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# Determination of Opioid-binding Cell Adhesion Molecule-related Clones in Humans

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**Human brain cDNA labrary was screened with probes (Cam 1 and Cam 2, Cam 1 and Cam 5) constructed from the common region of rat and bovine opioid-binding cell adhesion molecule (OBCAM) cDNA. A polymerase chain reaction product of 528 bp was obtained from a human brain cDNA library. We found that plaques contained probe sequence. Only one clone was isolated and sequenced. It has a 75.0% homology to known OBCAM bovine and 76.3% to known human cDNA. This study suggests that it may be a family of OBCAM-like genes.**

Opioid-binding cell adhesion molecule (OBCAM) was originally isolated and designated as a potential opiate receptor (Schofield *et al.*, 1989). The cDNA from bovine and rat brain have subsequently been found (Lippman *et al.*, 1992; Schofield *et al.*, 1989). Before then, Cho *et al.* (1986) purified a 58-kDa opioid binding protein to apparent homogeneity from rat brain. The sequence is homologous to various members of the immunoglobulin protein superfamily, especially to those molecules involved in cell adhesion (Schofield *et al.*, 1989). The precise role of OBCAM in opioid receptor function is not yet clear. However, alternative evidence suggests that OBCAM is not directly involved in opioid binding, but rather plays a role in coupling opioid receptors to G-protein (Govitrapong *et al.*, 1993). The stable NG108-15 cell line transfected with antisense to OBCAM showed a profound reduction in opioid binding activity (Roy *et al.*, 1988, 1989). The presence of antisense OBCAM cDNA in NG108-15 cells alters the signal transduction mechanism of δ-opioid receptors with a general down-regulation of these receptors (Ann *et al.*, 1992). OBCAM immunoreactivity was decreased by chronic agonist treatment of NG108 cells, similar to the *d*-opioid receptors present in these cell (Lane *et al.*, 1992). The structure of OBCAM does not possess a membrane-spanning domain but contains a C-terminal hydrophobic sequence characteristic of membrane attachment by phosphatidyl inositol linkage (Schofield *et al.*, 1989). It displays the highest degree of homology with several cell adhesion molecules, including neural cell adhesion molecule (NCAM) myelin-associated glycoprotein, and with several peptide receptors, including platelet-derived growth factor (PDGF) and interleukin-6 (Loh and Smith, 1991). Therefore, a recognization of the structure of OBCAM would provide very important evidence for understanding the opioid receptor properties.

Recently, OBCAM has been mapped in mouse chromosome 9 (Charkraborti *et al.*, 1993) and human OBCAM cDNA has been cloned, the chromosomal site of which was sought in chromosome 11 (Shark and Lee, 1995). The location is also known to carry the gene encoding NCAM (McConville *et al.*, 1990), and genes encoding the Thy-1 glycoprotein, the $D_2$ dopamine receptor (Grandy *et al.*, 1989) and the $R_4$ subtype of the kainate glutamate receptor (McNamara *et al.*, 1992). The purpose of this study was to characterize the true existence of OBCAM by our own approach on humans. We compared the sequence of human OBCAM to that of mammalian clones.

## Materials and Methods

*Materials*

A human brain cDNA library was obtained from the Clontech Co. (Palo Alto, U.S.A.). Enzymes and other materials were purchased from the following sources: restriction enzymes and T4 DNA ligase, KOSKO (Seoul, Korea); calf intestinal alkaline phosphatase, Promega (Madison, U.S.A.); T7 sequencing™ kit, pharmacia LKB (Uppsala, Sweden); sequenase kit, USB (Cleveland, U.S.A.); [α-$^{32}$P] dATP and [α-$^{35}$S]

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The abbreviations used are: bp, base pair; NCAM, neural cell adhesion molecule; OBCAM, opioid-binding cell adhesion molecule; PDGF, platelet-derived growth factor; PCR, polymerase chain reaction.

dATP, Amersham Co. (Chicago, U.S.A.); Agarose, acrylamide, bisacrylamide, ammonium persulfate, TEMED and urea, Sigma Chemical Co. (St. Louis, U. S.A.). All other chemicals were analytical grade.

*Primer design and probe synthesis*

Searching bovine and rat OBCAM cDNA via GenBank search, we compared the DNA homology between two cDNAs through the DNAsis program (Hitachi Co., Japan). The five primers are designed from the common region of two cDNAs, and the nucleotide sequences of synthesized primers are as follows.

Cam 1 5'-GCGCAGCGGAGAGATGCCACCTTTCCC-3'
Cam 2 5'-GAGGGTCTATGGACCACTTGTCATT-3'
Cam 3 5'-TAGACCAGAGCCTACGGTTAC-3'
Cam 4 5'-GGAAGGCCAGGGCTTTGTGAGTGAG-3'
Cam 5 5'-GAGTGAGCGCCTTGAATGATGT-3'

To synthesize the probe for cDNA library screening, we used the human brain cDNA library (Clontech, Palo Alto, U.S.A.) as the template in a polymerase chain reaction (PCR). The reaction mixture was made up of a standard PCR buffer (50 mM KCl, 10 mM Tris-HCl, pH 8.4, 1.5 mM $MgCl_2$, 0.1 mg/ml gelatin), 50 pmol of each primer, 200 μM of each deoxynucleotide triphosphate (dNTPs), and 20 μCi of [α-$^{32}$P] dATP. The amplification of cDNA was achieved by 30 cycles (95 ℃, 1 min; 55 ℃, 45 s; 72 ℃, 1 min).

*Cloning and sequencing of PCR products*

Directly ligating PCR product 528 bp to pT7 Blue vector (Novagene Co., U.S.A.), it was tranformed to a Novablue competent cell. After purification of plasmid from transformant by the alkaline lysis method, we identified the clones containing PCR products by electrophoresis of restriction fragments. The PCR products were sequenced by using a Sequenase V2.0 kit. A comparison of the nucleotide sequence of PCR the product with that of bovine OBCAM cDNA was done by using DNAsis.

*Screening of human brain cDNA library*

Approximately 300,000 plaques from human brain cDNA library were screened according to the procedure of molecular cloning (Sambrook *et al.*, 1989). Replicas of the plates were prepared using nitrocellulose membrane and these were screened by hybridization using a $^{32}$P-labeled 528 bp denatured DNA fragment, which was previously synthesized by the PCR as a probe. The hybridization was carried out in 6× SSC, 1× Denhardt's solution, 0.05% Na-pyrophosphate, 100 μg/ml boiled sperm DNA at 60 ℃ overnight. The filters were washed for 5-10 min three times in a solution of 6× SSC, 0.05% pyrophosphate at room temperature and then washed for 30 min in prewarmed 6× SSC, 0.05% pyrophosphate at 60 ℃. The second and third screening was carried out as the above method.

*Cloning and sequencing of the phage insert*

The overproduction of phage by Broth culture was followed by a concentration with PEG, and phage was isolated by centrifugation (25,000 rpm, 2 h, 4 ℃). We gained phage DNA by treatment with proteinase K and the ethanol down method. After ligation of the phage insert into the T-vector, we transformed to Novablue by the $CaCl_2$ procedure. The plasmid of the transformant was purified by an alkaline lysis method. Sequencing of the cloned DNA was performed by the dideoxynucleotide chain termination method using a single strand DNA as a template. We compared the sequence of the plasmid with that of OBCAM cDNA, and the homology was searched.

*Structure analysis of human OBCAM cDNA*

The DNA sequence of the phage insert was translated into the amino acid sequence. A structural analysis of DNA and the amino acid sequence, along with a homology search was done with DNAsis and prosis.

## Results and Discussion

Two kinds of PCR products, 167 bp and 528 bp, were obtained through PCR amplification of human brain cDNA library using oligo primers (Cam 1 and Cam 2, Cam 1 and Cam 5) derived from the common region of rat and bovine OBCAM cDNA (Figs. 1A and B).

The PCR products were ligated into T vector and transformated into *E. coli* followed by purification of plasmid, and PCR was done (Fig. 2). After sequencing the cloned 528 bp products with the dideoxynucleotide chain termination method, we found a 78.8% homology with that of bovine OBCAM cDNA. The human brain cDNA library was screened by using a 528 bp PCR product as a probe.

After purification of a positive plaque on the autoradiogram, we found the plaques contained a probe

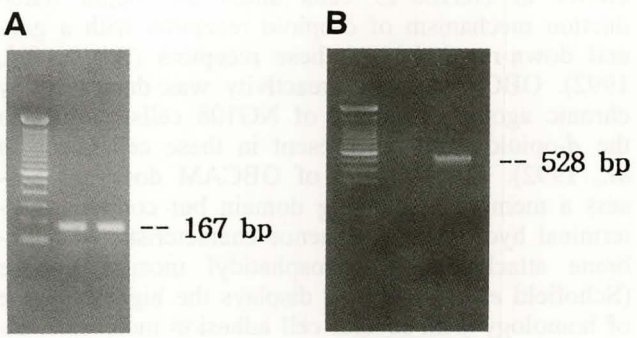


**Figure 1.** Amplification of the 167 bp and 528 bp fragment by PCR using a human brain cDNA library as a template and primer pairs of Cam 1-Cam 2 (A) and Cam 1-Cam 5 (B).

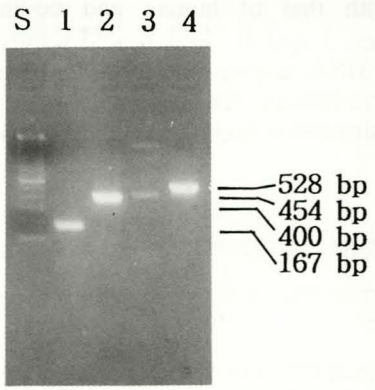


**Figure 2.** Cloning of the 528 bp fragment into the T-vector. PCR products using Cam 1-2 (lane 1), Cam 1-3 (lane 2), Cam 1-4 (lane 3), Cam 1-5 (lane 4) as primer pairs, and plasmid containing a 528 bp fragment as a template amplified four DNA fragments in expected size.

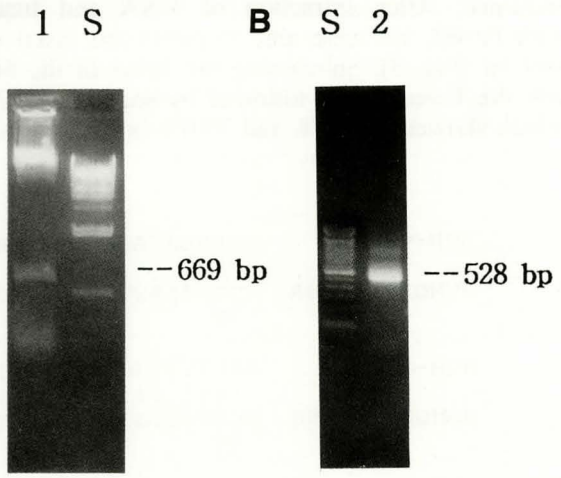


**Figure 3.** Digestion of phage DNA with *Eco*RI (A) showed a 669 bp insert containing a 528 bp probe fragment (B).

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*** DNA TRANSLATION ***

  1  CCC ACA GAA CCC TCT AAA TCC CCT TGT AAA TTT AAC TGT TAG TCC AAA

  1   M   H   N   S   I   S   W   A   I   F   T   G   L   A   A   L
 49  ATG CAC AAT TCT ATC TCT TGG GCA ATC TTC ACG GGG CTG GCT GCT CTG

 17   C   L   F   Q   G   V   P   V   R   T   G   D   A   T   F   P
 97  TGT CTC TTC CAA GGA GTG CCC GTG CGC ACG GGA GAT GCC ACC TTC CCC

 33   K   A   M   D   N   V   T   V   R   Q   G   E   S   A   T   L
145  AAA GCT ATG GAC AAC GTG ACG GTC CGG CAG GGG GAG AGC GCC ACC CTC

 49   R   C   T   I   D   N   R   V   T   X   V   A   W   L   N   R
193  AGG TGC ACT ATT GAC AAC CGG GTC ACC CNG GTG GCC TGG CTA AAC CGC

 65   S   T   I   L   Y   A   G   N   D   K   W   C   L   D   P   R
241  AGC ACC ATC CTC TAT GCT GGG AAT GAC AAG TGG TGC CTG GAT CCT CGC

 81   V   V   L   L   S   N   T   Q   T   Q   Y   S   I   E   I   Q
289  GTG GTC CTT CTG AGC AAC ACC CAA ACG CAG TAC AGC ATC GAG ATC CAG

 97   N   V   D   V   Y   D   E   G   P   Y   T   C   S   V   Q   T
337  AAC GTG GAT GTG TAT GAC GAG GGC CCT TAC ACC TGC TCG GTG CAG ACA

113   D   N   H   P   K   T   S   R   V   H   L   I   V   Q   V   S
385  GAC AAC CAC CCA AAG ACC TCT AGG GTC CAC CTC ATT GTG CAA GTA TCT

129   P   K   I   V   E   I   S   S   D   I   S   I   N   E   G   N
433  CCC AAA ATT GTA GAG ATT TCT TCA GAT ATC TCC ATT AAT GAA GGG AAC

145   N   I   S   L   T   C   I   A   T   G   R   P   E   P   T   V
481  AAT ATT AGC CTC ACC TGC ATA GCA ACT GGT AGA CCA GAG CCT ACG GTT

161   T   W   R   H   I   S   P   K   A   V   G   F   V   S   E   D
529  ACT TGG AGA CAC ATC TCT CCC AAA GCG GTT GGC TTT GTG AGT GAA GAC

177   E   Y   L   E   I   Q   G   I   T   R   E   Q   S   G   D   Y
577  GAA TAC TTG GAA ATT CAG GGC ATC ACC CGG GAG CAG TCA GGG GAC TAC

193   E   C   S   A   S   N   D   V   A   X   X   V   V   R   S
625  GAG TGC AGC GCC TCC AAT GAC GTG GCC G?G ?CC GTG GTA CGG AGC CC
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**Figure 4.** DNA and amino acid sequence of a phage insert (669 bp in size).

sequence. After extraction of DNA and treatment with *Eco*RI, we were able to purify the insert of the 669 bp (Fig. 3). Subcloning the insert of the 669 bp into the T-vector was followed by sequencing (Fig. 4) which showed a 76.3% and 75.0% homology in comparison with that of human and bovine OBCAM cDNA (Figs. 5 and 6). Amino acid sequence deduced from the cDNA sequence (669 bp) showed a 74.8% homology to human OBCAM.

It also showed a homology of cell adhesion mole-

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                  10         20         30         40         50         60
SUB-OB.SEQ        CCCACAGAACCCTCTAAATCCCCTTGTAAATTTAACTGTTAGTCCAAAATGCACAATTCT

HUMOBCAM.DNA      CTGGGAAGTTGTGGCTGTCGAGAATGGGGGTCTGTGGGTACCTGTTCCTGCCCTGGAAGT
                   30         40         50         60         70         80
                          70         80         90        100        110        120
SUB-OB.SEQ        ATCTCTTGGGCAATCTTCACGGGGCTGGCTGCTCTGTGTCTCTTCCAAGGAGTGCCCGTG
                    ::: ::: :     :: :  :::: :::: ::  ::  :   :  X:::::::::::
HUMOBCAM.DNA      GCCTCGTGGTCGTGTCTCTC-AGGCT-GCTGTTCCTTG--TACCCACAGGAGTGCCCGTG
                   90        100        110        120        130        140
                         130        140        150        160        170        180
SUB-OB.SEQ        CGCACGGGAGATGCCACCTTCCCCAAAGCTATGGACAACGTGACGGTCCGGCAGGGGGAG
                  ::::  ::::::::::::::::::::::::::::::::::::::::::::::::::::
HUMOBCAM.DNA      CGCAGCGGAGATGCCACCTTCCCCAAAGCTATGGACAACGTGACGGTCCGGCAGGGGGAG
                      150        160        170        180        190        200
                          190        200        210        220        230        240
SUB-OB.SEQ        AGCGCCACCCTCAGGTGCACTATTGACAACCGGGTCACCCNGGTGGCCTGGCTAAACCGC
                  ::::::::::::::::: :: :: ::  ::::::: ::::.:::::::::::::::::::
HUMOBCAM.DNA      AGCGCCACCCTCAGGTGTACCATAGATGACCGGGTAACCCGGGTGGCCTGGCTAAACCGC
                      210        220        230        240        250        260
                          250        260        270        280        290        300
SUB-OB.SEQ        AGCACCATCCTCTATGCTGGGAATGACAAGTGGTGCCTGGATCCTCGCGTGGTCCTTCTG
                  :::::::::::::: :::::::::::::::::::: : : :: ::::: ::: :: : :::
HUMOBCAM.DNA      AGCACCATCCTCTACGCTGGGAATGACAAGTGGTCCATAGACCCTCGTGTGATCATCCTG
                      270        280        290        300        310        320
                          310        320        330        340        350        360
SUB-OB.SEQ        AGCAACACCCAAACGCAGTACAGCATCGAGATCCAGAACGTGGATGTGTATGACGAGGGC
                    ::: :: : ::: ::::::::::::  :::::: :: ::::::::::::::::: ::
HUMOBCAM.DNA      GTCAATACACCAACCCAGTACAGCATCATGATCCAAAATGTGGATGTGTATGACGAAGGT
                      330        340        350        360        370        380
                          370        380        390        400        410        420
SUB-OB.SEQ        CCTTACACCTGCTCGGTGCAGACAGACAACCACCCAAAGACCTCTAGGGTCCACCTCATT
                  :: :::::::::: :::::::::::::: :: :: :: :: ::  :::: ::::: ::
HUMOBCAM.DNA      CCGTACACCTGCTCTGTGCAGACAGACAATCATCCCAAAACGTCCCGGGTTCACCTAATA
                      390        400        410        420        430        440
                          430        440        450        460        470        480
SUB-OB.SEQ        GTGCAAGTATCTCCCAAAATTGTAGAGATTTCTTCAGATATCTCCATTAATGAAGGGAAC
                  :::::::: :::: : :: : : :: :: ::::: ::: :  : :::::: :: : :
HUMOBCAM.DNA      GTGCAAGTTCCTCCTCAGATCATGAATATCTCCTCAGACATCACTGTGAATGAGGGAAGC
                      450        460        470        480        490        500
                          490        500        510        520        530        540
SUB-OB.SEQ        AATATTAGCCTCACCTGCATAGCAACTGGTAGACCAGAGCCTACGGTTACTTGGAGACAC
                  : : : : :::    ::  : :: : ::: ::::::::::: :: :: :: :::::::::
HUMOBCAM.DNA      AGTGTGACCCTGCTGTGTCTTGCTATTGGCAGACCAGAGCCAACTGTGACATGGAGACAC
                      510        520        530        540        550        560
                             550          560        570        580        590
SUB-OB.SEQ        ATCTCTCCCAA---AGCGGTTGGCTTTGTGAGTGAAGACGAATACTTGGAAATTCAGGGC
                   : ::   :::   ::     :::::::: ::::: :: :: ::: :::: ::    : :
HUMOBCAM.DNA      CTGTCAGTCAAGGAAGGCCAGGGCTTTGTAAGTGAGGATGAGTACCTGGAGATCTCTGAC
                      570        580        590        600        610        620
                  600        610        620        630        640        650
SUB-OB.SEQ        ATCACCCGGGAGCAGTCAGGGGACTACGAGTGCAGCGCCTCCAATGACGTGGC--CGGCC
                  ::::  :: :: ::::: ::::: ::::: :::::::: :  :: :: :: ::  :: ::
HUMOBCAM.DNA      ATCAAGCGAGACCAGTCCGGGGAGTACGAATGCAGCGCGTTGAACGATGTCGCTGCGCCC
                      630        640        650        660        670        680
                    660
SUB-OB.SEQ        GTGGTACGGAGCCC
                  :  :: v::: ^
HUMOBCAM.DNA      GATGTGCGGAAAGT
                      690
```

**Figure 5.** DNA sequence homology search between a phage insert and human OBCAM.

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                    10        20        30        40        50
OB668.AMI     MHNSISWA--IFTGLAALCLFQ-GVPVRTGDATFPKAMDNVTVRQGESATLRCTIDNRVT
                 ..:   . ..:  : : . X:::::::::::::::::::::::::::::::.:::
HUMOB.AMI     GYLFLPWKCLVVVSLRLLFLVPTGVPVRSGDATFPKAMDNVTVRQGESATLRCTIDDRVT
                  10        20        30        40        50        60
               60        70        80        90       100       110
OB668.AMI     XVAWLNRSTILYAGNDKWCLDPRVVLLSNTQTQYSIEIQNVDVYDEGPYTCSVQTDNHPK
              .:::::::::::::::::::..:::::..: ::.::::: ::::::::::::::::::::::
HUMOB.AMI     RVAWLNRSTILYAGNDKWSIDPRVIILVNTPTQYSIMIQNVDVYDEGPYTCSVQTDNHPK
                  70        80        90       100       110       120
              120       130       140       150       160       170
OB668.AMI     TSRVHLIVQVSPKIVEISSDISINEGNNISLTCIATGRPEPTVTWRHISPK-AVGFVSED
              ::::::::::.:.:..:::::..:::....: :.:.:::::::::::X.: : . ::::::
HUMOB.AMI     TSRVHLIVQVPPQIMNISSDITVNEGSSVTLLCLAIGRPEPTVTWRHLSVKEGQGFVSED
                 130       140       150       160       170       180
               180       190       200
OB668.AMI     EYLEIQGITREQSGDYECSASNDVAXXVVRS
              ::::: .:.:.:::.:::::: :::::.. ::
HUMOB.AMI     EYLEISDIKRDQSGEYECSALNDVAAPDVRK
                 190       200       210
```

**Figure 6.** Amino acid homology search between a phage insert and human OBCAM.

**Table 1.** Homology comparison of our clone with a cell adhesion molecule

| No. | Target file | Definition | Match% | Over |
|---|---|---|---|---|
| 1 | HUMOBCAM | Human (clone pHOM) opioid-binding cell | 76.3 | 608 |
| 2 | BTOBCAM | Bovine mRNA for opioid binding protein | 75.0 | 636 |
| 3 | RATCALMA | Rattus norvegicus cell adhesion-like m | 75.2 | 626 |
| 4 | RATCALMC | Rattus norvegicus cell adhesion-like m | 75.7 | 608 |
| 5 | RATCALMB | Rattus norvegicus cell adhesion-like m | 75.7 | 608 |

cules (Table 1). It contains an initiation codon and *Eco*RI restriction site in the 3' terminal but no termination codon. We think it may be produced by digestion with *Eco*RI during library construction.

Since OBCAM was characterized as an opioid-binding protein (Schofield *et al.*, 1989), its function on the opioid binding was of immediate interest. The OBCAM cDNA was recently reported in rats, bovines, and humans, but we also found another OBCAM cDNA, in spite of the 669 bp, which has a 75.0% homology to the known OBCAM cDNA of bovines and a 76.3% homology to known human cDNA.

We propose data in this report which shows that a possibly different kind of OBCAM family has been discovered by our approaches to humans. It will be necessary to characterize the clone more extensively and identify the function of the protein.

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