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The Gene Encoding the 5-HT$_{1A}$ Receptor Is Intact in Mood Disorders

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We studied the 5-HT$_{1A}$ receptor gene in 50 mood disorders and 50 normal volunteers. The 5-HT$_{1A}$ receptor gene was amplified by polymerase chain reaction and sequenced by the dideoxy method. The sequence of the 5-HT$_{1A}$ receptor encodes a protein of 422 amino acids, that is, one amino acid longer than the reported sequence (Kobilka et al. 1987). The DNA sequence at positions 454 to 459 is CGC GCC GCT, not CCG CGT, and the amino acids sequence at these positions is changing from proline arginine to arginine alanine alanine. These differences, however, were observed in both mood disorders and controls. One silent polymorphism, CTG to GTA at position 294, was found. These results suggest that the 5-HT$_{1A}$ receptor gene is intact in mood disorders.
[Neuropsychopharmacology 12:263–268, 1995]

KEY WORDS: *Mood disorders; 5-HT$_{1A}$ receptor; Polymerase chain reaction; Polymorphism*

The 5-HT$_{1A}$ receptor has been regarded to play a role in the pathogenesis of mood disorders. This hypothesis has been supported by several lines of evidence. Some studies have revealed that 5-HT$_{1A}$ agonists have an antidepressant effect. Schweizer et al. (1986) reported that buspirone, a 5-HT$_{1A}$ receptor agonist, had an antidepressant effect in nonmelancholic patients. Buspirone showed not only an antidepressive effect but also an antianxiety one (Goldberg and Finnerty 1979). The antidepressant-like action of 5-HT$_{1A}$ agonists in animal models of depression has also been reported (Glaser 1988; Kennet et al. 1987). Second, the long-term administration of monoamine oxidase inhibitors to rats decreased the density of 5-HT$_{1A}$ receptors (Palfreyman et al. 1987). The time course of this downregulation is similar to the time taken for a clinical response to occur in patients. Third, the number of 5-HT$_{1A}$ receptors in the frontal cortex of nonviolent suicide victims was found to be increased (Matsubara et al. 1991). The pathogeneses in suicide cases are heterogeneous, however, nearly half of them suffer from mood disorders (Roy 1989). These observations may also suggest that abnormal 5-HT$_{1A}$ receptors are associated with mood disorders.

The human 5-HT$_{1A}$ receptor has been cloned and revealed to have seven transmembrane domains that couple to guanine nucleotide-binding proteins (Fargin et al. 1988; Kobilka et al. 1987). One possible cause of mood disorders is that the gene encoding the 5-HT$_{1A}$ receptor has point mutation(s) that alter the character of the receptor. In fact, Guan et al. (1992) constructed by means of site-specific and random mutagenesis an artificial 5-HT$_{1A}$ receptor mutant, which had one amino acid substitution (i.e., valine for asparagine at position 385 in the VII transmembrane segment). They found that the mutant receptor had a 100-fold lower affinity for pindolol than the wild-type 5-HT$_{1A}$ receptor.

In this study we examined whether or not there is a point mutation in the DNA sequence of the 5-HT$_{1A}$ receptor gene in patients with mood disorders by means of polymerase chain reaction (PCR).

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Received June 15, 1994; revised October 17, 1994; accepted October 28, 1994.

Published by Elsevier Science Inc.
655 Avenue of the Americas, New York, NY 10010

0893-133X/95/$9.50
SSDI 0893-133X(94)00085-E

METHODS

Materials

The primers for PCR and sequencing were synthesized by Sawady Technology (Japan). A PCR reagent kit from Perkin Elmer Cetus (USA) was purchased from Takara Biomedicals (Japan). *Taq* polymerase, 2'-deoxynucleoside 5'-triphosphates (dNTPs), 2', 3'-dideoxynucleoside 5'-triphosphates (ddNTPs), T7 DNA polymerase, 100 Base-Pair Ladder were from Pharmacia (Japan). ^{35}S-dATP (1,200 Ci/mmol) was from Amersham (Japan). Scientific imaging films were from Kodak (Japan). Other chemicals were bought from Wako Pure Chemicals Ltd. (Japan).

Patients

Patients who were admitted to the inpatient and outpatient units of the Department of Psychiatry, Hamamatsu University School of Medicine Hospital, participated in this project. All gave informed consent. They were interviewed by psychiatrists who are Designated Physicians for Mental Health in Japan. The patient population consisted of 25 major depression (mean age, 40.4 ± 11.9; mean age at onset, 33.7 ± 13.0; male, 11; female, 14) and 25 bipolar disorders (mean age, 35.4 ± 18.0; mean age at onset, 28.8 ± 14.2; male, 14; female, 11) diagnosed according to the DSM-III-R criteria (American Psychiatric Association, 1987). The mean Hamilton depression rating scale (Hamilton 1960) were 15.5 ± 4.1 and 21.3 ± 6.5, respectively. Nine of the bipolar disorder patients were in manic phase. Seven of the major depression and seven of the bipolar disorder patients were family history-positive patients. Control genomic DNA was obtained from 50 normal volunteers (mean age, 30.1 ± 8.2; male, 25; female, 25).

Genomic DNA Preparation

Genomic DNA was obtained from blood using the method by Johns and Paulus-Thomas (1989). Briefly, 10 ml of whole blood was taken from the antecubital vein of each individual and then incubated on ice for 10 minutes with 35 ml of 0.32 M sucrose, 10 mM Tris-Cl (pH 8.0), 5 mM $MgCl_2$ and 1% Triton X-100. After centrifugation at 1,000 × g for 20 minutes, the remaining leukocytes were resuspended in 10 ml of 50 mM Tris-Cl (pH 8.0), 150 mM NaCl, 100 mM EDTA, 0.2% SDS and 1 mg RNaseA and then incubated at 37°C for 30 minutes. The solution was adjusted to 1.5% SDS and then heated at 60°C for 10 minutes. Following this step, 2.5 ml of freshly made 5 M sodium perchlorate was mixed in gently. Genomic DNA was extracted with chloroform, precipitated with ethanol, and then dissolved in 1 ml of 20 mM Tris-EDTA buffer (pH 8.0) and stored at 4°C.

DNA Amplification by the Polymerase Chain Reaction

Regions of the 5-HT_{1A} receptor gene were selectively amplified in vitro by PCR with selected primers. The sequences of the primers are depicted in Table 1. Polymerase chain reaction 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 Gene Amp buffer, in a final volume of 100 μl. Amplification was carried out for 30 cycles: each cycle consisted of incubations for 60 sec at 94°C for denaturation, 90 sec at 58°C for annealing, and 90 sec at 72°C for primer extension. At the beginning of the first cycle, the DNA was denatured at 94°C for 3 minutes; 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 electrophoresis in 1% agarose gel containing ethidium bromide.

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-amplified materials (about 1–5 μg) were purified from 1% low-melt agarose gel using phenol and chloroform. The purified DNA (about 100 ng) was mixed with 150 ng of one of the amplification primers in 6 μl

Table 1. Primers for an Amplification and Sequencing

Name	Position
1A-1	−66 to −47
1A-2	133 to 114
1A-3	25 to 44
1A-4	271 to 252
1A-5	206 to 225
1A-6	430 to 411
1A-7	337 to 356
1A-8	655 to 636
1A-9	569 to 588
1A-10	859 to 840
1A-11	770 to 789
1A-12	1099 to 1080
1A-13	1030 to 1049
1A-14	1266 to 1247
1A-15	1180 to 1199
1A-16	1405 to 1386

Nucleotide positions correspond to those of the 5-HT_{1A} receptor gene depicted in Figure 2. Primers 1A-2, 1A-4, 1A-6, 1A-8, 1A-10, 1A-12, 1A-14, and 1A-16 are complementary to DNA sequence in the 5-HT_{1A} receptor gene. The primers for PCR were combined and named as follows: primers 1A-1 and 1A-2, C1-2; 1A-3 and 1A-4, C3-4; 1A-5 and 1A-6, C5-6; 1A-7 and 1A-8, C7-8; 1A-9 and 1A-10, C9-10; 1A-11 and 1A-12, C11-12; 1A-13 and 1A-14, C13-14; 1A-15 and 1A-16, C15-16. See also Figure 2.

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-70  GGGGAAGGGG CGAGGCGAAT CTTCGCGCTG CTTTTTCTTC CCTCCCCCTT
-20  CCCGCGCCGG GCGCGCAGGC

1    ATGGATGTGCTCAGCCCTGGTCAGGGCAACAACACCACATCACCACCGGCTCCCTTTGAG
     METAspValLeuSerProGlyGlnGlyAsnAsnThrThrSerProProAlaProPheGlu

61   ACCGGCGGCAACACTACTGGTATCTCCGACGTGACCGTCAGCTACCAAGTGATCACCTCT
     ThrGlyGlyAsnThrThrGlyIleSerAspValThrValSerTyrGlnValIleThrSer

121  CTGCTGCTGGGCACGCTCATCTTCTGCGCGGTGCTGGGCAATGCGTGCGTGGTGGCTGCC
     LeuLeuLeuGlyThrLeuIlePheCysAlaValLeuGlyAsnAlaCysValValAlaAla

181  ATCGCCTTGGAGCGCTCCCTGCAGAACGTGGCCAATTATCTTATTGGCTCTTTGGCGGTC
     IleAlaLeuGluArgSerLeuGlnAsnValAlaAsnTyrLeuIleGlySerLeuAlaVal

241  ACCGACCTCATGGTGTCGGTGTTGGTGCTGCCCATGGCCGCGCTGTATCAGGTGCTCAAC
     ThrAspLeuMetValSerValLeuValLeuProMetAlaAlaLeuTyrGlnValLeuAsn

301  AAGTGGACACTGGGCCAGGTAACCTGCGACCTGTTCATCGCCCTCGACGTGCTGTGCTGC
     LysTrpThrLeuGlyGlnValThrCysAspLeuPheIleAlaLeuAspValLeuCysCys

361  ACCTCATCCATCTTGCACCTGTGCGCCATCGCGCTGGACAGGTACTGGGCCATCACGGAC
     ThrSerSerIleLeuHisLeuCysAlaIleAlaLeuAspArgTyrTrpAlaIleThrAsp

421  CCCATCGACTACGTGAACAAGAGGACGCCCCGGCGCGCCGCTGCGCTCATCTCGCTCACT
     ProIleAspTyrValAsnLysArgThrProArgArgAlaAlaAlaLeuIleSerLeuThr

481  TGGCTTATTGGCTTCCTCATCTCTATCCCGCCCATCCTGGGCTGGCGCACCCCGGAAGAC
     TrpLeuIleGlyPheLeuIleSerIleProProIleLeuGlyTrpArgThrProGluAsp

541  CGCTCGGACCCCGACGCATGCACCATTAGCAAGGATCATGGCTACACTATCTATTCCACC
     ArgSerAspProAspAlaCysThrIleSerLysAspHisGlyTyrThrIleTyrSerThr

601  TTTGGAGCTTTCTACATCCCGCTGCTGCTCATGCTGGTTCTCTATGGGCGCATATTCCGA
     PheGlyAlaPheTyrIleProLeuLeuMetLeuValLeuTyrGlyArgIlePheArg

661  GCTGCGCGCTTCCGCATCCGCAAGACGGTCAAAAAGGTGGAGAAGACCGGAGCGGACACC
     AlaAlaArgPheArgIleArgLysThrValLysLysValGluLysThrGlyAlaAspThr

721  CGCCATGGAGCATCTCCCGCCCCGCAGCCCAAGAAGAGTGTGAATGGAGAGTCGGGGAGC
     ArgHisGlyAlaSerProAlaProGlnProLysLysSerValAsnGlyGluSerGlySer

781  AGGAACTGGAGGCTGGGCGTGGAGAGCAAGGCTGGGGGTGCTCTGTGCGCCAATGGCGCG
     ArgAsnTrpArgLeuGlyValGluSerLysAlaGlyGlyAlaLeuCysAlaAsnGlyAla

841  GTGAGGCAAGGTGACGATGGCGCCGCCCTGGAGGTGATCGAGGTGCACCGAGTGGGCAAC
     ValArgGlnGlyAspAspGlyAlaAlaLeuGluValIleGluValHisArgValGlyAsn

901  TCCAAAGAGCACTTGCCTCTGCCCAGCGAGGCTGGTCCTACCCCTTGTGCCCCCGCCTCT
     SerLysGluHisLeuProLeuProSerGluAlaGlyProThrProCysAlaProAlaSer

961  TTCGAGAGGAAAAATGAGCGCAACGCCGAGGCGAAGCGCAAGATGGCCCTGGCCCGAGAG
     PheGluArgLysAsnGluArgAsnAlaGluAlaLysArgLysMetAlaLeuAlaArgGlu

1021 AGGAAGACAGTGAAGACGCTGGGCATCATCATGGGCACCTTCATCCTCTGCTGGCTGCCC
     ArgLysThrValLysThrLeuGlyIleIleMetGlyThrPheIleLeuCysTrpLeuPro

1081 TTCTTCATCGTGGCTCTTGTTCTGCCCTTCTGCGAGAGCAGCTGCCACATGCCCACCCTG
     PhePheIleValAlaLeuValLeuProPheCysGluSerSerCysHisMetProThrLeu

1141 TTGGGCGCCATAATCAATTGGCTGGGCTACTCCAACTCTCTGCTTAACCCCGTCATTTAC
     LeuGlyAlaIleIleAsnTrpLeuGlyTyrSerAsnSerLeuLeuAsnProValIleTyr

1201 GCATACTTCAACAAGGACTTTCAAAACGCGTTTAAGAAGATCATTAAGTGTAACTTCTGC
     AlaTyrPheAsnLysAspPheGlnAsnAlaPheLysLysIleIleLysCysAsnPheCys

1261 CGCCAGTGAT
     ArgGln---

1271 GACGGAGGAG TAGCCGGCCA GTCGAGGCTA CAGGATCCGT CCCATTCACT
1321 ATGCTTCCCC CAACCCTAGG GAATCCACAC TTAATATAAT TCGCCACTTC
1371 TCCTCTTTTT CTCTGCTCCG CTCACGGCTT GCAGACCTGG
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Figure 2. DNA and deduced amino acid sequence of the 5-HT_{1A} receptor. The DNA sequence at positions 454 to 462 (double underline) is different from as first reported (Fargin et al. 1988). Other polymorphisms at positions 292 to 294 and 1186 to 1188 are underlined.

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 ^{35}S-dATP, together with 2 units of T7 DNA polymerase, was added. Ten microliter of the resultant mixture was divided into four tubes each containing 2 µl each 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), or 8 µM ddTTP (tube T). The tubes were incubated at 37°C for 5 minutes, and then 2 µl each 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.

RESULTS

The whole 5-HT$_{1A}$ receptor gene was not amplified with several selected primers. Instead, the coding region of the 5-HT$_{1A}$ receptor gene and its 5'- and 3'- flanking regions were divided into eight fragments and amplified by PCR. Each interval of coupled primers was about 200 base pairs in length (Figure 1).

Direct sequencing confirmed the fragments encoding of the 5-HT$_{1A}$ receptor in both control and mood disorders. The human 5-HT$_{1A}$ receptor gene was originally reported to be intronless and 1,266 base pairs in length (Kobilka et al. 1987). We introduced different DNA sequences and three DNA insertions at positions 454 to 459. The DNA sequence, CGC GCC GCT, was found instead of CCG CGT. The corresponding amino acids, hence, changed from proline arginine to arginine alanine alanine (Figure 2). These differences, however, were observed not only in patients with mood disorders but also in normal volunteers (Figure 3A). Another substitution, GTG to GTA at position 294, was found in one major depression patient, M15 who also had GTG at position 294. However, the deduced amino acid sequence, valine^{98}, was unchanged (Figure 3B). Patient M15 is a 56-year-old female without a positive family history who developed her major depression within the last year. Her Hamilton depression rating scale was 10.

DISCUSSION

Recent PCR techniques have made it possible to amplify a specific target DNA millions-fold. Polymerase chain reaction is an in vitro method for the enzymatic synthesis of specific DNA sequences involving two oligonucleotide primers that hybridize to opposite strands and flank the region of interest in the target DNA. A repetitive series of cycles involving template denaturation, primer annealing, and extension of the annealed primers by DNA polymerase results in the exponential accumulation of a specific fragment. Because the primer extension products synthesized in one cycle can serve as templates in the next, 20 cycles of PCR lead to about a million-fold (2^{20}) amplification. This technique was first applied to the amplification of β-globulin DNA and to the prenatal diagnosis of sickle cell anemia, and it is now widely used in medicine (see Erlich 1992 for a review).

In this study DNA substitutions and insertions at positions 454 to 459 were found. The sequence is CGC GCC GCT instead of CCG CGT as originally reported, and corresponding amino acids changed from proline arginine to arginine alanine alanine. This sequence difference was observed not only in patients with mood disorders but also in normal volunteers. Thus, the human 5-HT$_{1A}$ receptor consists of 422 amino acids, the same as that of the rat (Albert et al. 1990). While preparing this paper, we learned that Chanda et al. (1993) also found such a discrepancy in the "normal" human genome. Their report also emphasized that this sequence difference is not related to the pathogenesis of mood disorders. They reported another substitution of tyrosine (TAC) for asparagine (AAC) at position 396, although our sequencing showed it was asparagine (AAC), the same as first reported (Kobilka et al. 1987). This mutation did not change the receptor character (Chanda et al. 1993). We also found a GTG to GTA change at position 294 in a major depression patient, M15, whereas it did not change the corresponding amino acid, valine. There should be several 5-HT$_{1A}$ receptors in the population. Actually, two different types of 5-HT$_{1A}$ receptors have been cloned in the rat.

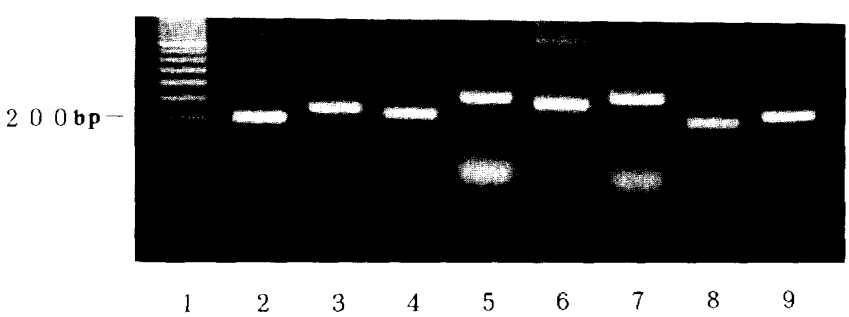

Figure 1. Agarose gel electrophoresis of the PCR products. Fifteen microliter of the amplified DNA, obtained with selected primers, was run in 2% agarose gel and visualized with ethidium bromide. Line 1, 100 Base-Pair Ladder; line 2, C1-2; line 3, C3-4; line 4, C5-6; Line 5, C7-8; line 6, C9-10; line 7, C11-12; line 8, C13-14; line 9, C15-16. See also Table 1.

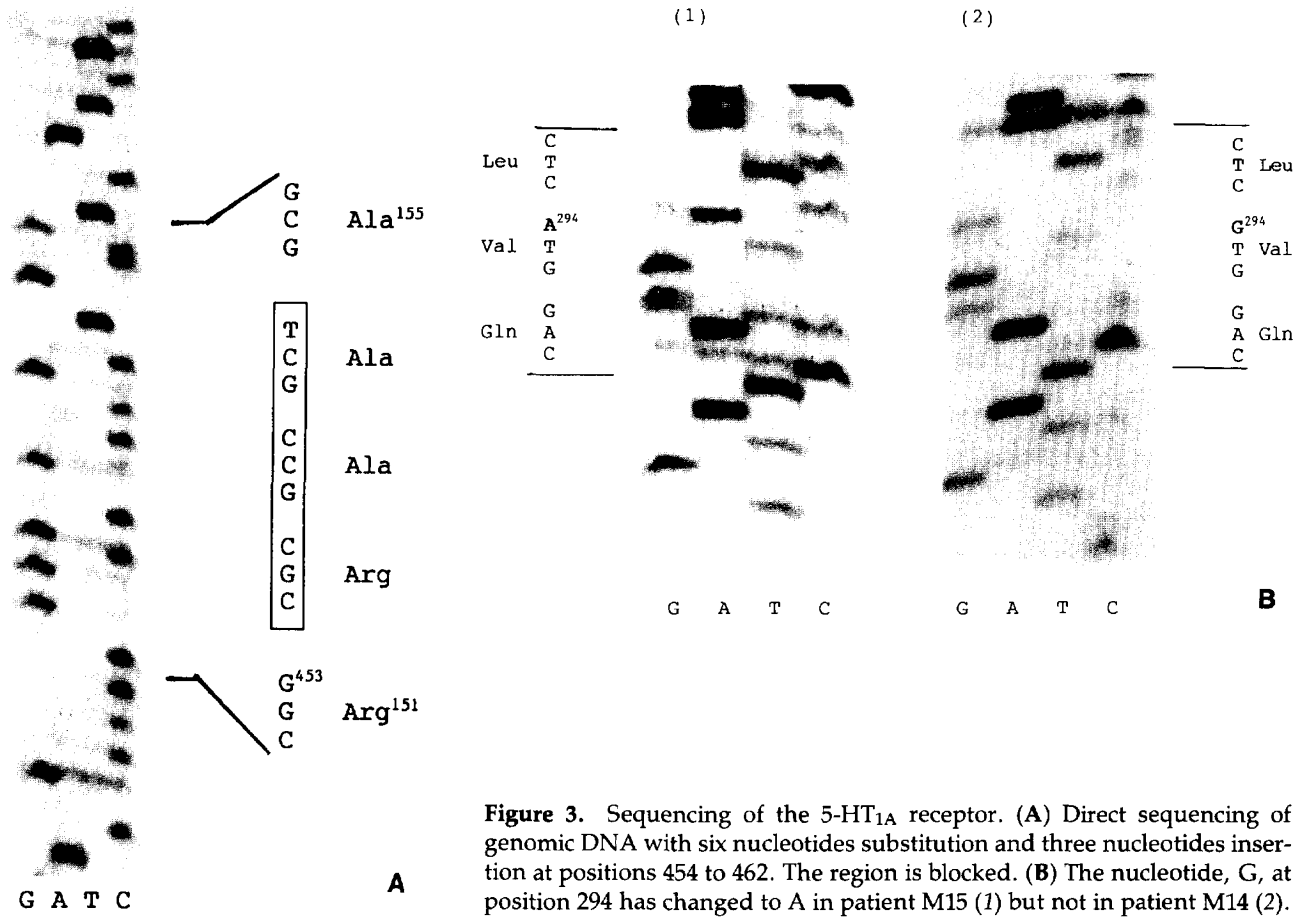

Figure 3. Sequencing of the 5-HT1A receptor. (**A**) Direct sequencing of genomic DNA with six nucleotides substitution and three nucleotides insertion at positions 454 to 462. The region is blocked. (**B**) The nucleotide, G, at position 294 has changed to A in patient M15 (*1*) but not in patient M14 (*2*).

They were two codons different and one amino acid different (asparagine or serine in the VI-VII cytoplasmic loop) (Albert et al. 1990; Fujiwara et al. 1990).

These results may suggest that the genome region encoding the 5-HT1A receptor is not involved in the pathogenesis of mood disorders. However, we must mention that the number of samples examined here was small and that only one-third of the patients had a positive family history. Therefore, the results do not rule out the role of this locus in multiplex families of mood disorders in which there are a number of affected first-degree relatives.

Furthermore, regulatory sequences, such as those of the promoter and the enhancer, may be involved in some cases of mood disorders. There may also be abnormalities in posttranslational processing in patients with mood disorders. Rausch et al. (1990) showed that gepirone, a 5-HT1A agonist, stimulated cortisol secretion more in depressed patients than in normal controls and that the cortisol level after gepirone challenge may be correlated with the depression severity. This may suggest an alteration in receptor–effector coupling.

ACKNOWLEDGMENTS

This work was supported by Grant in Aid for Scientific Research, Ministry of Education, Culture and Science of Japan (06770777), the Japan Foundation for Neuroscience and Mental Health, and the Mental Health Okamoto Memorial Foundation, Japan.

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