

Bioinformatic Analysis of the Human μ Opioid Receptor (OPRM1) Splice and Polymorphic Variants

Submitted: April 11, 2002; Accepted: June 28, 2002; Published: September 30, 2002

Lili Xin¹ and Zaijie Jim Wang¹

¹ Department of Biopharmaceutical Sciences, College of Pharmacy, University of Illinois, Chicago, IL 60612

ABSTRACT Mu opioid receptor (OPRM1), a member of the G-protein coupled receptor superfamily, mediates the analgesic and euphoric effects of opioid drugs. The sequences of OPRM1 cDNA and reported splice variants were used to search the public and Celera genomic databases. The matched sequences were analyzed to assemble an OPRM1 genomic contig. Human OPRM1 gene was estimated to span at least 90 kb in the chromosome 6q24-25 region. Four coding exons are separated by 3 introns. While intron 2 has only 773 bp, these databases for the first time provide the precise length of and other information about long introns 1 and 3, containing 50 and 27 kb, respectively. When a consensus exon/intron splice junction at the end of the coding exon 3 was not utilized, it may have resulted in continuous translation of the exon to yield the splice variant OPRM1A. The study did not identify human orthologs of other OPRM1 variants that had been reported for mouse OPRM1, although several proposed exons were found to be included in mouse genomic clones. Single nucleotide polymorphisms in the OPRM1 gene were also analyzed and summarized, which could provide potential polymorphic markers for molecular genetic studies.

KEYWORDS: OPRM1, MOR1, opioid receptor, ortholog, splice, variant, polymorphism.

INTRODUCTION Opioids (prototype: morphine) are primarily used as analgesics, although they have other pharmacological actions and are used to treat cough, diarrhea, and other conditions. These effects are mediated by opioid receptors. Three opioid receptors, μ , δ , and κ , have been identified by receptor binding,¹⁻³ pharmacological methods (see reviews, eg, Smith and Loh⁴ and Lewis et al,⁵ and molecular cloning.⁶⁻¹¹ The μ opioid receptor (OPRM1) is the primary receptor mediating the analgesic and rewarding effects of opioids, and thus it has been the focus of intensive research, with the hope of finding more potent analgesics with minimal undesired actions. Repeated use of opioids often leads

to the development of tolerance of these drugs, which greatly limits the effectiveness of pain control. In addition, drug dependence on opioids remains a medical and social problem. Large individual differences have been observed in the levels of brain OPRM1,¹² pain response,¹³⁻¹⁵ response to opioid analgesia,¹⁶⁻¹⁸ and vulnerability to drug addiction,^{15,19} and possibly the kinetics and severity of opioid tolerance.²⁰ Explanations of some of these differences will likely come from the studies of the genes involved. Pharmacological and genetic studies suggest that OPRM1 may be such a candidate gene.^{15,21}

The human OPRM1 complementary DNA (cDNA), also abbreviated MOR1, was cloned independently by different groups.^{22,23} However, the exact genomic arrangement of OPRM1 remained unclear largely because of its long introns.¹⁵ Using the human genome sequences available from the Human Genome Project and Celera database,^{24,25} we seek to assemble the OPRM1 genomic structure by identifying the chromosome locations of the gene and the exon and intron assignment and junctions.

Shortly after the cloning of OPRM1 cDNA, 2 splice variants were identified from human²⁶ and mouse²⁷ cell lines by reverse transcription-polymerase chain reaction (RT-PCR). Multiple subtypes of OPRM1 have previously been reported and characterized pharmacologically²⁸⁻³¹; therefore, splice variants could provide genetic evidence for receptor subtypes. Recently, another research group reported the discovery of 4 additional C-terminus variants of mouse OPRM1 using the rapid amplification of cDNA end and RT-PCR methods; however, no clones have been directly isolated from cDNA libraries.^{32,33} Just before the submission of this article, the same group further identified several N-terminal splice variants of mouse OPRM1 using similar approaches.³⁴ The current study attempts to map the locations of potential human orthologs of these variants in relation to that of the OPRM1 gene.

Another aim of this study is to analyze and determine the frequencies of the single nucleotide polymorphisms (SNPs) that have been reported in molecular genetic studies and sequencing efforts.

Correspondence to:

Zaijie Jim Wang
Telephone: (312) 996-0888
Facsimile: (312) 996-0098
E-mail: zjwang@uic.edu

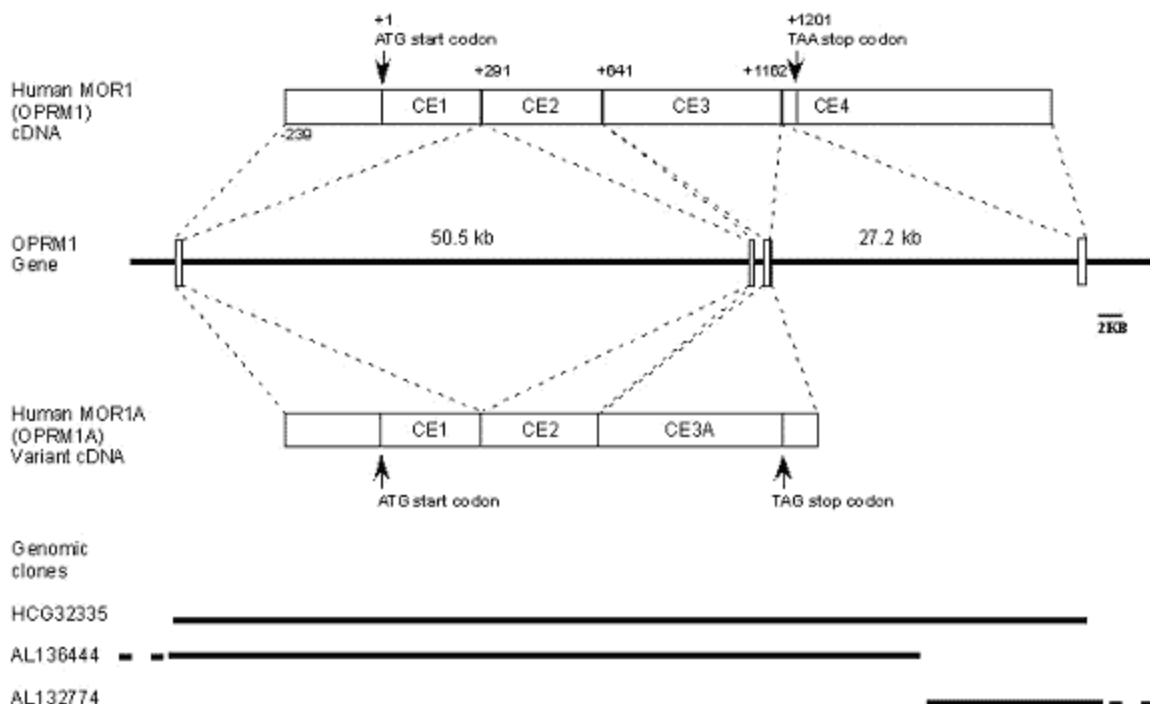


Figure 1. Genomic structure of human m opioid receptor (OPRM1) gene.

Coding exons (CEs) are separated by 3 introns to occupy over 80 kb genomic sequence in the chromosome 6q24-25 region (position: 161 380 kb-161 460 kb). The sizes of these 3 introns are 50 461, 773, and 27 207 bp, respectively. AL136444 extends 33 kb 5' of CE1. AL132774 contains 95-kb sequence beyond the translation stop. Only 3 coding exons are used in the splice variant OPRM1.

MATERIALS AND METHODS The sequences of the cloned human OPRM1 (Appendix 1) and reported splice variants (Appendix 2) were used to search GenBank (Release 124.0, June 2001,) and the Celera database () using the Blastn program.³⁵ A BLOSUM-62 matrix with default parameters was used for most analyses. Sequences matches were further analyzed by BESTFIT and FILEUP programs using the GCG package (Wisconsin Package Version 10.2, Genetics Computer Group, Madison, WI). From these analyses, a human OPRM1 contig was constructed based on the consensus sequence. This contig was then used to search for a variable number of tandem repeats using the COMPARE and DOTPLOT programs. Mouse OPRM1 variants were analyzed in a similar fashion except that all available sequences were used for comparisons with human EST, transcripts, and genomic sequences deposited in GenBank and the Celera database. For those sequences that failed to find significant homology in the human genome, we further used them and their

available intron sequences to search GenBank for all available sequences, including EST, unfinished high-throughput genomic sequences, genome survey sequence databases, and sequences deposited within the past month.

RESULTS Human OPRM1 cDNA sequence

Three human OPRM1 cDNA sequences have been published and deposited in GenBank (L25119, L29301, and U12569). In addition, a number of sequences were recorded via direct submission by sequencing laboratories (eg, NT_02345, XM_004341). These sequences were compared using BESTFIT and PILEUP programs to construct a consensus OPRM1 cDNA sequence (Appendix 1). The longest accountable human OPRM1 cDNA is 2187 bp in length, of which 1203 bp are used to encode 400 amino acid (aa) residues and the stop codon (**Figure 1**). To identify the sequence location, we assign the "+1" for the first nucleotide of the

Table 1. Human OPRM1 Coding Exon Arrangement*

| No | 5' Junction (Sequence at 3' Splice Site) | Total Size (bp) | Coding Size (bp) | Receptor Regions | 3' Junction (Sequence at 5' Splice Site) |
|----|---|-----------------------|---------------------|-----------------------|--|
| 1 | TBD | 527 | 290 | N-terminus, TM1 | ACTTCTGGTCATGTATGTGATTGTCAG |
| 2 | ATACACCAAGATGAAGACTGCCACCAACATCT | 353 | 353 | i1, TM2-4, part of e2 | TCTTCTGTAATGTTTCATGGCTACAACAAAATA CAGGCAAG |
| 3 | GTTCCATAGATTGTACACTAACATTCTCTCATC CAACCTGGTACTGGGAAAACCTGCTG | 521 | 521 | e2, TM5-7, C-terminus | CACCCCTCCACGGCCAATACAGTGGATAGAAC TAATCATCAG |
| 4 | CTAGAAAATCTGGAAGCAGAAAAGCTCCGTT GCCCTA | 786 | 39 | C-terminus last 13 aa | TBD |

*aa indicates amino acid; e2, extracellular loop 2; i1, intracellular loop 1; OPRM1, μ opioid receptor; TBD, to be determined; and TM, transmembrane domain. Consensus AG/GT sequences at 5' splice sites, and AG/NN at the 3' splice sites are in bold.

Table 2. Human OPRM1 Gene Intron Arrangement*

| No | 5' Junction (Sequence at 5' Splice Site) | Size (bp) | 3' Junction (Sequence at 3' Splice Site) |
|----|--|-----------|---|
| 1 | GT AAGGAAAGCGCCAGGGCTCCGAGCGGAGG G | 50,461 | TTTTACTGATTCTCACTCTTCTCCTTTATCTCCTAG |
| 2 | GT GAGTGATGTTACCAGCCTGAGGGAAGGAG G | 773 | TTGCTGCTAATTTTTCTTTAAATTCCTTTCTTCTAG |
| 3 | GT ACGCAGTCTCTAGAATTAGGTATATCTACTG | 27,207 | GAAATGTTCACTGTCTTTGCTCTTTCTCCTTTTCAG |

*OPRM1 indicates μ opioid receptor. Consensus AG/GT sequences at 5' splice sites, and AG/NN at the 3' splice sites are in bold.

translation initiation codon (ATG). Therefore, a negative number indicates the location 5' (upstream) to the translation start.

OPRM1 genomic sequence

The OPRM1 cDNA sequence was then used to search the genomic databases. A chromosome 6 clone, HCG32335 (Celera), was found to have significant nucleotide identity with the cDNA probe. This clone has perfect matches with the OPRM1 cDNA in 4 segments, corresponding to the 4 coding exons (**Figure 1**). Exon 1 has a 290-bp coding sequence encoding for 97 aa residues at the extracellular N-terminus and transmembrane domain (TM) 1 (**Tables 1** and **2**). Following coding exon 1 is a very large intron 1 of 50 461 bp in length. Coding exon 2 includes a sequence of 118 aa that make up the intracellular loop 1 to TM4 and part of the extracellular loop 2. Intron 2 contains 773 bp and is followed by exon 3. One hundred and seventy-four aa are encoded by exon 3, covering the regions including part of extracellular loop 2, TM5-TM7, and part of intracellular C-terminus. Before the short exon 4, responsible for 12 aa, is another long intron 3 of 27 207 bp. All exon-intron junctions match the consensus splicing requirements (**Tables 1** and **2**).

Adding up these exons and introns, OPRM1 is over 80 kb in chromosome 6 (161 380 kb-161 460 kb), which is

entirely covered by the clone HCG32335. At 80 591 bp in length, HCG32335 clone, however, does not provide much sequence information about the 5' UTR region, including the promoter regions.^{36,37} The search in GenBank turned up 2 clones: AL136444 and AL132774. AL136444 was deposited in the antisense direction; the reverse complementary sequence of AL136444 overlapped with HCG32335 for 63 620 bp. AL136444 also contained OPRM1 exons 1, 2, and 3 and part of intron 3. In addition, AL136444 included 32 690 bp 5' to the OPRM1 cDNA and HCG32335 (Appendix 1). OPRM1 exon 4 was found in another chromosome 6 clone, AL132774, which has an additional 95 kb beyond the OPRM1 translation stop codon. These 3 clones allow us to assemble a 200-kb OPRM1 contig (**Figure 1**); the overall sequence agreement in the overlapped regions was over 99%.

OPRM1 alternative splicing variants

Shortly after the cloning of the human OPRM1, a 3' splicing variant was reported and termed OPRM1A.²⁶ OPRM1A lacked the last 12 aa found in OPRM1. When the OPRM1A 3' unique sequence was used to search genome databases, it was found to be 100% identical with a region in the genomic clones HCG32335 and AL136444 (**Table 3**). The sequence alignment suggested that OPRM1A has the same coding exons 1

Table 3. Summary of the Proposed OPRM1 Exons*

| | Human Homologous Sequences (Homology) | Mouse Homologous Sequences (Homology) | Comments |
|---------------------------|---------------------------------------|---------------------------------------|--|
| CE1 (527 bp) [†] | AL136444, HC G32335 | eg, U10558 (78%) | |
| CE2 (353 bp) | AL136444, HC G32335 | eg, U10559 (90%) | |
| CE3 (521 bp) | AL136444, HC G32335 | eg, U10560 (86%) | |
| CE4 (786 bp) | AL136444, HC G32335 | eg, U10561 (84%) | 84% similarity in the first 110 bp |
| E3A (189 bp) [‡] | AL136444, HC G32335 | AF167565 [§] | |
| E5 (280 bp) [¶] | NF | AC055776 (85%) | AC055776: unfinished mouse chromosome 10 clone |
| E6 (121 bp) [¶] | NF | NF | |
| E7 (89 bp) [¶] | AJ310556, AL445220 (88%) | AC101798 (100%) | AJ310556: human PIP3-E gene AC101798: mouse working genomic sequence draft |
| E8 (66 bp) [¶] | NF | AZ365875 (100%) | AZ365875: mouse GSS [§] ; significant identity to a segment in AC101798 |
| E9 (389 bp) [¶] | NF | AC091451 (100%) | AC091451: mouse chromosome X PAC clone |
| E10 (186 bp) [¶] | NF | AC055776 (97%) | |
| E11 (185 bp) [¶] | NF | AC101942 (100%) | AC101942: significant identity to AL136444 |
| E12 (129 bp) [¶] | NF | BH121554 (99%) | BH121554: mouse GSS; no identity to AC101942 |
| E13 (149 bp) [¶] | NF | AC055776 (97%) | |
| E14 (108 bp) [¶] | NF | AC055776 (100%) | |

*GSS indicates genome survey sequence; NF, no significantly homologous sequence found; OPRM1, ? opioid receptor, and PIP3-E, phosphoinositide-binding protein, PAC clone, P1 artificial chromosome clone.

[†]CE: human OPRM1 coding exons. CE1-4 are included to show the overall homology between mouse and human OPRM1.

[‡]E3A: human OPRM1A third coding exon, which was initially reported by Bare and colleagues (26).

[§]AF167565: reported mouse OPRM1A sequence. Overall, AF167565 shares 86% nucleotide identity with human OPRM1A. However, most similarity is due to the shared core sequence of OPRM1. Mouse OPRM1A differs from the human ortholog by the last 3 amino acids. There is no homology in the 3'UTR between human and mouse OPRM1A either.

[¶]E5 was first reported by Zimprich and colleagues (27).

[¶]E6 to E9 were reported by Pan and colleagues (32).

[¶]E10 was reported by Pan and colleagues (33).

[¶]E11 to E14 were reported by Pan and colleagues (34).

and 2, and introns 1 and 2, as OPRM1. OPRM1A might have been formed when the consensus splice site AG/GT at the end of OPRM1 coding exon 3 was not used. Instead, it continued to transcribe, forming its own third coding exon (CE3A), which included 15 additional bp and encoded for 4 aa (V-R-S-L) before the stop codon TAG. All known 98-bp 3'UTR sequences²⁶ were found to follow immediately after the stop codon in the genomic clones (**Figure 1**).

At least 5 additional 3' and 8 5' splice variants have been proposed; however, no human orthologs are known. Since the exact exon numbers are not clear for OPRM1 at this point, we propose using coding exons for the currently known 4 exons. Other proposed exons are referred to as E5 to E14, keeping the same numerical numbers as were initially proposed.³²⁻³⁴ We first sought to identify these alternative variant exons, if any, in the human genome. For those sequences that failed to find significant homology in the human genome, we further used them and their available intron sequences to search GenBank for all available sequences. This approach yielded genomic structures of several proposed mouse exons.

MOR1B was initially reported for rat OPRM1.²⁷ It differed from OPRM1 by the last 12 aa, indicating the absence of coding exon 4. Instead, another exon (E5, Appendix 2) replaced the coding exon 4 and encoded 4 alternative aa (K-D-L-F).²⁷ The rat MOR1B was 91% homologous with a mouse sequence (AF167566) in a smaller region (125 bp). E5 (274 bp) was found to share 85% sequence identity with a sequence (AC055776)

from the unfinished mouse chromosome 10 clone RP23-11015 (**Table 3**). Unfortunately, AC055776 was still a working draft made up of 21 sequence contigs in no particular order. Analysis of AC055776 indicated that it contained all 4 mouse OPRM1 coding exons.³⁸ E10 was also found to be included in AC055776. However, E5 and E10 were found in separate contigs and not located near any of the coding exons; therefore, we were not able to estimate the distance between these exons.

Four other mouse OPRM1 3' variants require the use of 4 additional exons (E6-E9, see Appendix 2). These variants differ in their carboxyl terminal sequences. When expressed in Chinese hamster ovary (CHO) cells, all variants showed high affinity to OPRM1 agonists,^{31,32} which was not entirely unexpected. OPRM1 lacking large parts of the intracellular C-tail sequences exhibited a nearly identical ligand binding profile.³⁹ Antibodies raised to recognize specific variants revealed distinct expression regions from OPRM1, and within these variants.⁴⁰⁻⁴² Three proposed variants, MOR1C, E, and F, call for the presence of exon E7. We identified the genomic arrangement of E7 since it was found to be part of an unfinished mouse clone RP24-217J7 (AC101798). Interestingly, E7 also demonstrated 88% identity with a region in human phosphoinositide-binding protein (PIP3-E) gene (AJ310556), located in human genome clone AL445220 (**Table 3**) and a Celera sequence. The full-length PIP3-E gene was found in 3 human chromosome 6 clones: AL132774, AL445220, and AL033376 spanning ~90 kb. We then compared these PIP3-E-containing human clones with the mouse working

sequence AC101798. Sequences included in AC101798 were found to have significant matches (90%-100% in some regions) with regions in these human sequences and AL136444. No other human genomic sequences have significant identity with AC101798. These comparisons strongly suggested that mouse sequence AC101798 was from the mouse chromosome 10, most likely from the OPRM1 and PIP3-E gene locus.

E8 was reported to be composed of 66 bp, which was found to be part (443-508) of the mouse 10-kb plasmid UUGC1M library clone 1M0112B22R (**Table 3**). Unfortunately, only 545 bp of the latter sequence is deposited as AZ365875. We, nevertheless, used these 545 bp to search databases for homologous sequences, and we found that the first part (1-232) was identical with a segment (51028-51260) from a contig (50541-51265) in the clone AC101798. A portion (300 bp) of the same contig was found to match a segment from the rat genomic sequence AC114432 (80% identity), but there was no homology with any known human sequences.

The entire sequence of E9 (398 bp), the last putative exon reported for the mouse OPRM1, was found to share 99% nucleotide identity with an unfinished mouse PAC clone AC091451 from the chromosome X (**Table 3**).

The 8 newly reported 5' splice variants used 4 additional exons (E11-E14, Appendix 2). Three variants (MOR1H, I, J) can produce protein sequences identical to OPRM1. Other variants produce either 6 or no transmembrane-spanning proteins. The RT-PCR method revealed varying degrees of MOR1H, I, and J in various brain regions. E11 and E12 were reported to be the two most 5'-exons that are 1.8 kb apart.³⁴ We found E11 to be part of the mouse genomic sequence draft AC101942. Comparison of AC101942 with the human OPRM1 sequence AL136444 yielded multiple sequence alignments that were relatively well conserved (75%-91% similarity). Genomic search using E11 found no human homologous sequence. E11 was included in a single sequence readout of 534 bp in AC101942. When these 534-bp sequences were compared with AL136444, a moderate degree (82%) of identity was found in 2 segments, 30 kb from the OPRM1 coding exon 1. However, E11 was not included in either of the homologous segments. E12 had no significant identity with any sequences in AC101942. Intron regions surrounding E11 (AF062755), but not E12, were also found to be included in AC101942. E12 was found to be part of the mouse genomic clone RPCI-24; only the 538-bp sequence was known (BH121554). When the entire 538-bp sequence of BH121554 was used to search all available databases, no additional homologous sequence was retrieved.

Two other 5' variant exons, E13 and E14, were found in mouse chromosome 10 working draft AC055776. AC055776 included all 4 coding exons,³⁸ E5, and E10. Interestingly, both E13 and E14 were found in the same contig (3051-5112). If this contig was indeed reliable, the distance between E13 and E14 was estimated at 1448 bp, which was significantly shorter than what had been reported.³⁴ The previous estimate was around 21 kb between E13 and E14. Consensus intron/exon splicing sites were met at all these proposed exons (E5, E10, E13, and E14) and their intron junctions; however, none of these exons, and hence the variants composed of them, were found to have human orthologs available. The same mouse clone AC055776, however, did not contain E12.

E6 used by the proposed variant MOR1F failed to locate any significant similarity with all sequences searched.

OPRM1 SNPs

A number of SNPs have been identified in the OPRM1 gene through direct sequencing and other methods, which have been so far largely limited to the cDNA sequence and short intron 2.^{15, 19, 43-47} Ten coding region SNPs have been identified, of which 8 are nonsynonymous. Several rare SNPs in the I3 loop have been tested and shown to impair receptor signaling^{46,48}; however, the significance of these SNPs in heterozygous individuals carrying the SNPs (no homozygous case has been reported) remains to be demonstrated. Two coding region SNPs occur at relatively high frequencies, including 17 C/T and 118 A/G. The 118 A/G SNP leads to aa substitution, Asn/Asp, at the extracellular N-terminus, which can potentially eliminate a consensus N-glycosylation site. When MOR1 A118G mutation was expressed in AV12 cells, it exhibited nearly identical affinity and activation by opioid ligands, with the exception of the large endogenous peptide -endorphin.⁴⁴ A118G variant showed higher affinity and increased activity to -endorphin⁴⁴; however, the same results were not reproduced when A118G variant was expressed in COS-7 cells.⁴⁸ Molecular genetic studies employing polymorphism 118 A/G in drug and alcohol abuse have so far yielded conflicting results. Most studies found no significant association of 118 A/G with polysubstance abuse and alcoholism,^{43,44,47,49,50} although there was a trend of increased G allele in polysubstance abusers.⁴⁷ Two studies, however, found significant association of 118 A/G with alcoholism in a mixed population sample (primarily Caucasian subjects)⁵¹ and with opioid addiction in Chinese study subjects.⁵² A list of all reported human OPRM1 SNPs appears in **Table 4**.

Table 4. SNPs in OPRM1 Gene*

| Position | Gene Location | Amino Acid Change | Polymorphic Allele Frequency | References |
|--------------|---------------|-------------------|------------------------------|-------------|
| -1045 A/G | 5'UTR | — | ~1.4% | (45) |
| -995 C/A | 5'UTR | — | NA | (45) |
| -692 G/C | 5'UTR | — | 4.3% | (45) |
| -554 G/A | 5'UTR | — | NA | (45) |
| -488 G/T | 5'UTR | — | NA | (45) |
| -254 A/C | 5'UTR | — | <1% | (45) |
| -236 A/G | 5'UTR | — | <1% | (45) |
| -172 G/T | 5'UTR | — | 11.4% | (45) |
| -133 C/T | 5'UTR | — | <1% | (45) |
| -111 C/T | 5'UTR | — | ~1.4% | (45) |
| -54 G/T | 5'UTR | — | NA | (45) |
| -38 C/A | 5'UTR | — | 1.4%-5% | (19, 45) |
| 17 C/T | Exon 1 | 6 Ala/Val | 6.6%-19% | (19, 43-45) |
| 24 G/A | Exon 1 | Synonymous | ~2.0% | (44, 45) |
| 118 A/G | Exon 1 | Asn/Asp | 9.2%-13.9% | (43-45) |
| 440 C/G | Exon 2 | Ser/Cys | <1% | (43, 45) |
| 454 A/G | Exon 2 | 152 Asn/Asp | ~1.4% | (45, 53) |
| IVS2+31 G/A | Intron 2 | — | 14.3% | (45) |
| IVS2+106 T/C | Intron 2 | — | ~1.4% | (45) |
| IVS2+397 T/A | Intron 2 | — | ~1.4% | (45) |
| IVS2+438 G/A | Intron 2 | — | ~4.3% | (45) |
| IVS2+480 T/C | Intron 2 | — | NA | (45) |
| IVS2+534 C/T | Intron 2 | — | <1% | (45) |
| IVS2+691 C/G | Intron 2 | — | 40%-45% | (43, 45) |
| 779 G/A | Exon 3 | 260 Arg/His | <1% | (44, 45) |
| 794 G/A | Exon 3 | 265 Arg/His | <1% | (45, 46) |
| 802 T/C | Exon 3 | 268 Ser/Pro | <1% | (45) |
| 820 G/A | Exon 3 | 274 Asp/Asn | <1% | (45, 46) |
| 942 G/A | Exon 3 | Synonymous | <1% | (44, 45) |
| /S3+37 A/C | Intron 3 | — | NA | (45) |
| 401 G/C | 3'UTR | — | ~2.9% | (45) |

*NA indicates not applicable (SNPs with no reliable allele frequencies); OPRM1, mu opioid receptor; SNP, single nucleotide polymorphism.

DISCUSSION In this study we analyzed human OPRM1 sequences and identified ~2.2 kb of accountable cDNA sequence. Considering that human OPRM1 mRNA is over 12 kb (^{32, 37}, and Wang et al, unpublished results), there are additional cDNA sequences at either the 5' or the 3' end. The available human genomic data for the first time allowed a clear arrangement of the OPRM1 gene, with precise intron length and intron/exon junctions. Adding together the unaccountable ~10 kb cDNA and ~80 kb genomic sequences that 4 coding exons and introns occupy, OPRM1 is at least 90 kb. This number may be substantially longer if there are additional exons and introns to make up the ~10-kb additional cDNA sequence.

Human OPRM1A variant appears to be valid, given that the entire OPRM1A variant sequence can be found in intron 3 of OPRM1. However, this variant does not provide any cDNA usage information beyond what has been identified for OPRM1.

On the contrary, the other reported 5' and 3' splice variants ^{27, 32-34} may offer insights into additional cDNA sequence usage as well as OPRM1 multiple subtypes. However, the only human homologue of these additional exons identified in the study was E7, which may represent a sequence from the human PIP3-E gene (AJ310556). PIP3-E, previously identified as KIAA0403, is the first gene 3' from OPRM1 with known function. ⁵⁴ Another gene, LOC154045 (XM 094669), was identified by only a computer prediction program, GenomeScan, and located between OPRM1 and PIP3-E. Most likely, E7 also represents part of mouse PIP3-E, although such information is not available from the mouse genome. E7 was used in variant MOR1C, E, F, and M. ³²⁻³⁴ E9, which was required for MOR1C, D, E, F, M, and N, was found to have 100% identity with mouse chromosome X sequence (AC091451). Due to the draft nature of AC091451, it cannot be entirely ruled out that the E9-containing portion of AC091451 has sequencing or cloning errors. Antibodies made from the unique peptide sequences of MOR1C and MOR1D have been used to detect immunoactivities for these proposed variants in rat and mouse. ^{40,41} In both cases, the antibodies were

made from peptide sequences that were primarily encoded by E8, so the immunoreactivity was largely independent of the presence of E7 or E9. Our analysis indicated that E8 was located in the mouse sequence AC101798, a mouse chromosome 10 clone that also included OPRM1. In addition, part of a larger sequence containing E8 was found to have a rat homologue. There has been 1 study that found MOR1C-like immunoreactivity in human spinal tissue using the same MOR1C antibody.⁴² However, the epitope peptide was found to have no similarity to any known human protein sequences.

The 4 mouse coding exons, and E5, 10, 13, and 14, were found in the mouse genomic clone AC055776. The distance between E13 and E14 was estimated at 1.5 kb. A segment of a sequence contig containing E14, but not the E14 region, demonstrated significant similarity to human chromosome 6 clone AL136444. These data suggest that additional exons in the 5' variant MOR1G-L and 3' variant MOR1B are within a reasonable distance from the other coding exons in OPRM1. However, we found no human ortholog to support the presence of such variants in the human genome. This is not likely to be due to the lack of human genomic sequence information, as the regions surrounding OPRM1 are well covered with overlapping sequences; it may be due instead to the differences between the 2 genomes. Although the OPRM1 nucleotide sequence is relatively well conserved (**Table 3**), lower similarity was observed in OPRM1A unique 3' sequence and 3'UTR regions between the 2 species. However, the significance and function of the OPRM1 5' variants MOR1G-L are somewhat unclear. MOR1G-L produce proteins containing 6 or 0 TMs, uncharacteristic of a G-protein coupled receptor. When alternative translational start points are used by MOR1H, I and J, a protein identical to that encoded by OPRM1 cDNA is produced in all 3 variants. If this is the case, then the OPRM1 expression is under the control of multiple promoters.

In summary, this study was able to locate the human genomic sequence for the OPRM1 variant OPRM1A. We were able to identify the mouse genomic structures for several proposed variant exons. Little evidence was uncovered to support human orthologs of 5' variants and OPRM1B, whereas similarity to human PIP3-E gene and mouse chromosome X segment raised concern for other 3' OPRM1 variants.

ACKNOWLEDGEMENTS

This work was supported by a grant from the National Institutes of Health (DA00505).

REFERENCES

1. Pert CB, Snyder SH. Opiate receptor: demonstration in nervous tissue. *Science*. 1973;179:1011-1014.
2. Simon EJ, Hiller JM, Edelman I. Stereospecific binding of the potent narcotic analgesic (3H) etorphine to rat-brain homogenate. *Proc Natl Acad Sci U S A*. 1973;70:1947-1949.
3. Terenius L. Stereospecific interaction between narcotic analgesics and a synaptic plasma membrane fraction of rat cerebral cortex. *Acta Pharmacol Toxicol*. 1973;32:317-320.
4. Smith AP, Loh HH. Opioid peptide receptors and membrane biology. *Prog Clin Biol Res*. 1982;97:113-135.
5. Lewis J, Mansour A, Khachaturian H, Watson SJ, Akil H. Opioids and pain regulation. *Pain Headache*. 1987;9:129-159.
6. Evans CJ, Keith DE Jr, Morrison H, Magendzo K, Edwards RH. Cloning of a delta opioid receptor by functional expression. *Science*. 1992;258:1952-1955.
7. Kieffer BL, Befort K, Gaveriaux-Ruff C, Hirth CG. The delta-opioid receptor: isolation of a cDNA by expression cloning and pharmacological characterization. *Proc Natl Acad Sci U S A*. 1992;89:12048-12052.
8. Chen Y, Mestek A, Liu J, Hurley JA, Yu L. Molecular cloning and functional expression of a mu-opioid receptor from rat brain. *Mol Pharmacol*. 1993;44:8-12.
9. Meng F, Xie GX, Thompson RC, et al. Cloning and pharmacological characterization of a rat kappa opioid receptor. *Proc Natl Acad Sci U S A*. 1993;90:9954-9958.
10. Liu-Chen LY, Chen C, Phillips CA. Beta-[3H]funtaltrexamine-labeled mu-opioid receptors: species variations in molecular mass and glycosylation by complex-type, N-linked oligosaccharides. *Mol Pharmacol*. 1993;44:749-756.
11. Fukuda K, Kato S, Mori K, Nishi M, Takeshima H. Primary structures and expression from cDNAs of rat opioid receptor delta- and mu-subtypes. *FEBS Lett*. 1993;327:311-314.
12. Zubieta JK, Gorelick DA, Stauffer R, Ravert HT, Dannals RF, Frost JJ. Increased mu opioid receptor binding detected by PET in cocaine-dependent men is associated with cocaine craving. *Nat Med*. 1996;2:1225-1229.
13. Choiniere M, Melzack R. Acute and chronic pain in hemophilia. *Pain*. 1987;31:317-331.
14. Tyler DC, Pomietto M, Womack W. Variation in opioid use during PCA in adolescents. *Paediatr Anaesth*. 1996;6:33-38.
15. Uhl GR, Sora I, Wang Z. The mu opiate receptor as a candidate gene for pain: polymorphisms, variations in expression,

- nociception, and opiate responses. *Proc Natl Acad Sci U S A*. 1999;96:7752-7755.
16. Lim AT, Edis G, Kranz H, Mendelson G, Selwood T, Scott DF. Postoperative pain control: contribution of psychological factors and transcutaneous electrical stimulation. *Pain*. 1983;17:179-188.
17. Jonsson CE, Holmsten A, Dahlstrom L, Jonsson K. Background pain in burn patients: routine measurement and recording of pain intensity in a burn unit. *Burns*. 1998;24:448-454.
18. Dawson C, Vale JA, Corry DA, et al. Choosing the correct pain relief for extracorporeal lithotripsy. *Br J Urol*. 1994;74:302-307.
19. Berrettini WH, Hoehe MR, Ferraro TN, DeMaria PA, Gottheil E. Human mu opioid receptor gene polymorphisms and vulnerability to substance abuse. *Addiction Biol*. 1997;2:303-308.
20. Lehmann KA, Heinrich C, van Heiss R. Balanced anesthesia and patient-controlled postoperative analgesia with fentanyl: minimum effective concentrations, accumulation and acute tolerance. *Acta Anaesthesiol Belg*. 1988;39:11-23.
21. Berrettini WH, Ferraro TN, Alexander RC, Buchberg AM, Vogel WH. Quantitative trait loci mapping of three loci controlling morphine preference using inbred mouse strains. *Nat Genet*. 1994;7:54-58.
22. Wang JB, Johnson PS, Persico AM, Hawkins AL, Griffin CA, Uhl GR. Human mu opiate receptor: cDNA and genomic clones, pharmacologic characterization and chromosomal assignment. *FEBS Lett*. 1994;338:217-222.
23. Mestek A, Hurley JH, Bye LS, et al. The human mu opioid receptor: modulation of functional desensitization by calcium/calmodulin-dependent protein kinase and protein kinase C. *J Neurosci*. 1995;15:2396-2406.
24. Lander ES, Linton LM, Birren B, et al. Initial sequencing and analysis of the human genome. *Nature*. 2001;409:860-921.
25. Venter JC, Adams MD, Myers EW, et al. The sequence of the human genome. *Science*. 2001;291:1304-1351.
26. Bare LA, Mansson E, Yang D. Expression of two variants of the human mu opioid receptor mRNA in SK-N-SH cells and human brain. *FEBS Lett*. 1994;354:213-216.
27. Zimprich A, Simon T, Hollt V. Cloning and expression of an isoform of the rat mu opioid receptor (rMOR1B) which differs in agonist induced desensitization from rMOR1. *FEBS Lett*. 1995;359:142-146.
28. Gintzler AR, Pasternak GW. Multiple mu receptors: evidence for mu2 sites in the guinea pig ileum. *Neurosci Lett*. 1983;39:51-56.
29. Simone DA, Bodnar RJ, Goldman EJ, Pasternak GW. Involvement of opioid receptor subtypes in rat feeding behavior. *Life Sci*. 1985;36:829-833.
30. Pasternak GW. Insights into mu opioid pharmacology: the role of mu opioid receptor subtypes. *Life Sci*. 2001;68:2213-2219.
31. Pasternak GW. Incomplete cross tolerance and multiple mu opioid peptide receptors. *Trends Pharmacol Sci*. 2001;22:67-70.
32. Pan YX, Xu J, Bolan E, et al. Identification and characterization of three new alternatively spliced mu-opioid receptor isoforms. *Mol Pharmacol*. 1999;56:396-403.
33. Pan YX, Xu J, Bolan E, et al. Isolation and expression of a novel alternatively spliced mu opioid receptor isoform, MOR-1F. *FEBS Lett*. 2000;466:337-340.
34. Pan YX, Xu J, Mahurter L, Bolan E, Xu M, Pasternak GW. Generation of the mu opioid receptor (MOR-1) protein by three new splice variants of the OPRM gene. *Proc Natl Acad Sci U S A*. 2001;98:14084-14089.
35. Altschul SF, Madden TL, Schaffer AA, et al. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res*. 1997;25:3389-3402.
36. Ko JL, Minnerath SR, Loh HH. Dual promoters of mouse mu-opioid receptor gene. *Biochem Biophys Res Commun*. 1997;234:351-357.
37. Liang Y, Carr LG. Transcription of the mouse mu-opioid receptor gene is regulated by two promoters. *Brain Res*. 1997;769:372-374.
38. Min BH, Augustin LB, Felsheim RF, Fuchs JA, Loh HH. Genomic structure analysis of promoter sequence of a mouse mu opioid receptor gene. *Proc Natl Acad Sci U S A*. 1994;91:9081-9085.
39. Segredo V, Burford NT, Lameh J, Sadee W. A constitutively internalizing and recycling mutant of the mu-opioid receptor. *J Neurochem*. 1997;68(6):2395-2404.
40. Abbadie C, Pasternak GW, Aicher SA. Presynaptic localization of the carboxy-terminus epitopes of the mu opioid receptor splice variants MOR-1C and MOR-1D in the superficial laminae of the rat spinal cord. *Neuroscience*. 2001;106:833-842.
41. Abbadie C, Pan Y, Drake CT, Pasternak GW. Comparative immunohistochemical distributions of carboxy terminus epitopes from the mu-opioid receptor splice variants MOR-1D, MOR-1 and MOR-1C in the mouse and rat CNS. *Neuroscience*. 2000;100:141-153.
42. Abbadie C, Gultekin SH, Pasternak GW. Immunohistochemical localization of the carboxy terminus of the novel mu opioid receptor splice variant MOR-1C within the human spinal cord. *Neuroreport*. 2000;11:1953-1957.
43. Bergen AW, Kokoszka J, Peterson R, et al. Mu opioid receptor gene variants: lack of association with alcohol dependence. *Mol Psychiatr*. 1997;2:490-494.

44. Bond C, LaForge KS, Tian M, et al. Single-nucleotide polymorphism in the human mu opioid receptor gene alters beta-endorphin binding and activity: possible implications for opiate addiction. *Proc Natl Acad Sci U S A.* 1998;95:9608-9613.
45. Hoehe MR, Kopke K, Wendel B, et al. Sequence variability and candidate gene analysis in complex disease: association of mu opioid receptor gene variation with substance dependence. *Hum Mol Genet.* 2000;9:2895-2908.
46. Wang D, Quillan JM, Winans K, Lucas JL, Sadee W. Single nucleotide polymorphisms in the human mu opioid receptor gene alter basal G protein coupling and calmodulin binding. *J Biol Chem.* 2001;276:34624-34630.
47. Wang ZJ, Uhl GR. Human mu opioid receptor: gene structure and polymorphisms. Society for Neurosci, Abstract 337.7, Los Angeles, November 9, 1998.
48. Befort K, Filliol D, Decaillet FM, Gaveriaux-Ruff C, Hoehe MR, Kieffer BL. A single nucleotide polymorphic mutation in the human [micro]-opioid receptor severely impairs receptor signaling. *J Biol Chem.* 2001;276:3130-3137.
49. Sander T, Gscheidel N, Wendel B, et al. Human mu-opioid receptor variation and alcohol dependence. *Alcohol Clin Exp Res.* 1998;22:2108-2110.
50. Gelernter J, Kranzler H, Cubells J. Genetics of two mu opioid receptor gene (OPRM1) exon I polymorphisms: population studies, and allele frequencies in alcohol- and drug-dependent subjects. *Mol Psychiatry.* 1999;4:476-483.
51. Town T, Abdullah L, Crawford F, et al. Association of a functional mu-opioid receptor allele (+118A) with alcohol dependency. *Am J Med Genet.* 1999;88:458-461.
52. Szeto CY, Tang NL, Lee DT, Stadlin A. Association between mu opioid receptor gene polymorphisms and Chinese heroin addicts. *Neuroreport.* 2001;12:1103-1106.
53. Wendel B, Hoehe MR. The human mu opioid receptor gene: 5' regulatory and intronic sequences. *J Mol Med.* 1998;76:525-532.
54. Krugmann S, Anderson KE, Ridley SH, et al. Identification of ARAP3, a novel PI3K effector regulating both Arf and Rho GTPases, by selective capture on phosphoinositide affinity matrices. *Mol Cell.* 2002;9:95-108.

APPENDICES

APPENDIX 1. Human μ Opioid Receptor cDNA Sequence

```
1      GATGAGCCTC TGTGAACTAC TAAGGTGGGA GGGGGCTATA CGCAGAGGAG
51     AATGTCAGAT GCTCAGCTCG GTCCCCTCCG CCTGACGCTC CTCTCTGTCT
101    CAGCCAGGAC TGGTTTCTGT AAGAAACAGC AGGAGCTGTG GCAGCGGCGA
151    AAGGAAGCGG CTGAGGCGCT TGGAACCCGA AAAGTCTCGG TGCTCCTGGC
201    TACCTCGCAC AGCGGTGCCC GCCCGGCCGT CAGTACCATG GACAGCAGCG
251    CTGCCCCCAC GAACGCCAGC AATTGCACTG ATGCCTTGGC GTACTCAAGT
301    TGCTCCCCAG CACCCAGCCC CGGTTCTTGG GTCAACTTGT CCCACTTAGA
351    TGGCAACCTG TCCGACCCAT GCGGTCCGAA CCGCACCGAC CTGGGCGGGA
401    GAGACAGCCT GTGCCCTCCG ACCGGCAGTC CCTCCATGAT CACGGCCATC
451    ACGATCATGG CCCTCTACTC CATCGTGTGC GTGGTGGGGC TCTTCGGAAA
501    CTTCTCTGGT ATGTATGTGA TTGTCAGATA CACCAAGATG AAGACTGCCA
551    CCAACATCTA CATTTTCAAC CTTGCTCTGG CAGATGCCTT AGCCACCAGT
601    ACCCTGCCCT TCCAGAGTGT GAATTACCTA ATGGGAACAT GGCCATTTGG
651    AACCATCCTT TGCAAGATAG TGATCTCCAT AGATTACTAT AACATGTTCA
701    CCAGCATATT CACCCTCTGC ACCATGAGTG TTGATCGATA CATTGCAGTC
751    TGCCACCCTG TCAAGGCCTT AGATTTCCGT ACTCCCCGAA ATGCCAAAAT
801    TATCAATGTC TGCAACTGGA TCCTCTCTTC AGCCATTGGT CTTCTGTAA
851    TGTTTCATGGC TACAACAAAA TACAGGCAAG GTTCCATAGA TTGTACACTA
901    ACATTCTCTC ATCCAACCTG GTACTGGGAA AACCTGCTGA AGATCTGTGT
951    TTTTCATCTC GCCTTCATTA TGCCAGTGCT CATCATTACC GTGTGCTATG
1001  GACTGATGAT CTTGCGCCTC AAGAGTGTCC GCATGCTCTC TGGCTCCAAA
1051  GAAAAGGACA GGAATCTTCG AAGGATCACC AGGATGGTGC TGGTGGTGGT
1101  GGCTGTGTTC ATCGTCTGCT GGACTCCCAT TCACATTTAC GTCATCATTA
```

AAPS PharmSci 2002; 4 (4) article 23 (<http://www.aapspharmsci.org>).

1151 AAGCCTTGGT TACAATCCCA GAAACTACGT TCCAGACTGT TTCTTGGCAC
1201 TTCTGCATTG CTCTAGGTTA CACAAACAGC TGCCTCAACC CAGTCCTTTA
1251 TGCATTTCTG GATGAAAAC TCAAACGATG CTTTCAGAGAG TTCTGTATCC
1301 CAACCTCTTC CAACATTGAG CAACAAAACCT CCACTCGAAT TCGTCAGAAC
1351 ACTAGAGACC ACCCCTCCAC GGCCAATACA GTGGATAGAA CTAATCATCA
1401 GCTAGAAAAT CTGGAAGCAG AAAGTGTCTCC GTTGCCCTAA CAGGGTCTCA
1451 TGCCATTCCG ACCTTCACCA AGCTTAGAAG CCACCATGTA TGTGGAAGCA
1501 GGTGCTTCA AGAATGTGTA GGAGGCTCTA ATTCTCTAGG AAAGTGCCTG
1551 CTTTTAGGTC ATCCAACCTC TTTCTCTCT GGCCTCTG CTCTGCACAT
1601 TAGAGGGACA GCCAAAAGTA AGTGGAGCAT TTGGAAGGAA AGGAATATAC
1651 CACACCGAGG AGTCCAGTTT GTGCAAGACA CCCAGTGGAA CAAAACCCA
1701 TCGTGGTATG TGAATTGAAG TCATCATAAA AGGTGACCCT TCTGTCTGTA
1751 AGATTTTATT TTCAAGCAA TATTTATGAC CTCAACAAAG AAGAACCATC
1801 TTTTGTAAAG TTCACCGTAG TAACACATAA AGTAAATGCT ACCTCTGATC
1851 AAAGCACCTT GAATGGAAGG TCCGAGTCTT TTTAGTGTTT TTGCAAGGGA
1901 ATGAATCCAT TATTCTATTT TAGACTTTTA ACTTCAACTT AAAATTAGCA
1951 TCTGGCTAAG GCATCATTTT CACCTCCATT TCTTGGTTTT GTATTGTTTA
2001 AAAAAAATAA CATCTCTTTC ATCTAGCTCC ATAATTGCAA GGAAGAGAT
2051 TAGCATGAAA GGTAATCTGA AACACAGTCA TGTGTCANCT GTAGAAAGGT
2101 TGATTCTCAT GCACTNCAA TACTTCCAAA GAGTCATCAT GGGGATTTT
2151 TCATTCTTAG GCTTTCAGTG GTTTGTTCCT GGAATTC

AAPS PharmSci 2002; 4 (4) article 23 (<http://www.aapspharmsci.org>).
APPENDIX 2. Proposed Additional μ Opioid Receptor Exons

E5 LENGTH: 280 (based on sequences described in Pan et al. [32])

1 GAAAATAGTA TTATTTTGAA AAGGCATATA CACAGAACTG GGAGAAGCAC
51 GCCAGAGATA TTTGTGACC ATATGGCAA TGTAAGCCTA GAAACCAGGG
101 TACCTGATGC TGGTGCCAAAC CCCACTGACA ACCACTTGTG CAAGAACATC
151 CCATGCTGGT AGTTGTTGAA CAGATTTCTT CATGACCAGA AGATCATTGA
201 CCTCTAAGTT TGCTCTAGAG TTAGAACCTT GTTGATTACA TCATTCAGAC
251 TCTGGGCAAG CTCTCTCAA TGAACAAAGG

E6 LENGTH: 121 (based on sequences described in Pan et al. [32])

1 AAGAAAAAGC TGGACTCCCA GAGAGGGTGT GTACAGCATC CAGTGTGACC
51 TGTCCCTTGT CTTTGAGCCT GGGGGCCATC TTCTTTCACA GCATACCATT
101 TCCTTGATC CTCTCTGAAA G

E7 LENGTH: 89 (based on sequences described in Pan et al. [32])

1 CCAACCCTGG CAGTCAGCGT GGCCAGATC TTTACAGGAT ATCCTTCTCC
51 GACTCATGTT GAAAAACCCT GCAAGAGTTG CATGGACAG

E8 LENGTH: 66 (based on sequences described in Pan et al. [32])

1 AGGAATGAGG AACCTTCTTC CTGATGATGG CCAAGACAG GAATCCGGGG
51 AAGGCCAGCT TGGCAG

E9 LENGTH: 389 (based on sequences described in Pan et al. [32])

1 GTGAATGTCA TCCGAACACA GGGATGAGCT GGTGAGCAGT GTGGAGGTGC
51 AGGCTCTGCT AGAAGACAGC GAGAGGGAAG ATGCCTTTCA CTCATAAAGG
101 TTAGGAGTTG TAAGTGTAAT GTCTAATGTA TTGTCTGGTT TGCCGTATTG
151 CGTGTTTTCA TACGAATGAC TTGGCAATGT AGAGACAGCC TGTTAAACAT
201 GGCAATGTGG CTCTGTGGA CACCGCTCTC ATGCCACTGT GTGCAAATAA
251 ACAAGGGACT TCCTTAGGAG CAGCCTAGCC ACCTTACATG AAGAGTTTCC
301 TAATGTCACA GAAGAGGAAC GAAAGCCAGT ATTTGTTATG ACACTGTTTC

AAPS PharmSci 2002; 4 (4) article 23 (<http://www.aapspharmsci.org>).

351 TTCTAAATAA TGTAATTAAT AAAATAAAGA AAAACATGG

E10 LENGTH: 186 (based on sequences described in Pan et al. [33])

1 GCACCATGTG CATGCGTGCC TGGAGCCAAC AGAGGTCAAA CGAA

51 AGATCTTCTG GATCTGGAAT TGGAGACAGT TGGGAGCCAC CAGG

101 CTGAAACCAA CCCAGGTCCT TACGAAGGCA GCAAGTGC GC TGAA

151 GCCATCTCTC TGGTCCCGCT ATATTAGCAT TGTGCT

E11 LENGTH: 185 (based on sequences described in Pan et al. [34])

1 TTTTACTGTC CTTGAGAATG GAGAGGATCA GCAAAGCTGG GAAGCC

51 AGGCTCATTT CAGAGAGAAAT ATTCCACAGA GCTTGAAGGC GCGGGA

101 GGCCGATGAT GGAAGCTTTC TCTAAGTCTG CATTCCAAAA GCTCAG

151 AGAGATGGAA ATCAAGAGGG GAAGAGTTAC CTCAG

E12 LENGTH: 129 (based on sequences described in Pan et al. [34])

1 GTCTTGTGCA GGTGCACTGC TGCTGTGAAT TCATGAAGAC

51 CCTTTAGAAG ACAGTGCTTC ACAACACTCC CAACTAGCCT

101 TGTTCAC TTT GTCCCCTCTT CTGAAGCAG

E13 LENGTH: 149 (based on sequences described in Pan et al. [34])

1 ACACCTCATT CCAAGGAAGG AAATTATCTT TTTAAAACTG AAATAACTAG

51 GCATTCCAAG CACCTGGCGG TGAGCTGATA AAGACTGAGA GTGTAATGAG

101 TCAGAAAATT GTGTTGGGTT CCCCTCTGA GTGTGACTAA TGTCAAAAG

E14 LENGTH: 108 (based on sequences described in Pan et al. [34])

1 GCTCCCTCTG TCCATTCTTT TCCTGAACAA AGAGTCATGA CAACTCAAA

51 AATCAACTGA AAATCAAAAT AGAAAATGGG CTAAGGCAAC TGGTTCGACC

101 CCACAAAG