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(54) **HIGH AFFINITY CHOLINE TRANSPORTER**

HOCHAFFINITÄTS-CHOLINTRANSPORTER

TRANSPORTEUR DE CHOLINE A FORTE ACTIVITE

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Description

Technical Field

5 **[0001]** This invention relates to a protein having high-affinity choline transporter activity, a gene encoding said protein and the use of the same.

Prior Art

10 **[0002]** The autonomic nervous system which spreads to organs throughout a body and regulates the most basic functions of living organism including energy metabolism, circulation, respiration and reproduction along with endocrine system, is classified into the sympathetic and parasympathetic nervous systems. All autonomic nerve fibers excluding postganglionic fibers of the sympathetic nerve, motor nerve fiber, and sudoriferous gland/blood vessel dilative fiber in the sympathetic nerve are cholinergic, and acetylcholine is vital for the function of the autonomic nerve and the motor 15 nerve. It has been known that the cholinergic neuron, being observed also in the brain, is important for recognizing function of the brain and that it degenerates after the onset of Alzheimer's disease. In the cholinergic neuron, because of lack of biosynthetic ability for choline, choline, an acetylcholine decomposition product, is taken up into a cell by a high-affinity choline transporter at the presynaptic terminals to be reused for synthesizing acetylcholine. The high-affinity choline uptake is a rate-limiting step for acetylcholine synthesis and is presumed to regulate the efficiency of synaptic 20 transmission (J. Neurochem. 18, 781-798, 1971, Science 178, 626-628, 1972, Biochem. Biophys. Acta 291, 564-575, 1973, Mol. Pharmacol. 9, 630-639, 1973, J. Pharmacol. Exp. Ther. 192, 86-94, 1975, J. Neurochem. 30, 15-21, 1978, J. Neurochem. 44, 11-24, 1985, J. Neurochem. 60, 1191-1201, 1993, J. Neurochem. 20, 581-593, 1973, Eur. J. Pharmacol. 102, 369-370, 1984). To date, most of cDNAs of transporters for major neurotransmitters have been isolated, however, a cDNA of the high-affinity choline transporter, which is physiologically important, has not been identified.

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Disclosure of the Invention

[0003] So far, the existence of a protein being localized in the cholinergic neuron and having a function of taking up choline, a precursor of acetylcholine, into a cell has been expected, but molecular properties of said protein, a high-30 affinity choline transporter, have been unknown. An object of the present invention is to provide a physiologically important protein having the high-affinity choline transporter activity, a gene which encodes the protein, and a screening method of a high-affinity choline transporter activity promoter using the protein, the gene and the like.

[0004] The inventors have conducted intensive study to attain the above-mentioned object: with information of genomic project (Science 282, 2012-2018, 1998), Na^+-dependent transporter cDNAs being expected from the genomic sequence 35 of a nematode (C. elegans) were cloned one by one, and the high-affinity choline uptake activity of each cDNA was examined in the oocyte expression system of Xenopus, and the cDNA of nematode high-affinity choline transporter (cho-1) was identified on the basis of the above examination, then homologous molecules (CHT1) were cloned from rat spinal cord by using the homology of a base sequence to the cDNA as an index. This CHT1 had no homology to neurotransmitter transporters (J. Neurochem. 71, 1785-1803, 1998), but had 20 to 25% homology to molecules which belong to Na^+-de-40 pendent glucose transporter family (Nature 330, 379-381, 1987).

[0005] Northern blot analysis revealed that transcripts of CHT1 were confirmed only in spinal cord, basal forebrain, corpus striatum and brain stem, and CHT1 seemed to be expressed in cholinergic neurons. Accordingly, CHT1 was expressed in oocytes of Xenopus. As a result, choline uptake activity that is Na^+-dependent and completely inhibited by hemicholinium-3 was observed. These results indicate that CHT1 has high-affinity choline transporter activity. Further, 45 the inventors have cloned choline transporter cDNAs derived from a human and from a mouse, and determined their base sequences, and have confirmed that their expression products have high-affinity choline uptake activity. The present invention has thus completed.

[0006] The present invention relates to a DNA molecule which encodes a protein comprising an amino acid sequence represented by Seq. ID No. 6, or (b) a protein comprising an amino acid sequence where one amino acid is deleted, 50 substituted or added in the amino acid sequence represented by Seq. ID No.6, and having high-affinity choline transporter activity (claim 1), a DNA molecule containing a base sequence represented by Seq. ID No. 5 or its complementary sequence (claim 2), DNA derived from a human which hybridizes with DNA comprising a gene according to claim 1 under a stringent condition, and encodes a protein having high-affinity choline transporter activity (claim 3), a protein comprising an amino acid sequence represented by Seq. ID No. 6 (claim 4), a protein comprising an amino acid sequence 55 where one amino acid is deleted, substituted or added in the amino acid sequence represented by Seq. ID No.6, and having human high-affinity choline transporter activity (claim 5).

[0007] The present invention further relates to a fusion protein being constructed by expressing a cDNA encoding fusion proteins of a protein set out above having high-affinity choline transporter activity and a marker protein and/or a

peptide tag (claim 6).

[0008] The present invention still further relates to an antibody which specifically binds to a protein as set out above having high-affinity choline transporter activity (claim 7). The antibody may be a monoclonal antibody (claim 8).

[0009] The present invention also relates to a host cell into which has been introduced a DNA molecule as set out above (claim 9), in the host cell, the endogenous DNA which encodes a protein having high-affinity choline transporter activity may be inactive by mutation (claim 10).

[0010] The present invention further relates to a non-human animal in which function of a gene which expresses a protein as set out above.

[0011] The present invention still further relates to a preparing method of a cell having high-affinity choline transporter activity characterized in introducing the gene or the DNA as set out above into a cell whose endogenous DNA molecule which encodes a protein having high-affinity choline transporter activity is inactivated by mutation (claim 12), the preparing method of a cell having high-affinity choline transporter activity according to claim 12, wherein the DNA molecule is integrated into a chromosome of the cell, so the cell stably shows high-affinity choline transporter activity (claim 13).

[0012] The present invention also relates to a screening method of a promoter or a suppressor of high-affinity choline transporter activity characterized in measuring/evaluating high-affinity choline transporter activity of the protein set out above having high-affinity choline transporter activity in the presence of a candidate promoter/suppressor (claim 14), a screening method of a promoter or a suppressor of high-affinity choline transporter activity, or of high-affinity choline transporter expression characterized in comprising the steps of: contacting a cell membrane or a cell which expresses a protein having high-affinity choline transporter activity in vitro with a candidate promoter/suppressor; and measuring/evaluating any change in the activity and/or the expression of a protein as defined above in the cell membrane or the cell in the presence of the candidate promoter/suppressor (claim 15). In the screening method the cell membrane or the cell which expresses a protein having high-affinity choline transporter activity may be the host cell as defined above (claim 16) and the protein having high-affinity choline transporter activity may be a recombinant protein (claim 17), screening method of a promoter or a suppressor of high-affinity choline transporter activity, or of high-affinity choline transporter expression may be characterized in comprising the steps of: cultivating a cell obtained from the mouse as defined above in vitro in the presence of a candidate promoter/suppressor, and evaluating any change in; the activity and/or the expression amount of a protein having high-affinity choline transporter activity in the cell is the presence of the candidate promoter/suppressor (claim 18).

[0013] The present invention further relates to a medical constituent characterized in being used for a medical treatment for a patient who needs elevation of the activity or enhancement of the expression of a high-affinity choline transporter, and containing the protein as defined above as an active component (claim 19)

[0014] The present invention still further relates to a diagnostic method for diseases relating to the expression or the activity of a high-affinity choline transporter wherein the method comprises:

Obtaining a genomic DNA, RNA or cDNA sequence encoding a high-affinity choline transporter from a sample of blood urine saliva or tissue from a subject.
Comparing the genomic DNA, RNA or cDNA sequence obtained from the sample to a DNA sequence encoding the protein defined above; and
Detecting any mutation in the sequence obtained from the sample wherein mutation in the sequence obtained from the sample indicates the presence in the subject of a disease relating to the expression or the activity of a high-affinity choline transporter (claim 20).

[0015] The invention also related to a diagnostic probe for Alzheimer's disease comprising a part of an antisense strand of DNA or RNA encoding the protein defined above said part comprising at least 20 bases (claim 21), and a diagnostic drug for Alzheimer's disease characterized in containing the diagnostic probe defined above and/or the antibody defined above (claim 22.

Brief Explanation of the Drawings

[0016] Fig. 1 is a view showing the result of [^{3}H] choline uptake of oocytes from Xenopus of the present invention being injected with nematode cho-1 (C48D1.3 cRNA) or water.

[0017] Fig.2 is a view showing the result of the effect of Na^+ on choline uptake of oocytes from Xenopus of the present invention being injected with nematode cho-1 (C48D1.3 cRNA) or water.

[0018] Fig.3 is a view showing the result of the HC3-induced inhibition of choline uptake of oocytes from Xenopus of the present invention being injected with nematode cho-1 (C48D1.3 cRNA) or water.

[0019] Fig.4 is a view showing amino acid sequences of rat CHT1 and nematode CHO-1 of the present invention respectively.

Fig.5 is a view showing the distribution of neurons expressing cho-1::gfp of the present invention in the nervous system

of nematode.

[0020] Fig.6 is a view showing the phylogenetic tree of Na^+-dependent glucose transporter family.

[0021] Fig.7 is a view showing an expected topology of rat CHT1 of the present invention.

[0022] Fig.8 is a view showing the result of Northern blot analysis of CHT1 mRNA transcript in rat tissue of the present invention.

[0023] Fig.9 is a view showing the result of *in situ* hybridization analysis of CHT1 transcript in a rat brain of the present invention.

[0024] Fig.10 is a view showing the result of *in situ* hybridization analysis of CHT1 transcript in a spinal cord of the present invention.

[0025] Fig.11 is a view showing the result of [^{3}H] choline uptake of oocytes from Xenopus of the present invention being injected CHT1 cRNA of the present invention or water.

[0026] Fig.12 is a view showing the effect of choline concentration on choline uptake in CHT1 of the present invention.

[0027] Fig.13 is a view showing the result of HC3-induced inhibition of choline uptake of CHT1 of the present invention.

[0028] Fig.14 is a view showing the result of Na^+- and Cl^--dependent choline uptake of CHT1 of the present invention.

[0029] Fig.15 is a view showing the result of [^{3}H] HC3 binding to the membrane prepared from COS7 cells being introduced with CHT1 cDNA of the present invention or vector pcDNA 3.1 separately.

[0030] Fig. 16 is a view showing the result of saturation analysis of specific [^{3}H] HC3 binding to the membrane prepared from COS7 cells being introduced with CHT1 cDNA of the present invention or vector pcDNA 3.1 separately.

[0031] Fig.17 is a view showing the result of displacement of specific [^{3}H] HC3 binding by HC3 of the present invention, choline (Cho), acetylcholine (ACh).

Best Mode for Carrying out the Invention

[0032] The cDNA of nematode high-affinity choline transporter, being described in Seq . ID No. 1, can be obtained by injecting each cRNA prepared from candidate full-length cDNAs, which are expected as a member of Na^+-dependent transporter family according to C. elegans genome project, into oocytes of Xenopus, and examining the uptake of choline. The high-affinity uptake of choline in brain synaptosomes of mammals was completely inhibited by 1 μMhemicholinium-3 (HC3) (Ki=10-100 nM), while the low-affinity uptake of choline, which is distributed in every cells, was inhibited only by HC3 with higher concentration (Ki=50 μM). Therefore, the sensitivity to 1 μM HC3 can be used as criteria of high-affinity choline uptake during the process. For example, it is possible to confirm the identification, the expression, and the localization of an object gene from the candidate cDNA of a nematode (C. elegans) as follows.

[0033] It has been found that cDNA corresponding to the gene expected as C48D1.3 promotes significant choline uptake, being inhibited by 1 μM HC3, in the high-affinity choline uptake process. Fig. 1 shows the result of [^{3}H] choline uptake of oocytes from Xenopus being injected with C48D1.3 cRNA or water. In Fig. 1, the closed and the open columns indicate choline uptake in the absence or the presence of 1 μM HC3 respectively, and each column is shown by mean ± SEM (n=6 to 8 oocytes). Fig. 2 shows the effect of Na^+ on the choline uptake, and the closed columns indicate choline uptake measured in the standard solution ($[Na^+]$=100 mM), the open columns indicate choline uptake in the absence of Na^+ (Na^+ was substituted with Li^+). In addition. Fig. 3 shows the inhibition of choline uptake induced by HC3. Based on the above-mentioned Fig. 2 and 3, it is presumed that the uptake is Na^+-dependent, and that Ki of HC3 is 50 nM. The cDNA clone was designated as cho-1 (high-affinity choline transporter-1).

[0034] By comparing a base sequence of cDNA and that of genome, cho-1 gene was found to comprise 9 exons. A protein expected from a base sequence of cDNA of cho-1 includes 576 amino acid residues (see Fig. 4), and this protein, being represented by Seq. ID No. 2, can be constructed by a usual method. When the available data base was searched, the amino acid sequences of cho-1 showed weak, but significant homology to members of Na^+-dependent glucose transporter family. Hydrophobic analysis and comparison to other transporters suggest that there is a twelve-transmembrane region (see Fig. 7).

[0035] Then, in order to identify cells expressing cho-1 in the nervous system of a nematode (C. elegans), a gene of a green fluorescent protein (GFP) fused with a region 5.1kb upstream from cho-1 gene was introduced into a nematode, and distribution of neurons expressing cho-1::gfp was examined. A photograph of L1 larva possessing cho-1::gfp reporter DNA at the outside of chromosome is shown as Fig. 5 (scale bar; 50 μm). In Fig. 5, the arrowhead indicates nerve ring. In the ventral nerve cord, GFP is expressed only in cholinergic motor nerve, however, some of DA, DB nerve cells do not express GFP owing probably to deficiency of reporter DNA at the outside of chromosome. It supports the idea that cho-1 is a high-affinity choline transporter of the cholinergic neuron.

[0036] The cDNA of rat high-affinity choline transporter, being described in Seq. ID No. 3, can be prepared, for example, by a method comprising the steps of: paying attention to cho-1 homologous molecules of vertebrates and searching data base with amino acid sequences expected from cho-1, and identifying one candidate (GenBank accession number: AQ 316435) in human genomic survey sequence (GSS); amplifying cDNA fragments from rat spinal cord cDNA by PCR with degenerate primers on the basis of homology of base sequences between the human genome DNA and cho-1;

screening rat spinal cord cDNA library with this fragment, and a positive cDNA clone was obtained. A protein with 580 amino acid residues showing 51% identity and 70% similarity to cho-1 was expected from the base sequence of the longest reading frame (see Fig. 4). This rat cDNA clone was designated as CHT1. In Fig. 4, each amino acid sequence of rat CHT1 and nematode CHO-1 is shown, and the identical and the similar residues are indicated on a black ground and a gray ground respectively. The expected transmembrane region I-XII is underlined. This protein represented by Seq. ID No. 4 can be constructed by a usual method.

[0037] The above-mentioned amino acid sequence of CHT1 is significantly homologous to members of Na^+-dependent glucose transporter family (20 to 25%). The phylogenetic tree of Na^+-dependent glucose transporter family made by neighbor-joining method using a program CLUSTALW of National Institute of Genetics (Mishima, Japan) is shown in Fig. 6. In Fig. 6, the percentage of the identical amino acids, being contained in each protein, to rat CHT1 is shown on the right side. On the other hand, no homology was observed to a yeast choline transporter (J. Biol. Chem. 265, 15996-16003, 1990), acreatine transporter which had been originally reported as a high-affinity choline transporter (Biochem. Biophys. Res. Commun. 198, 637-645, 1994), and other neurotransmitter transporters.

[0038] The expected topology of CHT1 is thought to be the same as that of nematode CHO-1 fundamentally. Fig. 7 shows the expected topology of rat CHT1. In Fig. 7, the closed circles indicate the identical residues, the shadowed circles indicate highly conserved residues, and open circles indicate nonsimilar residues. The offshoots indicate the expected glycosylation sites. P among the circles shows the expected parts of phosphorylation induced by protein kinase C.

[0039] Next, the distribution of CHT1 mRNA expression was examined by Northern blot analysis and *in situ* hybridization. The expression of transcripts with the length of about 5 kb was confirmed by Northern blot analysis of various tissues of rats. Fig. 8 shows the result of Northern blot analysis of mRNA transcript of CHT1 in rat tissue, and the length of RNA standard (0.24 to 9.5 kb; GIBCO BRL) is exhibited on the left side. As shown in Fig. 8, an abundance of transcripts were confirmed in basal forebrain, brain stem and spinal cord, and a little of those were confirmed in corpus striatum. These tissues are known to contain cholinergic neurons. On the other hand, no transcript was observed in other regions of the brain or in tissues of non-nervous systems.

[0040] Consistent with these results, *in situ* hybridization confirmed the expression of CHT1 mRNA in cell groups of main cholinergic neurons including corpus striatum, cell population in basal forebrain and vental horn in spinal cord. Fig. 9 and 10 (scale bar; 1 mm) show micrographs of sections in bright-field, which were hybridized with a cRNA probe of an antisense labeled by digoxigenin. These micrographs relate to in situ hybridization analysis of CHT1 transcripts in rat brain and spinal cord. Fig. 9 indicates that mRNA transcripts of CHT1 were detected in vertical and horizontal limbs of the diagonal band (VDB, HDB), medial septal nucleus (MS), caudate and putamen (Cpu), and olfactory tubercle (Tu). Fig. 10 indicates that the expression was observed in ventral horn (VH) in spinal cord. Further, the adjacent section hybridized with a probe of vesicle acetylcholine transporter showed essentially same distribution. This expression distribution is essentially same as the reported distribution of cholineacetyl group transferase or vesicle acetylcholine transporter. These results show that the expression of CHT1 mRNA is limited to cholinergic neurons.

[0041] Next, choline uptake of CHT1 was examined by using oocytes of Xenopus. The choline uptake of the oocytes injected with CHT1 cRNA was 2 times to 4 times more than that of controls injected with water. Fig. 11 shows the result of [^{3}H] choline uptake of oocytes of Xenopus injected with CHT1 cRNA or water. In Fig. 11, the open and the closed columns respectively indicate choline uptake in the standard solutions containing 100 mM NaCl or LiCl, and each column is shown by mean $\pm$ SEM (n=6 to 8 oocytes). The effect of choline concentration on choline uptake is shown in Fig. 12. In Fig. 12, choline uptake of oocytes injected with water was subtracted from that of oocytes injected with cRNA in order to figure out CHT1-induced choline uptake, and the choline uptake was fitted to Michaelis-Menten curve. As shown in Fig. 12, choline uptake of CHT1 saturated when increasing choline concentration (Km=2.2 $\pm$ 0.2 μM, n=3). The Km of endogenous choline uptake of control is higher than 10 μM.

[0042] Then, the result of HC3-induced inhibition of choline uptake is shown in Fig. 13. Fig. 13 indicates that choline uptake of CHT1 is completely inhibited by 0.1 μM HC3 (Ki=2-3 nM), whereas 10 μM HC3 induced only slight inhibition in control. As shown in Fig. 14, ion-dependency of choline uptake of CHT1 was examined and found to be C1-dependent as well as Na^+-dependent. The closed and the open columns indicate choline uptake of oocytes injected with water and with cRNA respectively (100 mM NaCl in the standard solution is substituted with 100 mM of each salt) shown in the figure. These results indicate that CHT1 has the characteristics expected from high-affinity choline uptake in brain synaptosomes (high-affinity to choline, high sensitivity to HC3, and Na^+-Cl^--dependency) (J. Neurochem. 27, 93-99, 1976).

[0043] In addition, [^{3}H] HC3 binding activity of membranes prepared from COS7 cells introduced with CHT1 cDNA and a vector (control) respectively was examined. The result is shown in Fig. 15. As Fig. 15 indicates, Na^+-dependent [^{3}H HC3 binding was observed in a membrane of a cell where CHT1 was expressed, but not in a control membrane. Subsequently, a saturation analysis was conducted for specific [^{3}H] HC3 binding. As shown in Fig. 16, equilibrium dissociation constant (Kd) was estimated to be 1.6$\pm$0.2μM (n=3). This value was similar to that reported in brain synaptosomes (J. Neurochem. 60, 1191-1201, 1993, Life Sci. 35, 2335-2343, 1984, Brain Res. 348, 321-330, 1985). Further,

displacement of specific [^{3}H] HC3 binding by HC3, choline (Cho) and acetylcholine (Ach) was examined. Acetylcholine was measured in the presence of 1 μM physostigmine. The result is shown in Fig. 17. Fig. 17 indicates that specific [^{3}H] HC3 binding was displaced when the concentration of choline was at least about 10 times lower than that of acetylcholine. These results show that CHT1 is a HC3 binding site as well as a high-affinity choline transporter.

[0044] The cDNA of human high-affinity choline transporter of the present invention, being represented by Seq. ID No.5, can be prepared, for example, as follows: data base search was conducted with the amino acid sequence of nematode (C. elegans) CHO-1 to find a sequence of specific human genome DNA fragment having significant homology (R-107P12, a clone of human genomic survey sequence; GenBank accession number: AQ316435); a gene-specific primers for PCR were designed based on a base sequence of said DNA fragment; 5'-RACE (rapid amplification of cDNA ends) and 3'-RACE were conducted using Marathon-Ready™ cDNA (Clontech) of human whole brain, together with an attached adapter primer; the obtained PCR product was cloned into a cloning vector for PCR, and a base sequence of inserted DNA was determined. In addition, an amino acid sequence expected from this DNA sequence is represented by Seq. ID No. 6. A protein having human high-affinity choline transporter activity represented by said Seq. ID No. 6 can be constructed by a usual method on the basis of DNA sequence information shown in Seq. ID No. 5.

[0045] Examples of a protein having high-affinity choline transporter activity of the present invention include a protein derived from natural materials and a recombinant protein. In addition to the one represented by Seq. ID No. 6, which is specifically disclosed above, a protein comprising an amino acid sequence wherein one amino acid is deleted, substituted or added in amino acid sequence represented by Seq. ID No. 6, and having high-affinity choline transporter activity is also included. These proteins can be prepared by known methods. Further, examples of a gene or DNA encoding a protein having high-affinity choline transporter activity of the present invention include, in addition to the one represented by Seq. ID No. 5 which is specifically disclosed above, a gene or DNA which encodes a protein comprising an amino acid sequence wherein one amino acid is deleted, substituted or added in amino acid sequence represented by Seq. ID No. 6, and having high-affinity choline transporter activity, and DNA which encodes a protein hybridizing with said gene or DNA under a stringent condition and having high-affinity choline transporter activity. These genes and DNAs can be prepared by known methods.

[0046] Cholinergic neurons play an extremely important role in learning and memory. The damage of these neurons correlates to severity of dementia. The rate-limiting step in acetylcholine synthesis is presumed to be the uptake of choline, and its activity is controlled by neural activity or various kinds of stimuli. In the brains of patients who suffer Alzheimer's disease, the hyperfunction of high-affinity choline uptake and of HC3 binding activity are observed (Trends Neurosci. 15, 117-122, 1992, Ann. NY Acad. Sci. 777, 197-204, 1996, J. Neurochem. 69, 2441-2451, 1997). Cloning of said gene or DNA encoding a protein having high-affinity choline transporter activity and said protein having high-affinity choline transporter activity is important for elucidating the molecular mechanism of the high-affinity choline transporter and for developing new therapies for Alzheimer's disease.

The fusion protein of the present invention means a substance constructed by binding a protein from a human, which has high-affinity choline transporter activity, to a marker protein and/or a peptide tag. As the marker protein, any conventionally known marker protein can be used and the specific examples are alkaline phosphatase, Fc region of an antibody, HRP, and GFP. Conventionally known peptide tags, such as Myc tag, His tag, FLAG tag, GST tag, are exemplified as specific examples of the peptide tag of the present invention. Said fusion proteins can be constructed by a usual method, and are useful for the purification of a protein having high-affinity choline transporter activity utilizing the affinity between Ni-NTA and His tag, the detection of a protein having high-affinity choline transporter activity, the quantitation of an antibody to a protein having high-affinity choline transporter activity, and as a diagnostic marker for Alzheimer's disease, and an investigational reagent in the field concerned.

[0047] As an antibody that specifically combines with a protein having high-affinity choline transporter activity of the present invention, an immunospecific antibody such as a monoclonal antibody, a polyclonal antibody, a chimeric antibody, a single stranded antibody, a humanized antibody and the like are concretely exemplified. Though these antibodies can be constructed by a usual method with the above-mentioned protein having high-affinity choline transporter activity as an antigen, a monoclonal antibody is more preferable among them because of its specificity. Said antibody that specifically binds to a protein having high-affinity choline transporter activity, such as a monoclonal antibody or the like, is useful, for instance, for the diagnosis of Alzheimer's disease, and for elucidation of molecular mechanism of a high-affinity choline transporter.

[0048] An antibody to a protein having high-affinity choline transporter activity is developed by administering fragments containing the protein having high-affinity choline transporter activity or its epitope, or cells that express said protein on the surface of the membrane, to animals (preferably excluding human) with usual protocol. For instance, a monoclonal antibody can be prepared by an arbitrary method that brings antibodies developed by cultured materials of continuous cell line, such as hybridoma method (Nature 256, 495-497, 1975), trioma method, human B-cell hybridoma method (Immunology Today 4, 72, 1983), and EBV-hybridoma method (MONOCLONAL ANTIBODIES AND CANCER THERAPY, pp. 77-96, Alan R. Liss, Inc., 1985).

[0049] In order to develop a single stranded antibody to the above-mentioned protein having high-affinity choline

transporter activity of the present invention, the preparation method of single stranded antibodies (US Patent No. 4,946,778) can be applied. Further, in order to express a humanized antibody, it is possible to use transgenic mice, other mammalian animals or the like, and to isolate and identify the clones that express a protein having high-affinity choline transporter activity with the above-mentioned antibodies, and to purify the polypeptide by affinity chromatography. An antibody to a protein having high-affinity choline transporter activity could be used, in particular, for the diagnosis and the medical treatment of Alzheimer's disease, and the like.

[0050] This invention relates to a host cell which contains an expression system that can express said protein having high-affinity choline transporter activity. The gene that encodes a protein having high-affinity choline transporter activity can be introduced into a host cell by a number of methods described in many standard laboratory manuals such as by Davis et al. (BASIC METHODS IN MOLECULAR BIOLOGY, 1986), and by Sambrook et al. (MOLECULAR CLONING: A LABORATORY MANUAL, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N. Y., 1989). Examples of those methods include calcium phosphate transfection, DEAE-dextran-mediated transfection, transvection, microinjection, cationic lipid-mediated transfection, electroporation, transduction, scrape loading, ballistic introduction and infection. Examples of the host cells include bacterial procaryotic cells such as Escherichia coli, Streptomyces, Bacillus subtilis, Streptococcus, Staphylococcus and the like; fungous cells such as yeast, Aspergillus and the like; insect cells such as drosophila S2, spodptera Sf9 and the like; and animal or plant cells such as L cells, CHO cells, COS cells, HeLa cells, C127 cells, BALB/c3T3 cells (including mutant strains deficient in dihydrofolate reductase, thymidine kinase or the like), BHK21 cells, HEK293 cells, Bowes melanoma cells and the like.

[0051] As the expression system, any expression system that can express a protein having high-affinity choline transporter activity in a host cell will suffice. Examples of the expression system include expression systems derived from chromosome, episome and virus, for example, vectors derived from bacterial plasmid, yeast plasmid, papovavirus like SV40, vaccinia virus, adenovirus, chicken pox virus, pseudorabies virus, or retrovirus, vectors derived from bacteriophage, transposon, and the combination of these, for instance, vectors derived from genetic factors of plasmid and of bacteriophage such as cosmid or phagemid. These expression systems may contain a regulatory sequence that acts not only as a promoter but also as a controller of expressions.

[0052] A host cell that contains the above-mentioned expression system, cell membrane of said host cell, and a protein having high-affinity choline transporter activity which is obtainable by the cultivation of said host cell can be used in the screening method of the present invention as hereinafter described. For example, the method of F. Pietri-Rouxel et al. (Eur. J. Biochem. , 247, 1174-1179, 1997) or the like can be used as the method to obtain cell membranes, and publicly known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic-interaction chromatography, affinity chromatography, hydroxyapatite chromatography, and lectin chromatography, preferably high-speed liquid chromatography can be used to pick up said protein having high-affinity choline transporter activity from cell cultured material and purify it. As columns used for affinity chromatography, in particular, there are columns to which a protein antibody having anti-high-affinity choline transporter activity is bound, or in case that a normal peptide tag is added to said high-affinity choline transporter, there are columns to which materials having affinity to the peptide tag are bound. Proteins having high-affinity choline transporter activity can be obtained by using these columns.

[0053] In the present invention, said non-human animal whose function of a gene encoding a protein having high-affinity choline transporter activity is deficient on its chromosome means a non-human animal wherein a part or a whole of a gene encoding a protein having high-affinity choline transporter activity on chromosome is inactivated by gene mutation such as disruption, deficiency, substitution, etc. and function of expressing a protein having high-affinity choline transporter activity is lost. In addition, a non-human animal that overexpresses function of a gene that encodes a protein having high-affinity choline transporter activity on its chromosome means a non-human animal that produces larger amount of a protein having high-affinity choline transporter activity than a wild-type non-human animal does. Though specific examples of a non-human animal of the present invention include rodents, such as mice, rats and the like, a non-human animal of the present invention is not limited to these animals.

[0054] Homozygous non-human animals generated according to Mendelian ratio include a deficient type or an overexpression type for a protein having high-affinity choline transporter activity, and their littermate wild-type, and it is possible to carry out precise comparative experiments in individual level by using the deficient types, the overexpression types and the littermate wild-types of these homozygous non-human animals at the same time. Therefore, it is preferable to use animals of the same species, more preferably the littermates, as the wild-type non-human animals, in other words, the non-human animals being deficient in or overexpressing the function of a gene that encodes a protein having high-affinity choline transporter activity on their chromosome together in, for example, the screening hereinafter described in the present invention. The generating method of the non-human animals being deficient or overexpressing the function of a gene that encodes a protein having high-affinity choline transporter activity on their chromosome will be explained below, with an example of knockout mice and transgenic mice of a protein having high-affinity choline transporter activity.

[0055] For example, a mouse being deficient in the function of a gene that encodes a protein having high-affinity choline transporter activity on its chromosome, in other words, a knockout mouse of a protein having high-affinity choline

transporter activity on its chromosome can be constructed as follows. A gene that encodes a protein having high-affinity choline transporter activity is screened by using a gene fragment obtained from mouse gene library by a method like PCR. The screened gene that encodes a protein having high-affinity choline transporter activity is subcloned with a viral vector or the like, and specified by DNA sequencing. A target vector is constructed by substituting a whole or a part of a gene of this clone that encodes a protein having high-affinity choline transporter activity with pMC1 neo gene cassette or the like, and by introducing a diphteria toxin A fragment (DT-A) gene, a herpes simplex virus thymidine kinase (HSV-tk) gene or other such genes into 3'-terminal side.

[0056] This constructed target vector is linearized and introduced into ES cells by electroporation or the like to induce homologous recombination. The ES cells wherein homologous recombination is induced by an antibiotic such as G418, ganciclovir (GANC) or the like are selected from the homologous recombinants. It is preferable to confirm whether the selected ES cells are the recombinants of the object by Southern blot or the like. A chimeric mouse is constructed by microinjecting a clone of the confirmed ES cells into a blastocyst of a mouse and then transplanting the blastocyst into a recipient mouse. A heterozygous mouse can be obtained by interclossing the chimeric mouse with a wild-type mouse, and a knockout mouse of a protein having high-affinity choline transporter activity of the present invention can be constructed by interclossing the heterozygous mice. It is possible to confirm whether a knockout mouse of a protein having high-affinity choline transporter activity is constructed, for example, by isolating RNA from the mouse obtained by said method and examining it by Northern blot analysis or the like, or by examining the expression of the mouse by Western blot analysis or the like.

[0057] The transgenic mice of a protein having high-affinity choline transporter activity can be generated in following procedures. A transgene is constructed by fusing chicken β-actin, mouse neurofilament, SV40 or other such promoters, and rabbit β-globin, SV40 or other such poly A or introns with cDNA that encodes a protein having high-affinity choline transporter activity. The transgene is microinjected into the pronucleus of a fertilized egg of a mouse, and the obtained egg cell is cultured, then transplanted to the oviduct of a recipient mouse. After rearing up the recipient animal, baby mice that have the above-mentioned cDNA are selected from the mice born from the recipient animal. Thus transgenic mice can be generated. The baby mouse that has cDNA can be selected by extracting crude DNA from a tail or the like of a mouse, then carrying out methods like dot hybridization using the introduced gene that encodes a protein having high-affinity choline transporter activity as a probe, PCR method using a specific primer, and the like.

[0058] In addition, cells being useful for gene therapy of Alzheimer's disease and the like can be prepared by using a whole or a part of a gene or DNA that encodes a protein having high-affinity choline transporter activity of the present invention. As an example of a method for preparing these cells of the present invention, a method wherein a whole or a part of said gene or DNA of the present invention is introduced into a cell being deficient in the function of a gene that encodes a protein having high-affinity choline transporter activity on its chromosome by transfection or the like to obtain a cell having high-affinity choline transporter activity is exemplified. As the cell having high-affinity choline transporter activity, in particular, it is preferable to use a cell wherein said gene or DNA is integrated into a chromosome and high-affinity choline transporter activity is exhibited stably.

[0059] By using the above-mentioned gene or DNA that encodes a protein having high-affinity choline transporter activity, a protein having high-affinity choline transporter activity, a fusion protein created by combining a protein having high-affinity choline transporter activity and a marker protein and/or a peptide tag, an antibody to a protein having high-affinity choline transporter activity, a host cell which contains an expression system that can express a protein having high-affinity choline transporter activity, a cell having high-affinity choline transporter activity, or the like, it becomes possible to screen a pharmaceutical material useful for the treatment of symptoms as in Alzheimer's disease or the like, in other words, a material that promotes or suppresses the activity or the expression of a high-affinity choline transporter.

[0060] Examples of such screening methods include:

a method wherein the high-affinity choline transporter activity of the above-mentioned protein having high-affinity choline transporter activity of the present invention is measured/evaluated in the presence of a subject material; a method wherein a cell membrane or a cell which expresses a protein having high-affinity choline transporter activity of the present invention is cultivated in vitro in the presence of a subject material, and the activity and/or the expression amount of a protein having high-affinity choline transporter activity in the cell is measured/evaluated; and a method wherein a subject material is administered to said non-human animal whose function of a gene encoding a protein having high-affinity choline transporter activity is deficient or overexpresses on its chromosome and/or a wild-type non-human animal and then the activity and/or the expression amount of a protein having high-affinity choline transporter activity of the present invention is measured/evaluated. As said cell membrane or said cell, a cell such as a primary cultured cell obtained from said non-human animal whose function of a gene encoding a protein having high-affinity choline transporter activity is deficient or overexpresses on its chromosome or a wild-type non-human animal etc., a host cell containing an expression system which can express a protein having high-affinity choline transporter activity of the present invention, a cell having high-affinity choline transporter activity of the present invention, and cell membranes of these cells can be specifically exemplified.

[0061] The screening methods with said subject material and said protein having high-affinity choline transporter activity are now specifically explained together with examples, but the screening methods of the present invention are not limited to these examples. Cells expressing a protein having high-affinity choline transporter activity are cultured in the presence of a subject material, and the increase or the decrease of a protein having high-affinity choline transporter activity expressed on the cell surface after a certain period of cultivation can be immunochemically detected by ELISA or other such method with an antibody that specifically combines to a protein having high-affinity choline transporter activity of the present invention, or can be evaluated by using suppression or promotion of mRNA expression as an index. The mRNA can be detected by methods such as DNA chip, Northern hybridization or the like. Moreover, with a cell to which a gene wherein luciferase or other such reporter genes is linked to downstream of promoter of a gene that encodes high-affinity choline transporter is introduced, the suppression or the promotion of the expression of a gene that encodes a protein having high-affinity choline transporter activity induced by a subject material can be detected by using the activity of said reporter gene as an index.
A medical constituent which comprises a subject material which contains a protein having high-affnity choline transporter activity, a material which promotes the activity or the expression of a protein having high-affinity choline transporter activity, or a material which suppresses activity or expression of a protein having high-affinity choline transporter activity as an active component can be used for medical treatment for a patient who needs promotion of the activity or the expression of a protein having high-affinity choline transporter, or a medical constituent being used for medical treatment for a patient who needs suppression of the activity or the expression of a protein having high-affinity choline transporter. As a protein having high-affinity choline transporter activity is involved in many biological functions including many pathological ones, it is expected that a compound that can stimulate a protein having high-affinity choline transporter activity and a compound being able to inhibit the function of said protein can be used as pharmaceuticals.

[0062] As the material which promotes or suppresses the activity or the expression of a protein having high-affinity choline transporter activity, any material can be used as long as it binds to a protein having high-affinity choline transporter activity, or works on a signal transmitting molecule on upstream, and then promotes the activity or the expression of a protein having high-affinity choline transporter activity or inhibits/antagonizes the activity or the expression of the protein by itself. Specific examples include an antibody, a ligand of a protein having high-affinity choline transporter activity, a fragment of said protein, and an oligonucleotide encoding said fragment, and these materials can be used as pharmaceuticals for treatment, prevention or the like of symptoms observed in the case of Alzheimer's disease or other such diseases, but use of them is not limited to the above examples.

[0063] The present invention also relates to a diagnostic method for diseases relating to the activity or the expression of a protein having high-affinity choline transporter activity comprising a comparison of a DNA sequence encoding a protein having high-affinity choline transporter activity in a sample with a DNA sequence encoding a protein having high-affinity choline transporter activity of the present invention. The mutant type of DNA which encodes a protein having high-affinity choline transporter activity can be detected by finding gene-mutated individuals in DNA level, and this is useful for diagnosis of diseases caused by underexpression, overexpression or mutant expression of a protein having high-affinity choline transporter activity. Specific examples of a sample of said detection include cells of trial subjects, for example, genomic DNA, RNA or cDNA obtained from biopsy of blood, urine, saliva, tissue or the like, however said sample is not limited to these examples. It is also possible to use said sample being amplified by PCR or other such methods. Deficiency and insertion mutation of base sequences can be detected by the size change of the amplified product observed in comparison with normal genotype, and point mutation can be identified by hybridizing amplified DNA with a labeled gene that encodes a protein having high-affinity choline transporter activity. Thus, diagnosis or judgement of symptoms observed in the case of Alzheimer's disease or other such diseases can be made by detecting the mutation of a gene that encodes a protein having high-affinity choline transporter activity.

[0064] The present invention further relates to a diagnostic probe for diseases showing symptoms similar to those of Alzheimer's disease or the like comprising a part of an antisense chain of DNA or RNA encoding a protein of the present invention, said part comprising at least 20 bases and a diagnostic drug for diseases showing symptoms similar to those of Alzheimer's disease containing the diagnostic probe and/or an antibody which specifically binds to a protein having high-affinity choline transporter activity of the present invention.

[0065] In order to make a diagnostic drug for symptoms similar to those of Alzheimer's disease containing said probe and/or an antibody which specifically binds to a protein having high-affinity choline transporter activity of the present invention as active components, it is preferable to dissolve said probe into an appropriate buffer or sterilizing water for preventing said probe from decomposition. Further, it is also possible to diagnose diseases showing symptoms similar to those of Alzheimer's disease by methods using these diagnostic drugs, such as immunostaining (Dev. Biol. 170, 207-222, 1995, J. Neurobiol. 29, 1-17, 1996), in situ hybridization (J. Neurobiol. 29, 1-17, 1996), in situ PCR or the like.

[0066] Experimental methods or the like of the above-mentioned various experiments will now be explained in more detail below.

(Cloning of high-affinity choline transporter cDNA)

[0067] The candidate cDNA of nematode high-affinity choline transporter was isolated from poly (A)+RNA of nematode mixture from various stages in the development by reverse transcription PCR and 3' RACE. Marathon™ cDNA Amplification Kit (Clontech) was used according to its protocol. A primer for sense direction of PCR was designed at a provisional translation initiating point of a predicted gene based on a DNA base sequence obtained from C. elegans genomic project. The amplified PCR product was subcloned into Nco I (smoothing) site and Not I site of a modified pSPUTK vector (Stratagene), and the base sequence of inserted DNA was determined. CHT1 cDNA of rat was isolated from rat spinal cord cDNA library by using GeneTrapper cDNA Positive Selection System (GIBCO Bio-Rad Laboratory: GIBCO BRL) according to its protocol. The primer used was designed from the base sequence of a cDNA fragment obtained by degenerated PCR. The obtained cDNA clones were analyzed. Among them, positive clones were selected and subcloned into pSPUTK vector and pcDNA3.1+ vector (Invitrogen Corporation).

(Expression in oocytes of Xenopus)

[0068] In the presence of cap analog, cRNA was synthesized in vitro with SP6 or T7 RNA polymerase. 20 to 30 ng capped RNA was microinjected into oocytes (stage V to VI) of Xenopus. The uptake was measured in basically same manner as described previously (Nature 360, 467-471, 1992). Two or three days after the injection of RNA, choline uptake was conducted for 30 to 60 min. with oocytes (6 to 8) in 0.75 ml standard solution (0.01 to 1 μM [^{3}H]-choline, 100 mM NaCl, 2mM KCl, 1mM $MgCl_2$, 1mM $CaCl_2$, 10mM HEPES, 5mM Tris: pH 7.4). The oocytes completing uptake were solubilized with 10% SDS, and the amount of [^{3}H] was measured by a liquid scintillation counter.

(GFP expression construct)

[0069] The transcriptional fusion construct of cho-1::gfp was constructed by PCR in same manner as described previously (Gene 212, 127-135, 1998). A gene that encodes a green fluorescent protein (GFP) located on downstream of a nuclear localization signal sequence (NLS) was inserted into a position 3 residues downstream of cho-1 translation initiating point so that the reading frame was fitted. NLS and gfp gene were amplified from pPD104.53 vector. In order to prepare 5.1 kb upstream region of cho-1 translation initiating point, a PCR primer being designed to encompass the first 3 amino acid residues of cho-1 was used. By the same method as previously described (EMBO J. 10, 3959-3970, 1991), rol-6 (su1006) marker and generated DNA were injected into gonads of a nematode simultaneously.

(Northern blot analysis)

[0070] 6 μg poly(A)+RNA prepared from various tissues of rats was separated by formaldehyde-agarose electrophoresis, and transferred to a nylon membrane, then hybridized with CHT1 cDNA fragment being labeled with [^{32}P] by random prime method in hybridization solution (solution containing the final concentration of 50% formamido, 5 $\times$ SSPE, 5 $\times$ Denhardt's solution, 0.5% SDS, 100 μg/ml salmon sperm DNA) at 42° C for 16 hours. The nylon membrane was washed under final condition (0.1 $\times$ SSPE, 0.1% SDS: 65° C), and then autoradiography was conducted for 7 days together with an enhancing screen.

(In situ hybridization)

[0071] The transcript of an antisense labeled with digoxigenin was synthesized in vitro. Alkaline hydrolysis was repeated for the transcripts until their mean length was prepared to be 200 to 400 b. Cryostat sections of fresh frozen tissue (10 to 20 μm) were used. Hybridization was conducted with labeled cRNA probe (about 1 μg/ml) dissolved in 1 $\times$ Denhardt's solution [solution containing the final concentration of 50 mM Tris-HCl (pH 8.0), 2.5 mM EDTA, 0.3 M NaCl, 50% formamido, 10% dextran sulphate, 1 mg/ml E. coli tRNA] at 45° C for 20 hours. Then the sections were washed twice in 2 $\times$ SSC/50% formamido and once in 1 $\times$ SSC/50% formamido, at 45° C respectively. The hybridized probe was visualized by using anti-digoxigenin Fab fragment (Boehringer-Mannheim) and NBT/BCIP substrate. The sections were brought into reaction in substrate solution for 24 to 48 hours.

(Binding assay)

[0072] [^{3}H] hemicholinium-3 (HC3; 128Ci/mmol) was obtained from NEN Life Science Products. Either pcDNA3.1-CHT1 or pcDNA3.1 was transiently expressed in COS7 cells respectively. TransFast Reagent (Promega) was introduced and used according to the protocol. Membranes were prepared by following steps: homogenizing cells in 0.32 M sucrose; centrifuging the cells for 1 hour at 200, 000g; and suspending the precipitate. Binding assay was conducted in basically

same manner as described previously. Specific binding amount was calculated by subtracting non-specific binding amount determined in the presence of 10 μM HC3 from the whole binding amount. The Kd value was figured out by analyzing specific [^{3}H] HC3 binding amount from data of saturation binding assay with nonlinear approximation.

5

Industrial Applicability

[0073] The present invention makes it possible to provide a protein having high-affinity choline transporter activity, which is physiologically important, and gene DNA encoding said protein. In addition, by using the said protein and gene DNA, it becomes possible to screen materials being useful for prevention or treatment of Alzheimer's disease, and to prepare cells being useful for gene therapy.

10

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260 265 270
Ala Gly Val Gly Cys Ile Leu Met Ala Ile Pro Pro Ala Leu Ile Gly
275 280 285
Ala Ile Ala Arg Asn Thr Asp Trp Arg Met Thr Asp Tyr Ser Pro Trp
290 295 300
Asn Asn Gly Thr Lys Val Glu Ser Ile Pro Pro Asp Lys Arg Asn Met
305 310 315 320
Val Val Pro Leu Val Phe Gln Tyr Leu Thr Pro Arg Trp Val Ala Phe
325 330 335
Ile Gly Leu Gly Ala Val Ser Ala Ala Val Met Ser Ser Ala Asp Ser
340 345 350
Ser Val Leu Ser Ala Ala Ser Met Phe Ala His Asn Ile Trp Lys Leu
355 360 365
Thr Ile Arg Pro His Ala Ser Glu Lys Glu Val Ile Ile Val Met Arg
370 375 380
Ile Ala Ile Ile Cys Val Gly Ile Met Ala Thr Ile Met Ala Leu Thr
385 390 395 400
Ile Gln Ser Ile Tyr Gly Leu Trp Tyr Leu Cys Ala Asp Leu Val Tyr
405 410 415
Val Ile Leu Phe Pro Gln Leu Leu Cys Val Val Tyr Met Pro Arg Ser
420 425 430
Asn Thr Tyr Gly Ser Leu Ala Gly Tyr Ala Val Gly Leu Val Leu Arg
435 440 445
Leu Ile Gly Gly Glu Pro Leu Val Ser Leu Pro Ala Phe Phe His Tyr
450 455 460
Pro Met Tyr Thr Asp Gly Val Gln Tyr Phe Pro Phe Arg Thr Thr Ala
465 470 475 480
Met Leu Ser Ser Met Ala Thr Ile Tyr Ile Val Ser Ile Gln Ser Glu
485 490 495
Lys Leu Phe Lys Ser Gly Arg Leu Ser Pro Glu Trp Asp Val Met Gly
500 505 510
Cys Val Val Asn Ile Pro Ile Asp His Val Pro Leu Pro Ser Asp Val

515 520 525
Ser Phe Ala Val Ser Ser Glu Thr Leu Asn Met Lys Ala Pro Asn Gly
530 535 540
Thr Pro Ala Pro Val His Pro Asn Gln Gln Pro Ser Asp Glu Asn Thr
545 550 555 560
Leu Leu His Pro Tyr Ser Asp Gln Ser Tyr Tyr Ser Thr Asn Ser Asn
565 570 575

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<212> DNA
<213> Rattus norvegicus

<220>
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<222> (1)..(1743)

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ctt ata ttt ctg gtt gga ata tgg gct gca tgg aaa acc aaa aac agc 96
Leu Ile Phe Leu Val Gly Ile Trp Ala Ala Trp Lys Thr Lys Asn Ser
20 25 30

ggt aat gca gaa gaa cgc agc gaa gcc atc ata gtt ggg ggc cga gac 144
Gly Asn Ala Glu Glu Arg Ser Glu Ala Ile Ile Val Gly Gly Arg Asp
35 40 45

att ggt ttg ttg gtt ggt ggt ttt acc atg aca gcc acc tgg gtt gga 192
Ile Gly Leu Leu Val Gly Gly Phe Thr Met Thr Ala Thr Trp Val Gly
50 55 60

gga ggt tac atc aac ggg aca gct gaa gca gtt tat ggg cca ggt tgt 240
Gly Gly Tyr Ile Asn Gly Thr Ala Glu Ala Val Tyr Gly Pro Gly Cys
65 70 75 80

ggt cta gct tgg gct cag gca ccc att gga tat tct ctg agt ctg att 288
Gly Leu Ala Trp Ala Gln Ala Pro Ile Gly Tyr Ser Leu Ser Leu Ile
85 90 95

tta ggt ggc ctg ttt ttt gca aaa cct atg cgt tcc aag gga tat gtg 336
Leu Gly Gly Leu Phe Phe Ala Lys Pro Met Arg Ser Lys Gly Tyr Val
100 105 110

act atg tta gac ccg ttt caa cag atc tat gga aag cgc atg ggt ggg 384
Thr Met Leu Asp Pro Phe Gln Gln Ile Tyr Gly Lys Arg Met Gly Gly

115 120 125

ctg ctg ttc atc cct gca ctg atg gga gag atg ttc tgg gct gca gca 432
Leu Leu Phe Ile Pro Ala Leu Met Gly Glu Met Phe Trp Ala Ala Ala
130 135 140

att ttc tct gca tta ggg gct acc atc agc gta atc att gat gtg gat 480
Ile Phe Ser Ala Leu Gly Ala Thr Ile Ser Val Ile Ile Asp Val Asp
145 150 155 160

gtg aac ata tcg gtc att gtc tcc gca ctc att gcc att ctt tat acc 528
Val Asn Ile Ser Val Ile Val Ser Ala Leu Ile Ala Ile Leu Tyr Thr
165 170 175

ctc gtg gga ggg ctc tac tct gtg gca tat act gat gtt gta cag cta 576
Leu Val Gly Gly Leu Tyr Ser Val Ala Tyr Thr Asp Val Val Gln Leu
180 185 190

ttc tgc att ttt ata gga ttg tgg atc agt gtc cca ttt gcc ctg tca 624
Phe Cys Ile Phe Ile Gly Leu Trp Ile Ser Val Pro Phe Ala Leu Ser
195 200 205

cat cct gca gtc acc gac att gga ttc act gct gtg cat gct aaa tac 672
His Pro Ala Val Thr Asp Ile Gly Phe Thr Ala Val His Ala Lys Tyr
210 215 220

cag agt ccc tgg ctg gga acc att gaa tca gtt gaa gtc tac acc tgg 720
Gln Ser Pro Trp Leu Gly Thr Ile Glu Ser Val Glu Val Tyr Thr Trp
225 230 235 240

ctt gat aat ttt ctg ttg ttg atg ctg ggt gga ata cca tgg caa gcc 768
Leu Asp Asn Phe Leu Leu Leu Met Leu Gly Gly Ile Pro Trp Gln Ala
245 250 255

tac ttc cag agg gtc ctc tct tca tcg tca gcg acc tat gct cag gtg 816
Tyr Phe Gln Arg Val Leu Ser Ser Ser Ser Ala Thr Tyr Ala Gln Val
260 265 270

ctg tcc ttc ctg gca gct ttt ggg tgc ctg gtg atg gct cta cca gcc 864
Leu Ser Phe Leu Ala Ala Phe Gly Cys Leu Val Met Ala Leu Pro Ala
275 280 285

att tgc att ggg gcc att gga gcc tcc aca gac tgg aac caa act gca 912
Ile Cys Ile Gly Ala Ile Gly Ala Ser Thr Asp Trp Asn Gln Thr Ala
290 295 300

tat ggg ttt cca gat ccc aag acc aag gag gaa gca gac atg att ctc 960
Tyr Gly Phe Pro Asp Pro Lys Thr Lys Glu Glu Ala Asp Met Ile Leu
305 310 315 320

ccg att gtt cta cag tac ctc tgc cct gtg tac att tcc ttc ttt ggg 1008
Pro Ile Val Leu Gln Tyr Leu Cys Pro Val Tyr Ile Ser Phe Phe Gly
325 330 335

ctt ggt gct gtt tct gct gct gtc atg tcc tcg gct gac tca tcc atc 1056
Leu Gly Ala Val Ser Ala Ala Val Met Ser Ser Ala Asp Ser Ser Ile
340 345 350

cta tca gca agt tcc atg ttt gct cgg aat atc tac cag ctt tcc ttc 1104
Leu Ser Ala Ser Ser Met Phe Ala Arg Asn Ile Tyr Gln Leu Ser Phe
355 360 365

aga caa aat gca tca gac aag gaa att gtg tgg gtc atg agg atc act 1152
Arg Gln Asn Ala Ser Asp Lys Glu Ile Val Trp Val Met Arg Ile Thr
370 375 380

gtg ttt gtg ttt gga gca tct gca aca gcc atg gcc ttg ctc acg aag 1200
Val Phe Val Phe Gly Ala Ser Ala Thr Ala Met Ala Leu Leu Thr Lys
385 390 395 400

act gtg tat ggg ctc tgg tac ctg agc tct gac ctt gtc tac atc atc 1248
Thr Val Tyr Gly Leu Trp Tyr Leu Ser Ser Asp Leu Val Tyr Ile Ile
405 410 415

atc ttc cca cag ctg ctc tgt gta ctc ttc atc aaa gga acc aac act 1296
Ile Phe Pro Gln Leu Leu Cys Val Leu Phe Ile Lys Gly Thr Asn Thr
420 425 430

tat ggg gca gtt gct ggt tat att ttt gga ctt ttc ctg aga att acc 1344
Tyr Gly Ala Val Ala Gly Tyr Ile Phe Gly Leu Phe Leu Arg Ile Thr
435 440 445

gga gga gag cca tat cta tac ttg cag ccc tta atc ttc tac cct ggt 1392
Gly Gly Glu Pro Tyr Leu Tyr Leu Gln Pro Leu Ile Phe Tyr Pro Gly
450 455 460

tat tac cct gac aag aat ggt ata tac aat cag agg ttc cca ttt aaa 1440
Tyr Tyr Pro Asp Lys Asn Gly Ile Tyr Asn Gln Arg Phe Pro Phe Lys
465 470 475 480

act ctc tcc atg gtt acc tca ttc ttt acc aac att tgt gtt tcc tat 1488
Thr Leu Ser Met Val Thr Ser Phe Phe Thr Asn Ile Cys Val Ser Tyr
485 490 495

cta gcc aag tat cta ttt gaa agt gga acc ttg cct cca aaa tta gat 1536
Leu Ala Lys Tyr Leu Phe Glu Ser Gly Thr Leu Pro Pro Lys Leu Asp
500 505 510

ata ttt gat gct gtt gtc tca agg cac agt gaa gag aac atg gac aag 1584
Ile Phe Asp Ala Val Val Ser Arg His Ser Glu Glu Asn Met Asp Lys

515 520 525

acc att cta gtc aga aat gaa aac atc aaa tta aat gaa ctt gca cct 1632
Thr Ile Leu Val Arg Asn Glu Asn Ile Lys Leu Asn Glu Leu Ala Pro
530 535 540

gta aag cct cga cag agc cta acc ctc agt tca act ttc acc aat aaa 1680
Val Lys Pro Arg Gln Ser Leu Thr Leu Ser Ser Thr Phe Thr Asn Lys
545 550 555 560

gag gct ctc ctt gat gtt gat tcc agt cca gag gga tct ggg act gaa 1728
Glu Ala Leu Leu Asp Val Asp Ser Ser Pro Glu Gly Ser Gly Thr Glu
565 570 575

gat aac tta caa tga 1743
Asp Asn Leu Gln
580

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<211> 580
<212> PRT
<213> Rattus norvegicus

<400> 4

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Gly Asn Ala Glu Glu Arg Ser Glu Ala Ile Ile Val Gly Gly Arg Asp
35 40 45
Ile Gly Leu Leu Val Gly Gly Phe Thr Met Thr Ala Thr Trp Val Gly
50 55 60
Gly Gly Tyr Ile Asn Gly Thr Ala Glu Ala Val Tyr Gly Pro Gly Cys
65 70 75 80
Gly Leu Ala Trp Ala Gln Ala Pro Ile Gly Tyr Ser Leu Ser Leu Ile
85 90 95
Leu Gly Gly Leu Phe Phe Ala Lys Pro Met Arg Ser Lys Gly Tyr Val
100 105 110
Thr Met Leu Asp Pro Phe Gln Gln Ile Tyr Gly Lys Arg Met Gly Gly
115 120 125
Leu Leu Phe Ile Pro Ala Leu Met Gly Glu Met Phe Trp Ala Ala Ala
130 135 140
Ile Phe Ser Ala Leu Gly Ala Thr Ile Ser Val Ile Ile Asp Val Asp
145 150 155 160
Val Asn Ile Ser Val Ile Val Ser Ala Leu Ile Ala Ile Leu Tyr Thr
165 170 175
Leu Val Gly Gly Leu Tyr Ser Val Ala Tyr Thr Asp Val Val Gln Leu
180 185 190
Phe Cys Ile Phe Ile Gly Leu Trp Ile Ser Val Pro Phe Ala Leu Ser

195 200 205
His Pro Ala Val Thr Asp Ile Gly Phe Thr Ala Val His Ala Lys Tyr
210 215 220
Gln Ser Pro Trp Leu Gly Thr Ile Glu Ser Val Glu Val Tyr Thr Trp
225 230 235 240
Leu Asp Asn Phe Leu Leu Leu Met Leu Gly Gly Ile Pro Trp Gln Ala
245 250 255
Tyr Phe Gln Arg Val Leu Ser Ser Ser Ser Ala Thr Tyr Ala Gln Val
260 265 270
Leu Ser Phe Leu Ala Ala Phe Gly Cys Leu Val Met Ala Leu Pro Ala
275 280 285
Ile Cys Ile Gly Ala Ile Gly Ala Ser Thr Asp Trp Asn Gln Thr Ala
290 295 300
Tyr Gly Phe Pro Asp Pro Lys Thr Lys Glu Glu Ala Asp Met Ile Leu
305 310 315 320
Pro Ile Val Leu Gln Tyr Leu Cys Pro Val Tyr Ile Ser Phe Phe Gly
325 330 335
Leu Gly Ala Val Ser Ala Ala Val Met Ser Ser Ala Asp Ser Ser Ile
340 345 350
Leu Ser Ala Ser Ser Met Phe Ala Arg Asn Ile Tyr Gln Leu Ser Phe
355 360 365
Arg Gln Asn Ala Ser Asp Lys Glu Ile Val Trp Val Met Arg Ile Thr
370 375 380
Val Phe Val Phe Gly Ala Ser Ala Thr Ala Met Ala Leu Leu Thr Lys
385 390 395 400
Thr Val Tyr Gly Leu Trp Tyr Leu Ser Ser Asp Leu Val Tyr Ile Ile
405 410 415
Ile Phe Pro Gln Leu Leu Cys Val Leu Phe Ile Lys Gly Thr Asn Thr
420 425 430
Tyr Gly Ala Val Ala Gly Tyr Ile Phe Gly Leu Phe Leu Arg Ile Thr
435 440 445
Gly Gly Glu Pro Tyr Leu Tyr Leu Gln Pro Leu Ile Phe Tyr Pro Gly
450 455 460
Tyr Tyr Pro Asp Lys Asn Gly Ile Tyr Asn Gln Arg Phe Pro Phe Lys
465 470 475 480
Thr Leu Ser Met Val Thr Ser Phe Phe Thr Asn Ile Cys Val Ser Tyr
485 490 495
Leu Ala Lys Tyr Leu Phe Glu Ser Gly Thr Leu Pro Pro Lys Leu Asp
500 505 510
Ile Phe Asp Ala Val Val Ser Arg His Ser Glu Glu Asn Met Asp Lys
515 520 525
Thr Ile Leu Val Arg Asn Glu Asn Ile Lys Leu Asn Glu Leu Ala Pro
530 535 540
Val Lys Pro Arg Gln Ser Leu Thr Leu Ser Ser Thr Phe Thr Asn Lys
545 550 555 560
Glu Ala Leu Leu Asp Val Asp Ser Ser Pro Glu Gly Ser Gly Thr Glu
565 570 575
Asp Asn Leu Gln
580

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<211> 1743

<212> DNA
<213> Homo sapiens

<220>
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<222> (1)..(1743)

<400> 5

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1               5                   10                  15

cta att ttg ctg gtt gga ata tgg gct gcc tgg aga acc aaa aac agt    96
Leu Ile Leu Leu Val Gly Ile Trp Ala Ala Trp Arg Thr Lys Asn Ser
            20                  25                  30

ggc agc gca gaa gag cgc agc gaa gcc atc ata gtt ggt ggc cga gat    144
Gly Ser Ala Glu Glu Arg Ser Glu Ala Ile Ile Val Gly Gly Arg Asp
        35                  40                  45

att ggt tta ttg gtt ggt gga ttt acc atg aca gct acc tgg gtc gga    192
Ile Gly Leu Leu Val Gly Gly Phe Thr Met Thr Ala Thr Trp Val Gly
    50                  55                  60

gga ggg tat atc aat ggc aca gct gaa gca gtt tat gta cca ggt tat    240
Gly Gly Tyr Ile Asn Gly Thr Ala Glu Ala Val Tyr Val Pro Gly Tyr
65                  70                  75                  80

ggc cta gct tgg gct cag gca cca att gga tat tct ctt agt ctg att    288
Gly Leu Ala Trp Ala Gln Ala Pro Ile Gly Tyr Ser Leu Ser Leu Ile
                85                  90                  95

tta ggt ggc ctg ttc ttt gca aaa cct atg cgt tca aag ggg tat gtg    336
Leu Gly Gly Leu Phe Phe Ala Lys Pro Met Arg Ser Lys Gly Tyr Val
            100                 105                 110

acc atg tta gac ccg ttt cag caa atc tat gga aaa cgc atg ggc gga    384
Thr Met Leu Asp Pro Phe Gln Gln Ile Tyr Gly Lys Arg Met Gly Gly
        115                 120                 125

ctc ctg ttt att cct gca ctg atg gga gaa atg ttc tgg gct gca gca    432
Leu Leu Phe Ile Pro Ala Leu Met Gly Glu Met Phe Trp Ala Ala Ala
    130                 135                 140

att ttc tct gct ttg gga gcc acc atc agc gtg atc atc gat gtg gat    480
Ile Phe Ser Ala Leu Gly Ala Thr Ile Ser Val Ile Ile Asp Val Asp
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145                 150                 155                 160

atg cac att tct gtc atc atc tct gca ctc att gcc act ctg tac aca   528
Met His Ile Ser Val Ile Ile Ser Ala Leu Ile Ala Thr Leu Tyr Thr
                165                 170                 175

ctg gtg gga ggg ctc tat tct gtg gcc tac act gat gtc gtt cag ctc   576
Leu Val Gly Gly Leu Tyr Ser Val Ala Tyr Thr Asp Val Val Gln Leu
            180                 185                 190

ttt tgc att ttt gta ggg ctg tgg atc agc gtc ccc ttt gca ttg tca   624
Phe Cys Ile Phe Val Gly Leu Trp Ile Ser Val Pro Phe Ala Leu Ser
        195                 200                 205

cat cct gca gtc gca gac atc ggg ttc act gct gtg cat gcc aaa tac   672
His Pro Ala Val Ala Asp Ile Gly Phe Thr Ala Val His Ala Lys Tyr
    210                 215                 220

caa aag ccg tgg ctg gga act gtt gac tca tct gaa gtc tac tct tgg   720
Gln Lys Pro Trp Leu Gly Thr Val Asp Ser Ser Glu Val Tyr Ser Trp
225                 230                 235                 240

ctt gat agt ttt ctg ttg ttg atg ctg ggt gga atc cca tgg caa gca   768
Leu Asp Ser Phe Leu Leu Leu Met Leu Gly Gly Ile Pro Trp Gln Ala
                245                 250                 255

tac ttt cag agg gtt ctc tct tct tcc tca gcc acc tat gct caa gtg   816
Tyr Phe Gln Arg Val Leu Ser Ser Ser Ser Ala Thr Tyr Ala Gln Val
            260                 265                 270

ctg tcc ttc ctg gca gct ttc ggg tgc ctg gtg atg gcc atc cca gcc   864
Leu Ser Phe Leu Ala Ala Phe Gly Cys Leu Val Met Ala Ile Pro Ala
        275                 280                 285

ata ctc att ggg gcc att gga gca tca aca gac tgg aac cag act gca   912
Ile Leu Ile Gly Ala Ile Gly Ala Ser Thr Asp Trp Asn Gln Thr Ala
    290                 295                 300

tat ggg ctt cca gat ccc aag act aca gaa gag gca gac atg att tta   960
Tyr Gly Leu Pro Asp Pro Lys Thr Thr Glu Glu Ala Asp Met Ile Leu
305                 310                 315                 320

cca att gtt ctg cag tat ctc tgc cct gtg tat att tct ttc ttt ggt   1008
Pro Ile Val Leu Gln Tyr Leu Cys Pro Val Tyr Ile Ser Phe Phe Gly
                325                 330                 335

ctt ggt gca gtt tct gct gct gtt atg tca tca gca gat tct tcc atc   1056
Leu Gly Ala Val Ser Ala Ala Val Met Ser Ser Ala Asp Ser Ser Ile
            340                 345                 350
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ttg tca gca agt tcc atg ttt gca cgg aac atc tac cag ctt tcc ttc 1104
Leu Ser Ala Ser Ser Met Phe Ala Arg Asn Ile Tyr Gln Leu Ser Phe
355 360 365

aga caa aat gct tcg gac aaa gaa atc gtt tgg gtt atg cga atc aca 1152
Arg Gln Asn Ala Ser Asp Lys Glu Ile Val Trp Val Met Arg Ile Thr
370 375 380

gtg ttt gtg ttt gga gca tct gca aca gcc atg gcc ttg ctg acg aaa 1200
Val Phe Val Phe Gly Ala Ser Ala Thr Ala Met Ala Leu Leu Thr Lys
385 390 395 400

act gtg tat ggg ctc tgg tac ctc agt tct gac ctt gtt tac atc gtt 1248
Thr Val Tyr Gly Leu Trp Tyr Leu Ser Ser Asp Leu Val Tyr Ile Val
405 410 415

atc ttc ccc cag ctg ctt tgt gta ctc ttt gtt aag gga acc aac acc 1296
Ile Phe Pro Gln Leu Leu Cys Val Leu Phe Val Lys Gly Thr Asn Thr
420 425 430

tat ggg gcc gtg gca ggt tat gtt tct ggc ctc ttc ctg aga ata act 1344
Tyr Gly Ala Val Ala Gly Tyr Val Ser Gly Leu Phe Leu Arg Ile Thr
435 440 445

gga ggg gag cca tat ctg tat ctt cag ccc ttg atc ttc tac cct ggc 1392
Gly Gly Glu Pro Tyr Leu Tyr Leu Gln Pro Leu Ile Phe Tyr Pro Gly
450 455 460

tat tac cct gat gat aat ggt ata tat aat cag aaa ttt cca ttt aaa 1440
Tyr Tyr Pro Asp Asp Asn Gly Ile Tyr Asn Gln Lys Phe Pro Phe Lys
465 470 475 480

aca ctt gcc atg gtt aca tca ttc tta acc aac att tgc atc tcc tat 1488
Thr Leu Ala Met Val Thr Ser Phe Leu Thr Asn Ile Cys Ile Ser Tyr
485 490 495

cta gcc aag tat cta ttt gaa agt gga acc ttg cca cct aaa tta gat 1536
Leu Ala Lys Tyr Leu Phe Glu Ser Gly Thr Leu Pro Pro Lys Leu Asp
500 505 510

gta ttt gat gct gtt gtt gca aga cac agt gaa gaa aac atg gat aag 1584
Val Phe Asp Ala Val Val Ala Arg His Ser Glu Glu Asn Met Asp Lys
515 520 525

aca att ctt gtc aaa aat gaa aat att aaa tta gat gaa ctt gca ctt 1632
Thr Ile Leu Val Lys Asn Glu Asn Ile Lys Leu Asp Glu Leu Ala Leu
530 535 540

gtg aag cca cga cag agc atg acc ctc agc tca act ttc acc aat aaa 1680
Val Lys Pro Arg Gln Ser Met Thr Leu Ser Ser Thr Phe Thr Asn Lys

545 550 555 560

gag gcc ttc ctt gat gtt gat tcc agt cca gaa ggg tct ggg act gaa 1728
Glu Ala Phe Leu Asp Val Asp Ser Ser Pro Glu Gly Ser Gly Thr Glu
565 570 575

gat aat tta cag tga 1743
Asp Asn Leu Gln
580

<210> 6
<211> 580
<212> PRT
<213> Homo sapiens

<400> 6

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Leu Ile Leu Leu Val Gly Ile Trp Ala Ala Trp Arg Thr Lys Asn Ser
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Gly Ser Ala Glu Glu Arg Ser Glu Ala Ile Ile Val Gly Gly Arg Asp
35 40 45
Ile Gly Leu Leu Val Gly Gly Phe Thr Met Thr Ala Thr Trp Val Gly
50 55 60
Gly Gly Tyr Ile Asn Gly Thr Ala Glu Ala Val Tyr Val Pro Gly Tyr
65 70 75 80
Gly Leu Ala Trp Ala Gln Ala Pro Ile Gly Tyr Ser Leu Ser Leu Ile
85 90 95
Leu Gly Gly Leu Phe Phe Ala Lys Pro Met Arg Ser Lys Gly Tyr Val
100 105 110
Thr Met Leu Asp Pro Phe Gln Gln Ile Tyr Gly Lys Arg Met Gly Gly
115 120 125
Leu Leu Phe Ile Pro Ala Leu Met Gly Glu Met Phe Trp Ala Ala Ala
130 135 140
Ile Phe Ser Ala Leu Gly Ala Thr Ile Ser Val Ile Ile Asp Val Asp
145 150 155 160
Met His Ile Ser Val Ile Ile Ser Ala Leu Ile Ala Thr Leu Tyr Thr
165 170 175
Leu Val Gly Gly Leu Tyr Ser Val Ala Tyr Thr Asp Val Val Gln Leu
180 185 190
Phe Cys Ile Phe Val Gly Leu Trp Ile Ser Val Pro Phe Ala Leu Ser
195 200 205
His Pro Ala Val Ala Asp Ile Gly Phe Thr Ala Val His Ala Lys Tyr
210 215 220
Gln Lys Pro Trp Leu Gly Thr Val Asp Ser Ser Glu Val Tyr Ser Trp
225 230 235 240
Leu Asp Ser Phe Leu Leu Leu Met Leu Gly Gly Ile Pro Trp Gln Ala
245 250 255
Tyr Phe Gln Arg Val Leu Ser Ser Ser Ser Ala Thr Tyr Ala Gln Val

260 265 270
Leu Ser Phe Leu Ala Ala Phe Gly Cys Leu Val Met Ala Ile Pro Ala
275 280 285
Ile Leu Ile Gly Ala Ile Gly Ala Ser Thr Asp Trp Asn Gln Thr Ala
290 295 300
Tyr Gly Leu Pro Asp Pro Lys Thr Thr Glu Glu Ala Asp Met Ile Leu
305 310 315 320
Pro Ile Val Leu Gln Tyr Leu Cys Pro Val Tyr Ile Ser Phe Phe Gly
325 330 335
Leu Gly Ala Val Ser Ala Ala Val Met Ser Ser Ala Asp Ser Ser Ile
340 345 350
Leu Ser Ala Ser Ser Met Phe Ala Arg Asn Ile Tyr Gln Leu Ser Phe
355 360 365
Arg Gln Asn Ala Ser Asp Lys Glu Ile Val Trp Val Met Arg Ile Thr
370 375 380
Val Phe Val Phe Gly Ala Ser Ala Thr Ala Met Ala Leu Leu Thr Lys
385 390 395 400
Thr Val Tyr Gly Leu Trp Tyr Leu Ser Ser Asp Leu Val Tyr Ile Val
405 410 415
Ile Phe Pro Gln Leu Leu Cys Val Leu Phe Val Lys Gly Thr Asn Thr
420 425 430
Tyr Gly Ala Val Ala Gly Tyr Val Ser Gly Leu Phe Leu Arg Ile Thr
435 440 445
Gly Gly Glu Pro Tyr Leu Tyr Leu Gln Pro Leu Ile Phe Tyr Pro Gly
450 455 460
Tyr Tyr Pro Asp Asp Asn Gly Ile Tyr Asn Gln Lys Phe Pro Phe Lys
465 470 475 480
Thr Leu Ala Met Val Thr Ser Phe Leu Thr Asn Ile Cys Ile Ser Tyr
485 490 495
Leu Ala Lys Tyr Leu Phe Glu Ser Gly Thr Leu Pro Pro Lys Leu Asp
500 505 510
Val Phe Asp Ala Val Val Ala Arg His Ser Glu Glu Asn Met Asp Lys
515 520 525
Thr Ile Leu Val Lys Asn Glu Asn Ile Lys Leu Asp Glu Leu Ala Leu
530 535 540
Val Lys Pro Arg Gln Ser Met Thr Leu Ser Ser Thr Phe Thr Asn Lys
545 550 555 560
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565 570 575
Asp Asn Leu Gln
580

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<211> 1743
<211> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1)..(1743)

<400> 7

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  1               5                  10                  15

ctt ata ttt ctg gtt gga ata tgg gct gca tgg aaa acc aaa aac agc   96
Leu Ile Phe Leu Val Gly Ile Trp Ala Ala Trp Lys Thr Lys Asn Ser
             20                  25                  30

ggc aac cca gaa gag cac agt gaa gcc atc ata gtc ggg ggc cgt gac   144
Gly Asn Pro Glu Glu His Ser Glu Ala Ile Ile Val Gly Gly Arg Asp
         35                  40                  45

att ggt ttg ttg gtt ggt ggt ttt acc atg aca gcc acc tgg gtt gga   192
Ile Gly Leu Leu Val Gly Gly Phe Thr Met Thr Ala Thr Trp Val Gly
     50                  55                  60

gga ggc tac atc aat ggg aca gca gaa gca gtg tat ggg cca ggt tgt   240
Gly Gly Tyr Ile Asn Gly Thr Ala Glu Ala Val Tyr Gly Pro Gly Cys
 65                  70                  75                  80

ggt cta gct tgg gct cag gca ccc att gga tat tct ctg agt cta att   288
Gly Leu Ala Trp Ala Gln Ala Pro Ile Gly Tyr Ser Leu Ser Leu Ile
                 85                  90                  95

tta ggt ggt ctg ttt ttt gcg aaa cct atg cgt tcc aag gga tat gtg   336
Leu Gly Gly Leu Phe Phe Ala Lys Pro Met Arg Ser Lys Gly Tyr Val
            100                 105                 110

act atg tta gac cca ttt caa cag atc tat gga aag cgc atg ggt ggg   384
Thr Met Leu Asp Pro Phe Gln Gln Ile Tyr Gly Lys Arg Met Gly Gly
        115                 120                 125

ctg ctc ttc atc cct gca ctg atg gga gag atg ttc tgg gct gca gca   432
Leu Leu Phe Ile Pro Ala Leu Met Gly Glu Met Phe Trp Ala Ala Ala
    130                 135                 140

att ttc tct gca tta ggg gcc acc atc agc gtg atc att gat gtg gat   480
Ile Phe Ser Ala Leu Gly Ala Thr Ile Ser Val Ile Ile Asp Val Asp
145                 150                 155                 160

gtg aac ata tcg gtc att gtc tct gca ctc att gcc att ctt tat acc   528
Val Asn Ile Ser Val Ile Val Ser Ala Leu Ile Ala Ile Leu Tyr Thr
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Leu Val Gly Gly Leu Tyr Ser Val Ala Tyr Thr Asp Val Val Gln Leu
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            180                 185                 190

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cat cct gca gtc acc gac atc gga ttc aca gct gtg cat gct aaa tac   672
His Pro Ala Val Thr Asp Ile Gly Phe Thr Ala Val His Ala Lys Tyr
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cag agt ccc tgg ctg gga acc att gaa tca gtt gaa gtc tac acc tgg   720
Gln Ser Pro Trp Leu Gly Thr Ile Glu Ser Val Glu Val Tyr Thr Trp
225                 230                 235                 240

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Leu Asp Asn Phe Leu Leu Leu Met Leu Gly Gly Ile Pro Trp Gln Ala
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tac ttc cag agg gtc ctc tct tca tcc tca gcc acc tat gct cag gta   816
Tyr Phe Gln Arg Val Leu Ser Ser Ser Ser Ala Thr Tyr Ala Gln Val
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cta gcc aag tat cta ttt gaa agt gga acc ttg cct cca aaa tta gat   1536
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Val Phe Asp Ala Val Val Ala Arg His Ser Glu Glu Asn Met Asp Lys
        515                 520                 525

acc att cta gtc aga aat gaa aat atc aaa tta aat gaa ctt gca cct   1632
Thr Ile Leu Val Arg Asn Glu Asn Ile Lys Leu Asn Glu Leu Ala Pro
    530                 535                 540

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580

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20 25 30
Gly Asn Pro Glu Glu His Ser Glu Ala Ile Ile Val Gly Gly Arg Asp
35 40 45
Ile Gly Leu Leu Val Gly Gly Phe Thr Met Thr Ala Thr Trp Val Gly
20 50 55 60
Gly Gly Tyr Ile Asn Gly Thr Ala Glu Ala Val Tyr Gly Pro Gly Cys
65 70 75 80
Gly Leu Ala Trp Ala Gln Ala Pro Ile Gly Tyr Ser Leu Ser Leu Ile
25 85 90 95
Leu Gly Gly Leu Phe Phe Ala Lys Pro Met Arg Ser Lys Gly Tyr Val
100 105 110
Thr Met Leu Asp Pro Phe Gln Gln Ile Tyr Gly Lys Arg Met Gly Gly
115 120 125
30 Leu Leu Phe Ile Pro Ala Leu Met Gly Glu Met Phe Trp Ala Ala Ala
130 135 140
Ile Phe Ser Ala Leu Gly Ala Thr Ile Ser Val Ile Ile Asp Val Asp
145 150 155 160
Val Asn Ile Ser Val Ile Val Ser Ala Leu Ile Ala Ile Leu Tyr Thr
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Leu Val Gly Gly Leu Tyr Ser Val Ala Tyr Thr Asp Val Val Gln Leu
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Phe Cys Ile Phe Ile Gly Leu Trp Ile Ser Val Pro Phe Ala Leu Ser
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Pro Ile Val Leu Gln Tyr Leu Cys Pro Val Tyr Ile Ser Phe Phe Gly

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Val Leu Val Phe Gly Ala Ser Ala Thr Ala Met Ala Leu Leu Thr Lys
385                 390                 395                 400
Thr Val Tyr Gly Leu Trp Tyr Leu Ser Ser Asp Leu Val Tyr Ile Ile
                405                 410                 415
Ile Phe Pro Gln Leu Leu Cys Val Leu Phe Ile Lys Gly Thr Asn Thr
            420                 425                 430
Tyr Gly Ala Val Ala Gly Tyr Ile Phe Gly Leu Phe Leu Arg Ile Thr
        435                 440                 445
Gly Gly Glu Pro Tyr Leu Tyr Leu Gln Pro Leu Ile Phe Tyr Pro Gly
    450                 455                 460
Tyr Tyr Ser Asp Lys Asn Gly Ile Tyr Asn Gln Arg Phe Pro Phe Lys
465                 470                 475                 480
Thr Leu Ser Met Val Thr Ser Phe Phe Thr Asn Ile Cys Val Ser Tyr
                485                 490                 495
Leu Ala Lys Tyr Leu Phe Glu Ser Gly Thr Leu Pro Pro Lys Leu Asp
            500                 505                 510
Val Phe Asp Ala Val Val Ala Arg His Ser Glu Glu Asn Met Asp Lys
        515                 520                 525
Thr Ile Leu Val Arg Asn Glu Asn Ile Lys Leu Asn Glu Leu Ala Pro
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Val Lys Pro Arg Gln Ser Leu Thr Leu Ser Ser Thr Phe Thr Asn Lys
545                 550                 555                 560
Glu Ala Leu Leu Asp Val Asp Ser Ser Pro Glu Gly Ser Gly Thr Glu
                565                 570                 575
Asp Asn Leu Gln
            580
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Claims

1. A DNA molecule which encodes:

   (a) a protein comprising an amino acid sequence represented by *Seq. ID. No. 6;* or
   (b) a protein comprising an amino acid sequence where one amino acid is deleted, substituted or added in the amino acid sequence represented by *Seq. ID No. 6,* and having high-affinity choline transporter activity.

2. A DNA molecule containing a base sequence represented by *Seq. ID. No. 5* or its complementary sequence.

3. A DNA, molecule derived from a human which hybridizes with DNA comprising a gene according to claim 1 under stringent conditions, and encodes a protein having high-affuiity choline transporter activity.

4. A, protein comprising an amino acid sequence represented by *Seq. ID No. 6*,

5. A protein comprising an amino acid sequence where one amino acid is deleted, substituted or added in the amino acid sequence represented by *Seq. ID No. 6*, and having human high-affinity choline transporter activity.

6. A fusion protein being constructed by expressing a cDNA encoding fusion proteins of a protein according to claim

4 or 5 and a marker protein and/or a peptide tag.

7. An antibody which specifically binds to a protein according to claim 4 or 5.

8. The antibody according to claim 7, wherein the antibody is a monoclonal antibody.

9. A host cell into which has been introduced a DNA molecule according to any one of claims 1 to 3.

10. A host cell according to claim 9, whose endogenous DNA which encodes a protein having high-affinity choline transporter activity is inactivated by mutation.

11. A transgenic mouse which expresses a protein according to claim 4 or 5.

12. A method for preparing a cell having high-affinity choline transporter activity, **characterised in** introducing the DNA molecule according to any one of claims 1 to 3 into a cell whose endogenous DNA molecule which encodes a protein having high-affinity choline transporter activity is inactivated by mutation.

13. The method of claim 12, wherein the DNA molecule according to claim 1 or 2 is integrated into a chromosome, of the cell, so the cell stably shows high-affinity choline transporter activity.

14. A method for screening for a promoter or a suppressor of high-affinity choline transporter activity, **characterised in** measuring/evaluating any change in the activity of the protein having high-affinity choline transporter activity according to claim 4 or 5 in the presence of a candidate promoter/suppressor.

15. A method for screening for a promoter or a suppressor of high-affinity choline transporter activity, or of high-affinity choline transporter expression **characterised in** comprising the steps of: contacting a cell membrane or a cell which expresses a protein having high-affinity choline transporter activity in vitro with a candidate promoter/suppressor; and measuring/evaluating any change in the activity and/or the expression amount of a protein according to claim 4 or 5 in the cell membrane or the cell in the presence of the candidate promoter/suppressor.

16. The method according to claim 15, wherein the cell membrane or the cell which expresses a protein having high-affinity choline transporter activity is the host cell according to claim 9 or 10.

17. The method according to any one of claims 14 to 16, wherein the protein having high-affinity choline transporter activity is a recombinant protein.

18. A screening method of a promoter or a suppressor of high-affiruity choline transporter activity, or of high-affinity choline transporter expression **characterised in** comprising the steps of: cultivating a cell obtained from the mouse according to claim 11 in vitro in the presence of a candidate promoter/suppressor; and measuring/evaluating any change in the activity and/or the expression amount of a protein having high-affinity choline transporter activity in the cell in the presence of the candidate promoter/suppressor.

19. A medical constituent **characterised in** being used for a medical treatment for a patient who needs elevation of the activity or enhancement of the expression of a high-affinity choline transporter, and containing the protein according to claim 4 or 5, as an active component.

20. A diagnostic method for diseases relating to the expression or the activity of a high-affinity choline transporter wherein the method comprises:

obtaining a genomic DNA, RNA or cDNA sequence encoding a high-affinity choline transporter from a sample of blood, urine, saliva or tissue from a subject;
comparing the genomic DNA, RNA or cDNA sequence obtained from the sample to a DNA sequence encoding the protein according to claim 4 or 5; and
detecting any mutation in the sequence obtained from the sample;

wherein mutation in the sequence obtained from the sample indicates the presence in the subject of a disease relating to the expression or the activity of a high-affinity choline transporter.

21. A diagnostic probe for Alzheimer's disease comprising a part of an antisense strand of DNA or RNA encoding the protein according to claims 4 or 5, said part comprising at least 20 bases.

22. A diagnostic drug for Alzheimer's disease **characterised in** containing the diagnostic probe according to claim 21 and/or the antibody according to claim 7 or 8.

Patentansprüche

1. DNA-Molekül, welches:

(a) ein Protein, welches eine Aminosäuresequenz umfaßt, die repräsentiert wird durch *Seq. ID. No. 6*, oder
(b) ein Protein, das eine Aminosäuresequenz umfaßt, bei der eine Aminosäure in der Aminosäuresequenz, die durch *Seq. ID No. 6* repräsentiert wird, deletiert, substituiert oder hinzugefügt ist, und das eine hochaffine Cholintransporter-Aktivität aufweist,

codiert.

2. DNA-Molekül, welches eine Basensequenz enthält, die repräsentiert wird durch *Seq. ID No. 5* oder deren komplementärer Sequenz.

3. Von einem Menschen abgeleitetes DNA-Molekül, welches mit DNA, die ein Gen nach Anspruch 1 umfaßt, unter stringenten Bedingungen hybridisiert und welches ein Protein codiert, das eine hochaffine Cholintransporter-Aktivität aufweist.

4. Protein, welches eine Aminosäuresequenz umfaßt, die repräsentiert wird durch Seq. *ID No. 6.*

5. Protein, welches eine Aminosäuresequenz umfaßt, bei der eine Aminosäure in der Aminosäuresequenz, die durch *Seq. ID No. 6* repräsentiert wird, deletiert, substituiert oder hinzugefügt ist, und welches die Aktivität eines humanen hochaffinen Cholintransporters aufweist.

6. Fusionsprotein, welches erzeugt wird durch das Exprimieren einer cDNA, die Fusionsproteine von einem Protein nach Anspruch 4 oder 5 und einem Markerprotein und/oder einer Peptidmarkierung codiert.

7. Antikörper, welcher spezifisch an ein Protein nach Anspruch 4 oder 5 bindet.

8. Antikörper nach Anspruch 7, wobei der Antikörper ein monoklonaler Antikörper ist.

9. Wirtszelle, in welche ein DNA-Molekül nach einem der Ansprüche 1 bis 3 eingebracht wurde.

10. Wirtszelle nach Anspruch 9, deren endogene DNA, welche ein Protein codiert, das eine hochaffine Cholintransporter-Aktivität aufweist, durch Mutation inaktiviert wird.

11. Transgene Maus, die ein Protein nach Anspruch 4 oder 5 exprimiert.

12. Verfahren zur Herstellung einer Zelle mit einer hochaffinen Cholintransporter-Aktivität, **dadurch gekennzeichnet, daß** man das DNA-Molekül nach einem der Ansprüche 1 bis 3 in eine Zelle, deren endogenes DNA-Molekül, welches ein Protein mit einer hochaffinen Cholintransporter-Aktivität codiert, durch Mutation inaktiviert ist, einbringt.

13. Verfahren nach Anspruch 12, wobei das DNA-Molekül nach Anspruch 1 oder 2 in ein Chromosom der Zelle integriert wird, so daß die Zelle eine stabile hochaffine Cholintransporter-Aktivität zeigt.

14. Verfahren zum Screening nach einem Promotor oder einem Suppressor von hochaffiner Cholintransporter-Aktivität, **dadurch gekennzeichnet, daß** man jede Veränderung der Aktivität des Proteins mit einer hochaffinen Cholintransporter-Aktivität nach Anspruch 4 oder 5 in der Gegenwart eines potentiellen Promotors/Suppressors mißt/evaluiert.

15. Verfahren zum Screening nach einem Promotor oder einem Suppressor von hochaffiner Cholintransporter-Aktivität oder der Expression eines hochaffinen Cholintransporters, **dadurch gekennzeichnet, daß** es die Stufen aufweist,

bei denen man eine Zellmembran oder eine Zelle, die in vitro ein Protein mit einer hochaffinen Cholintransporter-Aktivität exprimiert, mit einem potentiellen Promotor/Suppressor in Kontakt bringt und jede Veränderung in der Aktivität und/oder der Expressionsmenge eines Proteins nach Anspruch 4 oder 5 in der Zellmembran oder der Zelle in der Gegenwart des potentiellen Promotors/Suppressors mißt/evaluiert.

16. Verfahren nach Anspruch 15, wobei die Zellmembran oder die Zelle, die ein Protein mit einer hochaffinen Cholintransporter-Aktivität exprimiert, die Wirtszelle nach Anspruch 9 oder 10 ist.

17. Verfahren nach einem der Ansprüche 14 bis 16, wobei das Protein mit der hochaffinen Cholintransporter-Aktivität ein rekombinantes Protein ist.

18. Screening-Verfahren nach einem Promotor oder einem Suppressor der hochaffinen Cholintransporter-Aktivität oder der Expression eines hochaffinen Cholintransporters, **dadurch gekennzeichnet, daß** es die Stufen umfaßt, bei denen man eine Zelle, die von einer Maus nach Anspruch 11 erhalten wurde, in vitro in der Gegenwart eines potentiellen Promotors/Suppressors kultiviert und jede Veränderung in der Aktivität und/oder der Expressionsmenge eines Proteins mit hochaffiner Cholintransporter-Aktivität in der Zelle in der Gegenwart des potentiellen Promotors/Suppressors mißt/evaluiert.

19. Arzneimittelbestandteil, **dadurch gekennzeichnet, daß** er für die medizinische Behandlung eines Patienten verwendet wird, der der Erhöhung der Aktivität oder der Verstärkung der Expression eines hochaffinen Cholintransporters bedarf, und dadurch, daß er das Protein nach Anspruch 4 oder 5 als eine aktive Komponente enthält.

20. Diagnoseverfahren für Erkrankungen, die mit der Expression oder der Aktivität eines hochaffinen Cholintransporters in Verbindung stehen, wobei das Verfahren umfaßt, daß man eine genomische DNA-, RNA- oder cDNA-Sequenz, die einen hochaffinen Cholintransporter codiert, aus einer Blut-, Urin-, Speichel- oder Gewebeprobe aus einem Patienten erhält, die aus der Probe erhaltene genomische DNA-, RNA- oder cDNA-Sequenz mit einer DNA-Sequenz, die das Protein nach Anspruch 4 oder 5 codiert, vergleicht und jede Mutation in der aus der Probe erhaltenen Sequenz erfaßt, wobei die Mutation in der aus der Probe erhaltenen Sequenz das Vorliegen einer Erkrankung, die mit der Expression oder der Aktivität eines hochaffinen Cholintransporters in Verbindung steht, in dem Patienten anzeigt.

21. Diagnosesonde für die Alzheimer-Krankheit, welche einen Teil eines Antisense-Stranges von DNA oder RNA, welche das Protein nach den Ansprüchen 4 oder 5 codiert, umfaßt, wobei dieser Teil wenigstens 20 Basen umfaßt.

22. Diagnosemittel für die Alzheimer-Krankheit, **dadurch gekennzeichnet, daß** sie die Diagnosesonde nach Anspruch 21 und/oder den Antikörper nach Anspruch 7 oder 8 enthält.

Revendications

1. Molécule d'ADN qui code pour :

(a) une protéine comprenant une séquence d'aminoacides représentée par la *SEQ ID N° 6 ;* ou
(b) une protéine comprenant une séquence d'aminoacides dans laquelle un aminoacide est éliminé par délétion de, substitué dans, ou ajouté à, la séquence d'aminoacides représentée par la *SEQ ID N° 6*, et ayant une activité de transporteur de choline à forte affinité.

2. Molécule d'ADN comprenant une séquence de bases représentée par la *SEQ ID N° 5* ou sa séquence complémentaire.

3. Molécule d'ADN dérivée d'un être humain, qui s'hybride avec l'ADN comprenant un gène suivant la revendication 1 dans des conditions drastiques, et qui code pour une protéine ayant une activité de transporteur de choline à forte affinité.

4. Protéine comprenant une séquence d'aminoacides représentée par la *SEQ ID N° 6*.

5. Protéine comprenant une séquence d'aminoacides dans laquelle un aminoacide est éliminé par délétion de, substitué dans, ou ajouté à, la séquence d'aminoacides représentée par la *SEQ ID N° 6,* et ayant une activité de transporteur

de choline à forte affinité chez l'homme.

6. Protéine de fusion construite par expression d'un ADNc codant pour des protéines de fusion d'une protéine suivant la revendication 4 ou 5 et d'une protéine servant de marqueur et/ou d'un marqueur peptidique.

7. Anticorps qui se lie spécifiquement à une protéine suivant la revendication 4 ou 5.

8. Anticorps suivant la revendication 7, l'anticorps étant un anticorps monoclonal.

9. Cellule hôte dans laquelle a été introduite une molécule d'ADN suivant l'une quelconque des revendications 1 à 3.

10. Cellule hôte suivant la revendication 9, dont l'ADN endogène qui code pour une protéine ayant une activité de transporteur de choline à forte affinité est inactivé par mutation.

11. Souris transgénique qui exprime une protéine suivant la revendication 4 ou 5.

12. Procédé pour la préparation d'une cellule ayant une activité de transporteur de choline à forte affinité, **caractérisé par** l'introduction de la molécule d'ADN suivant l'une quelconque des revendications 1 à 3 dans une cellule dont la molécule d'ADN endogène qui code pour une protéine ayant une activité de transporteur de choline à forte affinité est inactivée par mutation.

13. Procédé suivant la revendication 12, dans lequel la molécule d'ADN suivant la revendication 1 ou 2 est intégrée à un chromosome de la cellule, de telle sorte que la cellule présente de manière stable une activité de transporteur de choline à forte affinité.

14. Procédé pour sélectionner un promoteur ou un suppresseur d'activité de transporteur de choline à forte affinité, **caractérisé par** la mesure/l'évaluation de toute variation de l'activité de la protéine ayant une activité de transporteur de choline à forte affinité suivant la revendication 4 ou 5, en présence d'un promoteur/suppresseur candidat.

15. Procédé pour sélectionner un promoteur ou un suppresseur d'activité de transporteur de choline à forte affinité, ou d'expression du transporteur de choline à forte affinité, **caractérisé en ce qu'**il comprend les étapes consistant : à mettre en contact une membrane cellulaire ou une cellule qui exprime une protéine ayant une activité de transporteur de choline à forte affinité in vitro avec un promoteur/suppresseur candidat ; et à mesurer/évaluer toute variation de l'activité et/ou de la quantité exprimée d'une protéine suivant la revendication 4 ou 5, dans la membrane cellulaire ou la cellule en présence du promoteur/suppresseur candidat.

16. Procédé suivant la revendication 15, dans lequel la membrane cellulaire ou la cellule qui exprime une protéine ayant une activité de transporteur de choline à forte affinité est la cellule hôte suivant la revendication 9 ou 10.

17. Procédé suivant l'une quelconque des revendications 14 à 16, dans lequel la protéine ayant une activité de transporteur de choline à forte affinité est une protéine recombinante.

18. Procédé pour sélectionner un promoteur ou suppresseur d'activité de transporteur de choline à forte affinité, ou d'expression du transporteur de choline à forte affinité, **caractérisé en ce qu'**il comprend les étapes consistant : à cultiver une cellule obtenue à partir de la souris suivant la revendication 11 in vitro en présence d'un promoteur/suppresseur candidat ; à mesurer/évaluer toute variation de l'activité et/ou de la quantité exprimée d'une protéine ayant une activité de transporteur de choline à forte affinité dans la cellule en présence du promoteur/suppresseur candidat.

19. Constituant médical **caractérisé en ce qu'**il est utilisé pour un traitement médical d'un patient qui nécessite un accroissement de l'activité ou une augmentation de l'expression d'un transporteur de choline à forte affinité, et contenant la protéine suivant la revendication 4 ou 5 comme constituant actif.

20. Méthode de diagnostic pour des maladies liées à l'expression ou à l'activité d'un transporteur de choline à forte affinité, ladite méthode comprenant les étapes consistant à :

obtenir une séquence d'ADN génomique, d'ARN ou d'ADNc codant pour un transporteur de choline à forte affinité à partir d'un échantillon de sang, d'urine, de salive ou d'un tissu d'un sujet,

comparer la séquence d'ADN génomique d'ARN ou d'ADNc obtenue à partir de l'échantillon avec une séquence d'ADN codant pour la protéine suivant la revendication 4 ou 5 ; et
détecter toute mutation dans la séquence obtenue de l'échantillon ;

5 dans laquelle une mutation dans la séquence obtenue de l'échantillon indique la présence, chez le sujet, d'une maladie liée à l'expression ou à l'activité d'un transporteur de choline à forte affinité.

21. Sonde de diagnostic pour la maladie d'Alzheimer, comprenant une partie d'un brin antisens d'ADN ou d'ARN codant pour la protéine suivant la revendication 4 ou 5, ladite partie comprenant au moins 20 bases.

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22. Médicament de diagnostic pour la maladie d'Alzheimer, **caractérisé en ce qu'**il contient la sonde de diagnostic suivant la revendication 21 et/ou l'anticorps suivant la revendication 7 ou 8.

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Fig. 1

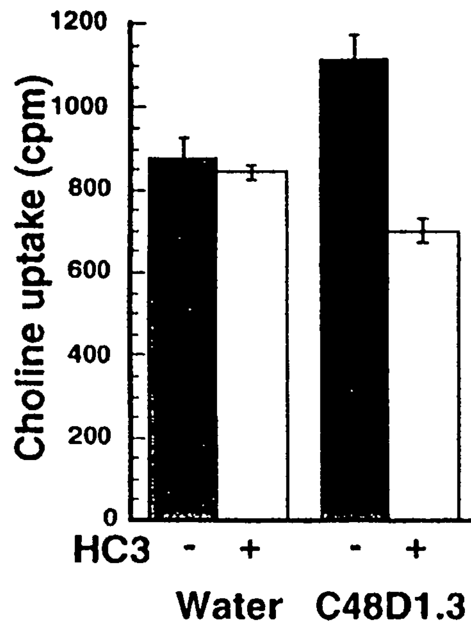

Fig. 2

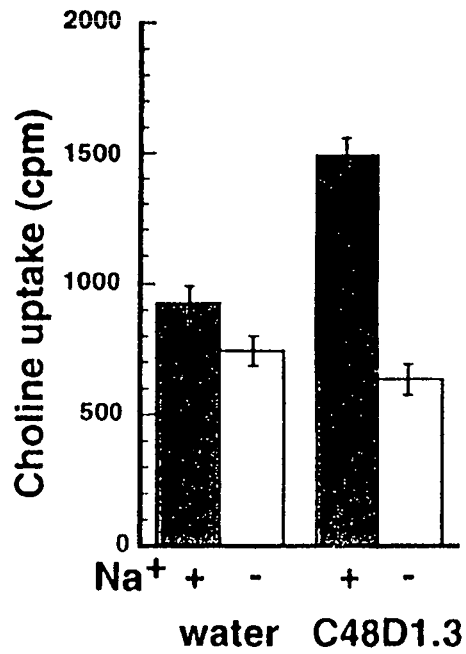

Fig. 3

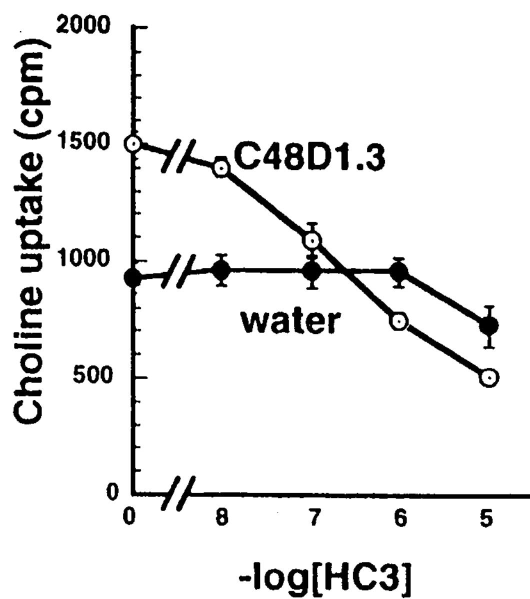

Fig. 4

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CHT1    MPFHVEGLVAIILFYLLILVVGIWAAWKTKNS----GNAEERSEAIIVGGRNIGLLVGGF     56
cht-1   -MADALGLVAIVLFYVLILVVGIWAGRKSKSSKELESLAGAALEEVMLAGRNIGTLVGIF     59
                    I

CHT1    TMTATWVGGGYINGTAEAVYGPGCGLAWAQAPLGYSLSLLGGLFFAKPMRSKGYVTMLD    116
cht-1   TMTATWVGGAYINGTAEALYNG--GLLGCQAPVGYAISLVVGGLIFAKKMREEGYITMLD    117
                                         II

CHT1    PFQCIYGKRYGGLLFLPALMGEMFWAAAIFSALGATISVIIDVDVNISVIVSALIALLYT    176
cht-1   PFQSKYGQRIGGLYMVPALLGETFWTAAILSALGATLSVILGLDYNASVTLSACIAVFYT    177
                                   III                           IV

CHT1    LVGGLYSVAYTDVVQLFCIFIGLWLSVPFALSHPVVTDIGFTAVHAKYQSPWLGTIES-V    235
cht-1   FTGGYYAVAYTDVVQLFCIFVGLWVCVPAAIVHDGAKDISRNAG------DWIGEIGGFK    231
                                  V

CHT1    EVYTWLDNFLLLMLGGIPWQAYFQRVLSSSSATYAQVLSFLAAFGCLVMAIPAICIGAIG    295
cht-1   ETSLWIDCVLLLVFGGIPWQVYFQRVLSSKTAHGAQTLSFVAGVGCLIMAIPPALIGAIA    291
                   VI                                 VII

CHT1    ASTDWNQTAYGFPDPKTKEEAD------MILPIVLQYLCPVYISFFGLGAVSAAVMSSAD    349
cht-1   RNTDWRMTDYSPWNNGTKVESIPPDKRNMVVPLVFQYLTPRWVAFLGLGAVSAAVMSSAD    351
                                                  VIII

CHT1    SSILSASSMFARNIYQLSFRQIASDKEIVWVMRITVFVFGASATAMALLTKTVYGLWYLS    409
cht-1   SSVLSAASMFAHNIWKLTIRPLASEKEVLIIVMRIATLCVGIMATIMALTIQSIYGLWYLC    411
                                                 IX

CHT1    SDLVYIIIFPQLLCVLFLKGTNTYGAVAGYIFGLFLRITGGEPYLYLQPLIFYPGYYPDK    469
cht-1   ADLVYVILFPQLLCVVYMPRSNTYGSVAGYAVGLVLRLIGGEPLVSLPAFHYPMYT--D    469
         X                           XI

CHT1    NGIYNQRFPFKTLSMVTSFFTNICVSYLAKYLFESGTLPPKLDIFDAVVSR---HSEENM    526
cht-1   G---VQYFPFRTTAMLSSMATIYIVSIQSEKLFKSGRLSPEWDVMGCVVNIPIDHVPLPS    526
                          XII

CHT1    DKTILVRNENIKLNELAPVKPRQSITLSSTFTNKEALLDVDSSPEGSGTEDNLQ    580
cht-1   DVSFAVSSE--TLNMKAPNGTPAPVHPNQQPSDENTLLHPYSDQSYYSTNSN--    576
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Fig. 5

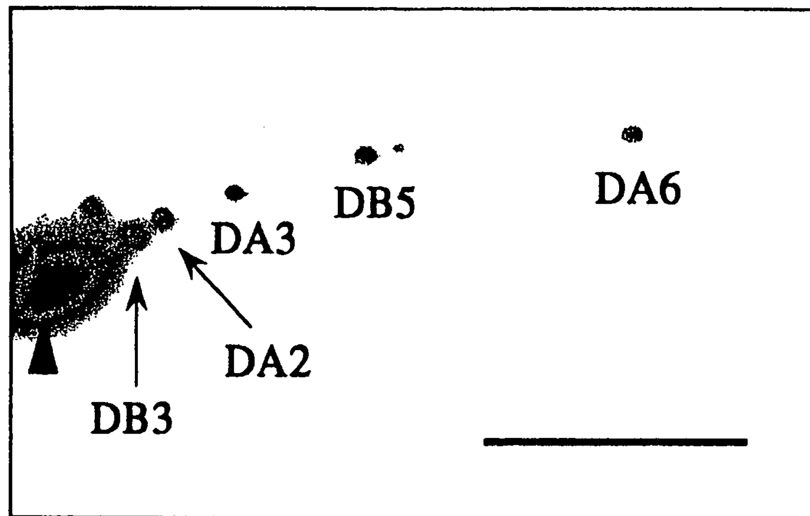


Fig. 6

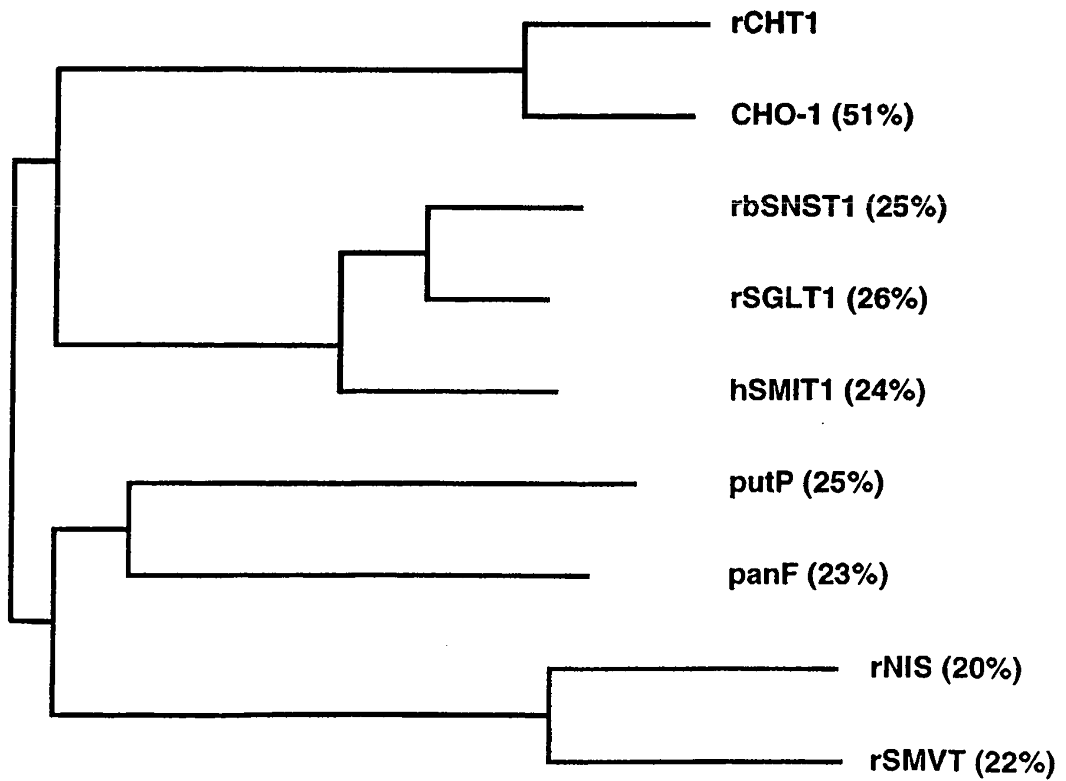


Fig. 7

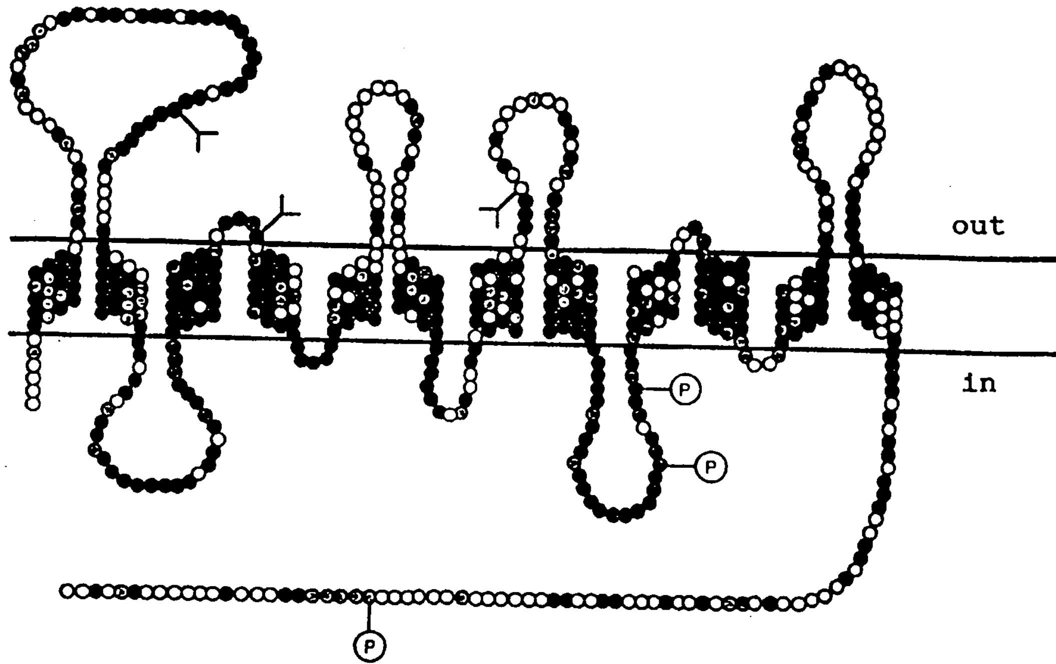


Fig. 8

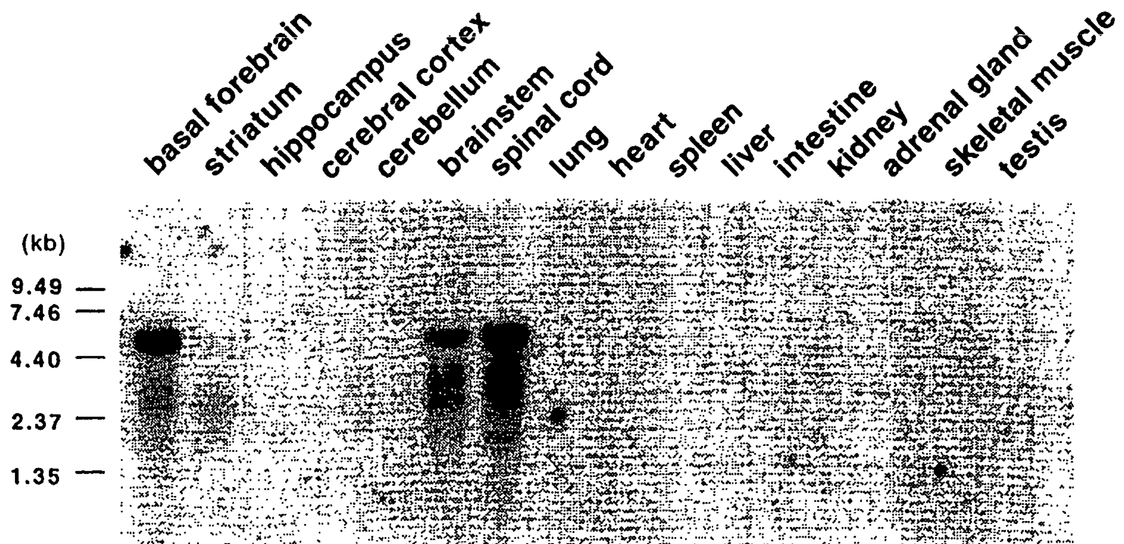


Fig. 9

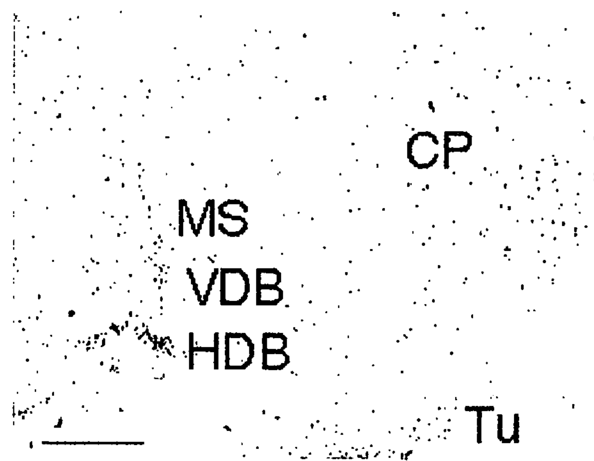


Fig. 10

Fig. 11

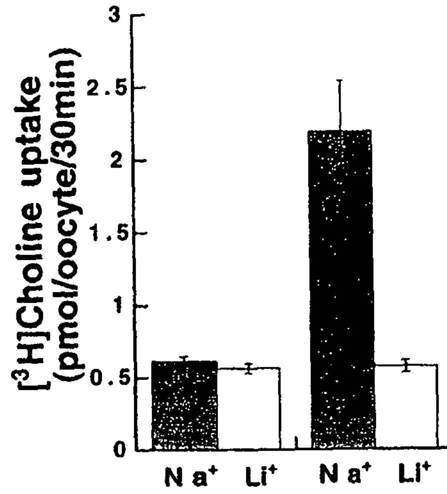


Fig. 12

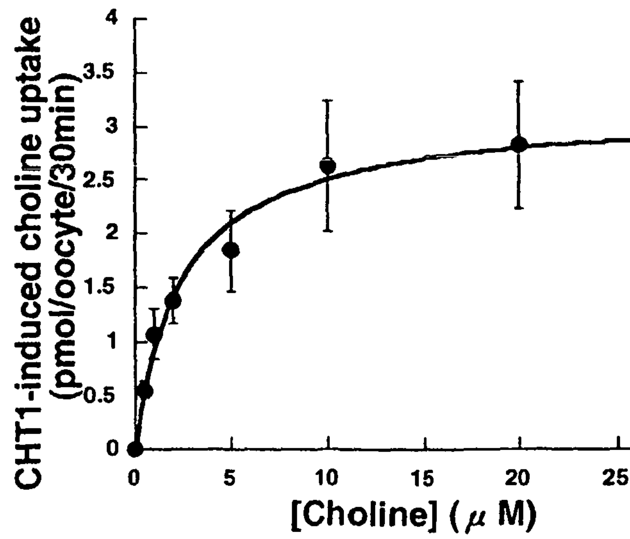


Fig. 13

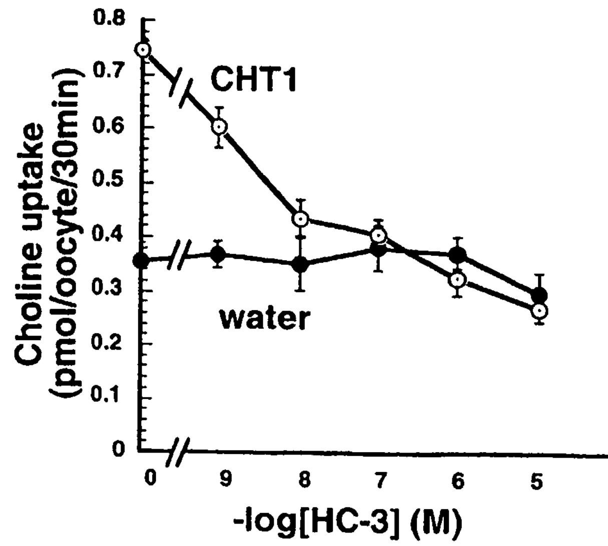


Fig. 14

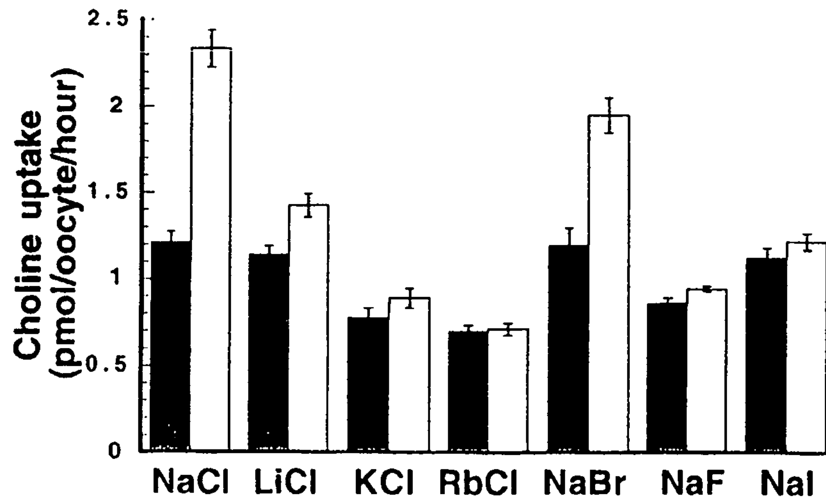


Fig. 15

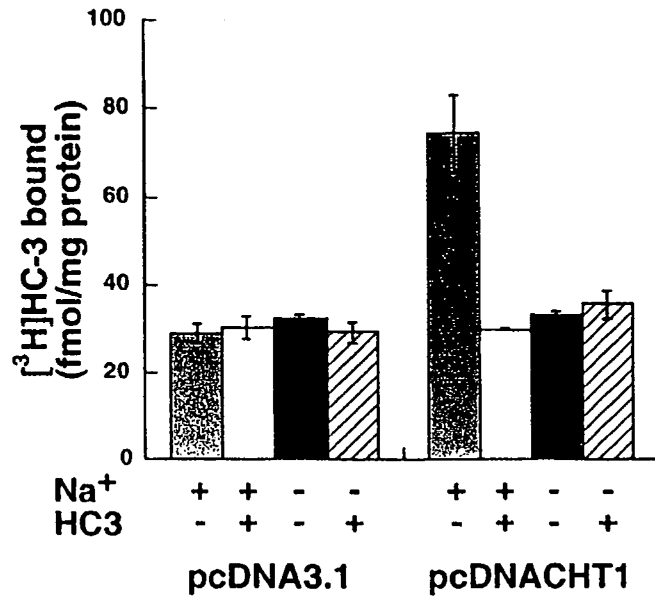


Fig. 16

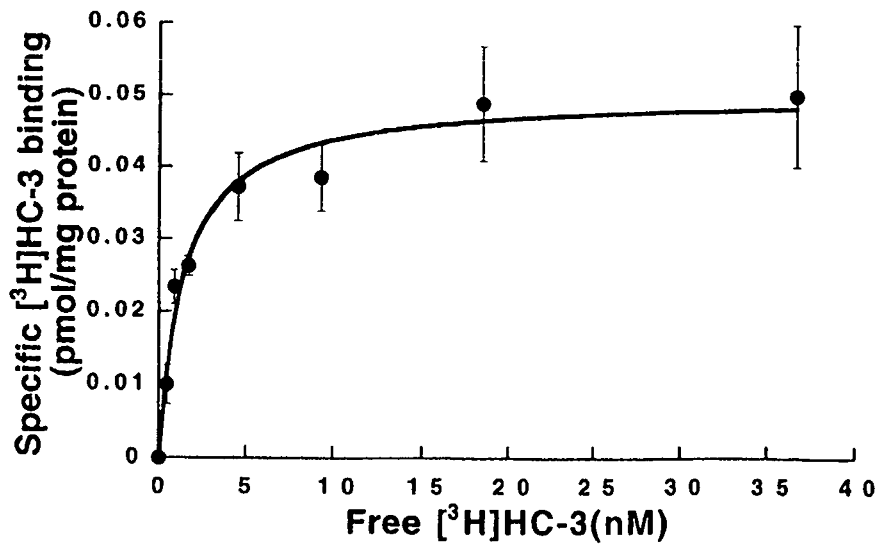
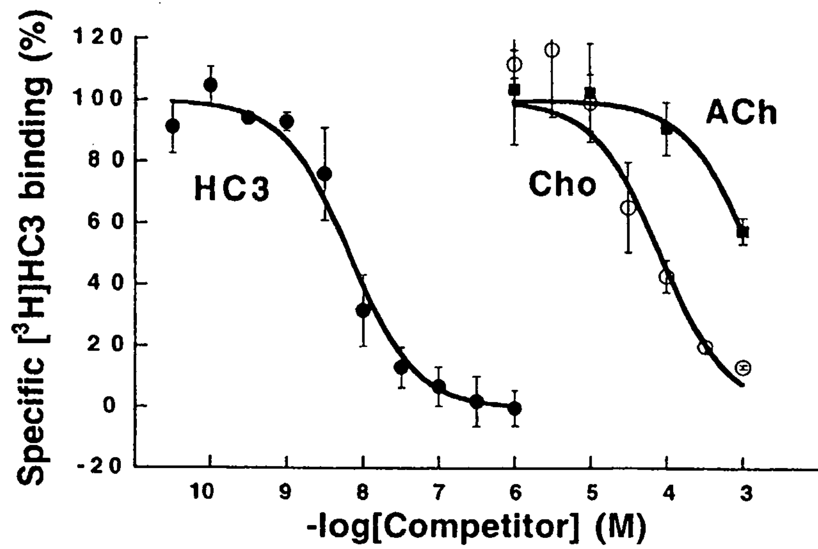


Fig. 17



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