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# Analysis of the 5′-Flanking Promoter Region of the 5-$HT_{2A}$ Receptor Gene in Schizophrenia

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*We studied the 5′-flanking promoter region comprising positions −1441 to +128 of the 5-$HT_{2A}$ receptor gene in 95 schizophrenics and 100 unrelated normal control subjects. The genes encoding the 5-$HT_{2A}$ receptor exons and the adjoining introl regions had already been studied in these subjects, but no disease specific polymorphism was found (Ishigaki et al., 1996). The DNA fragments were amplified by means of the polymerase chain reaction (PCR), and then analyzed by the single-stranded conformation polymorphism (SSCP) and sequencing methods. One patient had a substitution, from A to G, at position −668, and a 5 nucleotide deletion of TACTT at positions −646 to −642, however, the patient also had a normal sequence on the other allele. SSCP analysis showed that the other schizophrenics and the control subjects did not have any polymorphism in the 5′-flanking promoter region of the 5-$HT_{2A}$ receptor gene.* **[*Neuropsychopharmacology 17:274–278, 1997*]** *© 1997 American College of Neuropsychopharmacology. Published by Elsevier Science Inc.*

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The involvement of 5-HT in schizophrenia has been hypothesized for the last four decades (see Roth and Meltzer 1995, for a review). In particular, the 5-$HT_{2A}$ receptor has received much attention since it appears to be an important site of action of atypical antipsychotic agents (Kane 1994).

A decrease in the 5-HT receptor density in the frontal cortex of schizophrenics was found by Bennett et al. (1979) using tritiated lysergic acid diethylamide, which exhibits equal affinities for the 5-$HT_1$ and 5-$HT_2$ receptors, although others have failed to replicate their findings (Owen et al. 1981; Whitaker et al. 1981). Mita et al. (1986), using tritiated ketanserin, which is a more specific 5-$HT_{2A}$ receptor ligand, found that the number of 5-$HT_{2A}$ receptors was decreased in the prefrontal cortex of postmortem brains of schizophrenics. There was no difference in receptor density between patients taking neuroleptics until their death and those that had taken none for two months prior to their death. The same group also reported that the numbers of 5-$HT_{1A}$ receptors and D2 receptors were increased in the prefrontal cortex of schizophrenics (Mita et al. 1986; Hashimoto et al. 1993). Subsequently, Laruelle et al. (1993) found, using tritiated ketanserin, that the 5-$HT_{2A}$ receptor density in the prefrontal cortex was decreased in chronic psychotics dying of natural causes, as opposed to in psychotics dying of suicide, controls, and nonpsychotic suicide victims.

Analysis of linkage between the 5-$HT_{2A}$ receptor gene and schizophrenia in a large Swedish kindred showed that the gene itself is not a major susceptibility gene for schizophrenia (Hallmayer et al. 1992). Arranz et al. (1995) reported that homozygosity of the C at position 102 in the exon 1 allele was more frequent among patients who did not respond to clozapine than in the ones who did. We studied all exons and the flanking intron regions of the $5H_{2A}$ receptor gene in 100 schizo-

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phrenics by means of the polymerase chain reaction (PCR) and single-strand conformation polymorphism (SSCP) methods (Ishigaki et al. 1996) There was no association between the substitution of C for T at position 102 and the subdiagnosis, family history, age of onset, amounts of antipsychotics, or clinical symptoms. No other polymorphism was found.

Recently, the 5′-flanking promoter region of the 5-$HT_{2A}$ receptor gene was characterized in man (Zhu et al. 1995) and rat (Du et al. 1994, 1995). One possible reason for the decreased 5-$HT_{2A}$ receptor density in the brain in schizophrenia may be the aberrant 5-$HT_{2A}$ receptor gene promoter. Thus, in this study, we analyzed the 5′-flanking region of the 5-$H_{2A}$ receptor gene in schizophrenia.

## METHODS

### Subjects

Patients were collected from the Department of Psychiatry, Hamamatsu University School of Medicine Hospital, and affiliated hospitals. Each patient (or his/her legal guardian) gave informed consent to participate in the study. A structured diagnostic interview was given, using the Schedule for Affective Disorders and Schizophrenia (SADS) (Endicott and Spitzer 1978), by research psychiatrists who are Designated Physicians for Mental Health in Japan. All available medical records and information from relatives and medical staff were used to supplement the interviews. The patients were diagnosed according to the DSM-IV criteria (American Psychiatric Association, 1994). Patients with concurrent drug abuse, alcoholism, organic brain disorders, or mental retardation were excluded from the study. The positive and negative symptoms, before and after medication, were evaluated according to the Positive and Negative Syndrome Scale (PANSS) (Kay et al. 1991). The positive and negative symptoms were evaluated before medication and after conventional antipsychotic medication for at least six months. A positive family history was defined as the presence of at least one first- or second-degree relative with schizophrenia or schizoaffective disorder. The mean doses of antipsychotic drugs administered to the patients were expressed by the ratio of each antipsychotic drug to haloperidol (Toro 1984). The patient population consisted of 95 schizophrenics (mean age (SD), 50.8 (14.5) years; mean age at onset, 26.8 (9.63) years; male, 46; female, 49). Thirty-three schizophrenics had a positive family history. The mean (SD) scores of positive symptoms were 23.3 (6.00) before medication and 15.9 (6.03) after medication, respectively. The mean (SD) scores of negative symptoms were 25.0 (7.43) before medication and 27.5 (8.87) after medication, respectively. The gene encoding the 5-$HT_{2A}$ receptor had already been analyzed, but no disease specific polymorphism was found (Ishigaki et al. 1996).

**Table 1.** Primers for PCR, Sequencing, SSCP and RFLPs

| Name | Position | Sequence (5′ to 3′) |
|---|---|---|
| S2AP12-U | −1441 to −1422 | CCAGACACTCACAGCACTCC |
| S2AP12-L | −1226 to −1245 | GGACCAAACAGGCTTTTTCT |
| S2AP13-U | −1269 to −1250 | TAATATGTAGCAAAAAGAGG |
| S2AP13-L | −1036 to −1055 | ATTTAGAAATCATTCACGAG |
| S2AP14-U | −1077 to −1058 | TCTTTTGTGCGACTTTGAGG |
| S2AP14-L | −830 to −849 | TCACAGTAATTAAACTGGTG |
| S2AP15-U | −877 to −858 | GGTGGAAGATCAAGAAGAGG |
| S2AP15-L | −693 to −712 | ATTACATGTTGGCCAAGCAT |
| S2AP1-U | −743 to −724 | TTGCTGTGTAATTTTCTTTC |
| S2AP1-L | −543 to −562 | AGTTCTCAGCCATTCTTAGG |
| S2AP2-U | −600 to −581 | AGGAGCTGAAATTCCTGACA |
| S2AP2-L | −355 to −374 | ACTACTGGGACTCTTGTTTA |
| S2AP3-U | −422 to −403 | TGTTGCTAAACTAGTACCAT |
| S2AP3-L | −205 to −224 | AATTTTAGGAGAGTCCACTG |
| S2AP4-U | −258 to −239 | GTCCTCGGTTTGGTGAGAAA |
| S2AP4-L | −31 to −49 | TGTAGAAGGACTAACAGGTT |
| S2AP5-U | −102 to −83 | CCTCAGTGTTACAGAGTGTG |
| S2AP5-L | +128 to +109 | AATGCATCAGAAGTGTTAGC |

The nucleotide positions are quoted from Zhu et al. (1995). The sequences of primers S2AP12-L, S2AP13-L, S2AP14-L, S2AP15-L, S2AP1-L, S2AP2-L, S2AP3-L S2AP4-L, and S2AP5-L are the complementary sequences of the corresponding positions. The primers for PCR were combined and named as follows: primers S2AP12-U and S2AP12-L, C12; S2AP13-U and S2AP13-L, C13; S2AP14-U and S2AP14-L, C14; S2AP15-U and S2AP15-L, C15; S2AP1-U and S2AP1-L, C1; S2AP2-U and S2AP2-L, C2; S2AP3-U and S2AP3-L, C3; S2AP4-U and S2AP4-L, C4; and S2AP5-U and S2AP5-L, C5.

### DNA Amplification

Genomic DNA was obtained from whole blood by the method of Wang et al. (1994). A genomic DNA segment of 1569 base pairs (bp), positions −1441 to +128, of the 5-$HT_{2A}$ receptor was divided into nine fragments, which were amplified *in vitro* by PCR. The sequence for the human 5-$HT_{2A}$ receptor gene promoter was reported by Zhu et al. (1995). The positions of the primers are shown in Table 1. PCR was performed by the method of Saiki et al. (1988) in tubes containing 200 ng of genomic DNA, 100 ng of each primer, 200 μmol of each dNTP, and 2.5 U of *Taq* polymerase, in a final volume of 100 μl Gene Amp buffer. Amplification was carried out for 30 cycles: incubations for 30 sec at 94°C for denaturation; 30 sec at 48 to 51°C for annealing; and 60 sec at 72°C for primer extension. At the beginning of the first cycle, the DNA was denatured at 94°C for 2 min, and following the last cycle, the samples were incubated at 72°C for 7 min. The samples were stored at 4°C and then analyzed by gel electrophoresis on 1% agarose gels containing ethidium bromide.

### Sequence Analysis

The amplified DNA materials were ligated into plasmid pCR™II, according to the manufacturer's protocol (TA cloning kit, Invitrogen). The nucleotide sequences of the amplified samples from some subjects were determined

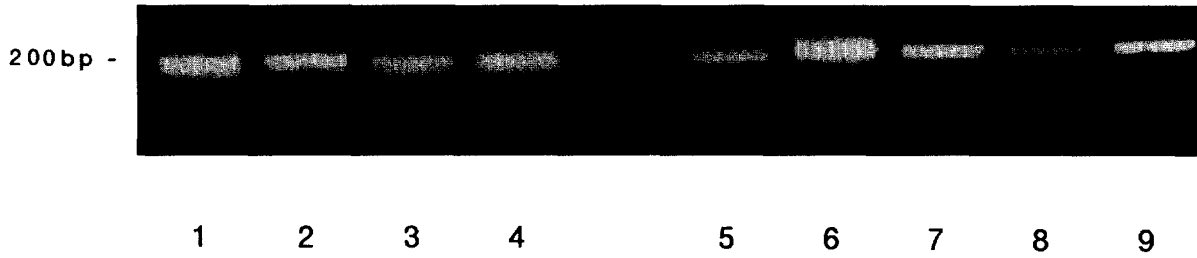


**Figure 1.** Agarose gel electrophoresis of the PCR products. Fifteen microliters of amplified DNA, obtained with selected primers, was run in a 1% agarose gel and then visualized with ethidium bromide. Lane 1, C12; lane 2, C13; lane 3, C14; lane 4, C15; lane 5, C1; lane 6, C2; lane 7, C3; lane 8, C4; lane 9, C5. See also Table 1.

by the dideoxy chain termination method (Sanger et al. 1977). We examined a total of 10 schizophrenic patients.

### Single-Strand Conformation Polymorphism Analysis

The samples from 95 schizophrenic patients were subjected to non-radioactive SSCP analysis method at 4°C to screen for DNA polymorphisms in the gene (Hongyo et al. 1993; Oto et al. 1993), as described elsewhere (Ishigaki et al. 1996).

## RESULTS

The 5′-flanking region of the 5-$HT_{2A}$ receptor gene, positions −1441 to +128, was divided into nine fragments, which were amplified by PCR. Each interval of coupled

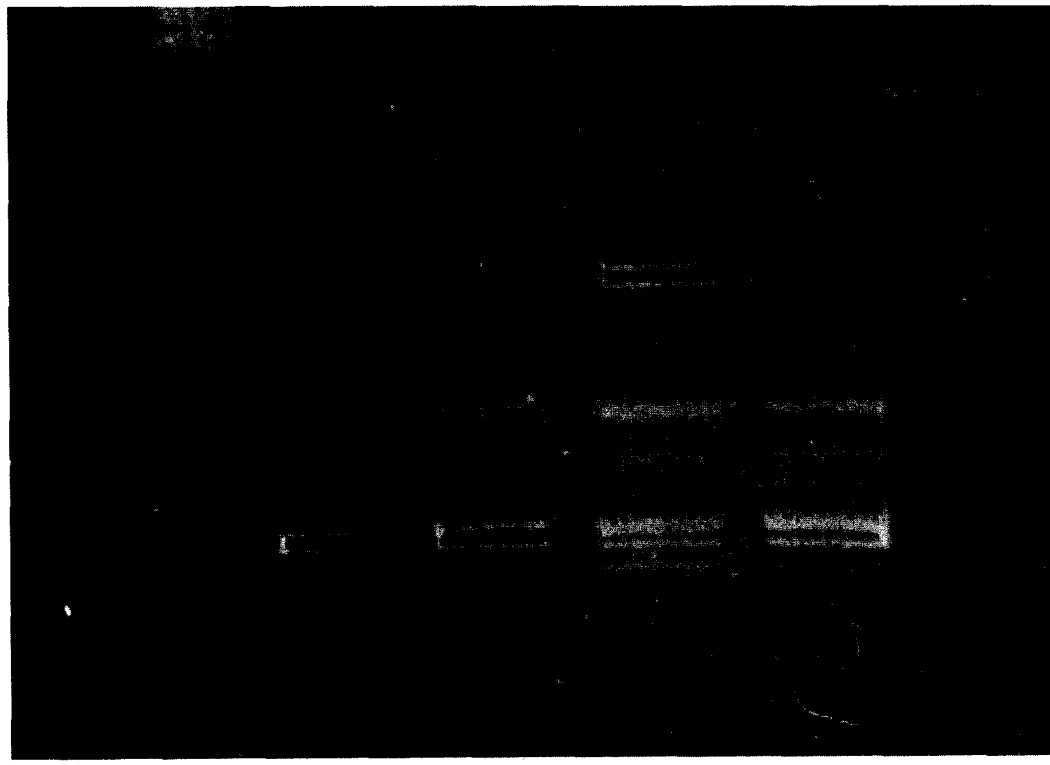


**Figure 2.** SSCP analysis of the S2P1 region. DNA samples were amplified with primers S2P1-U and S2P1-L, and subsequently analyzed by the SSCP method. Lanes 1, 2, and 4 show normal patterns, whereas lane 3, for patient No. 123, shows an abnormal pattern.

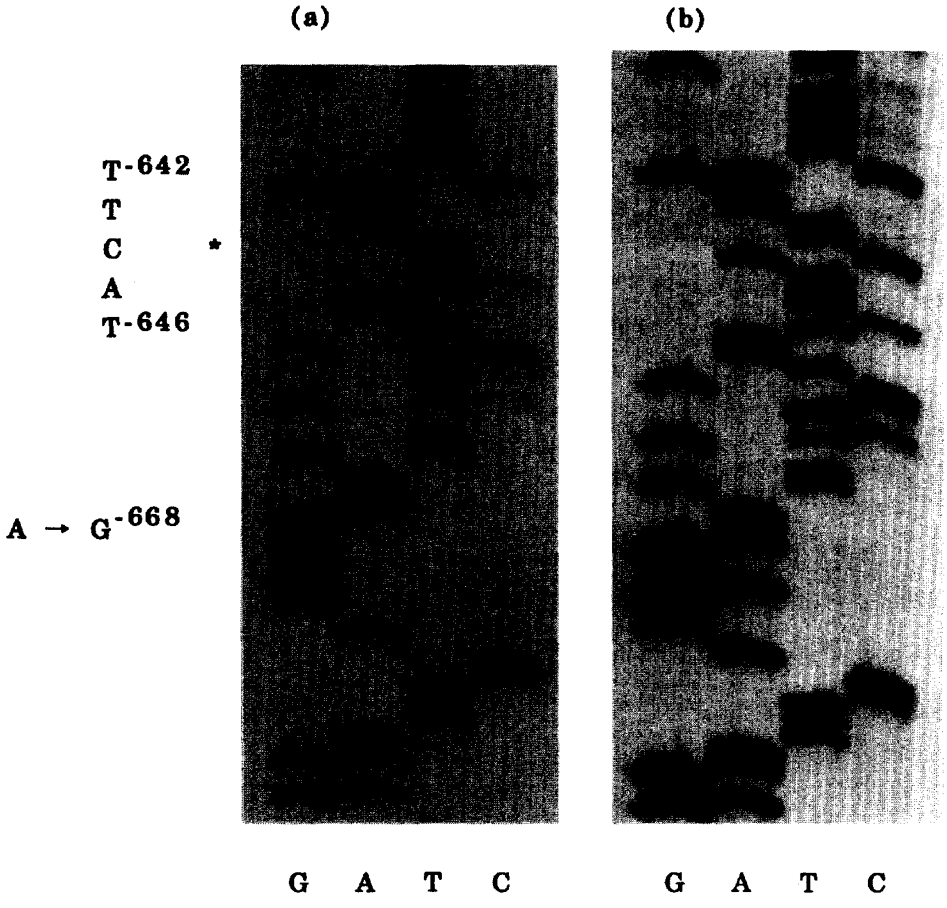


**Figure 3.** Sequence analysis of the S2P1 region. Sequence analysis revealed that patient No. 123 had a deletion of five nucleotides, TACTT, at positions −646 to −642, and a substitution, from A to G, at position −668 (a), in comparison with another subject (b).

primers was about 200 bp in length and thus suitable for SSCP analysis (Hayaski 1991) (Figure 1).

SSCP screening of S2AP1 revealed a different pattern in patient No. 123 (Figure 2). Sequence analysis revealed that patient No. 123 had a substitution from A to G at position −668, and a 5 nucleotide deletion of TACTT at positions −646 to −642 (Figure 3). The patient's other allele had a normal sequence. No other polymorphism was detected on SSCP analyses. Patient No. 123 was a 63-year-old woman, who developed an undifferentiated type of schizophrenia at age 27. She did not have a family history. Her total positive symptom scores were 30 before medication and 14 after medication, whereas her total negative symptom scores were 21 before medication and 35 after medication. She was in a psychiatric hospital for 35 years where she received 7 mg/day of neuroleptics. Her clinical course was defined, according to DSM-IV, as 'episodic with interepisode residual symptoms with prominent negative symptoms.'

## DISCUSSION

Recently, the 5'-flanking promoter region of the 5-$HT_{2A}$ receptor gene was described. In man, the 0.74 kilobase-pairs (kbp) HaeIII/PvuII fragment comprising positions −1316 to −577 exhibits significant promoter activity. This promoter activity is not affected by the sequence upstream of the 0.74 kbp fragment. The sequence downstream (the 0.45 kbp PvuII/SmaI fragment comprising positions −577 to −125) was found to strongly repress this promoter activity (Zhu et al. 1995). Du et al. (1995) suggested that in rat the basal promoter unit is included in positions −25 to +1, and that the upstream sequence comprising positions −1314 to −75 represses the basal promoter activity. Thus, as judged in our study, positions −1441 to +128 seem to cover the regulatory region of the 5-$H_{2A}$ receptor.

The substitution at position −668 in patient No. 123 may have affected the enhancer activity of the 5-$HT_{2A}$ receptor promoter, since the potential binding site for Ets proteins, i.e., AGGAAA, at positions −668 to −663 changed to GGGAAA. In fact, mutational analysis revealed that GGGAAA prevented the binding of Ets proteins (Wasylyk et al. 1991). These proteins regulate transcription initiation from a variety of cellular and viral gene promoter and enhancer elements (Macleod et al. 1992). Such a change, were it prevalent, could explain the decreased number of 5-$HT_{2A}$ receptors in the brains of some schizophrenics.

However, we must mention that in the rest of the 94 schizophrenics examined here, the sequence of the

5-$HT_{2A}$ receptor regulatory region was normal. The results may suggest that a decreased number of 5-$HT_{2A}$ receptors is not the primary etiology of schizophrenia but secondary to the disease process.

In our previous study (Ishigaki et al. 1996) and the present report, we screened, in schizophrenics, the 5-$HT_{2A}$ receptor coding region and its regulatory region at about 1.5 kb upstream of the translational initiation codon. However, this gene is greater than 20 kbp in length and contains three exons separated by two introns, so it is possible that other areas that affect expression of the gene may vary.

## ACKNOWLEDGMENTS

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