Identification of Multiple Serine Racemase (*SRR*) mRNA Isoforms and Genetic Analyses of *SRR* and *DAO* in Schizophrenia and D-Serine Levels

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Background: *We previously reported a reduction in serum levels of D-serine, an endogenous co-agonist of the N-methyl-D-aspartate (NMDA) receptor, in schizophrenia, supporting the hypofunction hypothesis of NMDA neurotransmission in schizophrenia. In this study, we examined the genetic roles of serine racemase (SRR), an enzyme catalyzing the formation of D-serine from L-serine, and D-amino-acid oxidase (DAO) in the susceptibility to schizophrenia and the regulation of serum D-serine levels.*

Methods: *We determined the complete cDNA and genomic structures of SRR and performed mutation screening. Single nucleotide polymorphisms (SNPs) in SRR and DAO were tested for their association with schizophrenia in both case–control and family-based designs and for correlation with serum levels of D-serine.*

Results: *Genomic analyses revealed that human brain SRR transcripts consist of four isoforms with one major species, which were derived from alternative use of various 5' end exons. Genetic association analyses showed no significant association between SRR/DAO and schizophrenia. We replicated the decreased serum D-serine levels in schizophrenia in the sample set, but D-serine levels did not correlate with SRR/DAO genotypes.*

Conclusions: *The SRR/DAO are not likely to be major genetic determinants in the development of schizophrenia or control of serum D-serine levels.*

Key Words: Alternative splicing, genotype–phenotype correlation, glycine site, mutation screening, N-methyl-D-aspartate receptor, polymorphism.

The precise etiology of schizophrenia remains largely unknown, and the genetic determinants of the disease are complex, making identification of definitive susceptibility genes a formidable task (Gottesman 1991; Kendler 2005). Converging evidence, including genetic studies, has, however, supported the hypofunction hypothesis of glutamatergic neurotransmission via the N-methyl-D-aspartate (NMDA)-type glutamate receptor in schizophrenic brains. This hypothesis originally stemmed from clinical observations that phencyclidine and its congener anesthetic ketamine, both acting as noncompetitive antagonists of the receptor, evoke a schizophrenia-like psychosis including positive and negative symptoms in healthy control subjects and that phencyclidine exacerbates schizophrenic symptoms in patients (Javitt and Zukin 1991; Krystal et al 1999). The cognate NMDA receptor is heteromeric, consisting of the indispensable NR1 subunit and one of six possible subunits, NR2A, NR2B, NR2C, NR2D, NR3A, or NR3B (Andersson et al 2001; Dingledine et al 1999). Genetic variations in the receptor molecules themselves (Itokawa et al 2003; Miyatake et al 2003), and other molecules that may indirectly influence NMDA receptor function (for review, see Harrison and Owen 2003) have been reported to confer a risk for schizophrenia.

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The receptor forms a cation channel, the opening of which is modulated by an arosteric glycine-binding site (Danysz and Parsons 1998; Leeson and Iversen 1994). The endogenous ligands for this strychnine-insensitive glycine site are deemed to include D-serine (Snyder and Ferris 2000). Importantly, therapeutic trials with D-serine have been shown to improve the positive and negative symptoms, and cognitive deficit, of patients with schizophrenia (Tsai et al 1998). These results suggest that endogenous D-serine could play an imperative role in the pathophysiology of schizophrenia (Goff and Coyle 2001). D-serine is synthesized by a glial serine racemase (SRR), a novel pyridoxal-5'-phosphate (vitamin B6)-dependent enzyme converting L-serine to D-serine in the mammalian brain (Schell 2004; Snyder and Ferris 2000; Wolosker et al 1999a, 1999b). Degradation of D-serine is mediated by D-amino acid oxidase (DAO), but this enzyme is not present in forebrain areas that are highly enriched for D-serine (Hashimoto et al, in press; Nagata 1992; Schell 2004). It is also known that glycine is converted to L-serine by the pyridoxal-5'-phosphate-dependent enzyme, serine hydroxymethyltransferase (Bauwe and Kolukisaoglu 2003). We recently reported that serum levels of D-serine and the ratio of D-serine to total serine were indeed significantly decreased in schizophrenia patients, suggesting that the activity of SRR may be reduced in schizophrenia (Hashimoto et al 2003). In this study, we first clarified the genomic architecture of the human *SRR* gene and then examined the genetic role of this gene in the susceptibility to schizophrenia and regulation of serum D-serine levels. We also studied the genetic contribution of the DAO, which catalyzes the oxidative deamination of D-amino acids, with the exception of D-aspartate and D-glutamate (which are oxidized by D-aspartate oxidase; Sacchi et al 2002), to the previously mentioned phenotypes.

Methods and Materials

Subjects

Fifty patients with schizophrenia (mean age 36.9 ± 14.2 years) and 52 healthy control subjects (mean age 30.3 ± 8.0

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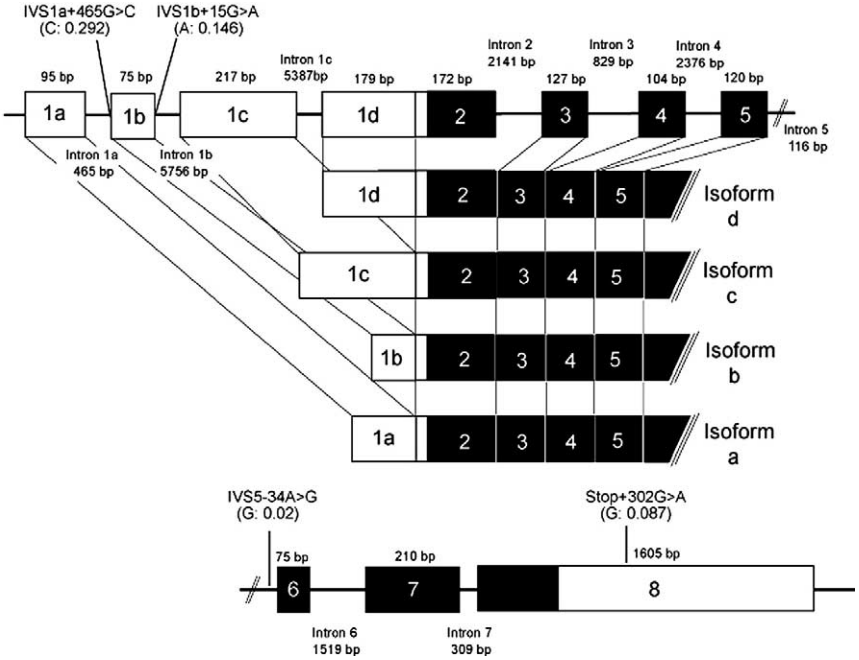

Figure 1. Genomic structure, transcript isoforms, and location of polymorphic sites for human *SRR*. Exons are denoted by boxes, with untranslated regions in white and translated regions in black. Note that the alternative use of 5′ end untranslated exons of 1a, 1b, 1c, and 1d generates four mRNA isoforms, of which isoform b is the major transcript in the brain.

years; hereafter referred to as sample set A) were used for evaluation of both serum levels of D and L serines and genotypes. Among these subjects, 31 schizophrenia patients (62%) and 44 normal control subjects (85%) were newly recruited in this study; the remainder were the same as those analyzed previously (Hashimoto et al 2003).

For a large-scale case–control genetic association study, an independent sample panel (referred to as sample set B) was used, which comprised 570 unrelated schizophrenia patients (285 men, 285 women; mean age 47.0 ± 11.4 years) and 570 age- and gender-matched control subjects who showed no history of mental illness in a brief psychiatric interview (285 men, 285 women; mean age 46.7 ± 11.1 years).

The third independent sample panel (referred to as sample set C), which was used for a family-based association test, consisted of 124 families with 376 members, of whom 163 were affected. This included 80 independent and complete trios (schizophrenic offspring and their parents), 15 probands with one parent, 13 probands with affected siblings, and 30 probands with discordant siblings.

All subjects resided in central Japan. A consensual diagnosis was made according to DSM-IV by at least two experienced psychiatrists on the basis of direct interviews, available medical records, and information from hospital staff and relatives. None of the patients had additional Axis I disorders as defined by DSM-IV.

The study was approved by the ethics committees of RIKEN and Chiba University Graduate School of Medicine. All control subjects and patients and family members gave informed written consent to participate in the study after provision and explanation of study protocols and purposes.

5′-RACE (Rapid Amplification of cDNA Ends) and Determination of the Genomic Structure for SRR

A partial cDNA sequence for *SRR* was obtained from GenBank (accession No. NM_021947) and the full length transcript was isolated by 5′-RACE using a brain-derived Marathon cDNA kit (BD Biosciences Clontech, Palo Alto, California), according to

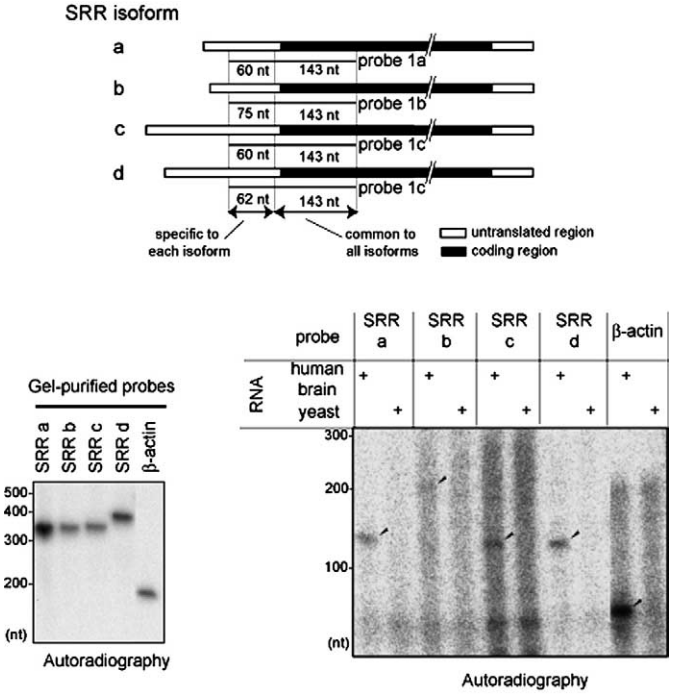

Figure 2. RNase protection assay of *SRR* transcripts. Upper panel shows *SRR* mRNA isoforms and positions of specific probes (probes 1a, 1b, 1c, and 1d). Filled boxes show the coding region of mRNA and open boxes indicate 5′- or 3′-untranslated regions. Note that all four probes consist of an isoform-specific region (60–75 nt) and common region (143 nt). Each probe is flanked by 71 nt at the 5′ end and 41 nt at 3′ end with vector derived sequences. The lower left panel is an autoradiogram, showing the integrity of radio-labeled riboprobes. Probes for *SRR* isoforms a–d and β-actin were gel-purified after synthesis and re-electrophoresed in a denaturing gel to evaluate the integrity of the probes. The lower right panel shows the results of the RNase protection assay. Each probe was hybridized to human total RNA or yeast total RNA (negative control), and then an RNase protection assay was performed. Arrowheads in the autoradiogram show the positions of specific signals.