A Genetic Variant of the Serine Racemase Gene Is Associated with Schizophrenia

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Background: Serine racemase (SRR) is a brain-enriched enzyme that converts L-serine to D-serine, which acts as an endogenous ligand of N-methyl D-aspartate (NMDA) receptors. Dysfunction of SRR may reduce the function of NMDA receptors and susceptibility to schizophrenia.

Methods: We genotyped three single-nucleotide polymorphisms (SNPs) of the 5′ region of the SRR gene in 525 patients with schizophrenia and 524 healthy controls. Effects of SNPs on the promoter activity and on serum levels of total and D-serine were examined.

Results: We found a significant excess of the IVS1a+465C allele of the SRR gene in schizophrenia, especially in the paranoid subtype ($p = .0028$). A reporter assay showed that the IVS1a+465C allele had 60% lower promoter activity than did the IVS1a+465G allele.

Conclusions: The IVS1a+465C allele of the SRR gene, which reduces expression of the gene, is a risk factor for schizophrenia, especially the paranoid subtype.

Key Words: Case–control study, column-switching high-performance liquid chromatography system, promoter activity, schizophrenia, serine racemase, single-nucleotide polymorphism

N-methyl D-aspartate (NMDA) receptors are an ionotropic subtype of glutamate receptors, which play important and indispensable roles in several higher brain functions such as learning, memory, and cognition (Chatterton *et al.* 2002; Das *et al.* 1998; Morris *et al.* 1986; Muller *et al.* 1988). Dysfunction of NMDA receptors has been suggested as one of the promising hypotheses for the pathophysiology of schizophrenia (Jackson et al 2004; Olney *et al.* 1989, 1991; Olney and Farber 1995; Qin et al 2005; Zhao *et al.* 2006). D-Serine is an endogenous co-agonist of the glycine site of NMDA receptors, and large amounts of D-serine have been found in mammalian brain regions that are enriched in NMDA receptors (Hashimoto *et al.* 1992; Wolosker et al 1999a, 1999b).

In 1999, Wolosker *et al.* (1999a, 1999b) purified serine racemase (SRR), which directly converts L-serine to D-serine, and is present in glial cells, especially in the forebrain, hippocampus, and corpus callosum (De Miranda *et al.* 2000; Panizzutti *et al.* 2001). Two previous genetic studies showed inconsistent findings of association between the SRR gene and patients with schizophrenia (Goltsov *et al.* 2006; Yamada *et al.* 2005). Therefore, we tried to confirm an association of the SRR gene with schizophrenia in a Japanese population by using a case–control study and tried to confirm the promoter activity by using an in vitro reporter assay system. We also measured serum concentrations of D- and L-serine.

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Methods and Materials

Human Subjects

We examined the first set of 238 patients (117 males and 121 females; mean age, 44.0 y; SD, 13.0 y; 115 were diagnosed with the paranoid subtype, and 117, with the hebephrenic subtype) fulfilling the ICD-10 diagnostic criteria for schizophrenia and 196 age-, gender-, and geographical origin–matched control subjects (99 males and 97 females; mean age, 40.6 y; SD 12.0 y; subject set A). For the second analysis, a different panel of subjects (subject set B), consisting of 287 patients (147 males and 140 females; mean age, 53.6 y; SD, 12.1 y; 120 were diagnosed with the paranoid subtype, and 143, with the hebephrenic subtype) with schizophrenia, and 328 age-, gender-, and geographical origin–matched control subjects (166 males and 162 females; mean age, 55.0 y; SD 14.3 y) was analyzed. Assessment for diagnosis of schizophrenia and determination of subtype was performed by two trained psychiatrists on the basis of all available information. This study was approved by the Ethics Committee of Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, and all subjects provided written informed consent.

Single-nucleotide Polymorphisms in the 5′ Region of the SRR Gene

We detected five kinds of single-nucleotide polymorphisms (SNPs) in the 5′ region but not of the other coding region of the SRR gene (GenBank accession number NT_010718) by direct sequencing. However, all SNPs had already been registered (http://www.ncbi.nlm.nih.gov/SNP/). They were SNP1, −1018T→C, rs2209073; SNP2, −962G→T rs2209072; SNP3, −757G→A, rs2224770; SNP4, −565C→T, rs3760229; and SNP5, IVS1a+465G→C, rs408067. We found that three SNPs, SNP1, SNP2, and SNP3, showed complete linkage disequilibrium in 48 controls and 24 case samples. Therefore, we selected SNP3, SNP4, and SNP5 for a case–control association study by PCR–restriction fragment length polymorphism assays.

Luciferase Assay for Promoter Activities

The fragments containing SNP5 in the 5′ region of the SRR gene were amplified from genome samples by using the primer pair 5′-CGGTGGCCGAGCTCGGAGGAAAAG-3′ (5′

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Table 1. Genotype and Allele Frequencies of SNP3–5 of the SAR Gene

Groups	*n*	Genotype (%)			*p* Value	Corrected *p* Value	Allele (%)		*p* Value	Corrected *p* Value
		G/G	G/A	A/A			G	A		
SNP3 (rs2224770)										
Controls	196	108 (55.1)	76 (38.8)	12 (6.1)		—	292 (74.5)	100 (25.5)		—
Schizophrenia	238	120 (50.4)	100 (42.0)	18 (7.6)	.59	—	340 (71.4)	136 (28.6)	.31	—
Paranoid type	115	49 (42.6)	57 (49.6)	9 (7.8)	.1	—	155 (67.4)	75 (32.6)	.06	—
Hebephrenic type	117	69 (59.0)	39 (33.3)	9 (7.7)	.59	—	177 (75.6)	57 (24.4)	.75	—
SNP4 (rs3760229)		C/C	C/T	T/T			C	T		
Controls	196	50 (25.5)	99 (50.5)	47 (24.0)			199 (50.8)	193 (49.2)		
Schizophrenia	238	43 (18.1)	118 (49.6)	77 (32.4)	.066	—	204 (42.9)	272 (57.1)	.021	—
Paranoid type	115	18 (15.7)	57 (49.6)	40 (34.8)	.044	—	93 (40.4)	137 (59.6)	.013	—
Hebephrenic type	117	25 (21.4)	58 (49.6)	34 (29.1)	.53	—	108 (46.2)	126 (53.8)	.26	—
SNP5 (rs408067)		G/G	G/C	C/C			G	C		
Controls	196	98 (50.0)	82 (41.8)	16 (8.2)			278 (70.9)	114 (29.1)		
Schizophrenia	238	99 (41.6)	99 (41.6)	40 (16.8)	.018	—	297 (62.4)	179 (37.6)	.0094	—
Paranoid type	115	47 (40.8)	42 (36.5)	26 (22.6)	.0019	.0171	136 (59.1)	94 (40.9)	.0028	.0252
Hebephrenic type	117	50 (42.7)	54 (46.2)	13 (11.1)	.39	—	154 (65.8)	80 (34.2)	.18	—

Statistical significances were assessed by a χ^2 test or Fisher's exact test. *p* values were corrected by Bonferroni correction.

end at IVS1a+380) and 5′-GGCCTCGCCTCACCTGAGCT-CACC-3′ (5′ end at exon 1b+65). The pGL3-basic plasmid with a 5′-region fragment of the SRR gene was cotransfected transiently into the human SH-SY5Y neuroblastoma cells and Hela cells (Japanese Collection of Research Bioresources, Tokyo, Japan). Measurements were performed in triplicate ($N = 9$).

Measurement of Total Serine, L-serine, and D-serine

We recruited 7 schizophrenic patients and 17 control subjects. Sample preparation and measurement of total, L-, and D-serine levels were performed according to a method described elsewhere (Morikawa *et al.* 2001).

Results

Genetic Association Analyses

The genotype distributions of patients and control groups did not deviate significantly from Hardy-Weinberg equilibrium at these polymorphic loci. We found significant differences in the frequency of the genotype and allele of SNP5 between patients with the paranoid subtype of schizophrenia and control subjects (Table 1; genotype, $p = .0019$; allele, $p = .0028$; odds ratio =1.69).

The positive association of SNP5 with paranoid subtype of schizophrenia was also demonstrated in subject set B, consisting of a larger number of subjects. We again found a significant difference between patients with schizophrenia and control subjects (Table 2). After Bonferroni correction, the associations of the genotype and allele of SNP5 with the paranoid subtype of schizophrenia remained significant (Tables 1 and 2).

Haplotype Association Analysis

Haplotype analyses showed that SNP3, -4, and -5 were in linkage disequilibrium. Multiloci analysis showed that the haplotype that consisted of SNP4 and -5 showed the strongest association with schizophrenia ($p = .00079$; Table 3).

Luciferase Assay for Promoter Activities

We investigated the effect of SNP5 (IVS1a+465G→C) on the promoter activity by using an in vitro reporter assay system. The promoter activity of the plasmid construct with IVS1a+465C allele was 30% and 60% lower than the plasmid construct with IVS1a+465G allele when Hela and SH-SY5Y cells, respectively, were used (Figure 1A and B).

Determination of Total Serine, L-serine, and D-serine

The concentrations of total (L and D) serine and L- and D-serine in the serum from subjects with G/G, G/C, and C/C genotypes of SNP5 (IVS1a+465G→C) were determined by HPLC system. We found that D-serine levels and the percentage of D-serine in total serine were not significantly different between G/G ($n = 10$), G/C ($n = 8$), and C/C genotypes ($n = 6$), even after correction by genotype, age, and gender (Figure 2).

Discussion

We found that the IVS1a+465C allele of SNP5 produces less expression of the SRR gene, which may result in less conversion of L-serine to D-serine and less activation of NMDA receptors, and that IVS1a+465C allele was a significant risk for schizophrenia, especially paranoid subtype. However, a previous study found

Table 2. Association Analysis of SNP5 in the Second Set of Subjects

SNP5 (rs408067)	*n*	Genotype (%)			*p* Value	Corrected *p* Value	Allele (%)		*p* Value	Corrected *p* Value
		G/G	G/C	C/C			G	C		
Controls	328	160 (48.8)	139 (42.4)	29 (8.8)			457 (69.7)	199 (30.3)		
Schizophrenia	287	123 (42.9)	116 (40.4)	48 (16.7)	.012	.036	362 (63.1)	212 (36.9)	.011	.033
Paranoid type	120	48 (40.0)	48 (40.0)	24 (20.0)	.0045	.014	144 (60.0)	96 (40.0)	.0049	.015
Hebephrenic type	143	61 (42.7)	61 (42.7)	21 (14.7)	.14	—	183 (64.0)	103 (36.0)	.070	—