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Intact 5-HT_{2A} Receptor Exons and the Adjoining Intron Regions in Schizophrenia

Tatsuya Ishigaki, M.D., Dar-Win Xie, M.D., Ph.D., Jian-Cheng Liu, M.D., Yuhei Nakamura, M.D., Hai-Yin Zhang, M.D., Kunihiko Tani, M.D., Yoshihiro Shimazu, M.D., Kevin Chen, Ph.D., Jean C. Shih, Ph.D., Katsumasa Miyasato, M.D., Ph.D., Kenshiro Ohara, M.D., Ph.D., and Koichi Ohara, M.D., Ph.D.

Genes that regulate serotonergic (5-HT) systems may underlie the etiology of schizophrenia. In this study the gene encoding the 5-HT_{2A} receptor in schizophrenics and healthy controls was examined. First, we sequenced all exons and the flanking introns of the 5-HT_{2A} receptor gene in 10 schizophrenics and 10 controls. The substitution of C for T at position 102 in exon 1, which had been reported by Warren et al. (1993), was confirmed. Restriction fragment length polymorphism (RFLP) analysis revealed no association between polymorphism and schizophrenia. There was no association between the polymorphism and subdiagnosis, family history, age of onset, amounts of antipsychotics, or positive and negative symptoms before or after medication. Other polymorphisms in the gene were screened in 100 schizophrenics by the single-strand conformation polymorphism method, but none was found. Our results suggest that an abnormality in the 5-HT_{2A} receptor gene in schizophrenia is unlikely.
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KEY WORDS: *Schizophrenia; 5-HT_{2A} receptor; Polymerase chain reaction; Restriction fragment length polymorphism; Single-strand conformation polymorphism; Polymorphism*

To date, three different serotonin type 2 receptors (5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C}) have been cloned and found to be G-protein linked type receptors the effect of which is mediated through activation of phosphoinositide metabolism (Julius 1991; Hoyer et al. 1994; Watson and Girdlestone (1994). In this article the official nomenclature of 5-HT receptors approved by IUPHAR is used (Humphrey et al. 1993). According to this classification, the historical 5-HT_2 receptor is termed the 5-HT_{2A} receptor.

Although the roles of dopamine D_2 receptors in the mechanisms of antipsychotics have received most attention since most antipsychotics were found to be D_2 receptor antagonists and their efficacy was found to be correlated with their affinity for D_2 receptors (Creese et al. 1976; Seeman et al. 1976), it has been regarded that the D_2 receptor antagonists are effective only on positive symptoms, and not on negative ones (Crow 1980a, 1980b). On the other hand, Leysen et al. (1978) showed that serotonergic receptors were also targets for antipsychotics. In addition, atypical antipsychotic drugs with strong 5-HT_{2A} receptor blocking properties, such as clozapine, risperidone, and ritanserin (Meltzer et al. 1989; Nordstrom et al. 1993; Nyberg et al. 1993; Leysen et al. 1994) may be efficacious in some treatment-resistant patients and may improve negative symptoms (Kane et al. 1988; Claus et al. 1992; Duinkerke et al. 1993). These findings suggest that blockading of the

From the Department of Psychiatry (TI; D-WX; J-CL; YN; KT; YS; KM; KO; KO), Hamamatsu University School of Medicine, Shizuoka, Japan; the Departments of Molecular Pharmacology and Toxicology (KC, JCS), School of Pharmacy, University of Southern California, Los Angeles, CA; and the Department of Psychiatry (H-YZ), Shanghai Second Medical University, Shanghai, China.

Address reprint requests to: Koichi Ohara, M.D., Ph.D., Department of Psychiatry, Hamamatsu University School of Medicine, 3600 Handa, Hamamatsu, Shizuoka, Japan 431-31.

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5-HT_{2A} receptors may be involved in the alleviation of negative symptoms of schizophrenia.

In a postmortem study Mita et al. (1986) showed that the number of tritiated ketanserin, a selective 5-HT_{2A} receptor antagonist, binding sites, was decreased, with no change in the dissociation constant in the prefrontal cortex of schizophrenics. Reynolds et al. (1983) found no such difference in a preliminary study. Mita et al. (1986) found there was no difference in 5-HT_{2A} receptor binding between medicated and nonmedicated patients. Subsequently, several studies were performed to replicate their findings (Arora and Meltzer 1991; Laruelle et al. 1993; Ohuoha et al. 1993). Joyce et al. (1993) showed an increase in 5-HT_{2A} receptors in the posterior cingulate, temporal cortex, hippocampus, nucleus accumbens, and ventral putamen of schizophrenics. These results suggest that 5-HT_{2A} receptors may be involved in the etiology of schizophrenia.

In the present study, therefore, we examined the gene encoding the 5-HT_{2A} receptor in schizophrenic patients and control subjects. First, we performed sequencing of all exons and the adjoining intron regions in 10 schizophrenic patients and 10 control subjects. Single-strand conformation polymorphism (SSCP) analyses were carried out in another 100 schizophrenic patients to screen for possible polymorphisms of the gene. The substitution from T to C at position 102 in exon 1, which had previously been reported by Warren et al. (1993), was evaluated by means of the restriction fragment length polymorphism (RFLP) method in 158 schizophrenics and 150 normal controls.

SUBJECTS AND METHODS

Subjects

Patients were recruited from the Department of Psychiatry, Hamamatsu University School of Medicine Hospital, and affiliated hospitals. All gave informed consent. They were interviewed by two authors who are psychiatrists, designated physicians for mental health in Japan, neither of whom knew the results of the DNA analyses. Best estimate diagnoses were made by reviewing these interviews, all available medical records, and information from relatives and medical staff. They were diagnosed according to the DSM-IV criteria (American Psychiatric Association 1994). Their positive and negative symptoms before and after medication were evaluated according to the Positive and Negative Syndrome Scale (PANSS) (Kay et al. 1991) by the psychiatrists, who had trained in the use of the scale with a videotape of patient interviews with a Japanese translation, provided by Janssen-Kyowa Co., Ltd. (Japan). A positive family history was defined as the presence of at least one second-degree relative with schizophrenia, schizoaffective disorder, mood disorder, or who had committed suicide. The mean doses of antipsychotic drugs administered to the patients were calculated by the ratio of each antipsychotic drug to haloperidol (Toru 1984). Healthy control subjects were unrelated medical staff and students living in the same area as the patients, and they were screened for a lifetime history of past or current psychiatric disorders. The patient population consisted of 158 schizophrenics (mean age ± SD, 44.61 ± 16.23; mean age at onset, 26.09 ± 9.71; male, 81; female, 77). The details are given in Table 1. The control subjects consisted of 150 normal volunteers (mean age, 37.07 ± 15.45; male, 65; female, 85). All patients and healthy control subjects were Japanese.

Materials

The primers for polymerase chain reaction (PCR) and sequencing were synthesized by Sawady Technology (Japan). A PCR reagent kit including *AmpliTaq* DNA polymerase (*Taq* polymerase) from Perkin Elmer Cetus

Table 1. Demographic and Clinical Characteristics of the Schizophrenics in the Study

Variable	Total (n = 158)	Paranoid (n = 35)	Disorganized (n = 27)	Catatonic (n = 5)	Undifferentiated (n = 81)	Residual (n = 10)
Sex (M/F)	81/77	19/16	10/17	2/3	45/36	5/5
Age of onset (yr)	26.1 ± 9.71	30.2 ± 11.6	22.3 ± 8.31	22.2 ± 7.56	25.4 ± 8.71	29.0 ± 10.3
Age (yr)	44.6 ± 16.2	40.8 ± 13.3	41.6 ± 16.0	29.2 ± 10.1	46.3 ± 16.9	59.9 ± 9.54
Positive family history	46	6	10	2	23	5
Antipsychotics (mg/d)	17.0 ± 18.6	8.87 ± 8.99	26.4 ± 21.4	28.4 ± 26.4	17.3 ± 19.4	12.4 ± 12.9
Positive symptoms						
Before medication	22.3 ± 6.01	24.1 ± 7.28	21.4 ± 6.10	24.0 ± 6.52	22.5 ± 5.08	16.2 ± 3.97
After medication	14.5 ± 5.72	14.1 ± 8.11	14.5 ± 5.49	12.0 ± 5.39	15.1 ± 4.82	13.1 ± 2.77
Negative symptoms						
Before medication	24.5 ± 7.91	16.5 ± 6.81	29.2 ± 7.60	13.8 ± 4.66	26.9 ± 5.37	26.3 ± 6.96
After medication	25.4 ± 9.17	15.5 ± 7.29	30.1 ± 7.81	12.6 ± 4.61	28.5 ± 6.92	28.6 ± 4.17

(USA) was purchased from Takara Biomedicals (Japan). 2'-, triphosphates (ddNTPs), T7 DNA polymerase, *Msp*I, and *Hha*I were obtained from Pharmacia (Japan). ^{35}S-dATP (1,200 Ci/mmol) was from Amersham (Japan). Scientific imaging films were from Kodak (Japan). Minislab gels (90 × 70 × 1 mm) and a Resolmax temperature controller were from ATTO Co., Ltd. (Japan). Other chemicals were purchased from Wako Pure Chemicals Ltd. (Japan).

Genomic DNA Preparation

Genomic DNA was obtained from whole blood by the method of Wang et al. (1994). Briefly, 0.5 ml of whole blood anticoagulated with EDTA-K_2 at 1 mg/ml blood was mixed with an equal volume of a lysis solution containing 1% Triton X-100 to lyse the cells, and then the nuclei were isolated. The isolated nuclei were suspended in an enzyme reaction solution containing 1% SDS and digested with 0.8 mg/ml proteinase K to liberate DNA from nuclear proteins. After a 1-hour incubation, a NaI solution was added to the nuclear lysate to give final concentrations of 4.5 M NaI and 0.4% SDS, followed by the addition of isopropanol. The contents of the tube were mixed well by inversion until a whitish precipitate appeared. The precipitate was collected by centrifugation and washed with an alcoholic solution. The washed precipitate was centrifuged and vacuum-dried and then dissolved in 50 μL of 20 mM Tris-EDTA buffer (pH 8.0) and stored at 4°C.

DNA Amplification by Polymerase Chain Reaction

The human 5-HT_{2A} receptor gene has been cloned and revealed to consist of three exons separated by two introns (Julius et al. 1990; Salzman et al. 1991; Chen et al. 1992). The genomic DNA region for the exons and the adjoining introns were amplified in vitro by PCR using *Taq* polymerase with selected primers. The positions of the primers are depicted in Table 2. PCR was performed by the method of Saiki et al. (1988) in tubes containing 200 ng of genomic DNA, 1 μg of each primer, 200 μmol each of dATP, dCTP, dGTP, and dTTP, and 2.5 U of *Taq* polymerase, in a final volume of 100 μL Gene Amp buffer. Amplification was carried out for 30 cycles: Each cycle consisted of incubations for 60 sec at 94°C for denaturation, 90 sec at 49 to 55°C for annealing, and 90 sec at 72°C for primer extension. At the beginning of the first cycle DNA was denatured at 94°C for 3 minutes; and following the last cycle, the samples were incubated at 72°C for 4 minutes. The samples were stored at 4°C and then analyzed by gel electrophoresis in 1% agarose gels containing ethidium bromide.

DNA Sequencing

We estimated 10 schizophrenic patients and 10 control subjects by means of direct sequencing. The nucleotide sequences of the amplified samples were determined by the dideoxy chain termination method (Sanger et al. 1977) as modified by Winship (1989). The PCR-ampli-

Table 2. Primers for PCR, Sequencing, SSCP, and RFLP

Primer No.	Position	Sequence (5' to 3')
1	−61 to −42	AGC AGA AAC TAT AAC CTG TT
2	131 to 112	TTA AAT GCA TCA GAA GTG TT
3	38 to 57	CAA CTA CGA ACT CCC TAA TG
4	349 to 330	CAA GTG ACA TCA GGA AAT AG
5	243 to 262	AGC CGT AGT GAT TAT TCT AA
6	I1/96 to I1/77	GTT TGT TTG CCC CCG GAG CC
7	I1/−65 to I1/−46	GGA TAG GGA TCC ATG TGC TC
8	I2/105 to I2/85	CAG TAG ATT GAG GAT GTC AGG
9	I2/−20 to I2/−39	TTC CTT AAT AAT CAT GTT TC
10	260 to 241	TGA AGA CAA AGA ACT CTG AG
11	177 to 196	GAA GCT ACT TTG TGT GTA AG
12	422 to 403	CAT GAT GTT TGT GAT GAA GA
13	364 to 383	GCA TCG TCT TCT TCC TGT TT
14	632 to 613	CGG TAT TGT GTT CAC TAA AA
15	528 to 547	ACA CTG TTC AAC AAG ACC TA
16	783 to 764	CAT TCA CTC CGT CGC TAT TG
17	692 to 711	TGC CAA GAC AAC AGA TAA TG
18	938 to 919	ATA AAA TGA GGC ATA CAG AT

Nucleotide positions are quated from Chen et al. (1992). The sequences of primers 2, 4, 6, 8, 10, 12, 14, 16, and 18 are complementary sequences of corresponding positions. I1 and I2 denote intron 1 and intron 2, respectively. The first nucleotides of introns 1 and 2 are numbered I1/1 and I2/1, respectively. The last nucleotides of introns 1 and 2 are numbered I1/−1 and I2/−1, respectively. The couples for PCR were 1 and 2; 3 and 4; 5 and 6; 7 and 8; 9 and 10; 11 and 12; 13 and 14; 15 and 16; and 17 and 18, respectively. PCR with primers 1 and 4 was performed for *Msp*I digestion.

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     .....gcatgtacaccagcctcagtgttacagagtgtgggtacatcaaggtgaatggtga
     gcagaaactataacctgttagtccttctacacctcatctgctacaagttctggcttagac
1    ATGGATATTCTTTGTGAAGAAAATACTTCTTTGAGCTCAACTACGAACTCCCTAATGCAA
     MetAspIleLeuCysGluGluAsnThrSerLeuSerSerThrThrAsnSerLeuMetGln
61   TTAAATGATGACACCAGGCTCTACAGTAATGACTTTAACTCTGGAGAAGCTAACACTTCT
     LeuAsnAspAspThrArgLeuTyrSerAsnAspPheAsnSerGlyGluAlaAsnThrSer
121  GATGCATTTAACTGGACAGTCGACTCTGAAAATCGAACCAACCTTTCCTGTGAAGGGTGC
     AspAlaPheAsnTrpThrValAspSerGluAsnArgThrAsnLeuSerCysGluGlyCys
181  CTCTCACCGTCGTGTCTCTCCTTACTTCATCTCCAGGAAAAAAACTGGTCTGCTTTACTG
     LeuSerProSerCysLeuSerLeuLeuHisLeuGlnGluLysAsnTrpSerAlaLeuLeu
241  ACAGCCGTAGTGATTATTCTAACTATTGCTGGAAACATACTCGTCATCATGGCAGTGTCC
     ThrAlaValValIleIleLeuThrIleAlaGlyAsnIleLeuValIleMetAlaValSer
301  CTAGAGAAAAAGCTGCAGAATGCCACCAACTATTTCCTGATGTCACTTGCCATAGCTGAT
     LeuGluLysLysLeuGlnAsnAlaThrAsnTyrPheLeuMetSerLeuAlaIleAlaAsp
361  ATGCTGCTGGGTTTCCTTGTCATGCCCGTGTCCATGTTAACCATCCTGTATGgtgagtgg
     MetLeuLeuGlyPheLeuValMetProValSerMetLeuThrIleLeuTyrG
     cattagtttcccagctatattcgcactggtaataaagagcat.....(Intron 1)...
     ..gctccaggaggcacagggttgctcactgataccaaccttctgcctcatagGGTACCGG
                                                        lyTyrArg
421  TGGCCTCTGCCGAGCAAGCTTTGTGCAGTCTGGATTTACCTGGACGTGCTCTTCTCCACG
     TrpProLeuProSerLysLeuCysAlaValTrpIleTyrLeuAspValLeuPheSerThr
481  GCCTCCATCATGCACCTCTGCGCCATCTCGCTGGACCGCTACGTCGCCATCCAGAATCCC
     AlaSerIleMetHisLeuCysAlaIleSerLeuAspArgTyrValAlaIleGlnAsnPro
541  ATCCACCACAGCCGCTTCAACTCCAGAACTAAGGCATTTCTGAAAATCATTGCTGTTTGG
     IleHisHisSerArgPheAsnSerArgThrLysAlaPheLeuLysIleIleAlaValTrp
601  ACCATATCAGTAGgtaagtggcaacatatttcagagtctcatttgaaatgacaggtcggg
     ThrIleSerValG
     ctt.....(Intron 2).....catatacttaattccttaataatcatgtttcatttttc
     tgttcaactccagGTATATCCATGCCAATACCAGTCTTTGGGCTACAGGACGATTCGAAG
                        lyIleSerMetProIleProValPheGlyLeuGlnAspAspSerLys
661  GTCTTTAAGGAGGGGAGTTGCTTACTCGCCGATGATAACTTTGTCCTGATCGGCTCTTTT
     ValPheLysGluGlySerCysLeuLeuAlaAspAspAsnPheValLeuIleGlySerPhe
721  GTGTCATTTTTCATTCCCTTAACCATCATGGTGATCACCTACTTTCTAACTATCAAGTCA
     ValSerPhePheIleProLeuThrIleMetValIleThrTyrPheLeuThrIleLysSer
781  CTCCAGAAAGAAGCTACTTTGTGTGTAAGTGATCTTGGCACACGGGCCAAATTAGCTTCT
     LeuGlnLysGluAlaThrLeuCysValSerAspLeuGlyThrArgAlaLysLeuAlaSer
841  TTCAGCTTCCTCCCTCAGAGTTCTTTGTCTTCAGAAAAGCTCTTCCAGCGGTCGATCCAT
     PheSerPheLeuProGlnSerSerLeuSerSerGluLysLeuPheGlnArgSerIleHis
901  AGGGAGCCAGGGTCCTACACAGGCAGGAGGACTATGCAGTCCATCAGCAATGAGCAAAAG
     ArgGluProGlySerTyrThrGlyArgArgThrMetGlnSerIleSerAsnGluGlnLys
961  GCATGCAAGGTGCTGGGCATCGTCTTCTTCCTGTTTGTGGTGATGTGGTGCCCTTTCTTC
     AlaCysLysValLeuGlyIleValPhePheLeuPheValValMetTrpCysProPhePhe
1021 ATCACAAACATCATGGCCGTCATCTGCAAAGAGTCCTGCAATGAGGATGTCATTGGGGCC
     IleThrAsnIleMetAlaValIleCysLysGluSerCysAsnGluAspValIleGlyAla
1081 CTGCTCAATGTGTTTGTTTGGATCGGTTATCTCTCTTCAGCAGTCAACCCACTAGTCTAC
     LeuLeuAsnValPheValTrpIleGlyTyrLeuSerSerAlaValAsnProLeuValTyr
1141 ACACTGTTCAACAAGACCTATAGGTCAGCCTTTTCACGGTATATTCAGTGTCAGTACAAG
     ThrLeuPheAsnLysThrTyrArgSerAlaPheSerArgTyrIleGlnCysGlnTyrLys
1201 GAAAACAAAAAAACCATTGCAGTTAATTTTAGTGAACACAATACCGGCTTTGGCCTACAAG
     GluAsnLysLysProLeuGlnLeuIleLeuValAsnThrIleProAlaLeuAlaTyrLys
1261 TCTAGCCAACTTCAAATGGGACAAAAAAAAGAATTCAAAGCAAGATGCCAAGACAACAGAT
     SerSerGlnLeuGlnMetGlyGlnLysLysAsnSerLysGlnAspAlaLysThrThrAsp
1321 AATGACTGCTCAATGGTTGCTCTAGGAAAGCAGCATTCTGAAGAGGCTTCTAAAGACAAT
     AsnAspCysSerMetValAlaLeuGlyLysGlnHisSerGluGluAlaSerLysAspAsn
1381 AGCGACGGAGTGAATGAAAAGGTGAGCTGTGTGtgataggctagttgccgtggcaactgt
     SerAspGlyValAsnGluLysValSerCysVal---
     ggaaggcacactgagcaagttttcac.....
```

Figure 2. Agarose gel electrophoresis of the amplified products for the 5-HT$_{2A}$ receptor gene. Twenty μL of the amplified products, obtained with selected primers, was run in 1% agarose gel and then visualized with ethidium bromide. *M*, size markers; *lane 1*, amplified products with primers 1 and 2; *lane* 2, 3 and 4; *lane* 3, 5 and 6; *lane* 4, 7 and 8; *lane 5*, 9 and 10; *lane* 6, 11 and 12; *lane* 7, 13 and 14; *lane* 8, 15 and 16; and *lane* 9, 17 and 18.

Figure 3. SSCP analysis of the PCR products with primers 1–2 of the 5-HT$_{2A}$ receptor gene. The PCR products with primers 1 and 2 were subjected to SSCP analysis to confirm the polymorphism from T to C at position 102 in exon 1. *Lanes 5, 6, 11, and 12* correspond to the genotype, A1/A1 (T/T); *lanes 1, 7, 8, 9, and 10* correspond to the genotype, A1/A2 (T/C); and *lanes 2, 3, and 4* correspond to the genotype, A2/A2 (C/C).

fied materials (about 1–5 μg) were purified from 1% low-melt agarose gel using phenol and chloroform. Purified DNA (about 100 ng) was mixed with 150 ng of one of the amplification primers in 6 μl of 40 mM Tris-HCl (pH 7.5), 25 mM $MgCl_2$, 50 mM NaCl, and 10% dimethyl sulfoxide (DMSO). After heat denaturation, 4 μL of a labeling mixture comprising 25 mM DTT and 10 μCi of ^{35}S-dATP together with two units of T7 DNA polymerase was added. Ten microliter of the mixture was divided into four tubes containing 2 μL of 80 μM dCTP, dGTP and dTTP, 50 mM NaCl, 10% DMSO, and 0.08 μM ddATP (tube A); 8 μM ddCTP (tube C); 8 μM ddGTP (tube G); and 8 μM ddTTP (tube T), respectively. The tubes were incubated at 37°C for 5 minutes, and then 2 μL of 0.25 mM dATP, dCTP, dGTP, and dTTP, 50 mM NaCl, and 10% DMSO was added, followed by a further 5-minute incubation at 37°C.

Single-Strand Conformation Polymorphism Analysis

One hundred samples from schizophrenic patients were studied by the nonradioactive SSCP method to screen DNA polymorphisms in the gene (Hongyo et al. 1993; Oto et al. 1993). A mixture consisting of 5 μL of PCR products diluted with the loading buffer, comprising 0.1% xylene cyanol, 0.1% bromphenol blue, 0.4 μL of 1M methylmercury hydroxide, 1.0 μL of 95% formamide, and 13.6 μL of 1 × TGE buffer (25 mM 2-amino-2 hydroxymethyl-1,3-propandiol, 192 mM glycine), was prepared. This mixture was heated at 95°C for 5 minutes, then chilled on ice for denaturation. The entire 20 μL of the mixture was loaded on a polyacrylamide gel (PAGEL; NGP-1020L, ATTO Co., Japan; 10%–20% gradient gel). Electrophoresis was carried out in 1 × TGE buffer at 200 V for 6 hours. A thermostatic refrigerated circulator was used to maintain a constant preset temperature. The gel was stained with ethidium bromide, with visualization by ultraviolet transillumination.

Statistical Calculations

A chi-square statistic or Fisher's exact test (2 x 2) was used to compare categorical measures. Analysis of variance (ANOVA) was used to compare continuous measures. Post hoc pairwise analysis by Bonferroni and Dunn's method was performed if an overall significant ($p < .05$) ANOVA was obtained.

RESULTS

The cDNA and genomic sequences of exons of the 5-HT$_{2A}$ receptor have been described previously (Saltzman et al. 1991; Chen et al. 1992). Here we present additional intron sequences of flanking exons (Figure 1). Fragments of the 5-HT$_{2A}$ receptor gene in controls and schizophrenics were amplified by means of PCR, as shown in Figure 2. The *X* in intron 1 at position 5 from the end of exon 1, which remained to be elucidated (Chen et al. 1992), is G in both patients and control subjects. The nucleotide at position 7 in intron 1 is G instead of C. The single base pair substitution from T to C at position 102 in exon 1, which had been reported by Warren et al. (1993), was confirmed in both schizophrenic patients and healthy controls. This substitution does not alter the amino acid, serine.

Subsequently, SSCP analyses with all PCR products were performed to screen the possible abnormal sequences in the 5-HT$_{2A}$ receptor gene in 100 schizophrenics. The PCR products with primer pair 7–8 were digested with *Hha*I to yield the appropriate sizes of 216 and 155 base pairs. The materials were subsequently subjected to SSCP analysis. Various preset temperatures

Figure 1. The DNA and amino acid sequences of the human 5-HT$_{2A}$ receptor. Lowercase letters indicate noncoding bases; capitals indicate coding bases. Only the coding bases are numbered, using the scheme of Chen et al. (1992). The nucleotides at position 102 in exon 1 and at positions 5 and 7 in intron 1 from the end of exon 1 are bold and underlined.

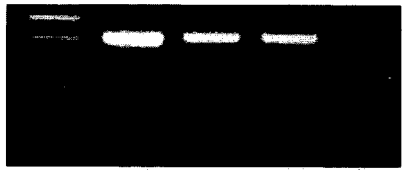

Figure 4. RFLP analysis of exon 1 of the 5-HT_{2A} receptor gene. Twenty μl of the amplified products with primers 1 and 4 was digested with 24 units of *MspI* in a final volume of 50 μl buffer (50 mM NaCl, 10 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$ and 1 mM DTT) at 37°C for 1 hour. Subsequently, the samples were electrophoresed in 1% agarose gel and visualized by ethidium bromide. A1/A1 is homozygous for allele 1, with a band of 410 bp. A1/A2 is heterozygous for alleles 1 and 2, with three bands of 410, 248, and 162 bp. A2/A2 is homozygous for allele 2, with two bands of 248 and 162 bp.

from 4°C to room temperature were examined to determine the appropriate condition for each primer pair. Two distinct bands were clearly obtained on SSCP at 8°C for the PCR products with primer pairs 3–4, 5–6, 9–10, 13–14, 15–16, and 17–18 and 4°C for the amplified products with primer pairs 1–2 and 11–12. Four distinct bands were observed with SSCP at 8°C for the PCR products with primer pair 7–8 digested with *Hha*I. The polymorphism from T to C at position 102 in exon 1 was also confirmed on SSCP of the PCR products with primer pairs 1–2 and 3–4. Figure 3 shows the different gel electrophoresis patterns of the amplified products with primers 1–2. Consequently, no novel polymorphisms were detected on SSCP analyses.

Because the substitution of C for T at position 102 in exon 1 created a *MspI* restriction enzyme site, we investigated the allele and genotype frequencies of the *MspI* polymorphism in schizophrenic patients and control subjects (Figure 4). Table 3 depicts the genotypic and allelic associations in subclinical categories of schizophrenia. No statistically significant differences were found between patients and control subjects in the genotype ($\chi^2 = 1.21$; df = 2; $p = .547$) or allele frequency ($\chi^2 = 0.020$; df = 1; p = .887). Furthermore, there were no differences between the genotype polymorphism and subdiagnosis ($\chi^2 = 7.44$; df = 8; $p = .490$), positive family history ($\chi^2 = 0.362$; df = 2; $p = 0.835$), age of onset (df = 2;155; $F = 1.68$; $p = .191$), amounts of antipsychotics (df = 2;155; $F = 2.55$; $p = .0813$), positive symptoms before medication (df = 2; 155; $F = 2.27$; $p = .106$) and after medication (df = 2; 155; $F = 0.319$; $p = .727$), or negative symptoms before medication (df = 2; 155; $F = 0.518$; $p = .727$) and after medication (df = 2; 155; $F = 0.049$, $p = .952$).

DISCUSSION

The linkage between schizophrenia and the 5-HT_{2A} receptor gene was excluded using the probe, phg53, for the *HTR2* locus in a large Swedish kindred (Hsieh et al. 1990; Hallmayer et al. 1992). However, the *HTR2* locus mentioned in Hallmayer's article was different from the polymorphic site at position 102 in exon 1. Moreover, a study concerning the association between schizophrenia and the 5-HT_{2A} receptor gene has not been previously reported. Herein we reported DNA sequencing and RFLP and SSCP analyses of the 5-HT_{2A} receptor gene in schizophrenia.

The previously unknown nucleotide at position 5 in intron 1 was found to be G. The nucleotide at position 7 in intron 1 was inconsistent with a previous report (Chen at al. 1992). Since these two nucleotides are located in an exon-intron junction, they may be involved

Table 3. Genotypes and Alleles of the MspI Polymorphism at Exon 1 of the 5-HT_{2A} Receptor Gene in Schizophrenic Patients and Control Subjects

	Genotype			Allele	
	A1/A1	A1/A2	A2/A2	Allele 1	Allele 2
Controls (n = 150)	45	69	36	159	141
Schizophrenics (n = 158)	54	63	41	171	145
Subdiagnosis					
Disorganized (27)	7	13	7	27	27
Catatonic (5)	1	3	1	5	5
Paranoid (35)	14	15	6	43	27
Undifferentiated (81)	26	30	25	82	80
Residual (10)	6	2	2	14	6
Family history					
Positive (46)	15	20	11	50	42
Negative (112)	39	43	30	121	103

in mRNA splicing. However, these findings were not disease specific and did not seem to contribute to the etiology of schizophrenia. The previously reported DNA polymorphism from T to C at position 12 at exon 1 (Warren et al. 1993) was observed on both DNA sequencing and SSCP analysis. Using this polymorphism as a marker, we can determine whether there is a linked mutation anywhere in the gene, even though the substitution does not alter the amino acid, serine, and the variation itself cannot be functionally significant. There were no statistically significant differences in the allele and genotype frequencies between patients and control subjects. In addition, there was no association between the polymorphism and subdiagnosis, family history, age of onset, amount of antipsychotics, or positive and negative symptoms in patients with schizophrenia.

All exons and the adjoining introns in the 5-HT_{2A} receptor gene were screened in the present study. However, this gene is greater than 20 kbp and contains three exons separated by two introns, so it remains possible that other areas that affect expression of the gene may show some variations. These include areas such as promoters, enhancers, and repressors that may operate at the level of gene activation, transcriptive initiation, or posttranslational processing. Several studies on the regulation of 5-HT_{2A} receptor gene expression have been reported. Recently, the 5′ flanking region of the 5-HT_{2A} receptor gene was cloned and sequenced, and its transcriptional regulatory functions was analyzed (Ding et al. 1993). Toth and Shenk (1994) reported that the 5-HT_{2A} receptor gene promoter contains multiple transcription initiation sites in a tumor cell line that does not show a mode of regulation like that found in vivo. It has also been shown that antagonist-mediated downregulation occurs at the level of transcription and that the downregulation is mediated by a specific DNA sequence in the 5′ flanking region of the receptor gene. These areas may be involved in the pathophysiology of schizophrenia.

Several methods, such as denaturing gradient gel electrophoresis, multiplex sequencing, and enzymatic mismatch scanning, allow efficient and large-scale molecular scanning. SSCP analysis could be useful, as a screening method because of its simplicity. SSCP analysis relies on the difference of the electrophoretic mobility in a single-strand DNA molecule (Orita et al. 1989). With this method 97% of the mutations in 100- to 300-base-long strands can be detected, the detection efficiency rate dropping to 67% for strands of 300 to 450 bases long (Hayashi 1991). In the present study all primer pairs other than pair 7–8 used for PCR were designed to amplify each PCR product of less than 300-base pairs. The PCR product with primer pair 7–8, which amounted to 371 base pairs, was digested with *Hha*I to yield 155- and 216-bp fragments that were suitable for SSCP analysis. Because the true sensitivity of SSCP analysis is not known, it might be possible that some mutations in the 5-HT_{2A} receptor gene were not identified.

Other 5-HT receptor genes (i.e., those for the 5-HT_{1A} and 5-HT_{2C} receptors), which have also been implicated in schizophrenia based on the results of pharmacological and postmortem studies, need to be analyzed. The abnormality in schizophrenia could be in dopaminergic-serotonergic interactions rather than in either transmitter alone (Meltzer 1989, 1992; Kahn et al. 1993). Moreover, noradrenergic or other transmitters systems may play a role in the pathophysiology of schizophrenia (Hsiao et al. 1993; Joyce 1993). These hypotheses must be tested by means of a molecular biological technique.

In summary, the results of DNA sequencing in 10 schizophrenics and 10 control subjects, SSCP analyses in 100 schizophrenics, and restriction enzyme analyses in 158 patients and 150 controls suggest that the 5-HT_{2A} receptor gene may not be responsible for the susceptibility to schizophrenia.

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