SNPs are available upon request.

Statistical analyses were performed using SNPAlyze V5.1.1 Pro software (DYNACOM, Yokohama, Japan) and SPSS 16.0J software (SPSS Japan Inc., Tokyo, Japan). Differences in clinical characteristics between patients and controls or between genotype groups were analyzed using χ^2 tests for categorical variables and the Mann–Whitney *U*-test for continuous variables. A deviation from Hardy–Weinberg equilibrium (HWE) was tested separately in cases and controls by using the χ^2 tests for goodness of fit. The allelic and genotypic distributions of the *BIK* polymorphisms between patients and controls were analyzed using χ^2 tests. The number of effective independent SNPs assayed was estimated by the spec-

Table 2
Marker-to-marker linkage disequilibrium for all the combinations of the four SNPs in the *BIK* gene.

	rs926328	rs4988372	rs4988374	rs2235316
rs926328	–	1.00	1.00	0.36
rs4988372	1.00	–	1.00	0.88
rs4988374	1.00	1.00	–	0.86
rs2235316	0.39	0.91	0.91	–

For each pair of markers, the standardized D' in controls is shown below the diagonal, and the standardized D' in patients with schizophrenia is shown above the diagonal.

tral decomposition method of Nyholt using SNPSpD software [14]. The pairwise linkage disequilibrium (LD) analyses, expressed by D', were applied to detect the intermarker relationship in each group using the SNPAlyze V5.1.1 Pro software. We performed power calculations using the Power Calculator for Two Stage Association Studies (http://www.sph.umich.edu/csg/abecasis/CaTS/ [18]). Power (>0.80) was calculated under prevalence of 0.01, several allele frequencies in patients (rs926328; 0.46, rs4988372; 0.06, rs4988374; 0.06, or rs2235316; 0.46) and an alpha level of 0.05 using a multiplicative model, assuming varying degrees of the odds ratio. All p-values reported are two tailed. Statistical significance was defined as $p < 0.05$.

The genotype and allele frequencies of four SNPs located in the *BIK* gene and the flanking regions are summarized in Table 1. Genotyping completeness ranged from 96.3% (rs2235316) to 99.3% (rs4988372). The genotype distributions of all examined SNPs in the *BIK* gene were in HWE for both controls and patients with schizophrenia ($p > 0.3$). We found no genotypic association between the four SNPs in the *BIK* gene and schizophrenia (Table 1). On the other hand, there were significant differences in allele frequencies of rs926328 and rs2235316 (Table 1). The major T allele of the rs926328 and the minor C allele of the rs2235316 in the *BIK* gene were in excess in patients with schizophrenia compared with controls (rs926328; $\chi^2 = 4.44$, $p = 0.035$, odds ratio = 1.13, 95% confidential interval 1.01–1.27, rs2235316; $\chi^2 = 4.41$, $p = 0.036$, odds ratio = 1.13, 95% confidential interval 1.01–1.27; Table 1). However, these associations did not survive after SNPSpD correction for multiple testing in this gene (the effective number of independent marker loci: 3.0; rs926328; corrected $p = 0.105$, rs2235316; corrected $p = 0.108$). There was no allelic association with schizophrenia for the other two SNPs, rs4988372 and rs4988374. The LD relationships between markers were shown in Table 2. The LD pattern observed in our patients was nearly identical to those in our controls. The strong LD patterns among four SNPs were observed in both groups ($D' > 0.8$), except for the weak LD between rs926328 and rs2235316 in both groups ($D' < 0.5$).

Nominal associations between the genetic variants, rs926328 and rs2235316, and schizophrenia in this study were no longer positive after correction for multiple testing. Power analysis showed that our subjects had sufficient power (>0.80) to detect an effect of the odds ratio (1.18 or more) for rs926328 or rs2235316. For other two SNPs, rs4988372 and rs4988374, our sample size had power (>0.80) to detect an effect of the odds ratio (1.38 or more). As the odds ratios of the rs926328 and rs2235316 were 1.13, our sample size was not enough to detect such a small contribution to risk for schizophrenia. This could lead to the type II error. There were other limitations in our study. Control samples were not matched by age and sex with the patient population or were not represent general population as they included substantial portion of hospital and institutional staffs. Although the psychiatric phenotypes of the controls were screened by the unstructured interview, we did not assess the family history of controls subjects. Thus, control subjects might carry the risk allele from affected parents. Careful interpretation of our results due to population stratification would be needed in our case control designed study, despite the precaution of ethnic matching of this study. Therefore, it is necessary to carry out further investigations to confirm our findings in other samples with age and sex matched case-control subjects and with much larger sample size.

In conclusion, we have examined a possible association between the *BIK* gene and schizophrenia for the first time and have not found the association with schizophrenia in the Japanese population. Two SNPs gave nominal evidence for association and this association did not survive after correction for multiple testing. *BIK* is not likely to be a major susceptibility gene for schizophrenia in this Japanese population. The diagnosis of schizophrenia itself is too non-specific and that the *BIK* might be more related to differences in patients with and without specific deficits in basic neurophysiological process such as sensory gating, executive function, etc. Further studies are needed to investigate the contribution of pro- or anti-apoptotic genes other than *BIK* in schizophrenia.

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ORIGINAL PAPER

No Association Between Polymorphisms of Neuronal Oxide Synthase 1 Gene (*NOS1*) and Schizophrenia in a Japanese Population

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Abstract The neuronal nitric oxide synthase gene (*NOS1*) is located on 12q24, in a susceptibility region for schizophrenia, and produces nitric oxide (NO) in the brain. NO plays a role in neurotransmitter release and is the second messenger of the *N*-methyl-D-aspartate (NMDA) receptor. Furthermore, it is connected to the dopaminergic and serotonergic neural transmission systems. Therefore, abnormalities in the NO pathway are thought to be involved in the pathophysiology of schizophrenia. Several genetic studies showed an association of *NOS1* with schizophrenia. However, results of replication studies have been inconsistent. Therefore, we conducted a replication study of *NOS1* with schizophrenia in a Japanese sample. We selected seven SNPs (rs41279104, rs3782221, rs3782219, rs561712, rs3782206, rs2682826, and rs6490121) in *NOS1* that were positively associated with schizophrenia in previous studies. Two SNPs showed an association with Japanese schizophrenic patients (542 cases and 519 controls, rs3782219: P allele $= 0.0291$ and rs3782206: P allele $= 0.0124$, P genotype $= 0.0490$), and almost these significances remained with an increased sample size (1154 cases and 1260 controls, rs3782219: P allele $= 0.0197$ and rs3782206: P allele $= 0.0480$). However, these associations also might have resulted from type I error on account of multiple testing (rs3782219: P allele $= 0.133$ and rs3782206: P allele $= 0.168$). In conclusion, we could not replicate the association between seven SNPs in *NOS1* and schizophrenia found in several earlier studies, using larger Japanese schizophrenia and control samples.

Keywords Schizophrenia · Neuronal nitric oxide syntase 1 gene (*NOS1*) · Case-control association study

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Introduction

Schizophrenia is a common psychiatric disease, seen in approximately 1% of the world population. It is characterized by delusions, hallucinations, and cognitive dysfunction. Genetic factors play an important role in susceptibility to schizophrenia (Cardno and Gottesman 2000), and several genetic studies have identified susceptibility genes (Ross et al. 2006).

The nitric oxide synthase 1 gene (*NOS1*) is located on 12q24, and consists of 12 alternative untranslated first exons, termed exon 1a_1l, and 28 exons in a genomic region spanning 149.404 Kb. *NOS1* is considered to be a likely candidate gene for schizophrenia owing to its

chromosomal location, 12q24, which has been reported to be a susceptibility locus from several linkage studies (Bailer et al. 2000, 2002; DeLisi et al. 2002), and to produce nitric oxide (NO). NO is synthesized from L-arginine by a family of isoformic enzymes known as nitric oxide synthases (NOSs). Three isoforms of NOS have been identified: neuronal (nNOS), endothelial (eNOS), and inducible (iNOS) (McLeod et al. 2001). NO is involved in a variety of mechanisms, such as neurotransmitter release, *N*-methyl-D-aspartate (NMDA) receptor activation (Joca et al. 2007; Snyder and Ferris 2000), and oxidative stress in the brain (Yao and Reddy 2005). Abnormalities in these mechanisms are thought to be involved in the pathophysiology of psychotic disorders (Bennett 2008). Moreover, evidence from pharmacological studies in animal and postmortem studies supports an association between NO and psychotic disorders (Wass et al. 2009; Yao et al. 2004).

A number of genetic association studies showed that single nucleotide polymorphisms (SNP) in *NOS1* were associated with schizophrenia. Shinkai et al. (2002) examined the association between a synonymous SNP (rs2682826) in exon 29 and schizophrenia in a Japanese population, and showed that it was significant. Fallin et al. (2005) identified a haplotype (rs3782221–rs3782219–rs561712–rs3782206) and reported it to be associated with schizophrenia and schizoaffective disorder. *NOS1* has a complex promoter–exon1 region. Expression of the different mRNA from distinct promoters in *NOS1* is controlled by the 5′ flanking region (Bros et al. 2006). Reif et al. (2006) reported a polymorphism (rs41279104) in the promoter region of exon 1c associated with schizophrenia and prefrontal brain function. Recently, a whole genome association study reported an association between rs6490121 in intron 2 of *NOS1* and schizophrenia (Moskvina et al. 2009).

In this study, we conducted a replication study of association between significant seven SNPs in *NOS1* and schizophrenia in a Japanese samples.

Materials and Methods

Subjects

A total of 542 patients with schizophrenia (276 males and 266 females; mean age ± standard deviation; 43.8 ± 14.8 years) and 519 healthy controls (264 males and 255 females; 36.5 ± 14.1 years) were recruited. For rs3782219 and rs3782206, which showed a significant association in the allele and/or genotype-wise analysis, additional samples were included for this association analysis, bringing the totals to 1154 schizophrenics (additional 612 cases) and 1260 controls (additional 741 controls). All subjects were unrelated to each other and ethnically Japanese. The patients were diagnosed according to DSM-IV criteria with the consensus of at least two experienced psychiatrists on the basis of unstructured interviews and a review of medical records, and who were outpatients or inpatients of psychiatric hospitals. The patients were grouped according to the following DSM-IV subtypes of schizophrenia: Paranoid Type ($n = 429$), Disorganized Type ($n = 441$), Catatonic Type ($n = 39$), Residual Type ($n = 138$), and Undifferentiated Type ($n = 107$). All healthy control subjects were also psychiatrically screened based on unstructured interviews. None had severe medical complications such as cirrhosis, renal failure, heart failure, or other Axis-I disorders according to DSM-IV. No structured methods were used to assess psychiatric symptoms in the controls (hospital staffs and medical students). None of the subjects were known to be related to each other, and all were ethnically Japanese. Written informed consent was obtained from each subject. This study was approved by the ethics committees at Fujita Health University, Okayama University, and Nagoya University Graduate School of Medicine.

SNP Selection and Genotyping

We selected seven SNPs (rs41279104, rs3782221, rs3782219, rs561712, rs3782206, rs2682826, and rs6490121) in *NOS1* shown by previous studies to have a positive association with schizophrenia (Fallin et al. 2005; Moskvina et al. 2009; Reif et al. 2006; Shinkai et al. 2002). We used TaqMan assays (Applied Biosystems, Foster City, CA, USA) for all SNPs. Detailed information is available on request.

Statistical Analysis

Genotype deviation from the Hardy–Weinberg equilibrium (HWE) was evaluated by the chi-square test (SAS/Genetics, release 8.2, SAS Japan Inc., Tokyo, Japan). Marker–trait association was also evaluated by the chi-square test in allele- and genotype-wise analyses. Haplotype frequencies were estimated in a two- to four-marker sliding window fashion and log likelihood ratio tests were performed for global *P* values with COCAPHASE program version 3.0.6 (Dudbridge 2003). In these haplotype-wise analyses, rare haplotypes (less than 0.05) in either of cases and controls were excluded from the association analysis. Power calculation was performed using a statistical program prepared by Genetic Power Calculator (http://pngu.mgh.harvard.edu/~purcell//gpc/). To correct for problems of multiple comparisons, we applied the Benjamini–Hochberg (BH) method, which is a procedure to control for false discovery

rate (FDR) (Dudbridge 2003). The level of significance for all statistical tests was 0.05.

Results

Genotype frequencies of subjects and controls did not deviate significantly from HWE. In the first-set of analysis, two SNPs (rs3782219 and rs3782206) showed a significant association with schizophrenia in allele and/or genotype-wise analysis (rs3782219: *P* allele = 0.0291 and rs3782206: *P* allele = 0.0124, *P* genotype = 0.0490). Five other SNPs did not show evidence of association with schizophrenia (Table 1). There was no evidence of association with schizophrenia in haplotype-wise analysis (Table 2).

To validate the significant association of rs3782219 and rs3782206 found in the first-set samples, and even some significances remained with an increased sample size (1154 cases and 1260 controls, rs3782219: *P* allele = 0.0197 and rs3782206: *P* allele = 0.0480); however, these associations also might have resulted from type I error on account of multiple testing (rs3782219: *P* allele = 0.133 and rs3782206: *P* allele = 0.168) (Table 1).

We obtained power of more than 80% for the detection of association when we set the genotype relative risk at 1.27–1.36 under a multiplicative model of inheritance in the first-set samples.

Discussion

We found marginal associations between two SNPs (rs3782219 and rs3782206) and schizophrenia in allele and/or genotype-wise analysis, and almost these significances remained with an increased sample size. However, we suggested that it might have resulted from type I error due to multiple testing. Fallin et al. (2005) reported that significant associations of haplotypes were identified with four SNPs in intron 2 (rs3782221, rs3782219, rs561712, and rs3782206). However, no association was found in our study. Shinkai et al. (2002) showed a strongly positive association between rs2682826 and schizophrenia in a Japanese population. However, although we examined more large Japanese samples than original study (Shinkai et al. 2002), we found no significant association with schizophrenia. The result of this study was in concordance with replication studies in other ethnic population samples (Liou et al. 2003; Tang et al. 2008). Recently, a whole genome association study reported a possible association between rs6490121 in *NOS1* and schizophrenia (O'Donovan et al. 2008). To avoid multiple testing problems, it is important to conduct replication study. Our samples were provided for replication study and showed a significant association and odds ratio that were opposite to UK samples (O'Donovan et al. 2008). However, although we performed a replication study using larger different samples

Table 1 Association study between *NOS1* and schizophrenia

SNP ID	Position	Phenotype[a]	MAF[b]	*N*	Genotype distribution[c]			*P* value[e]			Corrected *P* value[f]
					M/M	M/m	m/m	HWE[d]	Genotype	Allele	Allele
rs41279104	114886493	SCZ	0.197	542	355	162	25	0.243	0.436	0.230	
	Promoter region of exon 1c	CON	0.175	519	354	148	17	0.751			
rs3782221	114805000	SCZ	0.448	542	173	252	117	0.161	0.488	0.275	
	Intron 1	CON	0.424	519	175	247	97	0.550			
rs3782219	114797355	SCZ	0.411	1154	409	540	205	0.248	0.0655	0.0197	0.133
	Intron 1	CON	0.444	1260	394	611	255	0.518			
rs561712	114761232	SCZ	0.176	542	374	145	23	0.0677	0.128	0.856	
	Intron 2	CON	0.179	519	346	160	13	0.274			
rs3782206	114754240	SCZ	0.279	1154	610	443	101	0.111	0.133	0.0480	0.168
	Intron 3	CON	0.254	1260	706	467	87	0.415			
rs6490121	114717324	SCZ	0.390	542	203	255	84	0.790	0.244	0.0952	
	Intron 10	CON	0.425	519	175	246	98	0.484			
rs2682826	114662005	SCZ	0.353	542	223	255	64	0.491	0.469	0.228	
	Exon 29	CON	0.328	519	230	237	52	0.424			

[a] *SCZ* schizophrenia, *CON* control

[b] *MAF* minor allele frequency

[c] *M* major allele, *m* minor allele

[d] Hardy–Weinberg equilibrium

[e] Bold numbers represent significant *P* value

[f] Calculated using Benjamini–Hochberg (BH) method

Table 2 Haplotype-wise analysis between *NOS1* and schizophrenia

SNP ID	Global *P* value		
	2 Window	3 Window	4 Window
rs41279104			
	0.228		
rs3782221		0.187	
	0.051		0.106
rs3782219		0.223	
	0.180		0.203[a]
rs561712		0.0770	
	0.0620		0.112
rs3782206		0.0780	
	0.0600		0.223
rs2682826		0.211	
	0.131		
rs6490121			

[a] Fallin et al. reported

than original study, we could not replicate. In other recent study, Tang et al. (2008) reported a significant association with schizophrenia of rs3782206 in a Chinese population. This discordance of results may reflect problems in the replication study, such as population difference, in each sample. Therefore, it is necessary to evaluate a polymorphism for this association with schizophrenia in various ethnic populations.

A few points of caution should be noted in interpreting our results. First, we did not apply a LD-based approach and a mutation scan to detect rare variants with functional effects. Moreover, we did not examine a VNTR in exon 1f within the promoter region in *NOS1*. Reif et al. (2006) reported a significant association of the haplotype constructed by rs41279106 and this VNTR with schizophrenia. These problems are future topics for study. Second, our sample was not matched in terms of age. Moreover, our samples were not assessed by a standard structured interview, and thus there is a chance of false negatives due to misdiagnosis or sampling bias (Kishi et al. 2009).

In conclusion, we suggest that these seven SNPs in *NOS1* may not play a role in the susceptibility to schizophrenia in the Japanese population. However, other functional polymorphisms in *NOS1* may show important roles in the pathophysiology of schizophrenia, and further investigations will be necessary.

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