

## Short Sequence-Paper

## Cloning of the cDNA and the genomic DNA of the mouse angiotensin II type 2 receptor

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**Abstract**

Of the two major isoforms of the angiotensin II receptors, type 1 (AT<sub>1</sub>) and type 2 (AT<sub>2</sub>), little is known about the structure and features of AT<sub>2</sub>. We cloned a mouse AT<sub>2</sub> cDNA from a mouse fetus cDNA library and an AT<sub>2</sub> genomic DNA from a 129SV mouse genomic DNA library. The amino acid sequence of the mouse AT<sub>2</sub> (363 residues) deduced from a mouse cDNA clone showed seven membrane-spanning domains. Amino acid identity of the mouse AT<sub>2</sub> with mouse AT<sub>1</sub> is 37%, and 98% with rat AT<sub>2</sub>. The genomic DNA (4.4 kb) contained three exons and two introns and the entire coding region was contained in the third exon.

**Key words:** Angiotensin II; Type 2 receptor; cDNA; Genomic DNA sequence; (Mouse)

Reflecting the diverse actions of angiotensin II (Ang II), recent pharmacological studies indicated at least two isoforms of Ang II receptors, AT<sub>1</sub> and AT<sub>2</sub> [1–6]. AT<sub>1</sub> cDNAs have been cloned recently [7–10] and shown to mediate G-protein coupled functions such as the activation of phospholipase C, opening of Ca<sup>2+</sup> channel and inhibition of adenylyl cyclase. By contrast, little is known about the structure and functions of AT<sub>2</sub>. The use of AT<sub>2</sub>-specific inhibitors revealed the tissue distribution of AT<sub>2</sub> in mesenchymal tissue of rat fetus [11], healing wound [12], certain brain regions [13] and rat adrenal medulla [4]. The lack of effects of stable GTP-analogues on the ligand-binding of AT<sub>2</sub> [1] and absence of ligand-induced internalization [14] suggested its unique structural feature and signaling mechanism. Very recently we have expression cloned a cDNA for rat AT<sub>2</sub> from a rat pheochromocytoma cell line [15] and found it to be a receptor with seven membrane-spanning domains. However, little is known about its biological functions. With the objective of clarifying its biological significance by disrupting the AT<sub>2</sub> gene by a homologous recombination technique,

we undertook cloning of the genomic DNA of mouse AT<sub>2</sub> via cloning of its cDNA. In this paper, we report the nucleotide sequence of the mouse cDNA and the genomic DNA and the deduced amino acid sequence of the mouse Ang II type 2 receptor.

The cDNA for the mouse AT<sub>2</sub> receptor was cloned from a cDNA library prepared from two fetuses of Balb/C mice by conventional plaque hybridization with the rat AT<sub>2</sub> cDNA probe [15]. The nucleotide sequences were determined both in the sense and anti-sense directions. Of the two ATG sequences, the last one (position, 158 bp–160 bp) satisfied Kozak's rules (Fig. 1). The open reading frame between this initiation codon and the inframe termination codon, TAA (position, 1247 bp–1250 bp), consisted of 1089 bp equivalent to 363 amino acid residues. The nucleotide and amino acid sequence showed 97% and 98% sequence identity with the rat AT<sub>2</sub> cDNA cloned by Kambayashi et al. [15]. Hydropathy analysis according to Kyte-Doolittle indicated the presence of seven hydrophobic membrane-spanning domains as indicated by underlines, suggesting that this receptor belongs to the super family of seven membrane-spanning receptors. There is no signal sequence. The following structural features highly conserved and commonly found in

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|                                                                                   |       |
|-----------------------------------------------------------------------------------|-------|
| AGTAAGCTGATTTATGATAACTGCTTTAAACACTGGCACTAAAAAGGTGAAGAATTTGGAGTTGCTGCAGTTCAATATG   | 160   |
| Met                                                                               | (1)   |
| AAGGACAACTTCAGTTTGTGTCACCCAGCAGAAACATTACAGCAGCGCTCTTTTGATAATCTCAACGCACTGGCACC     | 241   |
| LysAspAnPheSerPheAlaAlaThrSerArgAsnIleThrSerSerArgProPheAspAnLeuAsnAlaThrGlyThr   | (28)  |
| AATGAGTCGCGCTTTAATGCTCACACAAACATCAGATAAGCATTGGAAGCAATCTGTTCTCTACTACATGATTTT       | 322   |
| AsnGluSerAlaPheAsnCysSerHisLysProSerAspLysHisLeuGluAlaIleProValLeuTyrTyrMetIlePhe | (55)  |
| TM1                                                                               |       |
| GTGATTGGGTTGCTGTTAATATTGTTGCTCTCACTGTTTGTGTCAAAAGGGCCCTAAAAAGGTGTCAGCATTTAC       | 403   |
| ValIleGlyPheAlaValAlaIleValValValSerLeuPheCysGlnLysGlyProLysLysValSerSerIleTyr    | (82)  |
| ATCTTCAATTCGGCTTGGCTGACTTACTCTCTTTGGCTACCCCTCCCTCTCTGGGCAACCTATTACTCTTATAGATATGAT | 484   |
| IlePheAsnLeuAlaLeuAlaAspLeuLeuLeuAlaThrLeuProLeuTrpAlaThrTyrTyrSerTyrArgTyrAsp    | (109) |
| TM2                                                                               |       |
| TGGCTTTTGGACCTGTGATGTGCAAGTGTGTTGTTCTTTCTGACTCTGAACATGTTTGAAGCATTTTATTTATACC      | 565   |
| TrpLeuPheGlyProValMetCysLysValPheGlySerPheLeuThrLeuAsnMetPheAlaSerIlePhePheIleThr | (136) |
| TM3                                                                               |       |
| TGCATGAGTGTGATAGGTACCAATCGTCTATCTACCTTTTCTGTCTCAAGAAGGAATCCCTGGCAAGCATCTTATGTA    | 646   |
| CysMetSerValAspArgTyrGlnSerValIleTyrProPheLeuSerGlnArgArgAsnProTrpGlnAlaSerTyrVal | (163) |
| GTTCCTCTTGTGTTGTTATGGCTTGTCTATCTCTATTCCTCAACATTTTATTTCCGGGATGTCAGAACCTTGAATACTTA  | 727   |
| ValProLeuValTrpCysMetAlaCysLeuSerSerLeuProThrPheTyrPheArgAspValArgThrIleGluTyrLeu | (190) |
| TM4                                                                               |       |
| GGTGTGAATGCTTGTATTATGGCTTTCCACCCGAGAAATATGCTCAGTGTCTGCTGGGATTGCTTAATGAAAAATATT    | 808   |
| GlyValAsnAlaCysIleMetAlaPheProProGluLysTyrAlaGlnTrpSerAlaGlyIleAlaLeuMetLysAsnIle | (217) |
| CTTGCTTTTATTTCTTTAATATTATAGCAACGTGTTACTTTGGAAACAGAAACATCTGCTGAAGACTAATAGCTAT      | 889   |
| LeuGlyPheIleIleProLeuIlePheIleAlaThrCysTyrPheGlyIleArgLysHisLeuLeuLysThrAsnSerTyr | (244) |
| TM5                                                                               |       |
| GGGAAGAACAGAAATTACCCGTCACCAAGTCTGAGTGGCAGTGTGTTGTTGGCATTTCATCTTGTGCTGGCTCC        | 970   |
| GlyLysAsnArgIleThrArgAspGlnValLeuLysMetAlaAlaAlaValValLeuAlaPheIleIleCysTrpLeuPro | (271) |
| TM6                                                                               |       |
| TTCCATGTTCTGACCTTCTTGGATGCTCTGACCTGGATGGGTATCATTAAAGCTGTGAAGTTATAGCAGTCATTGACCTG  | 1051  |
| PheHisValLeuThrPheLeuAspAlaLeuThrTrpMetGlyIleIleAsnSerCysGluValIleAlaValIleAspLeu | (298) |
| GCACCTCTCTTTGCCATCTCTGGGATTCACCAACAGCTGTGTTAATCCCTTCTGTTATGTTTGTGGAAACCGCTTC      | 1132  |
| AlaLeuProPheAlaIleLeuLeuGlyPheThrAsnSerCysValAsnProPheLeuTyrCysPheValGlyAsnArgPhe | (325) |
| TM7                                                                               |       |
| CAACAGAAAGCTCCGAGTGTGTTAGAGTTCCCACTTACTTGGCTCCAAAGGAGAGAGACTATGCTTGCAGAAAAGGC     | 1213  |
| GlnGlnLysLeuArgSerValPheArgValProIleThrTrpLeuGlnGlyLysArgGluThrMetSerCysArgLysGly | (352) |
| AGTTCTCTTAGAGAAATGGACACCTTTGTGCTTAAATCTGTTAGTGGATGCATGTAATCAGCCTAGCCATTGGTTTGG    | 1294  |
| SerSerLeuArgGluMetAspThrPheValSerSTOP                                             |       |
| Δ                                                                                 |       |
| GGCCACACAAATGATCTTAAAGTGCCATCAGTATAACAGCTTCTTTGCTTTATCTAATCTTTACTTACTCCCCGAGAA    | 1375  |
| CAGGAAGTCAAGTAGAAGTGAATCTTTATCTCCACAGCTTTCAGTGATAGTGCCTCTTTGCTGGTCTTTGGCAT        | 1456  |
| GAGATTGTGATATGTGAGCTAGATCTAATCTAGAAATATCTGGGGAATATCCCACTTATAATTAACAACAATTTAT      | 1537  |
| GAGTGTGATTTGACATCTCAGACTTCTCCCTGGAAAAATGCTGGCAATTTCTTAGTGGAGTTTGTGTCATTTTCATCAGAT | 1618  |
| TTCTTTTCTTGAACAAAGGCCAAATTTAAACTTCTTATCTAATCAACATATGATATAGCATGAGAGGTGAGCACTAA     | 1699  |
| GTGTGATGATATACCTCTCTATATATGCAATAGGTGGTGTGCTTATTCAGTCTCTAGGTATAGAGTTTCTCTTTTA      | 1780  |
| AAGAAATGTAAAGTTGTGTTCTTTTCCATTTCACTCAAGTATAGCTTTTGTACTTATTTCTACAGCTACACACTGAGCAGA | 1861  |
| TCTAGAAATGATTAATACACATCTGTCTTAGCTTATCTTGCAGTTATAGAAAGTACACTATTAGTAAACAGAAC        | 1942  |
| TGCAATGAAGAAGTATTTAGTATCCACAAACATGAATACACTTTGAAATTTTTCATCCATTTGACTCTTGTTTATTC     | 2023  |
| TATTTCTTCTGATGATTTTGAATACAAACAAACACTGTATTTAGCACTACGTAAGGTCACTTTTAAATTTTAA         | 2104  |
| ACCTTTTGAACATGTTGCTTTGATATATTCATGATGACTTGAGTTTAAATTTATTCATGCTTTTGTCTGGCTCTGCTCCA  | 2185  |
| AAATATCTCTTTGACCTGAAAGAGAGCACTTTTAAATCTTTAACTTTGTAATAAAGTCAAACTGGCATTGGGAAAG      | 2266  |
| GTATGTGACAGCTGGAAGTTTGTGCTCTTTGGGGTAAACAGACCCAGCAAAATGGCAAGTTTGGTGTCAACAAAGAAC    | 2347  |
| TTGTCAAGAACAAAGACTCCCTGGGGAGTAGTTTGAATCTGCAATTTCTGGGCACAGTCCAGAAATGTAAGAGTCTGTGAA | 2428  |
| GGTGATTTAAAGCAAGCCAGCTCCACAACTCACTTAAACAGAGTACATCTCTTACATTAGAGGAATATAATACCTG      | 2509  |
| AAGATGTGTACCTAAAGTTTACTCAAACTTCTCAATATAATTAATTCAGAAATTAAGATGTCAATCTCTGCTGCTCCC    | 2590  |
| ATATTATACAGGCTCACTAAGCACTTCTGGATTGCTGACCTATGAGGTAGATTCAAGTTTCTGGAACTTAACATT       | 2671  |
| TCTGTGATGATCCAGCGGTAGGTGGAAGATCTCTCATACCCCTCTTGGAAAAACCTGATTTTCATGTATTCATGTTA     | 2752  |
| ATTTTGTAGTAAAAAATAAGCTAAATATGTAATCAGTTATGACTTTGTGTTTAAAGCAATTTACACAAATCTCGTAAAA   | 2833  |
| TAATACTATTACTGGGAAAAAATTTTTTTTTT                                                  | 2871  |

Fig. 1. The nucleotide and deduced amino acid sequences of the mouse AT<sub>2</sub> cDNA. The amino acid sequence deduced from the nucleotide sequence of the open reading frame is given below the corresponding DNA sequence. Putative transmembrane domains are shown by underlines (TM1 to TM7). A potential poly(A)<sup>+</sup> signal is also underlined (position 2832 bp–2837 bp). Potential N-linked glycosylation sites and possible phosphorylation site by protein kinase C are indicated by asterisks (\*) and open triangle (Δ), respectively. Accession No. U00766.

the majority of members of this super family were also noted in the mouse AT<sub>2</sub> sequence, Asp<sup>141</sup>-Arg<sup>142</sup>-Tyr<sup>143</sup> (Fig. 1, indicated in italic) immediately after the third transmembrane domain, Asp<sup>90</sup> in the second transmembrane domain, and Pro<sup>98</sup>, Pro<sup>177</sup>, Pro<sup>223</sup>, Pro<sup>271</sup>, Pro<sup>315</sup> in the second, fourth, fifth, sixth and seventh transmembrane domain, respectively. Other notable features are five potential N-glycosylation sites which are located exclusively in N-terminal hydrophilic region

(Fig. 1, Asterisks) and short first and third cytosolic loops. There is a possible phosphorylation site by protein kinase C in C-terminal region (Fig. 1, open triangle). Important to note is only 37% amino acid homology with the mouse AT<sub>1</sub> [9,10], which is compatible the marked absence of functional similarity between AT<sub>1</sub> and AT<sub>2</sub>. Mouse AT<sub>2</sub> gene also has 35% homology with rat opioid receptor and 28% homology with mouse β<sub>2</sub>-adrenergic receptor according to the homology

AATTCTGTAGGTTGAAGGCTCCCAAGTGGACAGAGCGAATATATAAGGAAGAACAGAGATCTGGTGCACTTACATCTC 80  
 AGAGGCTGGGAGGGAGCTCGAAAGCTTACTTCAGCTGCATTTTAAAGTAAGGCAGAACAAATTTACACA 160  
 TGCTTGACTAGGGAGGGGAAACATACAGCAGTAAATGTTTCTGTGTGTGCGGTATGAATGTTTCTTTCTGTGTAT 240  
 TTTAAGCTGCAATTTTGAGAAATGCTTTAACTCTTAACTGCAATTTCTCTTGCAGTGTGATCGGGAGCTGAGTAAGCT 320  
 GATTATGATACTGCTTTAAACACTGGCACTTAAAGTCTGTAGTGGGATTTATTTTATTTCTTGCACTGTTAAATTTG 400  
 GAAGGTCATAGAAAGAAATGGGCTTTGACAGAAATGCTAGTTTCCCGTTCTGAGGAAGTTCTCTAGTGGTTACATTTT 480  
 AAATGAAATATGATGATGATGACTTTGAAATGATCTGGTGTAAATAGTTTGTCTCTGTGTGTGTTATCTTTAGTTTTC 560  
 TTTTGTCTCAAGTAATGTGTAGTTGAAAGAAACAGCAGCTTCCCACTGACCGCTGTGTTAATGCTGGAAAGTA 640  
 ATGACTATTCAGTTCTGTAAATTTGAAAGATAGCAAGCAAAAGCAGTAAACTATATCATAAITAGTCACTAATGTC 720  
 TAAITCTGATTTAAATTTGAGCTCCAAACCCATCTCTGTCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT 800  
 CTATTTAAATCTGAAATAGATGTTTCTTATGAAACAGATCAGATTCAAATATCCATATTTGAAACCTGGCATTGATTA 880  
 AAATTTTATAGTAAAGCTTTAAGAAATTCATCTATATCTTTTGTGTGTTAAAGCAAGGGAATAAATACATTAT 960  
 TTTTAAATAGCAAAACCCACCTGACTCTATCTTAATATGCACTTCTGCAATTTTCCAGCTCTCAGTACACTTAC 1040  
 CTATTTTCCCTATGGCTTATGTTACTTATATTTTCCATAGCTGCAACAAATTTGTATATAGTTACTTCAAGAAAGCTTT 1120  
 CTCTATGAAGCAGGGCAGCAGTGAATTTCTAGAAAGCACAGATGGAAGCTAGCCTTGTTTTGTGAGTATCAC 1200  
 ATTTGCTTTGGGCTCTCTATTTGTCTCATGACTCTACCTGATTAACTTTTAACTATTAAATTTGTCTCTGACTTTTC 1280  
 AAAGACATGACAGAGAAAGAAACACAAATGTTTGTTCAGAAATTTTCACTTAAGTCACTGAGGAAATGTTGTTT 1360  
 CTCTCTCTAGATGATATAGTAAATCCAGCCAGTAAAGCCAGGATGTTGTTTGTGTTTCTTCAACACCTTAG 1440  
 GTTATTTTGGCTCTGACAGATTTTCAAGTCTTATGACTCAAAATACCCCTTAGGCTTTGGGTATGCAATTAAGCTTTT 1520  
 CTTTCTTTTTCCTTAAAGGTGTAAGAAATTTGGAGTTGCTGCAATATGAAGGCAACCTCAGTTTTCGTGCTC 1600  
 ACCAGCAAGAACATTACAGCAGCCGCTCTTTGATAATCTCAACGCACTGGCAGCAATGAGTCCGCTTTAAATGCTC 1680  
 ACACAGAACTCAGATAAGCATTTGGAAGCAATCTGTTCTCTACTACATGATTTTGTGATTTGGTGTGCTGTTAATA 1760  
 TTGTTGTGCTCACTGTTTGTGTGCTCAAGGGCCCTAAAGGTGTCAGCATTACATCTTCAATCTGGCCTTGGCT 1840  
 GACTTACTCTCTTTGGCTACCTCCCTCTCTGGGCACTTATCTCTTATAGATATGATTTGCTTTTGGCCTGTGAT 1920  
 TGCTCAAGGTGTTTGGCTTTCTTCTGACTGCAACATGTTTGAAGCATTTTCTTATACCTGATGAGTGTGATGAT 2000  
 ACCAATCGGTCTATCCCTTTCTGTCTCAAGGAAGAAATCCCTGGCAAGCATCTTATGATTTCCCTTGTGTTGGT 2080  
 ATGCTGTGCTTCTCTCATTTGCCAATCTTTATTTCCGGGATGTCAGAACCTTGAATCTTATGTTGTGAATGCTTTGAT 2160  
 TATGGCTTTTCCACCCGAGAAATATCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT 2240  
 CTTTAAATATCTATAGCAACGTTTACTTTTGAATCAGAAACATCTGCTGAAGCTAATAGCTATCGGAAGACAGAAAT 2320  
 ACCCGTGACCAAGCTCTGAAGATGGCAGCTGCTGTTGTGTTGGCATTCATCTTTGCTGGCTTCCCTTCCATGTTCTGAC 2400  
 CTCTCTGAGATCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT 2480  
 CCATCTCTCTGGGATTCACCAACAGCTGTTGTTAACTCTCTGTTATGTTTGTGTTGTTGTTGTTGTTGTTGTTGTT 2560  
 CGCAGTGTGTTAGAGTTCCCATTTACTTGGCTCCTCAAGCAAGAGAGACTATGCTTTCAGAAAGGCAAGTCTCTT 2640  
 AGAAAATGGACACCTTTGTGCTTAAATCTGTTAGTGGGATGCTATGATCAGCTAGCCATTTGGTTGGAGGCCACACA 2720  
 AATGATCTTTAAGTGCAATCAGTATAATACAGTTCTTGTCTTATCTAAATCTTACTTACTCCCGGAGAACAGGAAGTC 2800  
 AAGTAGAAGCTGAAATCTTTATCTCCACAGCTTTCAGTGATAGTGCCTTCTTTGCTGGTCTTTGGCATGAGATTGT 2880  
 CATATGTGAGCTAGATCTATAATCTAGAAGTATCTGGGGAAATATCCCACTTATAATTAACAACAAATATGAGTGGT 2960  
 GATTGACATCTCAGACTTCTCCCTGGAAATGCTGGCATTTCTTAGTGGAGTTTGTGCTTATTTTATCAGATTTCTT 3040  
 TTTTCTTGAACAAAGGCCAATTTAACTTCTTATCTACTCAACCATATGATATAGCATGAGAGGTGAGCACTAAGTTTA 3120  
 GCATGATATCTCTTCTATATATGCCATAGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGT 3200  
 AAAATTTGAAGTTGTGCTTTTCCATTTCACTCAAGATAGCTTTTGTACTTATCTCAGCTACACACTGAGCAGATC 3280  
 TAGAATGTAGATTAATACACATCTGCTTCTAGCTTATCTTGCAGTTATAGAAAGTACACTTTTAGTAAACAGAACT 3360  
 GCAATGAAAGATTTTATGATCCCAAACTGAATATACACTTTGAAATTTTCAATCCATTTTGACTCTTGTTTATTC 3440  
 TATTTCTCTCTGATGATTTTGAATACAACAACAAACACTGTATTATGACACTACGTAAGGTCACCTTTTAAATTTT 3520  
 AACCTTTTGAACATGGTCTTTGATATATTTCAATGATGACTTGAGTTTAAATATTCATGCTTTTGTCTGGGCTTCGTC 3600  
 CAAATATCTCTTTGACCTGAAAGAGAGCATTTCTTAAATCTTTAACTTTGTAATAAGTGAACCTGGCATGGGAA 3680  
 AAGGTTATGTCAGACTGGAAGTTTGTGCTCTTCTGGGGTAAACAGACCCAGCAATGGCAAGTTTGTGTGTTCAACAG 3760  
 GAACTTGTGACAGAAAGACTCCCTGGGAGTAGTTTGAATCTGCATTTCTGGGCACAGTCCAGAAATGTATAAGAGTCT 3840  
 GTGAAGTGATTTAAAGCAAGCCAGGTCACAGAACTCATCTTAAACAGAGTACATCTTACATTAGAGGAATATAA 3920  
 TACCTGAAAGTGTGTTTACCTAAAGTTTACTCAAACTTCTCAATAAATATTAATTCAGAAAGTAAAGATGTCATTCTG 4000  
 CTGCTCCCATATTATACAGGTCACCTAAGACCTTCTGAGTTGATGCTGACCTATGAGGTAGATTTCAAGTCTTGGGAAC 4080  
 TTAACATTTCTGTCAGATTCAGGCGCTTTAGTTGAAGAAATCTCTCATACCTTCTTGGAAAACCTGATTTCATG 4160  
 TATTCATGTTTAAATTTTATGTAAGAAACAAATAGCTAAATATGTAATCAGTTATGACTTTTGTGTTTAAAGCAATTTACACA 4240  
 AAATCTGTAAGAAATTAATCATTTACTGGGAGACCATGTTGCTCTTATTTTAACTGCTTCCAGGTTTAGAGCTGCTG 4320  
 CTTCTCTTAGTCAGTCTCTCCAGCAATCCCATTCATAATTCAGTTTCAGCTACATGATTACTCATTGAGGTCA 4400  
 ATAAGAAACAGGCATATGAAAGCATATGTCGGAAATGAAT 4441

Fig. 2. The nucleotide sequence of the mouse AT<sub>2</sub> genomic DNA. Three exons are indicated by boxes. A putative TATAA box (position 42 bp–46 bp) and exon-intron boundary sequences (GT and AG) are underlined. Accession No. U00768.

search of GenBank data base. Homologous residues are mainly located in transmembrane portion. On the other hand, between rat and mouse AT<sub>2</sub>, only four amino acid residues were different out of the total 363 residues. DNA containing mouse AT<sub>2</sub> was cloned from 129SV mouse genomic library. The entire nucleotide sequence of the 4.4 kb *Eco*RI fragment was determined both in the sense and antisense directions (Fig. 2). Comparison with the nucleotide sequence of the AT<sub>2</sub> cDNA revealed that the 4.4 kb genomic DNA contained the entire transcribable sequence. Deduced from the 5' end of the AT<sub>2</sub> cDNA clone, there is a putative TATAA box 36 bp upstream of the first exon (position, 42 bp–46 bp). Two short exons (61 bp for the first exon and 60 bp for the second exon) were followed by a long third exon containing 2733 bp. Each of

the two intervening sequences contained the 5' boundary sequence gt and 3' boundary sequence ag. Open reading frame in the third exon was uninterrupted by any intron as is often found in many seven membrane-spanning receptor genes.

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