(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) **EP 2 444 814 B9**

(12) **CORRECTED EUROPEAN PATENT SPECIFICATION**

(15) Correction information:
Corrected version no 1 (W1 B1)
Corrections, see
Description Paragraph(s) 7, 16, 18, 19, 21, 24, 37, 65, 70, 83

(48) Corrigendum issued on:
09.08.2017 Bulletin 2017/32

(45) Date of publication and mention of the grant of the patent:
28.12.2016 Bulletin 2016/52

(21) Application number: **10777546.2**

(22) Date of filing: **17.05.2010**

(51) Int Cl.:
G01N 33/68 *(2006.01)* ***G01N 21/78*** *(2006.01)*
G01N 27/62 *(2006.01)* ***G01N 33/53*** *(2006.01)*
G01N 33/543 *(2006.01)* ***C07K 14/47*** *(2006.01)*

(86) International application number:
PCT/JP2010/003295

(87) International publication number:
WO 2010/134308 (25.11.2010 Gazette 2010/47)

(54) **BIOMARKER FOR MENTAL DISORDERS INCLUDING COGNITIVE DISORDERS, AND METHOD USING SAID BIOMARKER TO DETECT MENTAL DISORDERS INCLUDING COGNITIVE DISORDERS**

BIOMARKER FÜR PSYCHISCHE ERKRANKUNGEN EINSCHLIESSLICH KOGNITIVER STÖRUNGEN UND VERFAHREN UNTER VERWENDUNG SOLCHER BIOMARKER ZUR ERKENNUNG PSYCHISCHER ERKRANKUNGEN EINSCHLIESSLICH KOGNITIVER STÖRUNGEN

BIOMARQUEUR POUR TROUBLES MENTAUX Y COMPRIS LES TROUBLES COGNITIFS, ET MÉTHODE DE DÉPISTAGE DES TROUBLES MENTAUX Y COMPRIS DES TROUBLES COGNITIFS À L'AIDE DUDIT BIOMARQUEUR

(84) Designated Contracting States:
AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO SE SI SK SM TR

(30) Priority: **19.05.2009 JP 2009121226**

(43) Date of publication of application:
25.04.2012 Bulletin 2012/17

(60) Divisional application:
16171946.3 / 3 088 899

(73) Proprietor: **Mcbi Inc.**
Ibaraki 305-0035 (JP)

(72) Inventors:
- **UCHIDA, Kazuhiko**
  Tsukuba-shi
  Ibaraki 305-0035 (JP)
- **ISHII, Takashi**
  Tsukuba-shi
  Ibaraki 305-0035 (JP)
- **MENO, Kohji**
  Tsukuba-shi
  Ibaraki 305-0035 (JP)
- **SUZUKI, Hideaki**
  Tsukuba-shi
  Ibaraki 305-0035 (JP)

(74) Representative: **TBK**
Bavariaring 4-6
80336 München (DE)

(56) References cited:
WO-A2-02/088322
WO-A2-03/087768
WO-A2-2006/035237
WO-A2-2007/045865
WO-A2-2008/090319
WO-A2-2008/090319
JP-T- 2005 536 729
JP-T- 2007 513 337
JP-T- 2008 514 946
US-A1- 2004 072 261

Printed by Jouve, 75001 PARIS (FR)

(Cont. next page)

- THOMAS C. SUDHOF: 'Neuroligins and neurexins link synaptic function to cognitive disease' NATURE vol. 455, 16 October 2008, pages 903 - 911, XP008156907
- VED P. S. CHAUHAN ET AL.: 'Binding of Gelsolin, a Secretory Protein, to Amyloid Beta-Protein' BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS vol. 258, 1999, pages 241 - 246, XP001019672
- HARUHIKO AKIYAMA: 'Alzheimer-byo Kanja No ni Okeru Ketsueki Gyoko to Sen'yokei System' DEMENTIA vol. 9, 1995, pages 124 - 131, XP008157447
- AKIHITO HISHIMOTO ET AL.: 'MRXN1: Novel mRNA Transcripts, Differential Expression in and Association with Alzheimer Disease' THE WORLD JOURNAL OF BIOLOGICAL PSYCHIATRY vol. 9, no. SUPP1, 2008, page 155, XP008152740

Description

[Technical Field]

[0001] The present invention relates to novel biomarkers for cognitive impairment including mild cognitive impairment and Alzheimer disease and methods for detecting cognitive impairment using such biomarkers. Simultaneously, the present invention relates to novel biomarkers for non-demented neurological disease like depression, schizophrenia, etc. and methods for detecting non-demented neurological diseases using such biomarkers.

[Background Art]

[0002] The commonly used means to differentiate between normal and non-normal states of a human subject using his or her biological materials are mainly those which have been used in the field of diagnostics. Most frequently used are those methods which target biomarkers in blood, It has been practiced in this field to comparatively measure the amount of a specific protein or a peptide that is less than 10,000 in molecular weight or, in the case of enzyme protein, enzyme activities in samples from normal (healthy) subjects and those from diseased individuals to help diagnosis. Here, prior to testing real samples, measurements are done on a fixed number each of samples from healthy controls and patients with certain disease with respect to the amount (s) or activity (activities) of single or multiple specific proteins or peptides and the ranges of abnormal and normal values are respectively determined. The sample to be evaluated is then analyzed by the same method and the resultant value is judged with respect to whether it is in normal or abnormal range.

[0003] In the actual measurements, the amount(s) of specified protein(s) or peptide(s) in test samples, as such or after dilution, are determined by the use of enzyme-linked immunosorbent assay (ELISA) which uses a primary, or secondary, antibody labeled with an enzyme redacting with a substrate that yields a color upon reaction, chemilurninescent immunoassay (CLIA), radioimmunoassay (RIA) which uses a primary, or secondary, antibody labeled with a radioisotope, and, if the protein is an enzyme, the measurement of the activity of the enzyme by adding its substrate and determining the intensity of produced color, etc. These antibody-based methods are called as enzyme-, fluorescence- or radioisotope-labeled methods, respectively. In addition, there is a method where an enzyme reation product derived from the corresponding substrate is determined by high performance liquid chromatography (HPLC). In further addition, there is a method where HPLC is combined with mass spectrometer, called LC-MS/MS, and there is a method called selected reaction monitoring (SRM)/multiple reaction monitoring (MRM) that utilizes LC-MS/MS. In another method to determine the concentration in a sample, it is appropriately pretreated, and separation of proteins or peptides is attained by 2-dimensional polyacrylamide gel electrophoresis (2D-PAGE), and target protein or peptide is determined by silver staining, Coomassie blue staining or immunological staining (Western blotting) that uses an antibody to target protein or peptide. In still further addition, there is a method which utilizes mass spectrometry to determine the amount of target protein or peptide in samples fractionated by column chromatography. Instead of column chromatography, protein chips and magnetic beads may also be utilized for purpose of pretreatment.

[0004] Furthermore, these inventors have developed an immunoMS method, where target protein or peptide is captured by beads (including magnetic ones) with linked antibody to the protein or peptide, eluted from the beads, and determined by mass spectrometry. Further, intact proteins have been reported to be analyzed by mass spectrometry using above-mentioned methods after digestion with trypsin etc. (PTL 1). Here, intact target proteins are selected either by fractionation or by adsorption to an adsorbant specific to them and then determined by mass spectrometry.

[0005] Number of patients suffered from cognitive impairment like Alzheimer disease is increasing rapidly along with increasing of old-age population in Japan. It is estimated that number of patients is 1.3 million in 1995 and it will be 1.9 million in 2005 and will reach to about 3.0 million in 2020. It is reported that 60-90% of cognitive impairment is Alzheimer disease. As manifestation of Alzheimer disease is not only loss of memory but several disturbance in daily work, increase of patients of this disease is becoming an important social issue to be solved. In Japan, since Donepezil-hydrochloride, anti-acetylcholine esterase inhibitor has been available for medical treatment for Alzheimer disease from 1999, it let progress of cognitive impairment in these patients be 'slow-down' efficiently, if the patient is diagnosed at early stage. Thus, in medication of Alzheimer disease, most important issue is 'early diagnosis' to treat the patients effectively by present drug and new coming drug.

[0006] Followings are major criteria for diagnosis of Alzheimer disease described in DSM IV, which is published by American Psychiatric Association (NPL 1).

A. The development of multiple cognitive deficits manifested by both

(1) memory impairment (impaired ability to learn new information or to recall previously learned information)
(2) one (or more) of the following cognitive disturbances:

a) aphasia (language disturbance)
b) apraxia (impaired ability to carry out motor activities despite intact motor function)
c) agnosia (failure to recognize or identify objects despite intact sensory function)
d) disturbance in executive functioning (i.e., planning, organizing, sequencing, abstracting)

B. The cognitive deficits in Criteria A 1 and A2 each cause significant impairment in social or occupational functioning and represent a significant decline from a previous level of functioning.

[0007] There are several types of neurological disorders related to Alzheimer disease (AD). As cognitive dysfunction appears gradually in dementia including AD, there is a disease status like pre-stage of dementia. This stage is called as mild cognitive impairment (MCI). In United States, 10% MCI develops to AD within 1 year and 50% of MCI develops to AD within 4 years. MCI is defined as a condition characterized by newly acquired cognitive decline to an extent that is beyond that expected for age or educational background, yet not causing significant functional impairment, and show no disturbance in daily life. Frontotemporal dementia (frontotemporal lobar degeneration) (FTD) shows loss of personal awareness, loss of social awareness, hyperorality, and stereotyped, perseverative behavior. These clinical characteristics are different from AD. FTD include Pick's disease, which is characterized in microscopically Pick bodies usually found in limbic, paralimbic, and ventral temporal lobe cortex. Dementia with Lewy bodies (DLB) is characterized by progressive disease and psychiatric symptoms include anxiety, depression, hallucinations (usually visual) and delusions (false beliefs). DLB is thought to be the second most common subtype and 10-30% of dementia is DLB. The symptoms of DLB are caused by the build-up of Lewy bodies histologically. FTD and DLB belong to demented neurological disease as they also lose of memory, their ability to solve problems and maintain emotional control (NPL 1). In description in present patent, cognitive impairment includes AD, MCI and the demented neurological disease.

[0008] The screening tests for dementia widely used are the Hasegawa Dementia Scale-revised (HDS-R) and Mini-Mental State Examination (MMSE). In these screening tests, inspector asks several questions and evaluates level of cognitive impairment of each subject by scores. HDS-R is revised version of HDS published in 1991. In HDS-R, test consists 9 questions to analyses orientation, remembrance, calculation, retain and recall ability, and common sense. Full score is 30 and a person whose score is less than 23 is suspected as dementia. MMSE has been developed in United States to screen and diagnose dementia, and analyses global cognitive function, with items assessing orientation, word recall, attention and calculation, language abilities, and visuospatial (drawing) ability. This test consists of 11 questions, and full score is 30 and a person who has score less than 23 is suspected as dementia. The results of HDS-R and MMSE coincide with each other. Both are used for screening, not for diagnosis and not for disease progression staging of dementia (NPL 1).

[0009] Neuroimaging for dementia are Computed tomography (CT) and Magnetic resonance imaging (MRI) which evaluate morphological changes like brain atrophy and ventricular dilation and single-photon emission computed tomography (SPECT) which analyses regional cerebral blood flow and PET which shows brain metabolism by measurement of consumption of oxygen and sugar. SPECT and PET, nuclear imaging technologies, can identify neuronal dysfunction at preclinical stage (NPL 1). However, these neuroimaging can not be widely used in hospitals because they need special facilities to performe nuclear imaging, and neuroimaging may not be objective test as imaging diagnosis is completely depend on the skill of doctor who analyses the images.

[0010] Thus, present methods for screening and diagnosis of dementia including AD is dependent on tests lacking objectivity and is dependent on expensive instruments, and so it is very difficult to use present available tests for screening of early stage of cognitive impairment. If we have blood (serum/plasma) biomarker for cognitive impairment, which enables us objective test using specimens we easily obtain, we can identify cognitive impairment at early stage (preclinical stage) by blood test using such biomarker. Present patent provides novel biomarkers and a novel and potent diagnostic method for cognitive impairment by using such biomarkers and biomarkers described here. In addition, present patent provides diagnostic method and novel biomarkers for non-demented neurological disease like depression, schizophrenia, etc.

[Citation List]

[Patent Literature]

[0011]

PTL 1, JP-A-2004-333274
PTL 2, JP-A-2006-308533

[Non Patent Literature]

[0012]

NPL 1, "The better understanding of Alzheimer's disease.," edited by Imaharu Nakano and HIdehiro Mizusawa., Nagai Shoten Co., Ltd., 2004 (in Japanese)
NPL 2, Benkirane, N. et al., J. Biol. Chem. Vol. 268, 26279-26285, 1993

[0013] WO03087768 (A2) discloses the provision of mitochondrial targets for drug screening assays and for therapeutic intervention in the treatment of diseases associated with altered mitochondrial function. Complete amino acid sequences [SEQ ID NOS:1-3025] of polypeptides that comprise the human heart mitochondrial proteome are provided, using fractionated proteins derived from highly purified mitochondrial preparations, to identify previously unrecognized mitochondrial molecular components.

[Summary of Invention]

[Technical Problem]

[0014] The present invention aims to present methods to detect cognitive impairment including mild cognitive impairment and Alzheimer disease by using a protein or its partial peptide that differs in presence or absence, or in quantity between non-cognitive impairment subjects (Including healthy people, the human subjects that may be affected with any disease and unaffected with cognitive impairment) and patients with cognitive impairment and further aims to present biomarkers comprising said protein and said partial peptide to be used to detect cognitive impairment including mild cognitive impairment and Alzheimer disease. Simultaneously, the present invention aims to present novel biomarkers for non-demented neurological disease like depression, schizophrenia, etc. and methods for detecting cognitive impairment using such biomarkers.

[Solution to Problem]

[0015] These inventors investigated to find out means to detect cognitive impairment and found a peptide capable of detecting cognitive impairment and psychiatric disease including mild cognitive impairment and Alzheimer disease in the serum. Said peptides found in the present invention are those with significance as a biomarker to detecting in the case of serum not only other biological materials such as blood, plasma, cerebrospinal fluid, and urine. Simultaneously, protein or peptide is the origin of these peptides (hereinafter referred to as intact proteins or peptides) also has significance as biomarkers. The present invention provides a peptide or protein as defined in claim 1, an in vitro method for detection of psychiatric disease or cognitive impairment as defined in claim 5, and a kit as defined in claim 7 or 8. Further beneficial embodiments are disclosed in the respective dependent claims.

[0016] Specifically, these inventors found that a biomarker comprising at least one protein selected from the group consisting of Neurexin-2-beta precursor consisting of amino acid sequence expressed by SEQ ID NO: 1, Prothrombin precursor consisting of amino acid sequence expressed by SEQ ID NO: 3, Pendrin consisting of amino acid sequence expressed by SEQ ID NO: 6, Coatomer subunit zeta-1 consisting of amino acid sequence expressed by SEQ ID NO: 8, Retinoic acid receptor responder protein 2 precursor consisting of amino acid sequence expressed by SEQ ID NO: 1.0, Gelsolin precursor consisting of amino acid sequence expressed by SEQ ID NO: 13, Clusterin precursor consisting of amino acid sequence expressed by SEQ ID NO: 15, Eukaryotic translation initiation factor 3 subunit J consistiog of amino acid sequence expressed by SEQ ID NO: 18, and Leucine-rich repeat-containing protein 27 consisting of amino acid sequence expressed by SEQ ID NO: 20 could be used as biomarkers to detect psychiatric disease or cognitive impairment.

[0017] Furthermore, these inventors found that a biomarker comprising at least one peptide selected from the group consisting of Neurexin-2-beta precursor-derived peptide NRX2B consisting of amino acid sequence expressed by SEQ ID NO: 2, Prothrombin precursor-derived peptide THRB(R-) consisting of amino acid sequence expressed by SEQ ID NO: 4, Prothrombin precursor-derived peptide THRB(R+) consisting of amino acid sequence expressed by SEQ ID NO: 5, Pendrin-derived peptide S26A4 consisting of amino acid sequence expressed by SEQ ID NO: 7, Coatomer subunit zeta-1-derived peptide COPZ1 consisting of amino acid sequence expressed by SEQ ID NO: 9, Retinoic acid receptor responder protein 2 precursor-derived peptide RARR2(S-) consisting of amino acid sequence expressed by SEQ ID NO: 11, Retinoic acid receptor responder protein 2 precursor-derived peptide RARR2(S+) consisting of amino acid sequence expressed by SEQ ID NO: 12, Gelsolin precursor-derived peptide GELS consisting of amino acid sequence expressed by SEQ ID NO: 14, Clusterin precursor-derived peptide CLUS(N-term SDVP) consisting of amino acid sequence expressed by SEQ ID NO: 16, Clusterin precursor-derived peptide CLUS(N-term RFFT) consisting of amino

acid sequence expressed by SEQ ID NO: 17, Eukaryotic translation initiation factor 3 subunit J-derived peptide EIF3J consisting of amino acid sequence expressed by SEQ ID NO: 19, and Leucine-rich repeat-containing protein 27-derived peptide LRC27 consisting of amino acid sequence expressed by SEQ ID NO: 21 could be used as biomarkers to detect psychiatric disease or cognitive impairment.

[0018] These inventors brought the present invention to perfection by further succeeding in determining simultaneously these many proteins and its partial peptides by using two-dimensional high performance liquid chromatography-MALDI TOFMS method (mass spectrometry) and immunoMS method.

[1] A biomarker for detection of psychiatric disease or cognitive impairment comprising protein fragment or peptide of not less than 5 amino acid residues arising from at least one protein or peptide selected from the group consisting of Neurexin-2-beta precursor consisting of amino acid sequence expressed by SEQ ID NO: 1, Prothrombin precursor consisting of amino acid sequence expressed by SEQ ID NO: 3, Pendrin consisting of amino acid sequence expressed by SEQ ID NO: 6, Coatomer subunit zeta-1 consisting of amino acid sequence expressed by SEQ ID NO: 8, Retinoic acid receptor responder protein 2 precursor consisting of amino acid sequence expressed by SEQ ID NO: 10, Gelsolin precursor consisting of amino acid sequence expressed by SEQ ID NO: 13, Clusterin precursor consisting of amino acid sequence expressed by SEQ ID NO: 15, Eukaryotic translation initiation factor 3 subunit J consisting of amino acid sequence expressed by SEQ ID NO: 18, Leucine-rich repeat-containing protein 27 consisting of amino acid sequence expressed by SEQ ID NO: 20.

[2] A biomarker for detection of psychiatric disease comprising at least one peptide selected from the group consisting of Neurexin-2-beta precursor-derived peptide NRX2B consisting of amino acid sequence expressed by SEQ ID NO: 2, Prothrombin precursor-derived peptide THRB(R-) consisting of amino acid sequence expressed by SEQ ID NO: 4, Prothrombin precursor-derived peptide THRB(R+) consisting of amino acid sequence expressed by SEQ ID NO: 5, Pendrin-derived peptide S26A4 consisting of amino acid sequence expressed by SEQ ID NO: 7, Coatomer subunit zeta-1-derived peptide COPZ1 consisting of amino acid sequence expressed by SEQ ID NO: 9, Retinoic acid receptor responder protein 2 precursor-derived peptide RARR2(S-) consisting of amino acid sequence expressed by SEQ ID NO: 11, Retinoic acid receptor responder protein 2 precursor-derived peptide RARR2(S+) consisting of amino acid sequence expressed by SEQ ID NO: 12, Gelsolin precursor-derived peptide GELS consisting of amino acid sequence expressed by SEQ ID NO: 14, Clusterin precursor-derived peptide CLUS(N-term SDVP) consisting of amino acid sequence expressed by SEQ ID NO: 16, Clusterin precursor-derived peptide CLUS(N-term RFFT) consisting of amino acid sequence expressed by SEQ ID NO: 17, Eukaryotic translation initiation factor 3 subunit J-derived peptide EIF3J consisting of amino acid sequence expressed by SEQ ID NO: 19, and Leucine-rich repeat-containing protein 27-derived peptide LRC27 consisting of amino acid sequence expressed by SEQ ID NO: 21.

[3] A biomarker for detection of cognitive impairment comprising at least one peptide selected from the group consisting of Neurexin-2-beta precursor-derived peptide NRX2B consisting of amino acid sequence expressed by SEQ ID NO: 2, Prothrombin precursor-derived peptide THRB(R-) consisting of amino acid sequence expressed by SEQ ID NO: 4, Prothrombin precursor-derived peptide THRB(R+) consisting of amino acid sequence expressed by SEQ ID NO: 5, Pendrin-derived peptide S26A4 consisting of amino acid sequence expressed by SEQ ID NO: 7, Coatomer subunit zeta-1-derived peptide COPZ1 consisting of amino acid sequence expressed by SEQ ID NO: 9, Retinoic acid receptor responder protein 2 precursor-derived peptide RARR2(S-) consisting of amino acid sequence expressed by SEQ ID NO: 11, Retinoic acid receptor responder protein 2 precursor-derived peptide RARR2 (S+) consisting of amino acid sequence expressed by SEQ ID NO: 12, Gelsolin precursor-derived peptide GELS consisting of amino acid sequence expressed by SEQ ID NO: 14, Clusterin precursor-derived peptide CLUS(N-term SDVP) consisting of amino acid sequence expressed by SEQ ID NO: 16, Clusterin precursor-derived peptide CLUS(N-term RFFT) consisting of amino acid sequence expressed by SEQ ID NO: 17, Eukaryotic translation initiation factor 3 subunit J-derived peptide EIF3J consisting of amino acid sequence expressed by SEQ ID NO: 19, and Leucine-rich repeat-containing protein 27-derived peptide LRC27 consisting of amino acid sequence expressed by SEQ ID NO: 21.

[4] A biomarker of cognitive impairment comprising the peptides selected from the group consisting of amino acid sequence expressed by SEQ ID NO: 2, 5, 7, 9, 11, 12, 14, and 16 that is appeared or increased in biological material of patients of cognitive impairment as compared to biological material of subjects not suffering from psychiatric disease.

[5] A biomarker of cognitive impairment comprising the peptides selected from the group consisting of amino acid sequence expressed by SEQ ID NO: 4, 17, 19, and 21 that is disappeared or decreased in biological material of patients of cognitive impairment as compared to biological material of subjects not suffering from psychiatric disease.

[6] A biomarker of Alzheimer disease comprising the peptides selected from the group consisting of amino acid sequence expressed by SEQ ID NO: 2 that is appeared or increased in biological material of patients of Alzheimer disease as compared to biological material of subjects not suffering from non-demented neurological disease.

[7] A biomarker of Alzheimer disease comprising the peptides selected from the group consisting of amino acid sequence expressed by SEQ ID NO: 4 that is disappeared or decreased in biological material of patients of Alzheimer disease as compared to biological material of subjects not suffering from non-demented neurological disease.

[8] Method for detection of psychiatric disease involving determination in biological material of at least one biomarker for psychiatric disease as described in [1] or [2].

[9] Method for detection of cognitive impairment involving determination in biological material of at least one biomarker for cognitive impairment as described in [1] or [3].

[10] Method for detection of cognitive impairment in which patient is judged as suffering from cognitive impairment when, after determination in biological material of at least one biomarker for cognitive impairment as described in [4], said biomarker is found to be present in higher quantity than in subjects not suffering from psychiatric disease.

[11] Method for detection of cognitive impairment in which patient is judged as suffering from cognitive impairment when, after determination in biological material of at least one biomarker for cognitive impairment as described in [5], said biomarker is found to be present in lower quantity than in subjects not suffering from psychiatric disease.

[12] Method for detection of psychiatric disease as described in [8] wherein detection is made either by immunoblot procedure, Western blotting, enzyme-, fluorescence-, or radioisotope-labeled antibody method, mass spectrometry, immunoMS method or surface plasmon resonance method.

[13] Method for detection of cognitive impairment as described in any of [9] to [11] wherein detection is made either by immunoblot procedure, Western blotting, enzyme-, fluorescence-, or radioisotope-labeled antibody method, mass spectrometry, immunoMS method or surface plasmon resonance method.

[14] A kit for detection of psychiatric disease to determine at least one biomarker as described in [1] or [2].

[15] A kit for detection of cognitive impairment to determine at least one biomarker as described in any of claims [1], [3] to [5].

[16] A kit for detection of psychiatric disease containing antibody or aptamer to at least one biomarker as described in [1] or [2].

[17] A kit for detection of psychiatric disease containing antibody or aptamer to at least one biomarker as described in any of claims [1], [3] to [5].

[18] A kit for detection as described in [16] or [17] wherein antibody or aptamer is solidified on a plate or plates.

[Advantageous Effects of Invention]

[0019] According to the present invention, it is possible to diagnose a subject as to whether said subject has suffered from psychiatric disease or cognitive impairment by determining in biological material obtained from said subject the kind and amount of at least one peptide selected from the group consisting of Neurexin-2-beta precursor-derived peptide NRX2B consisting of amino acid sequence expressed by SEQ ID NO: 2, Prothrombin precursor-derived peptide THRB(R-) consisting of amino acid sequence expressed by SEQ ID NO: 4, Prothrombin precursor-derived peptide THRB(R+) consisting of amino acid sequence expressed by SEQ ID NO: 5, Pendrin-derived peptide S26A4 consisting of amino acid sequence expressed by SEQ ID NO: 7, Coatomer subunit zeta-1-derived peptide COPZ1 consisting of amino acide sequence expressed by SEQ ID NO: 9, Retinoic acid receptor responder protein 2 precursor-derived peptide RARR2(S-) consisting of amino acid sequence expressed by SEQ ID NO: 11, Retinoic acid receptor responder protein 2 precursor-derived peptide RARR2(S+) consisting of amino acid sequence expressed by SEQ ID NO: 12, Gelsolin precursor-derived peptide GELS consisting of amino acid sequence expressed by SEQ ID NO: 14, Clusterin precursor-derived peptide CLUS(N-term SDVP) consisting of amino acid sequence expressed by SEQ ID NO: 16, Clusterin precursor-derived peptide CLUS(N-term RFFT) consisting of amino acid sequence expressed by SEQ ID NO: 17, Eukaryotic translation

initiation factor 3 subunit J-derived peptide EIF3J consisting of amino acid sequence expressed by SEQ ID NO: 19, and Leucine-rich repeat-containing protein 27-derived peptide LRC27 consisting of amino acid sequence expressed by SEQ ID NO: 21. In addition, it is possible to diagnose a subject has suffering from Alzheimer's disease when compared with the increase in biological material of patients of non-demented neurological disease by determining amount of peptide consisting of amino acid sequence expressed by SEQ ID NO: 2, and it is possible to diagnose a subject has suffering from Alzheimer's disease when compared with the decrease in biological material of patients of non-demented neurological disease by determining amount of peptide consisting of amino acid sequence expressed by SEQ ID NO: 4.

[0020] The present invention presents a diagnostic system that is high in both accuracy and specificity. The present invention enables highly accurate diagnosis of cognitive impairment in which there have been no specific test methods for such biological materials as blood. Further, the biomarkers disclosed in the present invention are highly useful in judgement of drug efficacy.

[Brief Description of Drawings]

[0021]

[Fig. 1]Figure 1 illustrates the isolation of serum of Alzheimer's disease by 2D-LC-MALDI TOF-MS method. (Example 1)

[Fig. 2]Figure 2 illustrates the case of Marker A that is the one example of the result of differential analysis. As shown in Figure 3, Marker A is Neurexin-2-beta precursor-derived peptides NRX2B. Figure 2A) illustrates the comparison between ADN, MCI and AD, and Figure 2B) illustrates the comparison between ADN, AD, NDall, NDdem and NDnon. For each samples, the average value (devided by 1,000) and (SD) (devided by 1,000) are as follows. A) ADN 0.1 (0.1); MCI 45.8 (42.2); AD 41.7 (22.2). B) ADN 0.1 (0.2); AD 34.0 (27.8); NDall 19.2 (15.8); NDdem 24.3 (20.8); NDnon 14.0 (6.1). C), D) and E) illustrates respectively the ROC curve of the comparison of MCI vs. ADN, AD vs. ADN, and AD vs. NDnon. (Example 1)

[Fig. 3]Figure 3 illustrates the MS/MS spectrum of Marker A (SEQ ID NO: 2, NRX2B) obtained by using TOF/TOF mass spectrometer. (Example 1)

[Fig. 4]Figure 4 illustrates the comparison between non-psychiatric disease subjects (ADN) and patients of psychiatric disease including sementia for THRB(R-) (A) and B) of Figure 4) and THRB(R+) (C) and D) of Figure 4) in serum. (Example 1)

[Fig. 5]Figure 5A) illustrates the each individual comparison between non-psychiatric disease subjects (ADN) and patients of cognitive impairment (MCI, AD) patient for THRB(R-) and THRB(R+) in serum. Figure 5B) illustrates the ROC curve of the comparison of AD vs. NDdon for THRB(R-) in serum. (Example 1)

[Fig. 6]Figure 6 illustrates the comparison (A) and B) of Figure 6) between non-psychiatric disease subjects (ADN) and patients of psychiatric disease including dementia for S264A in serum, and the comparison (C) and D) of Figure 6) between non-psychiatric disease subjects (ADN) and patients of psychiatric disease including dementia for COPZ1 in serum. (Example 1)

[Fig. 7]Figure 7 illustrates the each individual comparison between non-psychiatric disease subjects (ADN) and patients of cognitive impairment (MCI, AD) patient for PARR2(S-) (A) of Figure 7) and PARR2(S+) (B) of Figure 7) in serum. (Example 1)

[Fig. 8]Figure 8 illustrates the comparison (A) and B) of Figure 8) between non-psychiatric disease subjects (ADN) and patients of psychiatric disease including dementia for GELS in serum. (Example 1)

[Fig. 9]Figure 9 illustrates the comparison (A) and B) of Figure 9) between non-psychiatric disease subjects (ADN) and patients of psychiatric disease including dementia for CLUS(N-term SDVP) in serum, and the comparison (C) and D) of Figure 9) between non-psychiatric disease subjects (ADN) and patients of psychiatric disease including dementia for CLUS(N-term RFFT) in serum. (Example 1)

[Fig. 10] Figure 10 illustrates the comparison (A) and B) of Figure 10) between non-psychiatric disease subjects (ADN) and patients of psychiatric disease including dementia for EIF3J in serum, and the comparison (C) and D) of Figure 10) between non-psychiatric disease subjects (ADN) and patients of cognitive impairment (MCI, AD) patient

for LRC27 in serum. (Example 1)

[Fig. 11] Figure 11 illustrates the mass spectrum of NRX2B peptide that captured and detected by immunoMS method using NRX2B-specific antibody from the serum of AD and MCI patients. The right figure is the enlarged view of arrow parts in the left figure. Endogenous NRX2B peptide (solid arrows) and stable isotope labeled-NRX2B synthetic peptide NRX2B (dashed arrows) are shown for each peak in right figure. (Example 4)

[Description of Embodiments]

[0022] The present invention is a method for determining the kind and the amount of intact protein and/or its partial peptide when test subject is suffering from cognitive impairment as well as for diagnosing whether test subject is suffering from cognitive impairment and, if test subject is diagnosed to be suffering from psychiatric disease. A peptide is generally said to be a chemical entity, made by polymerizing a number of amino acids, of less than 10,000 in molecular weight or by polymerizing several to less than about 50 amino acid residues. While in the present invention a partial peptide of an intact protein can be used as a biomarker for detection of cognitive impairment, such partial peptide is defined as a peptide of less than 10,000 in molecular weight consisting of a part of the amino acid sequence of the intact protein. Such peptide may arise as a partial peptide during the expression by transcription followed by synthesis by translation before maturing into an intact protein or as a peptide produced by enzyme digestion in the body after the intact protein has been synthesized. It is possible that, when the body is in abnormal state suffering from such disease as cognitive impairment, the mechanism for protein synthesis and regulation is de-regulated. In other words, the present invention is also a method for determining if test subject is in normal state or is suffering from cognitive impairment by using the degree of protein synthesis and/or protein digestion as an indicator. The detection of cognitive impairment in the present invention means evaluation and differentiation, i.e., diagnosis of test subject as to whether the subject is suffering from cognitive impairment. The present invention can also include the evaluation of patient's risk of suffering from more serious cognitive impairment.

[0023] Specifically, in a method, the examples of intact protein that can be used as a cognitive impairment include Neurexin-2-beta precursor consisting of amino acid sequence expressed by SEQ ID NO: 1, Prothrombin precursor consisting of amino acid sequence expressed by SEQ ID NO: 3, Pendrin consisting of amino acid sequence expressed by SEQ ID NO: 6, Coatomer subunit zeta-1 consisting of amino acid sequence expressed by SEQ ID NO: 8, Retinoic acid receptor responder protein 2 precursor consisting of amino acid sequence expressed by SEQ ID NO: 10, Gelsolin precursor consisting of amino acid sequence expressed by SEQ ID NO: 13, Clusterin precursor consisting of amino acid sequence expressed by SEQ ID NO: 15, Eukaryotic translation initiation factor 3 subunit J consisting of amino acid sequence expressed by SEQ ID NO: 18, and Leucine-rich repeat-containing protein 27 consisting of amino acid sequence expressed by SEQ ID NO: 20, and further, the peptide fragments that comprise of partial peptides of not less than 5 amino acid residues of these intact proteins can be used as same purpose.

[0024] Still further, an example of biomarkers for cognitive impairment includes the partial peptides consisting of amino acid sequence expressed by SEQ ID NO: 2 of Neurexin-2-beta precursor-derived peptide NRX2B, SEQ ID NO: 4 of Prothrombin precursor-derived peptide THRB(R-), SEQ ID NO: 5 of Prothrombin precursor-derived peptide THRB(R+), SEQ ID NO: 7 of Pendrin-derived peptide S26A4, SEQ ID NO: 9 of Coatomer subunit zeta-1-derived peptide COPZ 1, SEQ ID NO: 11 of Retinoic acid receptor responder protein 2 precursor-derived peptide RARR2(S-), SEQ ID NO: 12 of Retinoic acid receptor responder protein 2 precursor-derived peptide RARR2(S+), SEQ ID NO: 14 of Gelsolin precursor-derived peptide GELS, SEQ ID NO: 16 of Clusterin precursor-derived peptide CLUS(N-term SDVP), SEQ ID NO: 17 of Clusterin precursor-derived peptide CLUS(N-term RFFT), SEQ ID NO: 19 of Eukaryotic translation initiation factor 3 subunit J-derived peptide EIF3J, SEQ ID NO: 21 of Leucine-rich repeat-containing protein 27-derived peptide LRC27. In the present invention, proteins and peptides consisting of amino acid sequences derived from SEQ ID NO: 1 or 2 by deletion, exchange, and/or addition of one or a few amino acids can be used as biomarkers and are included in the present invention. "'One or a few" herein means "one or three," preferably "one or two", or "one." Furthermore, the partial peptides that can be used as biomarkers include those peptide fragments consisting of not less than 5 amino acid residues arising respectively from SEQ ID NO: 1 to 21. The basis for the limitation of peptide fragments consisting of not less than 5 amino acid residues is in the description below in Non-patent Document 2. The document reported that an antibody obtained by using the peptide IRGERA as immunogen, which was the C-terminus (130-135) of histone H3, recognized the peptide IKGERA derived by exchange of K for R and the peptide CGGGERA which was derived by deletion of IR followed by addition of CGG. This demonstrates that the immunogenicity (antigenicity) is recognized by a peptide of not less than 4 amino acid residues. In order to expand this finding to other peptides than the C-terminus of histone H3, the number of amino acid residue is defined as not less than 5 instead of 4. To make such a low molecular weight peptide is important when the method of detection and differentiation uses immunological means including immnoblot, ELISA and immunoMS.

[0025] It is to be noted that there are cases where a sugar chain or sugar chains have been added to an intact protein

or its partial peptide to form glycated entities. Proteins and partial peptides in glycated form can also be used as biomarkers for detection of cognitive impairment.

[0026] It is also to be noted that, in the present invention, biomarker can be quantified or its presence or absence can be determined qualitatively.

[0027] Two-dimensional electrophoresis (2-DE) or 2-dimensional chromatography (2-DC) can be used in the present invention to separate biomarkers in biological materials including serum. Known chromatographic methods can be selected from ion-exchange chromatography, reverse-phase chromatography and gel-filtration chromatography. It is also possible to make quantification with the SRM/MRM method in LC-MS/MS technology. Furthermore, the immunoMS method which these inventors have developed, where target protein or peptide is captured by beads (including magnetic ones) with antibody linked to the protein or peptide, eluted from the beads, and determined by mass spectrometry enables convenient determination of presence or absence or the amount of target protein, protein fragment or peptide without the use of 2-DE or chromatography.

[0028] It is possible with the use of the method disclosed in the present invention to evaluate at the stage of mild of cognitive dysfunction in test subject and therefore it can be useful in prophylactic medicine. Further, when psychotherapy and/or drug therapy is given to patients with cognitive impairment, it is reflected in the amount of proteins and partial peptides in biological materials such as serum if the progression of the disorder has been inhibited. Therefore, by measuring these proteins and partial peptides, it is possible to evaluate and determine therapeutic effect.

[0029] The kind and amount of a protein in biological materials can be determined by various methods. If target protein (including protein fragment and partial peptide) has been characterized and when an antibody (primary antibody) to it has already been obtained, the following methods can be used:

1. Immunoblot

[0030] This is one of the simplest methods. Test serum in a fixed amount (about 1 microliter) after stepwise dilution is dropped onto an approriate membrane such as of nitrocellulose and dried in air. The membrane is treated with a blocking solution containing a protein such as BSA, washed, reacted with primary antibody, and washed. Thereafter, the membrane is reacted with labeled secondary antibody to detect the primary antibody. The membrane is washed and the label is visualized to measure its density.

2. Western blotting

[0031] After separation with one-dimentional or two-dimentional electrophoresis involving isoelectric foucusing or SDS-PAGE, proteins are transferred onto such an approriate membrane as of nitrocellulose and their amounts are determined, as in above-mentioned immunoblot, using primary antibody and labeled secondary antibody.

3. ELISA

[0032] Antibody to protein or its partial peptide is fixed to such a plate as a chemically modified microtiter plate. Appropriate amounts of samples after stepwise dilution are applied to the plate and incubated. Proteins and peptides not captured are removed by washing. Next, the plate is incubated with secondary antibody labeled with fluorescent or chemiluminescent substance or enzyme. After addition of respective substrate, fluorescence, chemiluminescence or visible light due to enzyme reaction is measured for evaluation and judgement.

[0033] Additional examples of methods are illustrated below (see PTL 2) but the invention is not limited by these examples.

4. Methods that use microarray (microchip)

[0034] A microarray is a general term for devices where solidified materials with affinity for target substances are arrayed on solid support (plate). In the present invention, antibodies or aptamers to proteins and partial peptides are arrayed. A sample of biological material is placed on the microarray for fixation of target proteins or partial peptides and the microarray is then incubated with secondary antibody labeled with fluorescent or chemiluminescent substance or enzyme. After addition of respective substrate, fluorescence, chemiluminescence or visible light due to enzyme reaction is measured.

5. Mass Spectrometry

[0035] In mass spectrometry, for example, antibody to a specified protein or partial peptide is attached to chemically modified microbeads or plate (protein chip). The microbeads could be magnetic beads. There are no requirements for

the material of the plate. The antibody to be used could be (1) an antibody which recognizes the full length form of the specified protein only, (2) an antibody which recognizes a partial peptide only, (3) all of antibodies which recognizes both the specified protein and its partial peptide, or a combination of (1) and (2), (1) and (3), or (2) and (3). Samples after stepwise dilution with original solvent or buffer are added to the microbeads or plate carrying antibody or antibodies and incubated. Those proteins and partial peptides not captured are removed by washing. The protein or partial peptide captured by micorbeads or plate is eluted, and analyzed by mass spectrometry with MALDI-TOF-MS, SELDI-TOF-MS, etc. Measurements are made with respect to the mass and intensity of the peak due to the protein, protein fragment or partial peptide. Prior to the measurements a fixed amount of substance serving as the internal standard is added to the original biological material and the intensity of its peak is also measured. The concentration of the target in the original biological material can be calculated from the ratio of peak intensity of the target to the peak intensity of the internal standard. This is called immunoMS method. Further, it is possible to make quantification, after the sample is diluted with original solvent or buffer, or after part of proteins are removed, by separation with HPLC followed by mass spectrometry with electrospray ionization (ESI) method. Therein the SRM/MRM method can be utilized for absolute quantification with the use of an isotope-labeled internal standard peptide.

[0036] Furthermore, in addition to the above-mentioned methods, it is possible to analyze proteins and partial peptides by using 2-DE, surface plasmon resonance, etc.

[0037] The present invention includes the method to detect cognitive impairment from the presence or absence or amount of the above-mentioned biomarker after applying biological material obtained from test subject to 2-DE or surface plasmon resonance.

[Example 1]

[0038] Discovery of a marker peptide for detection of cognitive impairment using two-dimensional liquid chromatography (2D-LC)-MALDI TOF-MS.

(1) Serum samples.

[0039] Followings, the characters before the parenthesis are an abbreviation.

[0040] A sera obtained from 20 AD (Alzheimer's disease), 20 ADN (subjects not suffering from psychiatric disease and age and sex-matched patients with AD, "N" means normal), and 20 NDall (neurological disease) were used. NDall consists of 10 NDdem (demented neurological disease) and 10 NDnon (non-demented neurological disease). Furthermore, NDdem consists of dementia with Lewy body and frontotemporal dementia each consisting of 5 cases, and NDnon consists of schizophrenia and depression each consisting of 5 cases.

(2) Methods

[0041] After 475 μl of 0.1 % trifluoroacetic acid (TFA) were added in each of 25 μl of sera, samples were boiled for 15 min at 100 degrees. Subsequently, In order to recover peptides of molecular weight of 10,000 or less, ultrafiltration were performed by using YM-10 filter unit (Millipore Corp.). Then the analysis using 2D-LC-MALDI TOF-MS were performed as follows. In other words, recovering samples were fractionated to 1,146 fractions per sample by using two-dimensional HPLC (SCX cation exchange column and C18 reverse-phase column). All fractionated samples were spotted on MALDI target plate for MALDI TOF/TOF mass spectrometer (ultraflex TOF/TOF, Bruker Daltonics), and matrix solution (alpha-cyano-hydroxycinnamic acid, CHCA) were mixed and crystallized, and the mass and the peak area of the mass were measured automatically in refrectron mode by irradiating to crystallised sample by laser. Peak area was normalized with 250 fmole of per each well of bradykinin 1-7 fragment that was added into matrix solution in advance. In other words, the area value was calculated in 10,000 times of the value dividing the peak area in specific mass of sample by the peak area obtained from 250 fmole of bradykinin1-7 fragment. This area value is corresponding in 25 μl of sample serum. Detection of difference in abundance of peptides in serum between groups (called differential analysis) was performed using multi-group statistical analysis software DeView developed by us. Peptide that was observed to difference in abundance was directly determined amino acid sequence in MS/MS analysis by ultraflex TOF/TOF, and intact proteins or peptides of their origin were identified.

(3) Results

[0042] Figure 1 shows the result that was obtained from serum of one case of AD patient that was applied to 2D-LC-MALDI TOF-MS. Sample was fractionated into 6 fractions by SCX cation exchange column in the first dimension, then each of fractions were fractionated into 191 fractions by C18 reverse-phase column. Mass spectra of 191 fractions were obtained by MALDI TOF-MS measuring. As the horizontal axis is the m/z and the vertical axis is the fractions of reverse-

phase column chromatography, Figure 1 was visualized by Deview. SCX 1 shows flow-through fractions, SCX 2 shows fractions eluted in 10% salt concentration, SCX 3 shows fractions eluted in 20% salt concentration, SCX 4 shows fractions eluted in 30% salt concentration, SCX 5 shows fractions eluted in 50% salt concentration, SCX 6 shows fractions eluted in 100% salt concentration. As seen in Figure 1, many peptides in many sera are present in fractions of SCX1, SCX 3, SCX 4, and SCX 5. Total numbers of peptides fractionated by 2D-LC and detected by MALDI TOF-MS were about 4,000.

[0043] As one example of the results of differential analysis, Figure 2 shows the case of Marker A. Marker A, as shown later in Figure 3, is Neurexin-2-beta precursor-derived peptide NRX2B. A) of Figure 2 shows a comparison between ADN, MCI, AD. A) and B) shows the result of experiments carried out separately, ADN and AD were used the same samples in both experiments (ie, for ADN and AD, the measurement results would indicate the reproducibility.). In A) of Figure 2, it was found that Marker A is increased in MCI and AD patients than ADN patient. In B) of Figure 2, it was found that Marker A is increased in AD, NDall, NDdem, and NDnon patients than ADN patient. In particular, in comparison of AD and NDnon, AD was significantly higher than NDnon (t-test, $p = 0.035$). From these results, it was found that Marker A was useful to distinguish between cognitive impairment (MCI, AD, NDdem) patient and non-demented neurological disease (NDnon).

[0044] From the results of A) and B) in Figure 2, in order to evaluate the extent to which the Marker A is useful as biomarker, the analysis by receiver operating characteristic (ROC) curve was performed. C), D) and E) in Figure 2 shows respectively the ROC curve of the comparison of MCI vs. ADN, AD vs. ADN, and AD vs. NDnon. If the area value (hereinafter referred to as the ROC value) of under the ROC curve is close to 1, the usefulness as biomarker of Marker A will be higher. In C), D), E) of Figure 2, the typical values of sensitivity and specificity are the values of the point (open square in the figure) of the coordinate on ROC curve that the distance is minimized when a straight line was drawn to ROC curve from the point of 100% on y-axis. The value of cut-off giving this point becomes a useful threshold to distinguish between the different groups, and the values of sensitivity and specificity at that time (ie, above the typical values) becomes an indicator of the usefulness of biomarkers together with ROC values. In C) of Figure 2, as typical values in MCI vs. ADN, the sensitivity was 90%, the specificity was 100%, and the ROC value was 0.99. In D) of Figure2, as typical values in AD vs. ADN, the sensitivity was 100%, the specificity was 100%, and the ROC value was 1. In E) of Figure 2, it was comparing between AD vs. CCC, the sensitivity was 100%, the specificity was 50%, and the ROC value was 0.710. Thus, it was revealed that Marker A (NRX2B) was useful to distinguish MCI and AD with ADN. And also, it was revealed that Marker A was useful to distinguish AD with non- demented neurological disease (NDnon). In particular, since MCI is the state of previous stage of AD, Marker A (NRX2B) is considered to be a extremely useful marker to detect MCI for early diagnosis of potential subjects to migrate to AD.

[0045] Figure 3, for Marker A, illustrates the results of MS/MS spectrum using ultraflex TOF/TOF. The signals that show y-ions and b-ions have enough appeared, and the amino acid sequence could be readily identified. Mascot search was performed on this result, and the protein of origin or the peptide (hereinafter referred to as intact proteins or peptides) is Neurexin-2-beta precursor, and the detected peptide was found that the sequence is RSGGNATLQVDSWP (SEQ ID NO: 2). NRX2B of entry name of Swiss-Prot against Neurexin-2-beta precursor will use as an abbreviation of the peptide name. Also as for other peptides that were detected, the entry names of Swiss-Prot will be used as abbreviations of the peptide names in the following descriptions.

[0046] Including the Marker A, the peptides that have difference in abundance between the groups in serum were measured MS/MS spectra using ultraflex TOF/TOF,and in addition to determining the amino acid sequence, the results identified intact proteins or peptides were shown below. For peptides other than Marker A, the signals that show y-ions and b-ions has enough appeared, and the amino acid sequence could be readily identified. The following amino acid sequence that shows a set of two sequences, the entire sequence of the first sequence shows the amino acid sequence of intact proteins or peptides. The peptide comprising of the underlined portion of the first sequence and the second sequence is peptide detected by 2D-LC-MALDI TOF-MS. 001 represents N-terminus. About the peptide that was sequenced with the state of oxidation of methionine in the peptides consisting of the second sequence, was marked (+ Oxidation (M)) at the end of the amino acid sequence. For the protein with mutation of amino acid by gene mutation, applicable amino acid residue was expressed with (X).

(1) Neurexin-2-beta precursor-derived peptide NRX2B

[0047] NRX2B shown as SEQ ID NO: 2 was not detected in ADN patient, and was detected in MCI, AD, NDall, NDdem, and NDnon patients. Furthermore, in comparison of AD and NDnon, AD shown higher value than NDnon, NRX2B was shown distinction ability (previously described in Figure 2).

Intact protein / peptide

001 MPPGGSGPGG CPRRPPALAG PLPPPPPPPP PPLLPLLPLL LLLLLGAAEG
051 ARVSSSLSTT HHVHHFHSKH GTVPIAINRM PFLTRGGHAG TTYIFGKGGA
101 LITYTWPPND RPSTRMDRLA VGFSTHQRSA VLVRVDSASG LGDYLQLHID
151 QGTVGVIFNV GTDDITIDEP NAIVSDGKYH VVRFTRSGGN ATLQVDSWPV

201 NERYPAGNFD NERLAIARQR IPYRLGRVVD EWLLDKGRQL TIFNSQAAIK
251 IGGRDQGRPF QGQVSGLYYN GLKVLALAAE SDPNVRTEGH LRLVGEGPSV
301 LVASAECPSD DEDLEECEPS TGGELILPII TEDSLDPPPV ATRSPFVPPP
351 PTFYPFLTGV GATQDTLPPP AARRPPSGGP CQAERDDSDC EEPIEASGFA
401 SGEVFDSSLP PTDDEDFYTT FPLVTDRTTL LSPRKPAPRP NLRTDGATGA
451 PGVLFAPSAP APNLPAGKMN HRDPLQPLLE NPPLGPGAPT SFEPRRPPPL
501 RPGVTSAPGF PHLPTANPTG PGERGPPGAV EVIRESSSTT GMVVGIVAAA
551 ALCILILLYA MYKYRNRDEG SYQVDQSRNY ISNSAQSNGA VVKEKAPAAP
601 KTPSKAKKNK DKEYYV (SEQ ID NO: 1)

[0048] Neurexin-2-beta precursor-derived peptide NRX2B

RSGGNATLQVDSWP (SEQ ID NO: 2)

(2) Prothrombin precursor-derived peptide (THRB(R-))

[0049] Prothrombin precursor-derived peptides are two types, and (R-) means the peptide lacking of R (Arginine residue) of C-terminus. THRB(R-) shown as SEQ ID NO: 4 was detected specifically in ADN patient, and was detected extremely low value in MCI, AD, NDall, NDdem, NDnon patients. Diagrams of THRB(R-) and THRB(R+) showed side by side in Figure 4 and Figure 5. Figure 4 shows scatter plot. Figure 5A) shows that the appearance of THRB(R-) and THRB(R+) how is different in every individual of ADN, MCI and AD. In the same individual, THRB(R-) has appeared overwhelmingly in ADN, THRB(R+) has appeared overwhelmingly in MCI and AD. It can be said that both peptides are extremely useful markers determining MCI and ADN. Figure 5B) shows ROC curve comparing AD and NDnon of THRB(R-). ROC value indicated a high value of 0.815. the value in AD was lower compared to NDnon. In other words, it has been found that THRB(R-) as well as NRX2B is useful marker to distinguish between patients of cognitive impairment (MCI, AD, NDdem) and patients of non-demented neurological disease (NDnon).

Intact protein / peptide

001 MAHVRGLQLP GCLALAALCS LVHSQHVFLA PQQARSLLQR VRRANTFLEE
051 VRKGNLEREC VEETCSYEEA FEALESSTAT DVFWAKYTAC ETARTPRDKL
101 AACLEGNCAE GLGTNYRGHV NITRSGIECQ LWRSRYPHKP EINSTTHPGA
151 DLQENFCRNP DSSTTGPWCY TTDPTVRRQE CSIPVCGQDQ VTVAMTPRSE
201 GSSVNLSPPL EQCVPDRGQQ YQGRLAVTTH GLPCLAWASA QAKALSKHQD
251 FNSAVQLVEN FCRNPDGDEE GVWCYVAGKP GDFGYCDLNY CEEAVEEETG
301 DGLDEDSDRA IEGRTATSEY QTFFNPRTFG SGEADCGLRP LFEKKSLEDK
351 TERELLESYI DGRIVEGSDA EIGMSPWQVM LFRKSPQELL CGASLISDRW
401 VLTAAHCLLY PPWDKNFTEN DLLVRIGKHS RTRYERNIEK ISMLEKIYIH
451 PRYNWRENLD RDIALMKLKK PVAFSDYIHP VCLPDRETAA SLLQAGYKGR
501 VTGWGNLKET WTANVGKGQP SVLQVVNLPI VERPVCKDST RIRITDNMFC
551 AGYKPDEGKR GDACEGDSGG PFVMKSPFNN RWYQMGIVSW GEGCDRDGKY
601 GFYTHVFRLK KWIQKVIDQF GE (SEQ ID NO: 3)

[0050] Prothrombin precursor-derived peptide THRB(R-)

GLDEDSDRAIEG (SEQ ID NO: 4)

(3) Prothrombin precursor-derived peptide (THRB(R+))

[0051] THRB(R+) shows as SEQ ID NO: 5 was not detected in ADN patient, and was detected in MCI, AD, NDall, NDdem, and NDnon patients (Figure 4). (R+) means that the peptide having of R (Arginine residue) of C-terminus. For explanation, refer to (2) Prothrombin precursor-derived peptide (THRB(R-)).

Intact protein / peptide

001 MAHVRGLQLP GCLALAALCS LVHSQHVFLA PQQARSLLQR VRRANTFLEE
051 VRKGNLEREC VEETCSYEEA FEALESSTAT DVFWAKYTAC ETARTPRDKL
101 AACLEGNCAE GLGTNYRGHV NITRSGIECQ LWRSRYPHKP EINSTTHPGA
151 DLQENFCRNP DSSTTGPWCY TTDPTVRRQE CSIPVCGQDQ VTVAMTPRSE
201 GSSVNLSPPL EQCVPDRGQQ YQGRLAVTTH GLPCLAWASA QAKALSKHQD
251 FNSAVQLVEN FCRNPDGDEE GVWCYVAGKP GDFGYCDLNY CEEAVEEETG
301 DGLDEDSDRA IEGRTATSEY QTFFNPRTFG SGEADCGLRP LFEKKSLEDK
351 TERELLESYI DGRIVEGSDA EIGMSPWQVM LFRKSPQELL CGASLISDRW
401 VLTAAHCLLY PPWDKNFTEN DLLVRIGKHS RTRYERNIEK ISMLEKIYIH
451 PRYNWRENLD RDIALMKLKK PVAFSDYIHP VCLPDRETAA SLLQAGYKGR
501 VTGWGNLKET WTANVGKGQP SVLQVVNLPI VERPVCKDST RIRITDNMFC
551 AGYKPDEGKR GDACEGDSGG PFVMKSPFNN RWYQMGIVSW GEGCDRDGKY
601 GFYTHVFRLK KWIQKVIDQF GE (SEQ ID NO: 3)

[0052] Prothrombin precursor-derived peptide THRB(R+)

GLDEDSDRAIEGR (SEQ ID NO: 5)

(4) Pendrin-derived peptide (S26A4)

[0053] S26A4 shows as SEQ ID NO: 7 was not detected in ADN patient, and was detected in MCI, AD, NDall, NDdem, and NDnon patients (Figure 6).

Intact protein / peptide

001 MAAPGGRSEP PQLPEYSCSY MVSRPVYSEL AFQQQHERRL QERKTLRES
051 AKCCSCSRKR AFGVLKTLVP ILEWLPKYRV KEWLLSDVIS GVSTGLVATL
101 QGMAYALLAA VPVGYGLYSA FFPILTYFIF GTSRHISVGP FPVVSLMVGS
151 VVLSMAPDEH FLVSSSNGTV LNTTMIDTAA RDTARVLIAS ALTLLVGIIQ
201 LIFGGLQIGF IVRYLADPLV GGFTTAAAFQ VLVSQLKIVL NVSTKNYNGV
251 LSIIYTLVEI FQNIGDTNLA DFTAGLLTIV VCMAVKELND RFRHKIPVPI
301 PIEVIVTIIA TAISYGANLE KNYNAGIVKS IPRGFLPPEL PPVSLFSEML
351 AASFSIAVVA YAIAVSVGKV YATKYDYTID GNQEFIAFGI SNIFSGFFSC
401 FVATTALSRT AVQESTGGKT QVAGIISAAI VMIAILALGK LLEPLQKSVL
451 AAVVIANLKG MFMQLCDIPR LWRQNKIDAV IWVFTCIVSI ILGLDLGLLA
501 GLIFGLLTVV LRVQFPSWNG LGSIPSTDIY KSTKNYKNIE EPQGVKILR
551 SSPIFYGNVD GFKKCIKSTV GFDAIRVYNK RLKALRKIQK LIKSGQLRAT
601 KNGIISDAVS TNNAFEPDED IEDLEELDIP TKEIEIQVDW NSELPVKVNV
651 PKVPIHSLVL DCGAISFLDV VGVRSLRVIV KEFQRIDVNV YFASLQDYV
701 EKLEQCGFFD DNIRKDTFFL TVHDAILYLQ NQVKSQEGQG SILETITLIQ
751 DCKDTLELIE TELTEEELDV QDEAMRTLAS (SEQ ID NO: 6)

[0054] Pendrin-derived peptide S26A4

LAGLIFGLLTVVLR (SEQ ID NO: 7)

(5) Coatomer subunit zeta-1-derived peptide (COPZ1)

[0055] COPZ1 shows as SEQ ID NO: 9 was shown low value in ADN patient, was shown high value in MCI, AD, and NDdem patients (Figure 6).

Intact protein / peptide

001 MEALILEPSL YTVKAILILD NDGDRLFAKY YDDTYPSVKE QKAFEKNIFN
051 KTHRTDSEIA LLEGLTVVYK SSIDLYFYVI GSSYENELML MAVLNCLFDS
101 LSQMLRKNVE KRALLENMEG LFLAVDEIVD GGVILESDPQ QVVHRVALRG
151 EDVPLTEQTV SQVLQSAKEQ IKWSLLR (SEQ ID NO: 8)

[0056] Coatomer subunit zeta-1-derived peptide COPZ1

AILILDNDGDRLFAKYYDD (SEQ ID NO: 9)

(6) Retinoic acid receptor responder protein 2 precursor-derived peptide (RARR2(S-))

[0057] RARR2(S-) shows as SEQ ID NO: 11 was not detected in ADN patient, and was detected in AD and MCI patients (Figure 7). Retinoic acid receptor responder protein 2 precursor-derived peptides are two types, and (S-) means the peptide lacking of S (Serine residue) of C-terminus.

Intact protein / peptide

001 MRRLLIPLAL WLGAVGVGVA ELTEAQRRGL QVALEEFHKH PPVQWAFQET
051 SVESAVDTPF PAGIFVRLEF KLQQTSCRKR DWKKPECKVR PNGRKRKCLA
101 CIKLGSEDKV LGRLVHCPIE TQVLREAEEH QETQCLRVQR AGEDPHSFYF
151 PGQFAFSKAL PRS (SEQ ID NO: 10)

[0058] Retinoic acid receptor responder protein 2 precursor-derived peptide RARR2 (S-)

PHSFYFPGQFAFSKALPR (SEQ ID NO: 11)

(7) Retinoic acid receptor responder protein 2 precursor-derived peptide (RARR2(S+))

[0059] RARR2(S+) shows as SEQ ID NO: 12 was not detected in ADN patient as well as RARR2(S-), and was detected in AD and MCI patients (Figure 7). (S+) means that the peptide having of S (Serine residue) of C-terminus.

Intact protein / peptide

001 MRRLLIPLAL WLGAVGVGVA ELTEAQRRGL QVALEEFHKH PPVQWAFQET
051 SVESAVDTPF PAGIFVRLEF KLQQTSCRKR DWKKPECKVR PNGRKRKCLA
101 CIKLGSEDKV LGRLVHCPIE TQVLREAEEH QETQCLRVQR AGEDPHSFYF
151 PGQFAFSKAL PRS (SEQ ID NO: 10)

[0060] Retinoic acid receptor responder protein 2 precursor-derived peptide RARR2 (S+)

PHSFYFPGQFAFSKALPRS (SEQ ID NO: 12)

(8) Gelsolin precursor-derived peptide (GELS)

[0061] GELS shows as SEQ ID NO: 14 was shown low value in ADN patient, and was shown relatively high value in MCI and AD patients (Figure 8).

Intact protein / peptide

001 MAPHRPAPAL LCALSLALCA LSLPVRAATA SRGASQAGAP QGRVPEARPN
051 SMVVEHPEFL KAGKEPGLQI WRVEKFDLVP VPTNLYGDFF TGDAYVILKT
101 VQLRNGNLQY DLHYWLGNEC SQDESGAAAI FTVQLDDYLN GRAVQHREVQ
151 GFESATFLGY FKSGLKYKKG GVASGFKHVV PNEVVVQRLF QVKGRRVVRA
201 TEVPVSWESF NNGDCFILDL GNNIHQWCGS NSNRYERLKA TQVSKGIRDN
251 ERSGRARVHV SEEGTEPEAM LQVLGPKPAL PAGTEDTAKE DAANRKLAKL
301 YKVSNGAGTM SVSLVADENP FAQGALKSED CFILDHGKDG KIFVWKGKQA
351 NTEERKAALK TASDFITKMD YPKQTQVSVL PEGGETPLFK QFFKNWRDPD
401 QTDGLGLSYL SSHIANVERV PFDAATLHTS TAMAAQHGMD DDGTGQKQIW
451 RIEGSNKVPV DPATYGQFYG GDSYIILYNY RHGGRQGQII YNWQGAQSTQ
501 DEVAASAILT AQLDEELGGT PVQSRVVQGK EPAHLMSLFG GKPMIIYKGG
551 TSREGGQTAP ASTRLFQVRA NSAGATRAVE VLPKAGALNS NDAFVLKTPS
601 AAYLWVGTGA SEAEKTGAQE LLRVLRAQPV QVAEGSEPDG FWEALGGKAA
651 YRTSPRLKDK KMDAHPPRLF ACSNKIGRFV IEEVPGELMQ EDLATDDVML
701 LDTWDQVFVW VGKDSQEEEK TEALTSAKRY IETDPANRDR RTPITVVKQG
751 FEPPSFVGWF LGWDDDYWSV DPLDRAMAEL AA (SEQ ID NO: 13)

[0062] Gelsolin precursor-derived peptide GELS

PVRAATASRGAS (SEQ ID NO: 14)

(9) Clusterin precursor-derived peptide (CLUS(N-term SDVP))

[0063] CLUS(N-term SDVP) shows as SEQ ID NO: 16 was shown low value in ADN patient, and was shown relatively high value in MCI and AD patients (Figure 9). Clusterin precursor-derived peptides are two types, and (N-termSDVP) means that the amino acid sequence of N-terminus in peptide is SDVP.

Intact protein / peptide

001 MMKTLLLFVG LLLTWESGQV LGDQTVSDNE LQEMSNQGSK YVNKEIQNAV
051 NGVKQIKTLI EKTNEERKTL LSNLEEAKKK KEDALNETRE SETKLKELPG
101 VCNETMMALW EECKPCLKQT CMKFYARVCR SGSGLVGRQL EEFLNQSSPF
151 YFWMNGDRID SLLENDRQQT HMLDVMQDHF SRASSIIDEL FQDRFFTREP
201 QDTYHYLPFS LPHRRPHFFF PKSRIVRSLM PFSPYEPLNF HAMFQPFLEM
251 IHEAQQAMDI HFHSPAFQHP PTEFIREGDD DRTVCREIRH NSTGCLRMKD
301 QCDKCREILS VDCSTNNPSQ AKLRRELDES LQVAERLTRK YNELLKSYQW
351 KMLNTSSLLE QLNEQFNWVS RLANLTQGED QYYLRVTTVA SHTSDSDVPS
401 GVTEVVVKLF DSDPITVTVP VEVSRKNPKF METVAEKALQ EYRKKHREE (SEQ ID NO: 15)

[0064] Clusterin precursor-derived peptide CLUS(N-term SDVP)

SDVPSGVTEVVVKLFDS (SEQ ID NO: 16)

(10) Clusterin precursor-derived peptide (CLUS(N-term RFFT))

[0065] CLUS(N-term RFFT) shows as SEQ ID NO: 17 was detected in ADN patient, and was not completely detected in AD patient (Figure 9). (N-term RFFT) means that the amino acid sequence of N-terminus in peptide is RFFT.

Intact protein / peptide

001 MMKTLLLFVG LLLTWESGQV LGDQTVSDNE LQEMSNQGSK YVNKEIQNAV
051 NGVKQIKTLI EKTNEERKTL LSNLEEAKKK KEDALNETRE SETKLKELPG
101 VCNETMMALW EECKPCLKQT CMKFYARVCR SGSGLVGRQL EEFLNQSSPF
151 YFWMNGDRID SLLENDRQQT HMLDVMQDHF SRASSIIDEL FQDRFFTREP
201 QDTYHYLPFS LPHRRPHFFF PKSRIVRSLM PFSPYEPLNF HAMFQPFLEM
251 IHEAQQAMDI HFHSPAFQHP PTEFIREGDD DRTVCREIRH NSTGCLRMKD
301 QCDKCREILS VDCSTNNPSQ AKLRRELDES LQVAERLTRK YNELLKSYQW
351 KMLNTSSLLE QLNEQFNWVS RLANLTQGED QYYLRVTTVA SHTSDSDVPS
401 GVTEVVVKLF DSDPITVTVP VEVSRKNPKF METVAEKALQ EYRKKHREE (SEQ ID NO: 15)

[0066] Clusterin precursor-derived peptide CLUS(N-term RFFT)

RFFTREPQDTYHYLPFSLPH (SEQ ID NO: 17)

(11) Eukaryotic translation initiation factor 3 subunit J-derived peptide (EIF3J)

[0067] EIF3J shows as SEQ ID NO: 19 was detected in ADN patient, and was not at all or almost detected in MCI, AD, NDall, NDdem, and NDnon patients (Figure 10).

Intact protein / peptide

001 MAAAAAAAGD SDSWDADAFS VEDPVRKVGG GGTAGGDRWE GEDEDEDVKD
051 NWDDDDDEKK EEAEVKPEVK ISEKKKIAEK IKEKERQQKK RQEEIKKRLE
101 EPEEPKVLTP EEQLADKLRL KKLQEESDLE LAKETFGVNN AVYGIDAMNP
151 SSRDDFTEFG KLLKDKITQY EKSLYYASFL EVLVRDVCIS LEIDDLKKIT
201 NSLTVLCSEK QKQEKQSKAK KKKKGVVPGG GLKATMKDDL ADYGGYDGGY

251 VQDYEDFM (SEQ ID NO: 18)

[0068] Eukaryotic translation initiation factor 3 subunit J-derived peptide EIF3J

GVVPGGGLKATMKDDLADYGGYDGG + Oxidation (M) (SEQ ID NO: 19)

(12) Leucine-rich repeat-containing protein 27-derived peptide (LRC27)

[0069] LRC27 shows as SEQ ID NO: 21 was detected in ADN patient, and was not completely detected in AD patient (Figure 10).

Intact protein / peptide

001 MEGSSSYEVP SVAAADLEEG AGQTRSLPAT PSKDVHKGVG GIIFSSSPIL
051 DLSESGLCRL EEVFRIPSLQ QLHLQRNALC VIPQDFFQLL PNLTWLDLRY
101 NRIKALPSGI GAHQHLKTLL LERNPIKMLP VELGSVTTLK ALNLRHCPLE
151 FPPQLVVQKG LVAIQRFLRM WAVEHSLPRN PTSQEAPPVR EMTLRDLPSP
201 GLELSGDHAS NQGAVNAQDP EGAVMKEKAS FLPPVEKPDL SELRKSADSS
251 ENWPSEEEIR RFWKLRQEIV EHVKADVLGD QLLTRELPPN LKAALNIEKE
301 LPKPRHVFRR KTASSRSILP DLLSPYQMAI RAKRLEESRA AALRELQEKQ
351 ALMEQQRREK RALQEWRERA QRMRKRKEEL SKLLPPRRSM VASKIPSATD
401 LIDNRKVPLN PPGKMKPSKE KSPQASKEMS ALQERNLEEK IKQHVLQMRE
451 QRRFHGQAPL EEMRKAAEDL EIATELQDEV LKLKLGLTLN KDRRRAALTG
501 NLSLGLPAAQ PQNTFFNTKY GESGNVRRYQ (SEQ ID NO: 20)

[0070] Leucine-rich repeat-containing protein 27-derived peptide LRC27

SSPILDLSESGLCRLEEVFRIPS (SEQ ID NO: 21)

[0071] There have already quoted, but for the peptides of SEQ ID NO: 2 (NRX2B) to SEQ ID NO: 21 (LRC27), the scatter plots of comparison between ADN, MCI and AD patients, and the scatter plot of comparison between ADN, AD, NDall, NDdem and NDnon patients, and the p-value of t-test in each comparison were showed in Figure 2, Figure 4 and Figure 6 through Figure 10.

[0072] Table 1 shows the list of ROC values for comparison of MCI vs. ADN and AD vs. ADN about 12 marker peptides shown above.

[Table 1]

Marker peptides		MCI vs. ADN		AD vs. ADN	
Swiss-Prot Entry	Sequence No.	ROC value	MCI was	ROC value	AD was
NRX2B	2	0.99	up	1	up
THRB (R-)	4	0.854	down	0.841	down
THRB (R+)	5	0.94	up	0.985	up
S26A4	7	0.925	up	0.95	up
COPZ1	9	0.786	up	0.767	up
RARR2 (S-)	11	0.885	up	0.914	up
RARR2 (S+)	12	0.95	up	0.919	up
GELS	14	0.716	up	0.762	up
CLUS (N-term SDVP)	16	0.739	up	0.717	up
CLUS (N-term RFFT)	17	0.675	down	0.75	down
EIF3J	19	0.748	down	0.775	down
LRC27	21	0.699	down	0.755	down

[0073] Table 1 shows the usefulness of each marker peptides in the detection of cognitive impairment (MCI and AD). Using these marker peptides in singly or in combination, using or without using liquid chromatography and/or any other suitable separation methods, directly measuring the abundance in serum using other methods such as mass spectrometry or immunological methods or enzymatic methods, it is possible to distinguish between non-dementia and dementia in neurological disease and diagnose cognitive impairment like AD and MCI. The marker peptide that is not detected in ADN patient and is detected in MCI, AD, NDall, NDdem and NDnon patients, or vice versa, the marker peptide that is detected in ADN patient and is not detected in MCI, AD, NDall, NDdem and NDnon patients, are also useful for the detection of psychiatric diseases.

[Example 2]

Example 2. Synthesis of a marker peptide, and preparation of a marker peptide specific polyclonal antibody

[0074] The antigenic peptide was synthesized to prepare the specific antibody that recognizes Neurexin-2-beta precursor-derived peptide NRX2B of SEQ ID NO: 2. The synthetic peptide for coupling to a carrier protein was added the cysteine residue (labeled as C or Cys) in C-terminus. The peptide that was combined with carrier protein (RSGGNATC-KLH, see below) was mixed with an adjuvant, and the mixture was immunized in rabbit. Total eight times immunizations is performed every 1-2 weeks, and the test blood collection performed twice every 4 weeks, and the antibody titers were measured by enzyme immunoassay (EIA). After three months from the start of the immunization, the whole blood was collected from rabbit and the antiserum was obtained, furthermore the purification of the specific antibody was performed using the peptide column that antigen peptide was bound as ligand.

[0075] The sequence of synthetic antigen peptide for preparation of peptide specific antibody is shown below.

[0076]

RSGGNAT+Cys (SEQ ID NO: 22)

[Example 3]

Example 3. Preparation of antibody-beads

(1) Method

(1-1) Preparation of antibody, and binding to magnetic-beads

[0077] The antibody solution, 1 mg of the antibody (anti-NRX2B antibody, Rabbit IgG) that specifically recognizes the peptide of amino acid sequence expressed by SEQ ID NO: 22 was dissolved with 3 ml of 0.1 M MES. After washing 1 ml (10 mg beads) of the magnetic-beads (Magnosphere MS300/carboxyl, JSR Corporation) by using 0.1 M MES, the magnetic-beads were mixed with the antibody solution and were gently shaken for 30 min at room temperature.

(1-2) Cross-linking of antibody and magnetic-beads

[0078] 400 μl of EDC solution (10 mg/ml 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride in 0.1M MES) was added in antibody-beads solution and was suspended gently for 3 hours to bind antibody with beads by covalent bond.

(1-3) Blocking

[0079] 1 ml of 200 mM ethanolamine (pH8.0) was added to wash beads, and further 1 ml of 200 mM ethanolamine (pH8.0) was added and was shaken gently for 1 hours at room temperature to block amine groups.

(1-4) Washing

[0080] After removal of 200 mM 200m M ethanolamine (pH8.0), the beads were washed three times by 1 ml of TBST solution (25 mM Tris-HCl (pH7.2) containing 0.15M NaCl and 0.05% Tween 20).

(1-5) Storage

[0081] After suspending the beads by adding with 1 ml of TBST solution, and stored at 4 °C.

[Example 4]

[0082] Example 4. The proof by immunoMS method that the peak of m/z 1,488 in patient serum detected by 2D-LC-MALDI-TOF-MS is NRX2B.

(1) Methods

[0083] As the control for comparison, stable isotope-labeled NRX2B synthetic peptide (12C and 13C5 of V has been replaced by 15N and 14N) greater than mass of NRX2B was used. The mass difference between NRX2B and its stable isotope peptide is 6 u. Both the endogenous peptide and the stable isotope-labeled peptide are captured by anti-NRX2B antiboby. 1μl of 200 fmol/μl stable isotope-labeled NRX2B synthetic peptide was added in 25 μl of each of patient serum in AD and MCI, and incubated for 10 min at 4 °C. Then, 475 μl of 0.1% trifluoroacetic acid (TFA) was added and boiled for 5 min at 100 °C After centrifugation for 15 min at 14,000×g, 500 μl of 100 mM Tris-HCl buffer (pH 7.5) containing 0.3 M NaCl and 0.2 % *n*-octyl glycoside was added in supernatant as peptide solutions. 20 μl of anti-NRX2B antibody-beads created in Example 3 was added in the peptide solutions, and was shaken gently for 2 hours. Then,after standing for 1 min on the magnetic stand, the supernatant was removed. 1 ml of 50 mM Tris-HCl buffer (pH 7.5) (TBS) containing 0.15 M NaCl and 0.1 % *n*-octyl glycoside was added, and was shaken gently for 10 min. After standing for 1 min on the magnetic stand, the supernatant was removed. In addition, after adding 500 μl of TBS, and standing for 1 min on the magnetic stand, the supernatant was removed. This procedure was repeated three times. Furthermore, after adding 500 μl of 50 mM ammonium carbonate, and standing for 1 min on the magnetic stand, the supernatant was removed. This procedure was repeated three times. 50 μl of 2-propanol: H2O: formic acid (4:4:1) solution was added, and was stood for 10 min, and then after standing for 1 min on the magnetic stand, the filtrate was recovered. This procedure was repeated twice. The filtrates were completely dried using vacuum centrifuge. Then, 20 μl of 0.095 %TFA containing 5% acetonitrile was added and was re-dissolved by sonication. The peptides were concentrated using C18 pipette tip (PerfectPure C-18 Tip, Eppendorf), and were spotted on MALDI target plate (MTP AnchorChipTM 600/384 plate, BRUKER DALTONICS) by eluting from C18 pipette tip, and then the peptides were analyzed using MALDI TOF mass spec-

trometer (AXIMA CFRplus, SHIMADZU).

(2) Results

[0084] Figure 11 shows the result of the mass spectrum of NRX2B peptide detected from the patient serum in AD and MCI using the above method. Figure 11 A) shows the overall mass spectrum, and Figure 11 B) shows the enlarged view of the arrow parts in Figure 11A). The signal indicated by dashed arrows in Figure 11 B) is stable isotope-labeled NRX2B synthetic peptide that was spiked, and the signal indicated by solid arrows is endogenous NRX2B peptide. The observed mass value was within the measurement error of the expected value. And also the mass difference between endogenous NRX2B peptide and its stable isotope-labeled peptide was 6 u. Therefore, it was demonstrated that the trapped peptide is NRX2B.

[0085] In this experiment, NRX2B which is the peptide marker was detected from serum by using immunoMS method that developed originally by these inventors, and it could be shown that it is possible to distinguish between AD and MCI patients from ADN. At the same time, in this experiment, it has also shown that the specific antibody against NRX2B is useful in detecting its peptide marker. In addition, it also shows that immunological detection method could be effective against the peptide or protein comprised in the amino acid sequence of NRX2B using the specific antibody against NRX2B. In addition, in this experiment, it was determined by using the specific antibodies that recognize one peptide marker, but the combination of biomarkers specific antibodies that recognize other peptides that were found in Example 1, is expected to further increase the accuracy of diagnosis of the pathosis.

[Industrial Applicability]

[0086] As cognitive impairment including mild cognitive impairment and Alzheimer disease and cognitive impairment and non-psychiatric disease can be detected by using the biomarkers disclosed in the present invention, the invention is applicable to the use in the field of medical diagnosis including that of diagnostic agents.

[Sequence Listing]

[0087] 09P01007_Sequence.txt

SEQUENCE LISTING

[0088]

<110> MCBI Inc.

<120> New Biomarkers for detecting Alzheimer Disease

<130> 09P01007

<160> 25

<170> PatentIn version 3.5

<210> 1
<211> 616
<212> PRT
<213> Homo sapiens

<400> 1

```
Met Pro Pro Gly Gly Ser Gly Pro Gly Gly Cys Pro Arg Arg Pro Pro
1               5                  10                  15

Ala Leu Ala Gly Pro Leu Pro Pro Pro Pro Pro Pro Pro Pro Pro Pro
            20                  25                  30

Leu Leu Pro Leu Leu Pro Leu Leu Leu Leu Leu Leu Gly Ala Ala
        35                  40                  45

Glu Gly Ala Arg Val Ser Ser Ser Leu Ser Thr Thr His His Val His
    50                  55                  60

His Phe His Ser Lys His Gly Thr Val Pro Ile Ala Ile Asn Arg Met
65                  70                  75                  80

Pro Phe Leu Thr Arg Gly Gly His Ala Gly Thr Thr Tyr Ile Phe Gly
                85                  90                  95

Lys Gly Gly Ala Leu Ile Thr Tyr Thr Trp Pro Pro Asn Asp Arg Pro
            100                 105                 110

Ser Thr Arg Met Asp Arg Leu Ala Val Gly Phe Ser Thr His Gln Arg
        115                 120                 125

Ser Ala Val Leu Val Arg Val Asp Ser Ala Ser Gly Leu Gly Asp Tyr
    130                 135                 140

Leu Gln Leu His Ile Asp Gln Gly Thr Val Gly Val Ile Phe Asn Val
145                 150                 155                 160

Gly Thr Asp Asp Ile Thr Ile Asp Glu Pro Asn Ala Ile Val Ser Asp
                165                 170                 175

Gly Lys Tyr His Val Val Arg Phe Thr Arg Ser Gly Gly Asn Ala Thr
            180                 185                 190
```

```
Leu Gln Val Asp Ser Trp Pro Val Asn Glu Arg Tyr Pro Ala Gly Asn
        195                 200                 205

Phe Asp Asn Glu Arg Leu Ala Ile Ala Arg Gln Arg Ile Pro Tyr Arg
    210                 215                 220

Leu Gly Arg Val Val Asp Glu Trp Leu Leu Asp Lys Gly Arg Gln Leu
225                 230                 235                 240

Thr Ile Phe Asn Ser Gln Ala Ala Ile Lys Ile Gly Gly Arg Asp Gln
                245                 250                 255

Gly Arg Pro Phe Gln Gly Gln Val Ser Gly Leu Tyr Tyr Asn Gly Leu
            260                 265                 270

Lys Val Leu Ala Leu Ala Ala Glu Ser Asp Pro Asn Val Arg Thr Glu
        275                 280                 285

Gly His Leu Arg Leu Val Gly Glu Gly Pro Ser Val Leu Val Ala Ser
    290                 295                 300

Ala Glu Cys Pro Ser Asp Asp Glu Asp Leu Glu Glu Cys Glu Pro Ser
305                 310                 315                 320

Thr Gly Gly Glu Leu Ile Leu Pro Ile Ile Thr Glu Asp Ser Leu Asp
                325                 330                 335

Pro Pro Pro Val Ala Thr Arg Ser Pro Phe Val Pro Pro Pro Pro Thr
            340                 345                 350

Phe Tyr Pro Phe Leu Thr Gly Val Gly Ala Thr Gln Asp Thr Leu Pro
        355                 360                 365

Pro Pro Ala Ala Arg Arg Pro Pro Ser Gly Gly Pro Cys Gln Ala Glu
    370                 375                 380

Arg Asp Asp Ser Asp Cys Glu Glu Pro Ile Glu Ala Ser Gly Phe Ala
385                 390                 395                 400

Ser Gly Glu Val Phe Asp Ser Ser Leu Pro Pro Thr Asp Asp Glu Asp
                405                 410                 415

Phe Tyr Thr Thr Phe Pro Leu Val Thr Asp Arg Thr Thr Leu Leu Ser
            420                 425                 430

Pro Arg Lys Pro Ala Pro Arg Pro Asn Leu Arg Thr Asp Gly Ala Thr
        435                 440                 445
```

```
Gly Ala Pro Gly Val Leu Phe Ala Pro Ser Ala Pro Ala Pro Asn Leu
    450                 455                 460

Pro Ala Gly Lys Met Asn His Arg Asp Pro Leu Gln Pro Leu Leu Glu
465                 470                 475                 480

Asn Pro Pro Leu Gly Pro Gly Ala Pro Thr Ser Phe Glu Pro Arg Arg
                485                 490                 495

Pro Pro Pro Leu Arg Pro Gly Val Thr Ser Ala Pro Gly Phe Pro His
            500                 505                 510

Leu Pro Thr Ala Asn Pro Thr Gly Pro Gly Glu Arg Gly Pro Pro Gly
        515                 520                 525

Ala Val Glu Val Ile Arg Glu Ser Ser Ser Thr Thr Gly Met Val Val
    530                 535                 540

Gly Ile Val Ala Ala Ala Ala Leu Cys Ile Leu Ile Leu Leu Tyr Ala
545                 550                 555                 560

Met Tyr Lys Tyr Arg Asn Arg Asp Glu Gly Ser Tyr Gln Val Asp Gln
                565                 570                 575

Ser Arg Asn Tyr Ile Ser Asn Ser Ala Gln Ser Asn Gly Ala Val Val
            580                 585                 590

Lys Glu Lys Ala Pro Ala Ala Pro Lys Thr Pro Ser Lys Ala Lys Lys
        595                 600                 605

Asn Lys Asp Lys Glu Tyr Tyr Val
    610                 615
```

<210> 2
<211> 14
<212> PRT
<213> Homo sapiens

<400> 2

```
Arg Ser Gly Gly Asn Ala Thr Leu Gln Val Asp Ser Trp Pro
1               5                   10
```

<210> 3
<211> 622
<212> PRT
<213> Homo sapiens

<400> 3

```
Met Ala His Val Arg Gly Leu Gln Leu Pro Gly Cys Leu Ala Leu Ala
1               5                  10                  15
```

```
Ala Leu Cys Ser Leu Val His Ser Gln His Val Phe Leu Ala Pro Gln
            20                  25                  30

Gln Ala Arg Ser Leu Leu Gln Arg Val Arg Arg Ala Asn Thr Phe Leu
        35                  40                  45

Glu Glu Val Arg Lys Gly Asn Leu Glu Arg Glu Cys Val Glu Glu Thr
    50                  55                  60

Cys Ser Tyr Glu Glu Ala Phe Glu Ala Leu Glu Ser Ser Thr Ala Thr
65                  70                  75                  80

Asp Val Phe Trp Ala Lys Tyr Thr Ala Cys Glu Thr Ala Arg Thr Pro
                85                  90                  95

Arg Asp Lys Leu Ala Ala Cys Leu Glu Gly Asn Cys Ala Glu Gly Leu
            100                 105                 110

Gly Thr Asn Tyr Arg Gly His Val Asn Ile Thr Arg Ser Gly Ile Glu
        115                 120                 125

Cys Gln Leu Trp Arg Ser Arg Tyr Pro His Lys Pro Glu Ile Asn Ser
    130                 135                 140

Thr Thr His Pro Gly Ala Asp Leu Gln Glu Asn Phe Cys Arg Asn Pro
145                 150                 155                 160

Asp Ser Ser Thr Thr Gly Pro Trp Cys Tyr Thr Thr Asp Pro Thr Val
                165                 170                 175

Arg Arg Gln Glu Cys Ser Ile Pro Val Cys Gly Gln Asp Gln Val Thr
            180                 185                 190

Val Ala Met Thr Pro Arg Ser Glu Gly Ser Ser Val Asn Leu Ser Pro
        195                 200                 205

Pro Leu Glu Gln Cys Val Pro Asp Arg Gly Gln Gln Tyr Gln Gly Arg
    210                 215                 220

Leu Ala Val Thr Thr His Gly Leu Pro Cys Leu Ala Trp Ala Ser Ala
225                 230                 235                 240

Gln Ala Lys Ala Leu Ser Lys His Gln Asp Phe Asn Ser Ala Val Gln
                245                 250                 255

Leu Val Glu Asn Phe Cys Arg Asn Pro Asp Gly Asp Glu Glu Gly Val
            260                 265                 270
```

```
Trp Cys Tyr Val Ala Gly Lys Pro Gly Asp Phe Gly Tyr Cys Asp Leu
        275                 280                 285

Asn Tyr Cys Glu Glu Ala Val Glu Glu Glu Thr Gly Asp Gly Leu Asp
    290                 295                 300

Glu Asp Ser Asp Arg Ala Ile Glu Gly Arg Thr Ala Thr Ser Glu Tyr
305                 310                 315                 320

Gln Thr Phe Phe Asn Pro Arg Thr Phe Gly Ser Gly Glu Ala Asp Cys
                325                 330                 335

Gly Leu Arg Pro Leu Phe Glu Lys Lys Ser Leu Glu Asp Lys Thr Glu
            340                 345                 350

Arg Glu Leu Leu Glu Ser Tyr Ile Asp Gly Arg Ile Val Glu Gly Ser
        355                 360                 365

Asp Ala Glu Ile Gly Met Ser Pro Trp Gln Val Met Leu Phe Arg Lys
    370                 375                 380

Ser Pro Gln Glu Leu Leu Cys Gly Ala Ser Leu Ile Ser Asp Arg Trp
385                 390                 395                 400

Val Leu Thr Ala Ala His Cys Leu Leu Tyr Pro Pro Trp Asp Lys Asn
                405                 410                 415

Phe Thr Glu Asn Asp Leu Leu Val Arg Ile Gly Lys His Ser Arg Thr
            420                 425                 430

Arg Tyr Glu Arg Asn Ile Glu Lys Ile Ser Met Leu Glu Lys Ile Tyr
        435                 440                 445

Ile His Pro Arg Tyr Asn Trp Arg Glu Asn Leu Asp Arg Asp Ile Ala
    450                 455                 460

Leu Met Lys Leu Lys Lys Pro Val Ala Phe Ser Asp Tyr Ile His Pro
465                 470                 475                 480

Val Cys Leu Pro Asp Arg Glu Thr Ala Ala Ser Leu Leu Gln Ala Gly
                485                 490                 495

Tyr Lys Gly Arg Val Thr Gly Trp Gly Asn Leu Lys Glu Thr Trp Thr
            500                 505                 510

Ala Asn Val Gly Lys Gly Gln Pro Ser Val Leu Gln Val Val Asn Leu
        515                 520                 525
```

```
Pro Ile Val Glu Arg Pro Val Cys Lys Asp Ser Thr Arg Ile Arg Ile
    530                 535                 540

Thr Asp Asn Met Phe Cys Ala Gly Tyr Lys Pro Asp Glu Gly Lys Arg
545                 550                 555                 560

Gly Asp Ala Cys Glu Gly Asp Ser Gly Gly Pro Phe Val Met Lys Ser
                565                 570                 575

Pro Phe Asn Asn Arg Trp Tyr Gln Met Gly Ile Val Ser Trp Gly Glu
            580                 585                 590

Gly Cys Asp Arg Asp Gly Lys Tyr Gly Phe Tyr Thr His Val Phe Arg
        595                 600                 605

Leu Lys Lys Trp Ile Gln Lys Val Ile Asp Gln Phe Gly Glu
    610                 615                 620
```

<210> 4
<211> 12
<212> PRT
<213> Homo sapiens

<400> 4

```
Gly Leu Asp Glu Asp Ser Asp Arg Ala Ile Glu Gly
1               5                   10
```

<210> 5
<211> 622
<212> PRT
<213> Homo sapiens

<400> 5

```
Met Ala His Val Arg Gly Leu Gln Leu Pro Gly Cys Leu Ala Leu Ala
1               5                  10                  15

Ala Leu Cys Ser Leu Val His Ser Gln His Val Phe Leu Ala Pro Gln
            20                  25                  30

Gln Ala Arg Ser Leu Leu Gln Arg Val Arg Arg Ala Asn Thr Phe Leu
        35                  40                  45

Glu Glu Val Arg Lys Gly Asn Leu Glu Arg Glu Cys Val Glu Glu Thr
    50                  55                  60

Cys Ser Tyr Glu Glu Ala Phe Glu Ala Leu Glu Ser Ser Thr Ala Thr
65                  70                  75                  80

Asp Val Phe Trp Ala Lys Tyr Thr Ala Cys Glu Thr Ala Arg Thr Pro
                85                  90                  95
```

```
Arg Asp Lys Leu Ala Ala Cys Leu Glu Gly Asn Cys Ala Glu Gly Leu
            100                 105                 110

Gly Thr Asn Tyr Arg Gly His Val Asn Ile Thr Arg Ser Gly Ile Glu
        115                 120                 125

Cys Gln Leu Trp Arg Ser Arg Tyr Pro His Lys Pro Glu Ile Asn Ser
    130                 135                 140

Thr Thr His Pro Gly Ala Asp Leu Gln Glu Asn Phe Cys Arg Asn Pro
145                 150                 155                 160

Asp Ser Ser Thr Thr Gly Pro Trp Cys Tyr Thr Thr Asp Pro Thr Val
                165                 170                 175

Arg Arg Gln Glu Cys Ser Ile Pro Val Cys Gly Gln Asp Gln Val Thr
            180                 185                 190

Val Ala Met Thr Pro Arg Ser Glu Gly Ser Ser Val Asn Leu Ser Pro
        195                 200                 205

Pro Leu Glu Gln Cys Val Pro Asp Arg Gly Gln Gln Tyr Gln Gly Arg
    210                 215                 220

Leu Ala Val Thr Thr His Gly Leu Pro Cys Leu Ala Trp Ala Ser Ala
225                 230                 235                 240

Gln Ala Lys Ala Leu Ser Lys His Gln Asp Phe Asn Ser Ala Val Gln
                245                 250                 255

Leu Val Glu Asn Phe Cys Arg Asn Pro Asp Gly Asp Glu Glu Gly Val
            260                 265                 270

Trp Cys Tyr Val Ala Gly Lys Pro Gly Asp Phe Gly Tyr Cys Asp Leu
        275                 280                 285

Asn Tyr Cys Glu Glu Ala Val Glu Glu Glu Thr Gly Asp Gly Leu Asp
    290                 295                 300

Glu Asp Ser Asp Arg Ala Ile Glu Gly Arg Thr Ala Thr Ser Glu Tyr
305                 310                 315                 320

Gln Thr Phe Phe Asn Pro Arg Thr Phe Gly Ser Gly Glu Ala Asp Cys
                325                 330                 335

Gly Leu Arg Pro Leu Phe Glu Lys Lys Ser Leu Glu Asp Lys Thr Glu
            340                 345                 350
```

```
Arg Glu Leu Leu Glu Ser Tyr Ile Asp Gly Arg Ile Val Glu Gly Ser
        355                 360                 365

Asp Ala Glu Ile Gly Met Ser Pro Trp Gln Val Met Leu Phe Arg Lys
    370                 375                 380

Ser Pro Gln Glu Leu Leu Cys Gly Ala Ser Leu Ile Ser Asp Arg Trp
385                 390                 395                 400

Val Leu Thr Ala Ala His Cys Leu Leu Tyr Pro Pro Trp Asp Lys Asn
                405                 410                 415

Phe Thr Glu Asn Asp Leu Leu Val Arg Ile Gly Lys His Ser Arg Thr
            420                 425                 430

Arg Tyr Glu Arg Asn Ile Glu Lys Ile Ser Met Leu Glu Lys Ile Tyr
        435                 440                 445

Ile His Pro Arg Tyr Asn Trp Arg Glu Asn Leu Asp Arg Asp Ile Ala
    450                 455                 460

Leu Met Lys Leu Lys Lys Pro Val Ala Phe Ser Asp Tyr Ile His Pro
465                 470                 475                 480

Val Cys Leu Pro Asp Arg Glu Thr Ala Ala Ser Leu Leu Gln Ala Gly
                485                 490                 495

Tyr Lys Gly Arg Val Thr Gly Trp Gly Asn Leu Lys Glu Thr Trp Thr
            500                 505                 510

Ala Asn Val Gly Lys Gly Gln Pro Ser Val Leu Gln Val Val Asn Leu
        515                 520                 525

Pro Ile Val Glu Arg Pro Val Cys Lys Asp Ser Thr Arg Ile Arg Ile
    530                 535                 540

Thr Asp Asn Met Phe Cys Ala Gly Tyr Lys Pro Asp Glu Gly Lys Arg
545                 550                 555                 560

Gly Asp Ala Cys Glu Gly Asp Ser Gly Gly Pro Phe Val Met Lys Ser
                565                 570                 575

Pro Phe Asn Asn Arg Trp Tyr Gln Met Gly Ile Val Ser Trp Gly Glu
            580                 585                 590

Gly Cys Asp Arg Asp Gly Lys Tyr Gly Phe Tyr Thr His Val Phe Arg
        595                 600                 605

Leu Lys Lys Trp Ile Gln Lys Val Ile Asp Gln Phe Gly Glu
```

610 615 620

<210> 6
<211> 13
<212> PRT
<213> Homo sapiens

<400> 6

Gly Leu Asp Glu Asp Ser Asp Arg Ala Ile Glu Gly Arg
1 5 10

<210> 7
<211> 780
<212> PRT
<213> Homo sapiens

<400> 7

```
Met Ala Ala Pro Gly Gly Arg Ser Glu Pro Pro Gln Leu Pro Glu Tyr
1               5                  10                  15

Ser Cys Ser Tyr Met Val Ser Arg Pro Val Tyr Ser Glu Leu Ala Phe
            20                  25                  30

Gln Gln Gln His Glu Arg Arg Leu Gln Glu Arg Lys Thr Leu Arg Glu
        35                  40                  45

Ser Leu Ala Lys Cys Cys Ser Cys Ser Arg Lys Arg Ala Phe Gly Val
    50                  55                  60

Leu Lys Thr Leu Val Pro Ile Leu Glu Trp Leu Pro Lys Tyr Arg Val
65                  70                  75                  80

Lys Glu Trp Leu Leu Ser Asp Val Ile Ser Gly Val Ser Thr Gly Leu
                85                  90                  95

Val Ala Thr Leu Gln Gly Met Ala Tyr Ala Leu Leu Ala Ala Val Pro
            100                 105                 110

Val Gly Tyr Gly Leu Tyr Ser Ala Phe Phe Pro Ile Leu Thr Tyr Phe
        115                 120                 125

Ile Phe Gly Thr Ser Arg His Ile Ser Val Gly Pro Phe Pro Val Val
    130                 135                 140

Ser Leu Met Val Gly Ser Val Val Leu Ser Met Ala Pro Asp Glu His
145                 150                 155                 160

Phe Leu Val Ser Ser Ser Asn Gly Thr Val Leu Asn Thr Thr Met Ile
                165                 170                 175
```

```
Asp Thr Ala Ala Arg Asp Thr Ala Arg Val Leu Ile Ala Ser Ala Leu
            180                 185                 190

Thr Leu Leu Val Gly Ile Ile Gln Leu Ile Phe Gly Gly Leu Gln Ile
        195                 200                 205

Gly Phe Ile Val Arg Tyr Leu Ala Asp Pro Leu Val Gly Gly Phe Thr
    210                 215                 220

Thr Ala Ala Ala Phe Gln Val Leu Val Ser Gln Leu Lys Ile Val Leu
225                 230                 235                 240

Asn Val Ser Thr Lys Asn Tyr Asn Gly Val Leu Ser Ile Ile Tyr Thr
                245                 250                 255

Leu Val Glu Ile Phe Gln Asn Ile Gly Asp Thr Asn Leu Ala Asp Phe
            260                 265                 270

Thr Ala Gly Leu Leu Thr Ile Val Val Cys Met Ala Val Lys Glu Leu
        275                 280                 285

Asn Asp Arg Phe Arg His Lys Ile Pro Val Pro Ile Pro Ile Glu Val
    290                 295                 300

Ile Val Thr Ile Ile Ala Thr Ala Ile Ser Tyr Gly Ala Asn Leu Glu
305                 310                 315                 320

Lys Asn Tyr Asn Ala Gly Ile Val Lys Ser Ile Pro Arg Gly Phe Leu
                325                 330                 335

Pro Pro Glu Leu Pro Pro Val Ser Leu Phe Ser Glu Met Leu Ala Ala
            340                 345                 350

Ser Phe Ser Ile Ala Val Val Ala Tyr Ala Ile Ala Val Ser Val Gly
        355                 360                 365

Lys Val Tyr Ala Thr Lys Tyr Asp Tyr Thr Ile Asp Gly Asn Gln Glu
    370                 375                 380

Phe Ile Ala Phe Gly Ile Ser Asn Ile Phe Ser Gly Phe Phe Ser Cys
385                 390                 395                 400

Phe Val Ala Thr Thr Ala Leu Ser Arg Thr Ala Val Gln Glu Ser Thr
                405                 410                 415

Gly Gly Lys Thr Gln Val Ala Gly Ile Ile Ser Ala Ala Ile Val Met
            420                 425                 430

Ile Ala Ile Leu Ala Leu Gly Lys Leu Leu Glu Pro Leu Gln Lys Ser
```

435 440 445

Val Leu Ala Ala Val Val Ile Ala Asn Leu Lys Gly Met Phe Met Gln
450 455 460

Leu Cys Asp Ile Pro Arg Leu Trp Arg Gln Asn Lys Ile Asp Ala Val
465 470 475 480

Ile Trp Val Phe Thr Cys Ile Val Ser Ile Ile Leu Gly Leu Asp Leu
485 490 495

Gly Leu Leu Ala Gly Leu Ile Phe Gly Leu Leu Thr Val Val Leu Arg
500 505 510

Val Gln Phe Pro Ser Trp Asn Gly Leu Gly Ser Ile Pro Ser Thr Asp
515 520 525

Ile Tyr Lys Ser Thr Lys Asn Tyr Lys Asn Ile Glu Glu Pro Gln Gly
530 535 540

Val Lys Ile Leu Arg Phe Ser Ser Pro Ile Phe Tyr Gly Asn Val Asp
545 550 555 560

Gly Phe Lys Lys Cys Ile Lys Ser Thr Val Gly Phe Asp Ala Ile Arg
565 570 575

Val Tyr Asn Lys Arg Leu Lys Ala Leu Arg Lys Ile Gln Lys Leu Ile
580 585 590

Lys Ser Gly Gln Leu Arg Ala Thr Lys Asn Gly Ile Ile Ser Asp Ala
595 600 605

Val Ser Thr Asn Asn Ala Phe Glu Pro Asp Glu Asp Ile Glu Asp Leu
610 615 620

Glu Glu Leu Asp Ile Pro Thr Lys Glu Ile Glu Ile Gln Val Asp Trp
625 630 635 640

Asn Ser Glu Leu Pro Val Lys Val Asn Val Pro Lys Val Pro Ile His
645 650 655

Ser Leu Val Leu Asp Cys Gly Ala Ile Ser Phe Leu Asp Val Val Gly
660 665 670

Val Arg Ser Leu Arg Val Ile Val Lys Glu Phe Gln Arg Ile Asp Val
675 680 685

Asn Val Tyr Phe Ala Ser Leu Gln Asp Tyr Val Ile Glu Lys Leu Glu
690 695 700

```
Gln Cys Gly Phe Phe Asp Asp Asn Ile Arg Lys Asp Thr Phe Phe Leu
705                 710                 715                 720

Thr Val His Asp Ala Ile Leu Tyr Leu Gln Asn Gln Val Lys Ser Gln
                725                 730                 735

Glu Gly Gln Gly Ser Ile Leu Glu Thr Ile Thr Leu Ile Gln Asp Cys
            740                 745                 750

Lys Asp Thr Leu Glu Leu Ile Glu Thr Glu Leu Thr Glu Glu Glu Leu
        755                 760                 765

Asp Val Gln Asp Glu Ala Met Arg Thr Leu Ala Ser
    770                 775                 780
```

<210> 8
<211> 14
<212> PRT
<213> Homo sapiens

<400> 8

```
Leu Ala Gly Leu Ile Phe Gly Leu Leu Thr Val Val Leu Arg
1               5                   10
```

<210> 9
<211> 177
<212> PRT
<213> Homo sapiens

<400> 9

Met Glu Ala Leu Ile Leu Glu Pro Ser Leu Tyr Thr Val Lys Ala Ile
1 5 10 15

Leu Ile Leu Asp Asn Asp Gly Asp Arg Leu Phe Ala Lys Tyr Tyr Asp
20 25 30

Asp Thr Tyr Pro Ser Val Lys Glu Gln Lys Ala Phe Glu Lys Asn Ile
35 40 45

Phe Asn Lys Thr His Arg Thr Asp Ser Glu Ile Ala Leu Leu Glu Gly
50 55 60

Leu Thr Val Val Tyr Lys Ser Ser Ile Asp Leu Tyr Phe Tyr Val Ile
65 70 75 80

Gly Ser Ser Tyr Glu Asn Glu Leu Met Leu Met Ala Val Leu Asn Cys
85 90 95

Leu Phe Asp Ser Leu Ser Gln Met Leu Arg Lys Asn Val Glu Lys Arg
100 105 110

Ala Leu Leu Glu Asn Met Glu Gly Leu Phe Leu Ala Val Asp Glu Ile
115 120 125

Val Asp Gly Gly Val Ile Leu Glu Ser Asp Pro Gln Gln Val Val His
130 135 140

Arg Val Ala Leu Arg Gly Glu Asp Val Pro Leu Thr Glu Gln Thr Val
145 150 155 160

Ser Gln Val Leu Gln Ser Ala Lys Glu Gln Ile Lys Trp Ser Leu Leu
165 170 175

Arg

<210> 10
<211> 19
<212> PRT
<213> Homo sapiens

<400> 10

Ala Ile Leu Ile Leu Asp Asn Asp Gly Asp Arg Leu Phe Ala Lys Tyr
1 5 10 15

Tyr Asp Asp

<210> 11
<211> 163
<212> PRT
<213> Homo sapiens

<400> 11

Met Arg Arg Leu Leu Ile Pro Leu Ala Leu Trp Leu Gly Ala Val Gly
1 5 10 15

Val Gly Val Ala Glu Leu Thr Glu Ala Gln Arg Arg Gly Leu Gln Val
20 25 30

Ala Leu Glu Glu Phe His Lys His Pro Pro Val Gln Trp Ala Phe Gln
35 40 45

Glu Thr Ser Val Glu Ser Ala Val Asp Thr Pro Phe Pro Ala Gly Ile
50 55 60

Phe Val Arg Leu Glu Phe Lys Leu Gln Gln Thr Ser Cys Arg Lys Arg
65 70 75 80

Asp Trp Lys Lys Pro Glu Cys Lys Val Arg Pro Asn Gly Arg Lys Arg
85 90 95

Lys Cys Leu Ala Cys Ile Lys Leu Gly Ser Glu Asp Lys Val Leu Gly
100 105 110

Arg Leu Val His Cys Pro Ile Glu Thr Gln Val Leu Arg Glu Ala Glu
115 120 125

Glu His Gln Glu Thr Gln Cys Leu Arg Val Gln Arg Ala Gly Glu Asp
130 135 140

Pro His Ser Phe Tyr Phe Pro Gly Gln Phe Ala Phe Ser Lys Ala Leu
145 150 155 160

Pro Arg Ser

<210> 12
<211> 18
<212> PRT
<213> Homo sapiens

<400> 12

```
Pro His Ser Phe Tyr Phe Pro Gly Gln Phe Ala Phe Ser Lys Ala Leu
1               5                   10                  15

Pro Arg
```

<210> 13
<211> 163
<212> PRT
<213> Homo sapiens

<400> 13

```
Met Arg Arg Leu Leu Ile Pro Leu Ala Leu Trp Leu Gly Ala Val Gly
1               5                   10                  15

Val Gly Val Ala Glu Leu Thr Glu Ala Gln Arg Arg Gly Leu Gln Val
            20                  25                  30

Ala Leu Glu Glu Phe His Lys His Pro Pro Val Gln Trp Ala Phe Gln
        35                  40                  45

Glu Thr Ser Val Glu Ser Ala Val Asp Thr Pro Phe Pro Ala Gly Ile
    50                  55                  60

Phe Val Arg Leu Glu Phe Lys Leu Gln Gln Thr Ser Cys Arg Lys Arg
65                  70                  75                  80

Asp Trp Lys Lys Pro Glu Cys Lys Val Arg Pro Asn Gly Arg Lys Arg
                85                  90                  95

Lys Cys Leu Ala Cys Ile Lys Leu Gly Ser Glu Asp Lys Val Leu Gly
            100                 105                 110

Arg Leu Val His Cys Pro Ile Glu Thr Gln Val Leu Arg Glu Ala Glu
        115                 120                 125

Glu His Gln Glu Thr Gln Cys Leu Arg Val Gln Arg Ala Gly Glu Asp
    130                 135                 140

Pro His Ser Phe Tyr Phe Pro Gly Gln Phe Ala Phe Ser Lys Ala Leu
145                 150                 155                 160

Pro Arg Ser
```

<210> 14
<211> 19

<212> PRT
<213> Homo sapiens

<400> 14

```
Pro His Ser Phe Tyr Phe Pro Gly Gln Phe Ala Phe Ser Lys Ala Leu
1               5                   10                  15

Pro Arg Ser
```

<210> 15
<211> 782
<212> PRT
<213> Homo sapiens

<400> 15

```
Met Ala Pro His Arg Pro Ala Pro Ala Leu Leu Cys Ala Leu Ser Leu
1               5                   10                  15

Ala Leu Cys Ala Leu Ser Leu Pro Val Arg Ala Ala Thr Ala Ser Arg
            20                  25                  30

Gly Ala Ser Gln Ala Gly Ala Pro Gln Gly Arg Val Pro Glu Ala Arg
        35                  40                  45

Pro Asn Ser Met Val Val Glu His Pro Glu Phe Leu Lys Ala Gly Lys
    50                  55                  60

Glu Pro Gly Leu Gln Ile Trp Arg Val Glu Lys Phe Asp Leu Val Pro
65                  70                  75                  80
```

```
Val Pro Thr Asn Leu Tyr Gly Asp Phe Phe Thr Gly Asp Ala Tyr Val
                85                  90                  95

Ile Leu Lys Thr Val Gln Leu Arg Asn Gly Asn Leu Gln Tyr Asp Leu
            100                 105                 110

His Tyr Trp Leu Gly Asn Glu Cys Ser Gln Asp Glu Ser Gly Ala Ala
        115                 120                 125

Ala Ile Phe Thr Val Gln Leu Asp Asp Tyr Leu Asn Gly Arg Ala Val
    130                 135                 140

Gln His Arg Glu Val Gln Gly Phe Glu Ser Ala Thr Phe Leu Gly Tyr
145                 150                 155                 160

Phe Lys Ser Gly Leu Lys Tyr Lys Lys Gly Gly Val Ala Ser Gly Phe
                165                 170                 175

Lys His Val Val Pro Asn Glu Val Val Val Gln Arg Leu Phe Gln Val
            180                 185                 190

Lys Gly Arg Arg Val Val Arg Ala Thr Glu Val Pro Val Ser Trp Glu
        195                 200                 205

Ser Phe Asn Asn Gly Asp Cys Phe Ile Leu Asp Leu Gly Asn Asn Ile
    210                 215                 220

His Gln Trp Cys Gly Ser Asn Ser Asn Arg Tyr Glu Arg Leu Lys Ala
225                 230                 235                 240

Thr Gln Val Ser Lys Gly Ile Arg Asp Asn Glu Arg Ser Gly Arg Ala
                245                 250                 255

Arg Val His Val Ser Glu Glu Gly Thr Glu Pro Glu Ala Met Leu Gln
            260                 265                 270

Val Leu Gly Pro Lys Pro Ala Leu Pro Ala Gly Thr Glu Asp Thr Ala
        275                 280                 285

Lys Glu Asp Ala Ala Asn Arg Lys Leu Ala Lys Leu Tyr Lys Val Ser
    290                 295                 300

Asn Gly Ala Gly Thr Met Ser Val Ser Leu Val Ala Asp Glu Asn Pro
305                 310                 315                 320

Phe Ala Gln Gly Ala Leu Lys Ser Glu Asp Cys Phe Ile Leu Asp His
                325                 330                 335
```

```
Gly Lys Asp Gly Lys Ile Phe Val Trp Lys Gly Lys Gln Ala Asn Thr
            340                 345                 350

Glu Glu Arg Lys Ala Ala Leu Lys Thr Ala Ser Asp Phe Ile Thr Lys
        355                 360                 365

Met Asp Tyr Pro Lys Gln Thr Gln Val Ser Val Leu Pro Glu Gly Gly
    370                 375                 380

Glu Thr Pro Leu Phe Lys Gln Phe Phe Lys Asn Trp Arg Asp Pro Asp
385                 390                 395                 400

Gln Thr Asp Gly Leu Gly Leu Ser Tyr Leu Ser Ser His Ile Ala Asn
                405                 410                 415

Val Glu Arg Val Pro Phe Asp Ala Ala Thr Leu His Thr Ser Thr Ala
            420                 425                 430

Met Ala Ala Gln His Gly Met Asp Asp Asp Gly Thr Gly Gln Lys Gln
        435                 440                 445

Ile Trp Arg Ile Glu Gly Ser Asn Lys Val Pro Val Asp Pro Ala Thr
    450                 455                 460

Tyr Gly Gln Phe Tyr Gly Gly Asp Ser Tyr Ile Ile Leu Tyr Asn Tyr
465                 470                 475                 480

Arg His Gly Gly Arg Gln Gly Gln Ile Ile Tyr Asn Trp Gln Gly Ala
                485                 490                 495

Gln Ser Thr Gln Asp Glu Val Ala Ala Ser Ala Ile Leu Thr Ala Gln
            500                 505                 510

Leu Asp Glu Glu Leu Gly Gly Thr Pro Val Gln Ser Arg Val Val Gln
        515                 520                 525

Gly Lys Glu Pro Ala His Leu Met Ser Leu Phe Gly Gly Lys Pro Met
    530                 535                 540

Ile Ile Tyr Lys Gly Gly Thr Ser Arg Glu Gly Gly Gln Thr Ala Pro
545                 550                 555                 560

Ala Ser Thr Arg Leu Phe Gln Val Arg Ala Asn Ser Ala Gly Ala Thr
                565                 570                 575

Arg Ala Val Glu Val Leu Pro Lys Ala Gly Ala Leu Asn Ser Asn Asp
            580                 585                 590
```

```
Ala Phe Val Leu Lys Thr Pro Ser Ala Ala Tyr Leu Trp Val Gly Thr
        595                 600                 605

Gly Ala Ser Glu Ala Glu Lys Thr Gly Ala Gln Glu Leu Leu Arg Val
    610                 615                 620

Leu Arg Ala Gln Pro Val Gln Val Ala Glu Gly Ser Glu Pro Asp Gly
625                 630                 635                 640

Phe Trp Glu Ala Leu Gly Gly Lys Ala Ala Tyr Arg Thr Ser Pro Arg
                645                 650                 655

Leu Lys Asp Lys Lys Met Asp Ala His Pro Pro Arg Leu Phe Ala Cys
            660                 665                 670

Ser Asn Lys Ile Gly Arg Phe Val Ile Glu Glu Val Pro Gly Glu Leu
        675                 680                 685

Met Gln Glu Asp Leu Ala Thr Asp Asp Val Met Leu Leu Asp Thr Trp
    690                 695                 700

Asp Gln Val Phe Val Trp Val Gly Lys Asp Ser Gln Glu Glu Glu Lys
705                 710                 715                 720

Thr Glu Ala Leu Thr Ser Ala Lys Arg Tyr Ile Glu Thr Asp Pro Ala
                725                 730                 735

Asn Arg Asp Arg Arg Thr Pro Ile Thr Val Val Lys Gln Gly Phe Glu
            740                 745                 750

Pro Pro Ser Phe Val Gly Trp Phe Leu Gly Trp Asp Asp Asp Tyr Trp
        755                 760                 765

Ser Val Asp Pro Leu Asp Arg Ala Met Ala Glu Leu Ala Ala
    770                 775                 780
```

<210> 16
<211> 12
<212> PRT
<213> Homo sapiens

<400> 16

```
Pro Val Arg Ala Ala Thr Ala Ser Arg Gly Ala Ser
1               5                   10
```

<210> 17
<211> 449

<212> PRT
<213> Homo sapiens

<400> 17

```
Met Met Lys Thr Leu Leu Leu Phe Val Gly Leu Leu Leu Thr Trp Glu
1               5                  10                  15

Ser Gly Gln Val Leu Gly Asp Gln Thr Val Ser Asp Asn Glu Leu Gln
            20                  25                  30

Glu Met Ser Asn Gln Gly Ser Lys Tyr Val Asn Lys Glu Ile Gln Asn
        35                  40                  45

Ala Val Asn Gly Val Lys Gln Ile Lys Thr Leu Ile Glu Lys Thr Asn
    50                  55                  60

Glu Glu Arg Lys Thr Leu Leu Ser Asn Leu Glu Glu Ala Lys Lys Lys
65                  70                  75                  80

Lys Glu Asp Ala Leu Asn Glu Thr Arg Glu Ser Glu Thr Lys Leu Lys
                85                  90                  95

Glu Leu Pro Gly Val Cys Asn Glu Thr Met Met Ala Leu Trp Glu Glu
            100                 105                 110

Cys Lys Pro Cys Leu Lys Gln Thr Cys Met Lys Phe Tyr Ala Arg Val
        115                 120                 125

Cys Arg Ser Gly Ser Gly Leu Val Gly Arg Gln Leu Glu Glu Phe Leu
    130                 135                 140

Asn Gln Ser Ser Pro Phe Tyr Phe Trp Met Asn Gly Asp Arg Ile Asp
145                 150                 155                 160

Ser Leu Leu Glu Asn Asp Arg Gln Gln Thr His Met Leu Asp Val Met
                165                 170                 175

Gln Asp His Phe Ser Arg Ala Ser Ser Ile Ile Asp Glu Leu Phe Gln
            180                 185                 190

Asp Arg Phe Phe Thr Arg Glu Pro Gln Asp Thr Tyr His Tyr Leu Pro
        195                 200                 205

Phe Ser Leu Pro His Arg Arg Pro His Phe Phe Phe Pro Lys Ser Arg
    210                 215                 220

Ile Val Arg Ser Leu Met Pro Phe Ser Pro Tyr Glu Pro Leu Asn Phe
225                 230                 235                 240

His Ala Met Phe Gln Pro Phe Leu Glu Met Ile His Glu Ala Gln Gln
                245                 250                 255
```

```
Ala Met Asp Ile His Phe His Ser Pro Ala Phe Gln His Pro Pro Thr
            260                 265                 270

Glu Phe Ile Arg Glu Gly Asp Asp Asp Arg Thr Val Cys Arg Glu Ile
        275                 280                 285

Arg His Asn Ser Thr Gly Cys Leu Arg Met Lys Asp Gln Cys Asp Lys
    290                 295                 300

Cys Arg Glu Ile Leu Ser Val Asp Cys Ser Thr Asn Asn Pro Ser Gln
305                 310                 315                 320

Ala Lys Leu Arg Arg Glu Leu Asp Glu Ser Leu Gln Val Ala Glu Arg
                325                 330                 335

Leu Thr Arg Lys Tyr Asn Glu Leu Leu Lys Ser Tyr Gln Trp Lys Met
            340                 345                 350

Leu Asn Thr Ser Ser Leu Leu Glu Gln Leu Asn Glu Gln Phe Asn Trp
        355                 360                 365

Val Ser Arg Leu Ala Asn Leu Thr Gln Gly Glu Asp Gln Tyr Tyr Leu
    370                 375                 380

Arg Val Thr Thr Val Ala Ser His Thr Ser Asp Ser Asp Val Pro Ser
385                 390                 395                 400

Gly Val Thr Glu Val Val Val Lys Leu Phe Asp Ser Asp Pro Ile Thr
                405                 410                 415

Val Thr Val Pro Val Glu Val Ser Arg Lys Asn Pro Lys Phe Met Glu
            420                 425                 430

Thr Val Ala Glu Lys Ala Leu Gln Glu Tyr Arg Lys Lys His Arg Glu
        435                 440                 445

Glu
```

<210> 18
<211> 17
<212> PRT
<213> Homo sapiens

<400> 18

Ser Asp Val Pro Ser Gly Val Thr Glu Val Val Val Lys Leu Phe Asp
1 5 10 15

Ser

<210> 19
<211> 449
<212> PRT
<213> Homo sapiens

<400> 19

```
Met Met Lys Thr Leu Leu Leu Phe Val Gly Leu Leu Leu Thr Trp Glu
1               5                  10                  15

Ser Gly Gln Val Leu Gly Asp Gln Thr Val Ser Asp Asn Glu Leu Gln
            20                  25                  30

Glu Met Ser Asn Gln Gly Ser Lys Tyr Val Asn Lys Glu Ile Gln Asn
        35                  40                  45

Ala Val Asn Gly Val Lys Gln Ile Lys Thr Leu Ile Glu Lys Thr Asn
    50                  55                  60

Glu Glu Arg Lys Thr Leu Leu Ser Asn Leu Glu Glu Ala Lys Lys Lys
65                  70                  75                  80

Lys Glu Asp Ala Leu Asn Glu Thr Arg Glu Ser Glu Thr Lys Leu Lys
                85                  90                  95

Glu Leu Pro Gly Val Cys Asn Glu Thr Met Met Ala Leu Trp Glu Glu
            100                 105                 110

Cys Lys Pro Cys Leu Lys Gln Thr Cys Met Lys Phe Tyr Ala Arg Val
        115                 120                 125

Cys Arg Ser Gly Ser Gly Leu Val Gly Arg Gln Leu Glu Glu Phe Leu
    130                 135                 140

Asn Gln Ser Ser Pro Phe Tyr Phe Trp Met Asn Gly Asp Arg Ile Asp
145                 150                 155                 160

Ser Leu Leu Glu Asn Asp Arg Gln Gln Thr His Met Leu Asp Val Met
                165                 170                 175

Gln Asp His Phe Ser Arg Ala Ser Ser Ile Ile Asp Glu Leu Phe Gln
            180                 185                 190

Asp Arg Phe Phe Thr Arg Glu Pro Gln Asp Thr Tyr His Tyr Leu Pro
        195                 200                 205

Phe Ser Leu Pro His Arg Arg Pro His Phe Phe Phe Pro Lys Ser Arg
    210                 215                 220
```

Ile Val Arg Ser Leu Met Pro Phe Ser Pro Tyr Glu Pro Leu Asn Phe
225 230 235 240

His Ala Met Phe Gln Pro Phe Leu Glu Met Ile His Glu Ala Gln Gln
245 250 255

Ala Met Asp Ile His Phe His Ser Pro Ala Phe Gln His Pro Pro Thr
260 265 270

Glu Phe Ile Arg Glu Gly Asp Asp Asp Arg Thr Val Cys Arg Glu Ile
275 280 285

Arg His Asn Ser Thr Gly Cys Leu Arg Met Lys Asp Gln Cys Asp Lys
290 295 300

Cys Arg Glu Ile Leu Ser Val Asp Cys Ser Thr Asn Asn Pro Ser Gln
305 310 315 320

Ala Lys Leu Arg Arg Glu Leu Asp Glu Ser Leu Gln Val Ala Glu Arg
325 330 335

Leu Thr Arg Lys Tyr Asn Glu Leu Leu Lys Ser Tyr Gln Trp Lys Met
340 345 350

Leu Asn Thr Ser Ser Leu Leu Glu Gln Leu Asn Glu Gln Phe Asn Trp
355 360 365

Val Ser Arg Leu Ala Asn Leu Thr Gln Gly Glu Asp Gln Tyr Tyr Leu
370 375 380

Arg Val Thr Thr Val Ala Ser His Thr Ser Asp Ser Asp Val Pro Ser
385 390 395 400

Gly Val Thr Glu Val Val Val Lys Leu Phe Asp Ser Asp Pro Ile Thr
405 410 415

Val Thr Val Pro Val Glu Val Ser Arg Lys Asn Pro Lys Phe Met Glu
420 425 430

Thr Val Ala Glu Lys Ala Leu Gln Glu Tyr Arg Lys Lys His Arg Glu
435 440 445

Glu

<210> 20
<211> 20
<212> PRT

<213> Homo sapiens

<400> 20

```
Arg Phe Phe Thr Arg Glu Pro Gln Asp Thr Tyr His Tyr Leu Pro Phe
1               5                   10                  15

Ser Leu Pro His
            20
```

<210> 21
<211> 258
<212> PRT
<213> Homo sapiens

<400> 21

```
Met Ala Ala Ala Ala Ala Ala Ala Gly Asp Ser Asp Ser Trp Asp Ala
1               5                  10                  15

Asp Ala Phe Ser Val Glu Asp Pro Val Arg Lys Val Gly Gly Gly Gly
            20                  25                  30

Thr Ala Gly Gly Asp Arg Trp Glu Gly Glu Asp Glu Asp Glu Asp Val
        35                  40                  45

Lys Asp Asn Trp Asp Asp Asp Asp Asp Glu Lys Lys Glu Glu Ala Glu
    50                  55                  60

Val Lys Pro Glu Val Lys Ile Ser Glu Lys Lys Lys Ile Ala Glu Lys
65                  70                  75                  80

Ile Lys Glu Lys Glu Arg Gln Gln Lys Lys Arg Gln Glu Glu Ile Lys
                85                  90                  95

Lys Arg Leu Glu Glu Pro Glu Glu Pro Lys Val Leu Thr Pro Glu Glu
            100                 105                 110

Gln Leu Ala Asp Lys Leu Arg Leu Lys Lys Leu Gln Glu Glu Ser Asp
        115                 120                 125

Leu Glu Leu Ala Lys Glu Thr Phe Gly Val Asn Asn Ala Val Tyr Gly
    130                 135                 140

Ile Asp Ala Met Asn Pro Ser Ser Arg Asp Asp Phe Thr Glu Phe Gly
145                 150                 155                 160

Lys Leu Leu Lys Asp Lys Ile Thr Gln Tyr Glu Lys Ser Leu Tyr Tyr
                165                 170                 175

Ala Ser Phe Leu Glu Val Leu Val Arg Asp Val Cys Ile Ser Leu Glu
            180                 185                 190
```

```
Ile Asp Asp Leu Lys Lys Ile Thr Asn Ser Leu Thr Val Leu Cys Ser
        195                 200                 205

Glu Lys Gln Lys Gln Glu Lys Gln Ser Lys Ala Lys Lys Lys Lys Lys
    210                 215                 220

Gly Val Val Pro Gly Gly Gly Leu Lys Ala Thr Met Lys Asp Asp Leu
225                 230                 235                 240

Ala Asp Tyr Gly Gly Tyr Asp Gly Gly Tyr Val Gln Asp Tyr Glu Asp
                245                 250                 255

Phe Met
```

<210> 22
<211> 25
<212> PRT
<213> Homo sapiens

<220>
<221> MOD_RES
<222> (12)..(12)
<223> Oxidation of Methionine

<400> 22

```
Gly Val Val Pro Gly Gly Gly Leu Lys Ala Thr Met Lys Asp Asp Leu
1               5                   10                  15

Ala Asp Tyr Gly Gly Tyr Asp Gly Gly
            20                  25
```

<210> 23
<211> 530
<212> PRT
<213> Homo sapiens

<400> 23

```
Met Glu Gly Ser Ser Ser Tyr Glu Val Pro Ser Val Ala Ala Ala Asp
1               5                  10                  15

Leu Glu Glu Gly Ala Gly Gln Thr Arg Ser Leu Pro Ala Thr Pro Ser
            20                  25                  30

Lys Asp Val His Lys Gly Val Gly Gly Ile Ile Phe Ser Ser Ser Pro
        35                  40                  45

Ile Leu Asp Leu Ser Glu Ser Gly Leu Cys Arg Leu Glu Glu Val Phe
    50                  55                  60

Arg Ile Pro Ser Leu Gln Gln Leu His Leu Gln Arg Asn Ala Leu Cys
```

65 70 75 80

Val Ile Pro Gln Asp Phe Phe Gln Leu Leu Pro Asn Leu Thr Trp Leu
85 90 95

Asp Leu Arg Tyr Asn Arg Ile Lys Ala Leu Pro Ser Gly Ile Gly Ala
100 105 110

His Gln His Leu Lys Thr Leu Leu Leu Glu Arg Asn Pro Ile Lys Met
115 120 125

Leu Pro Val Glu Leu Gly Ser Val Thr Thr Leu Lys Ala Leu Asn Leu
130 135 140

Arg His Cys Pro Leu Glu Phe Pro Pro Gln Leu Val Val Gln Lys Gly
145 150 155 160

Leu Val Ala Ile Gln Arg Phe Leu Arg Met Trp Ala Val Glu His Ser
165 170 175

Leu Pro Arg Asn Pro Thr Ser Gln Glu Ala Pro Pro Val Arg Glu Met
180 185 190

Thr Leu Arg Asp Leu Pro Ser Pro Gly Leu Glu Leu Ser Gly Asp His
195 200 205

Ala Ser Asn Gln Gly Ala Val Asn Ala Gln Asp Pro Glu Gly Ala Val
210 215 220

Met Lys Glu Lys Ala Ser Phe Leu Pro Pro Val Glu Lys Pro Asp Leu
225 230 235 240

Ser Glu Leu Arg Lys Ser Ala Asp Ser Ser Glu Asn Trp Pro Ser Glu
245 250 255

Glu Glu Ile Arg Arg Phe Trp Lys Leu Arg Gln Glu Ile Val Glu His
260 265 270

Val Lys Ala Asp Val Leu Gly Asp Gln Leu Leu Thr Arg Glu Leu Pro
275 280 285

Pro Asn Leu Lys Ala Ala Leu Asn Ile Glu Lys Glu Leu Pro Lys Pro
290 295 300

Arg His Val Phe Arg Arg Lys Thr Ala Ser Ser Arg Ser Ile Leu Pro
305 310 315 320

Asp Leu Leu Ser Pro Tyr Gln Met Ala Ile Arg Ala Lys Arg Leu Glu
325 330 335

```
Glu Ser Arg Ala Ala Ala Leu Arg Glu Leu Gln Glu Lys Gln Ala Leu
            340                 345                 350

Met Glu Gln Gln Arg Arg Glu Lys Arg Ala Leu Gln Glu Trp Arg Glu
        355                 360                 365

Arg Ala Gln Arg Met Arg Lys Arg Lys Glu Glu Leu Ser Lys Leu Leu
    370                 375                 380

Pro Pro Arg Arg Ser Met Val Ala Ser Lys Ile Pro Ser Ala Thr Asp
385                 390                 395                 400

Leu Ile Asp Asn Arg Lys Val Pro Leu Asn Pro Pro Gly Lys Met Lys
                405                 410                 415

Pro Ser Lys Glu Lys Ser Pro Gln Ala Ser Lys Glu Met Ser Ala Leu
            420                 425                 430

Gln Glu Arg Asn Leu Glu Glu Lys Ile Lys Gln His Val Leu Gln Met
        435                 440                 445

Arg Glu Gln Arg Arg Phe His Gly Gln Ala Pro Leu Glu Glu Met Arg
    450                 455                 460

Lys Ala Ala Glu Asp Leu Glu Ile Ala Thr Glu Leu Gln Asp Glu Val
465                 470                 475                 480

Leu Lys Leu Lys Leu Gly Leu Thr Leu Asn Lys Asp Arg Arg Arg Ala
                485                 490                 495

Ala Leu Thr Gly Asn Leu Ser Leu Gly Leu Pro Ala Ala Gln Pro Gln
            500                 505                 510

Asn Thr Phe Phe Asn Thr Lys Tyr Gly Glu Ser Gly Asn Val Arg Arg
        515                 520                 525

Tyr Gln
    530
```

<210> 24
<211> 23
<212> PRT
<213> Homo sapiens

<400> 24

Ser Ser Pro Ile Leu Asp Leu Ser Glu Ser Gly Leu Cys Arg Leu Glu
1 5 10 15

Glu Val Phe Arg Ile Pro Ser
20

<210> 25
<211> 8
<212> PRT
<213> Homo sapiens

<400> 25

Arg Ser Gly Gly Asn Ala Thr Cys
1 5

Claims

1. A peptide or protein for use as a biomarker for detection of psychiatric disease or cognitive impairment, wherein

   the protein is Neurexin-2-beta precursor consisting of amino acid sequence expressed by SEQ ID NO: 1 or the protein is a protein consisting of amino acid sequence derived from SEQ ID NO: 1 by deletion, exchange and/or addition of one to three amino acids, and
   the peptide is Neurexin-2-beta precursor-derived peptide NRX2B consisting of amino acid sequence expressed by SEQ ID NO: 2 or the peptide is a peptide consisting of an amino acid sequence derived from SEQ ID No. 2 by deletion, exchange and/or addition of one to three amino acids.

2. The peptide or protein according to claim 1, wherein the peptide or protein is the peptide for use as a biomarker of cognitive impairment consisting of amino acid sequence expressed by SEQ ID NO: 2 that is appeared or increased in biological material of patients of cognitive impairment as compared to biological material of subjects not suffering from psychiatric disease.

3. The peptide or protein according to claim 1, wherein the peptide or protein is the peptide for use as a biomarker of Alzheimer disease consisting of amino acid sequence expressed by SEQ ID NO: 2 that is appeared or increased in biological material of patients of Alzheimer disease as compared to biological material of subjects not suffering from non-demented neurological disease.

4. The peptide or protein according to any one of claims 1 to 3, wherein the peptide has a molecular weight of less than 10000 or has less than 50 amino acids.

5. An in vitro method for detection of psychiatric disease or cognitive impairment involving determination in biological material of at least one peptide or protein as described in claim 1.

6. An in vitro method according to claim 5 for detection of cognitive impairment in which patient is judged as suffering from cognitive impairment when, after determination in biological material of the peptide as biomarker for cognitive impairment as described in claim 2, said peptide is found to be present in higher quantity than in subjects not suffering from psychiatric disease.

7. A kit for detection of psychiatric disease containing antibody or aptamer specific to at least one peptide or protein as described in claim 1, to determine at least one peptide or protein as described in claim 1.

8. A kit for detection of cognitive impairment containing antibody or aptamer specific to at least one peptide or protein

as described in claim 1, to determine at least one peptide or protein as described in claims 1 or 2.

Patentansprüche

1. Peptid oder Protein zur Verwendung als ein Biomarker zum Detektieren einer psychiatrischen Krankheit oder kognitiven Beeinträchtigung, wobei

   das Protein ein Neurexin-2-beta Vorläufer ist, der aus der durch SEQ ID NR: 1 dargestellten Aminosäuresequenz besteht, oder das Protein ein Protein ist, das aus einer Aminosäuresequenz besteht, die durch Deletion, Austausch und/oder Addition von einer bis drei Aminosäuren von SEQ ID NR: 1 abgeleitet ist, und
   das Peptid ein Neurexin-2-beta Vorläufer-abgeleitetes Peptid NRX2B ist, das aus der durch SEQ ID NR: 2 dargestellten Aminosäuresequenz besteht, oder das Peptid ein Peptid ist, das aus einer Aminosäuresequenz besteht, die durch Deletion, Austausch und/oder Addition von einer bis drei Aminosäuren von SEQ ID NR: 2 abgeleitet ist.

2. Peptid oder Protein nach Anspruch 1, wobei das Peptid oder Protein das Peptid zur Verwendung als ein Biomarker einer kognitiven Beeinträchtigung ist, das aus der durch SEQ ID NR: 2 dargestellten Aminosäuresequenz besteht, das in biologischem Material von Patienten mit kognitiver Beeinträchtigung verglichen mit biologischem Material von Subjekten, die nicht an einer psychiatrischen Krankheit leiden, aufgetreten oder erhöht ist.

3. Peptid oder Protein nach Anspruch 1, wobei das Peptid oder Protein das Peptid zur Verwendung als ein Biomarker für die Alzheimer-Krankheit ist, das aus der durch SEQ ID NR: 2 dargestellten Aminosäuresequenz besteht, das in biologischem Material von Patienten mit Alzheimer-Krankheit verglichen mit biologischem Material von Subjekten, die nicht an nichtdementen neurologischen Krankheiten leiden, aufgetreten oder erhöht ist.

4. Peptid oder Protein nach einem der Ansprüche 1 bis 3, wobei das Peptid ein Molekulargewicht von weniger als 10000 aufweist oder weniger als 50 Aminosäuren aufweist.

5. In vitro Verfahren zum Detektieren einer psychiatrischen Krankheit oder kognitiver Beeinträchtigung, das die Bestimmung von zumindest einem Peptid oder Protein, wie im Anspruch 1 beschrieben, in biologischem Material einschließt.

6. In vitro Verfahren nach Anspruch 5 zum Detektieren einer kognitiven Beeinträchtigung, bei welchem ein Patient als an einer kognitiven Beeinträchtigung leidend beurteilt wird, wenn, nach der Bestimmung des Peptids als Biomarker für eine kognitive Beeinträchtigung, wie in Anspruch 2 beschrieben, in biologischem Material, herausgefunden wird, dass das Peptid in größerer Menge als in Subjekten, die nicht an einer psychiatrischen Krankheit leiden, vorhanden ist.

7. Kit zum Detektieren einer psychiatrischen Krankheit, das Antikörper oder Aptamer enthält, der/das zumindest gegen ein Peptid oder Protein, wie in Anspruch 1 beschrieben, spezifisch ist, um zumindest ein Peptid oder Protein, wie in Anspruch 1 beschrieben, festzustellen.

8. Kit zum Detektieren einer kognitiver Beeinträchtigung, das Antikörper oder Aptamer enthält, der/das zumindest gegen ein Peptid oder Protein, wie in Anspruch 1 beschrieben, spezifisch ist, um zumindest ein Peptid oder Protein, wie in Anspruch 1 oder 2 beschrieben, festzustellen.

Revendications

1. Peptide ou protéine pour une utilisation en tant que biomarqueur pour la détection d'une maladie psychiatrique ou d'une altération de la cognition, où
   la protéine est le précurseur de la neurexine-2-bêta constitué d'une séquence d'acides aminés exprimée par SEQ ID NO : 1 ou la protéine est une protéine constituée de la séquence d'acides aminés dérivée de SEQ ID NO : 1 par délétion, échange et/ou addition d'un à trois acides aminés, et
   le peptide est le peptide NRX2B dérivé du précurseur de la neurexine-2-bêta constitué de la séquence d'acides aminés exprimée par SEQ ID NO : 2 ou le peptide est un peptide constitué d'une séquence d'acides aminés dérivée de SEQ ID NO : 2 par délétion, échange et/ou addition d'un à trois acides aminés.

2. Peptide ou protéine selon la revendication 1, le peptide ou la protéine étant le peptide pour une utilisation en tant que biomarqueur d'une altération de la cognition, constitué de la séquence d'acides aminés exprimée par SEQ ID NO : 2 qui est apparu ou augmenté dans du matériau biologique de patients souffrant d'une altération de la cognition comparativement au matériau biologique de sujets ne souffrant pas d'une maladie psychiatrique.

3. Peptide ou protéine selon la revendication 1, le peptide ou la protéine étant le peptide pour une utilisation en tant que biomarqueur de la maladie d'Alzheimer constitué de la séquence d'acides aminés exprimée par SEQ ID NO : 2 qui est apparu ou augmenté dans du matériau biologique de patients souffrant de la maladie d'Alzheimer comparativement au matériau biologique de sujets ne souffrant pas d'une maladie neurologique non démente.

4. Peptide ou protéine selon l'une quelconque des revendications 1 à 3, le peptide ayant un poids moléculaire inférieur à 10 000 ou comportant moins de 50 acides aminés.

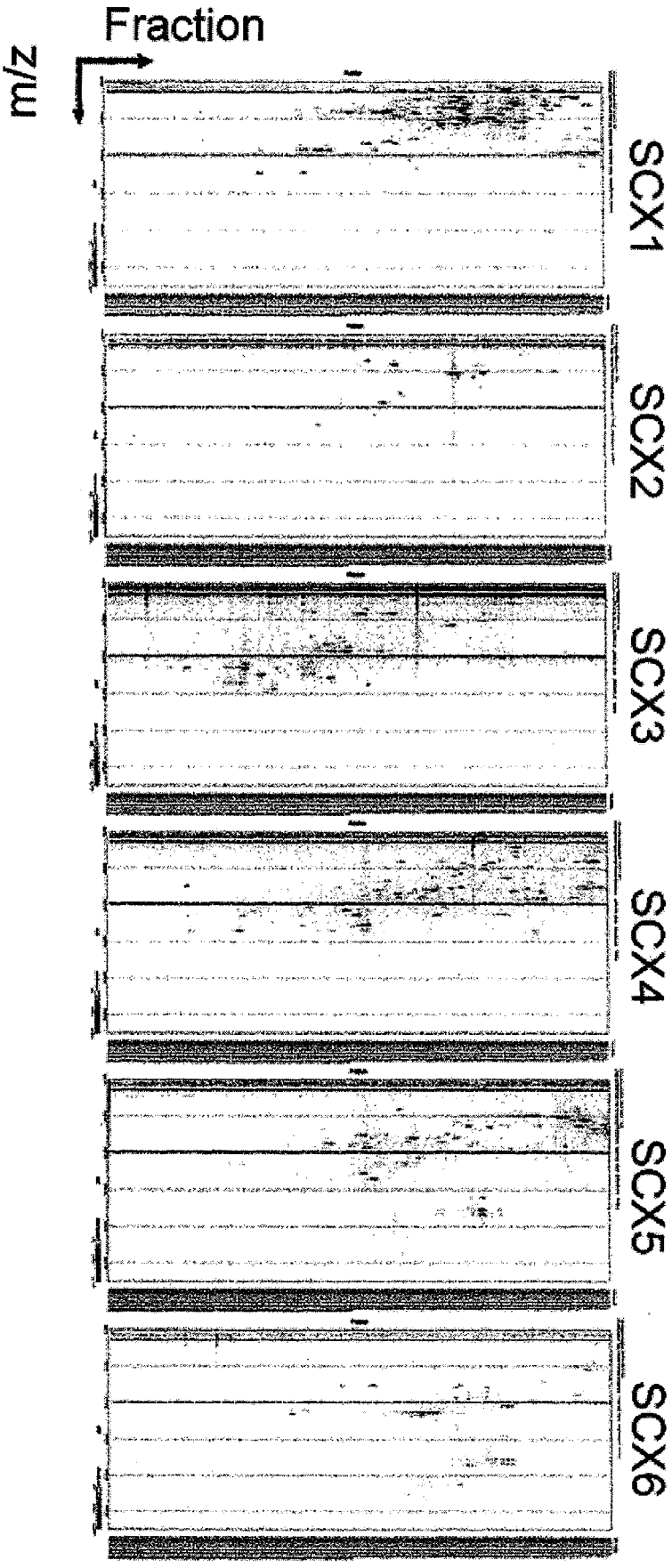
5. Procédé *in vitro* de détection d'une maladie psychiatrique ou d'une altération de la cognition impliquant la détermination dans du matériau biologique d'au moins un peptide ou une protéine tel que décrit dans la revendication 1.

6. Procédé *in vitro* selon la revendication 5 pour la détection d'une altération de la cognition dans lequel le patient est jugé comme souffrant d'une altération de la cognition lorsque, après détermination dans le matériau biologique du peptide en tant que biomarqueur pour une altération de la cognition tel que décrit dans la revendication 2, ledit peptide étant trouvé être présent dans une quantité supérieure par rapport à des sujets ne souffrant pas d'une maladie psychiatrique.

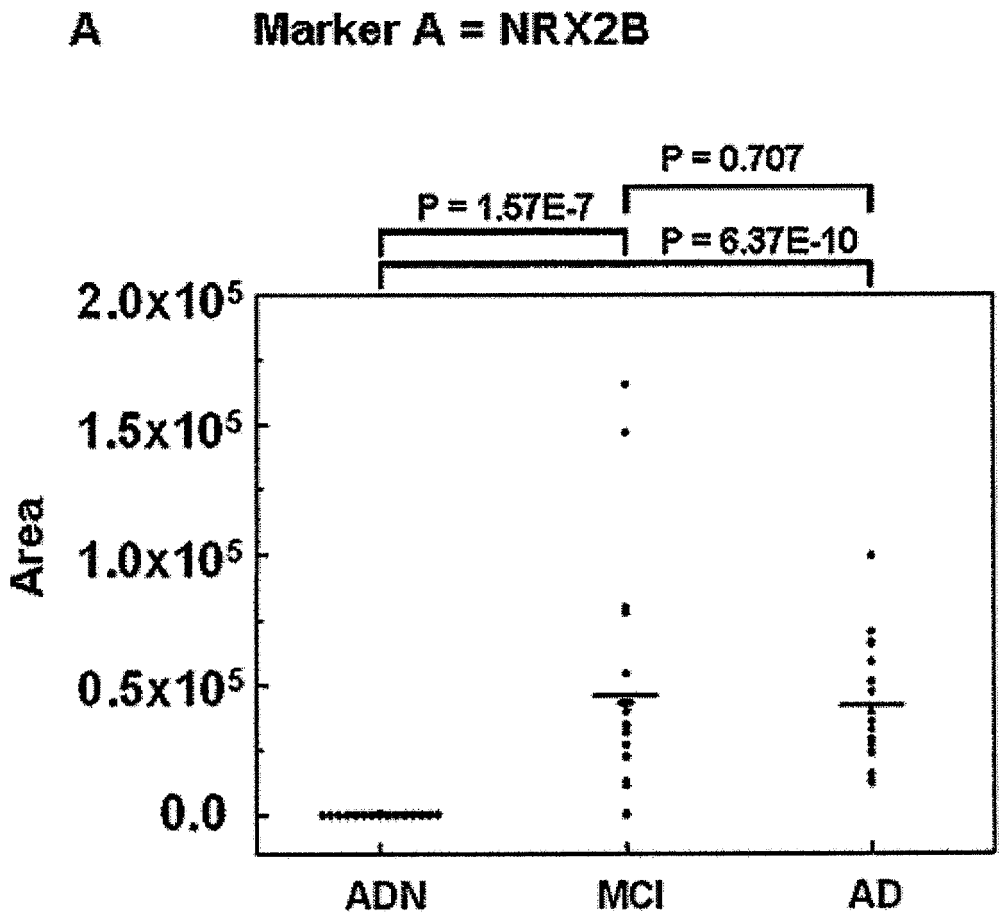
7. Kit pour la détection d'une maladie psychiatrique contenant un anticorps ou un aptamère spécifique d'au moins un peptide ou d'une protéine tel que décrit dans la revendication 1, pour déterminer au moins un peptide ou une protéine tel que décrit dans la revendication 1.

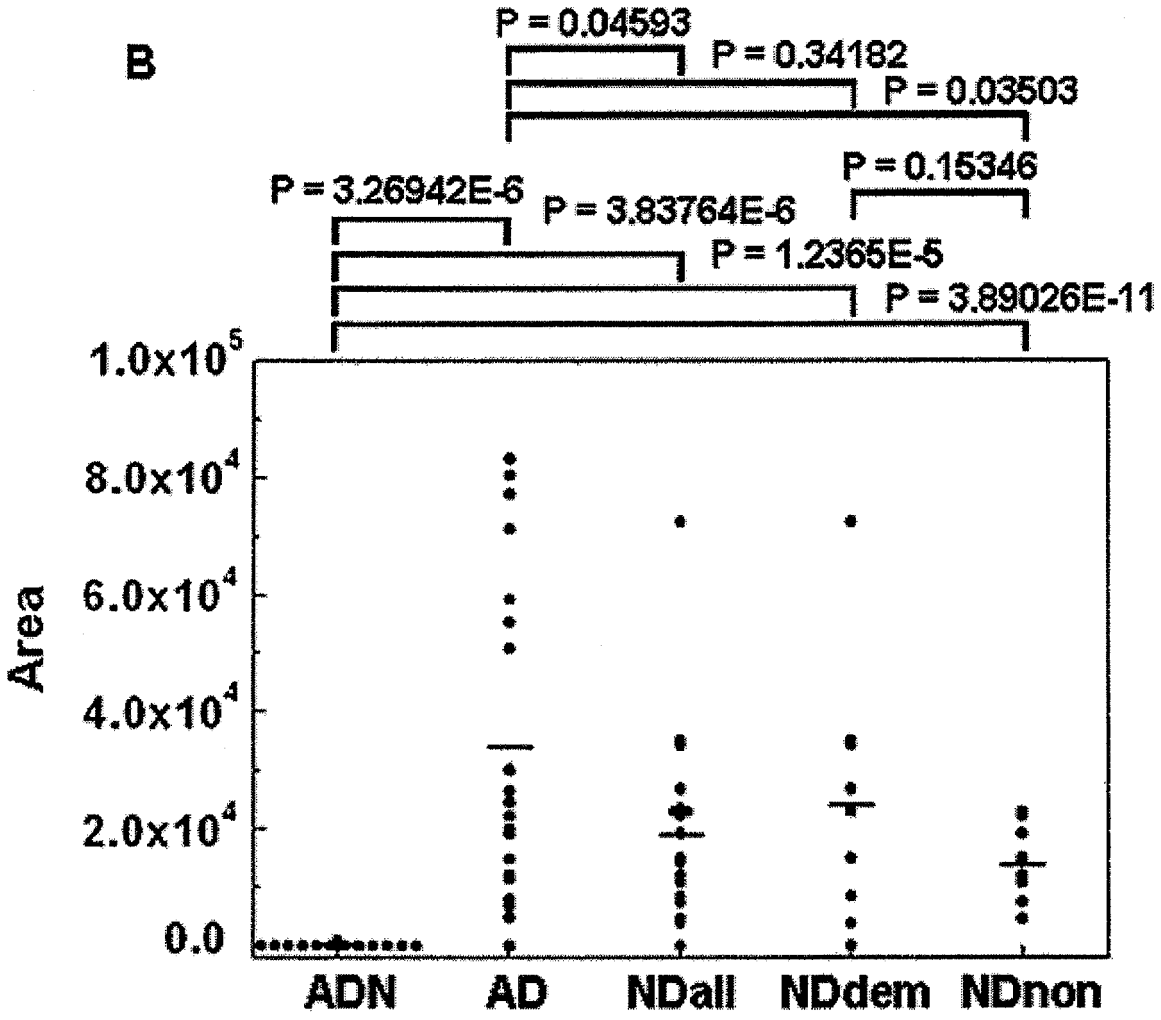
8. Kit pour la détection d'une altération de la cognition contenant un anticorps ou un aptamère spécifique d'au moins un peptide ou d'une protéine tel que décrit dans la revendication 1, pour déterminer au moins un peptide ou une protéine tel que décrit dans les revendications 1 ou 2.

[Fig. 1]

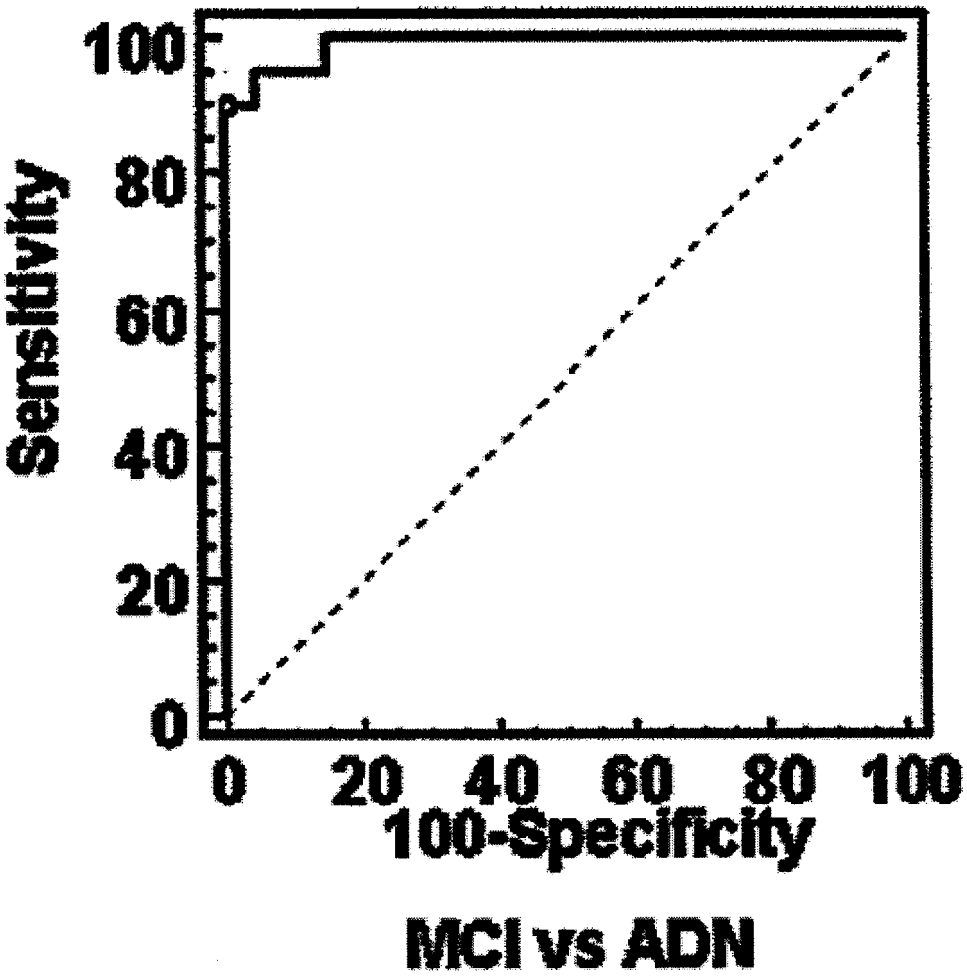


[Fig. 2]

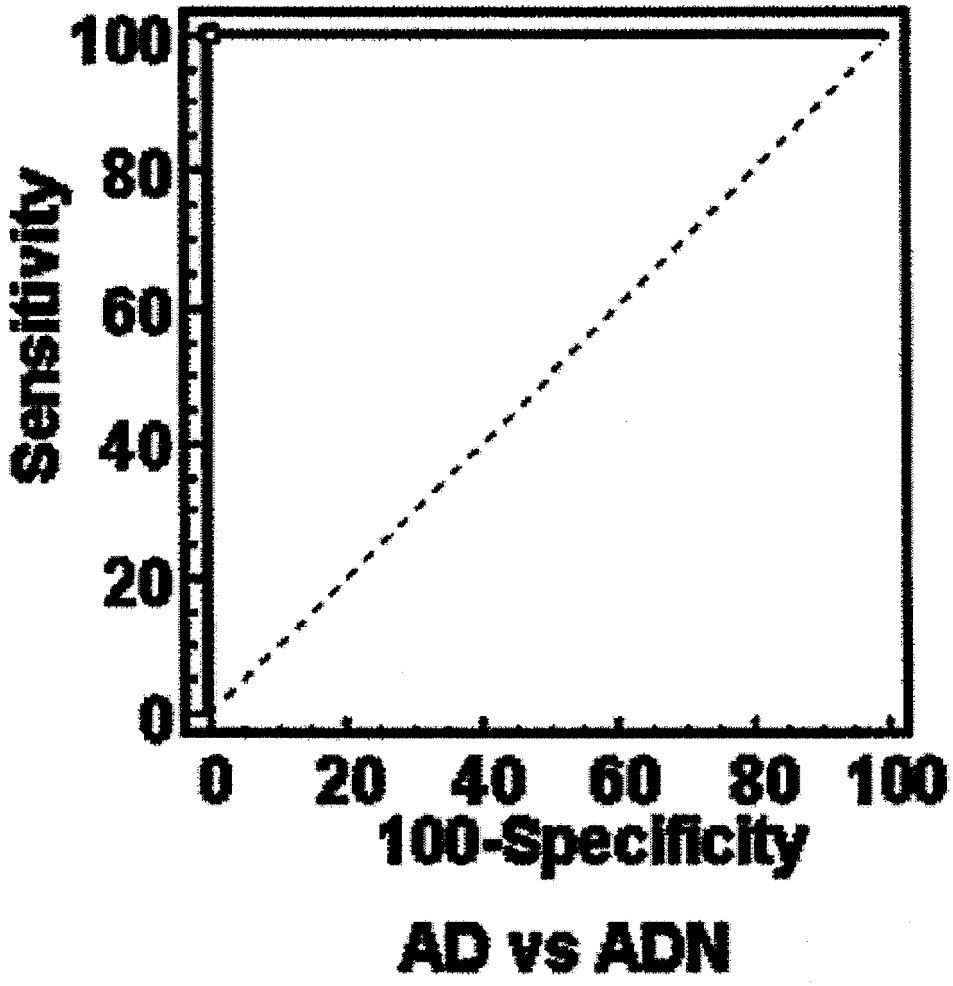

A
Marker A = NRX2B
P = 0.707
P = 1.57E-7
P = 6.37E-10
2.0x10^5
1.5x10^5
1.0x10^5
0.5x10^5
0.0
Area
ADN
MCI
AD


B
P = 0.04593
P = 0.34182
P = 0.03503
P = 0.15346
P = 3.26942E-6
P = 3.83764E-6
P = 1.2365E-5
P = 3.89026E-11
1.0x10^5
8.0x10^4
6.0x10^4
4.0x10^4
2.0x10^4
0.0
Area
ADN
AD
NDall
NDdem
NDnon

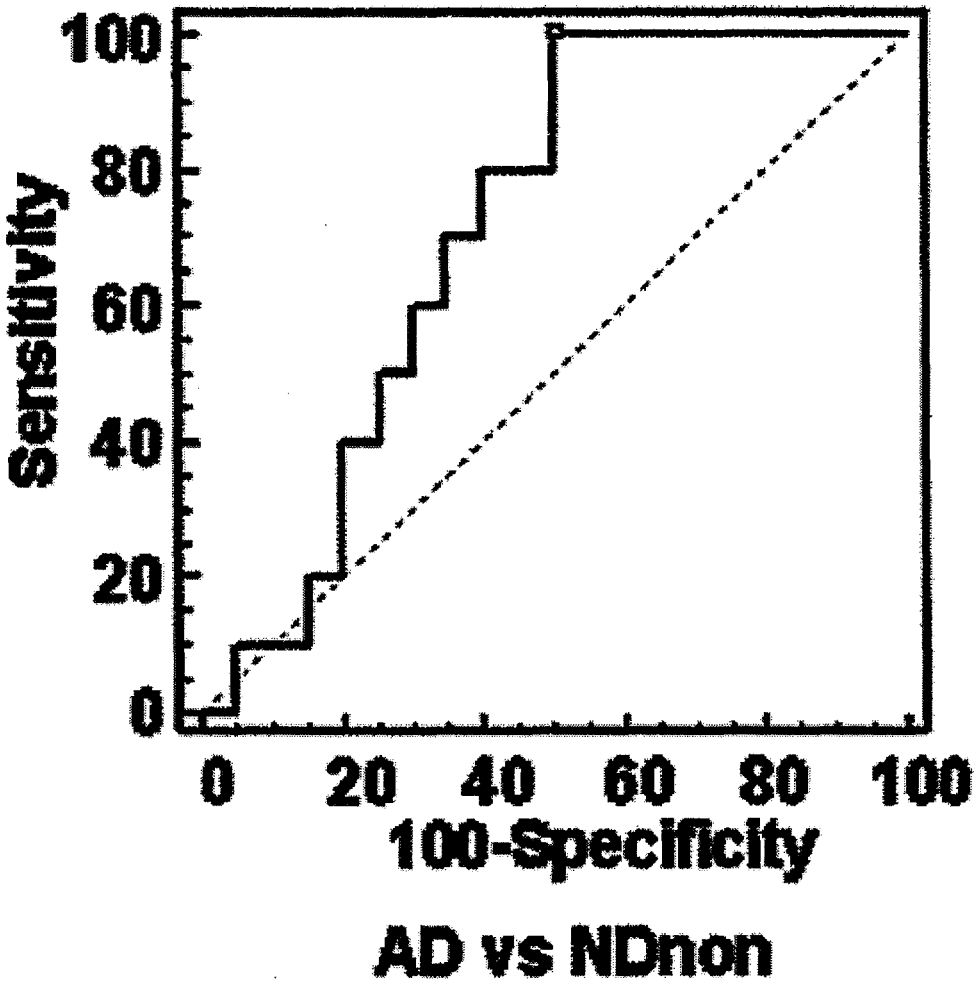
C


100
80
60
40
20
0
Sensitivity
0
20
40
60
80
100
100-Specificity
MCI vs ADN

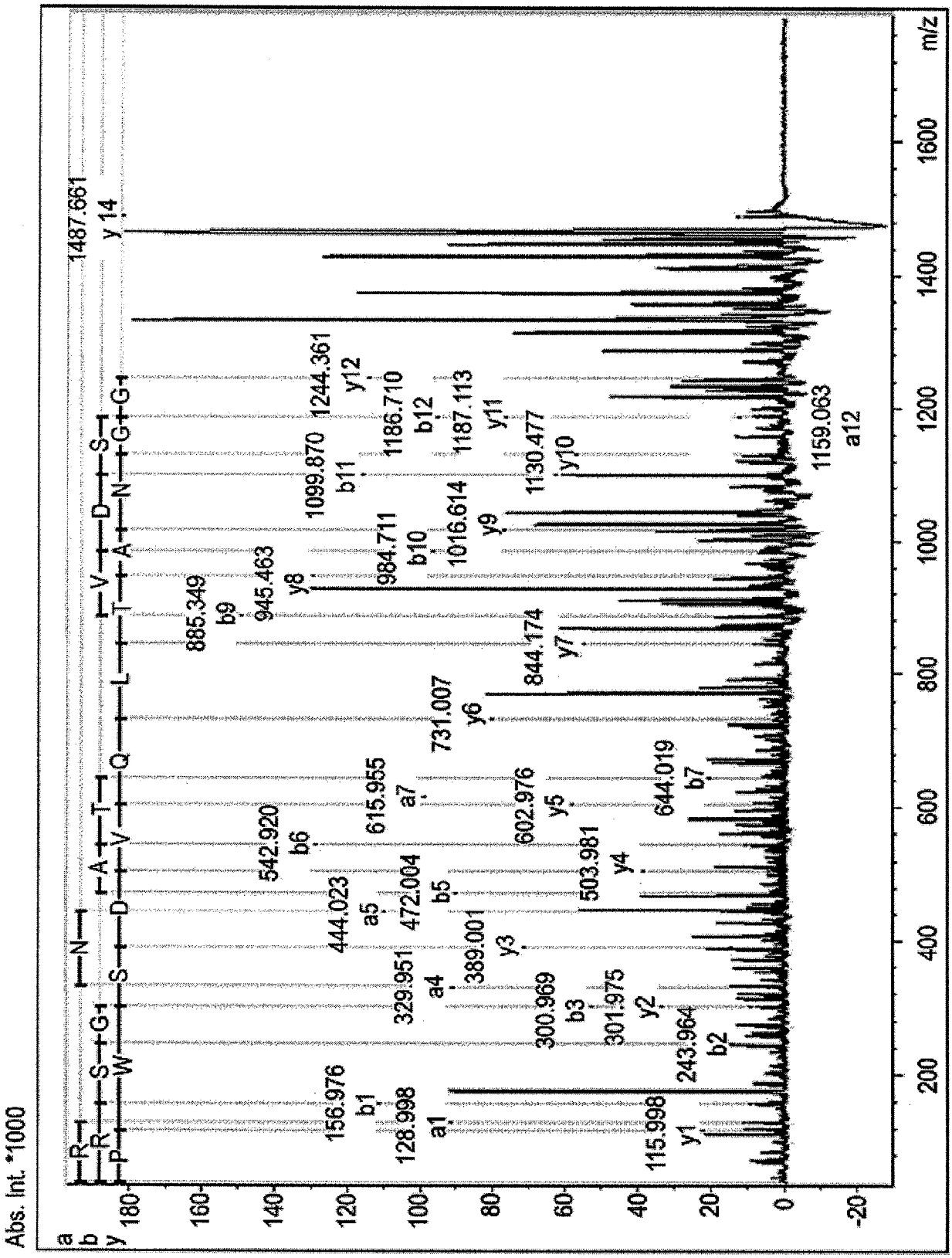
D


100
80
60
40
20
0
Sensitivity
0
20
40
60
80
100
100-Specificity
AD vs ADN

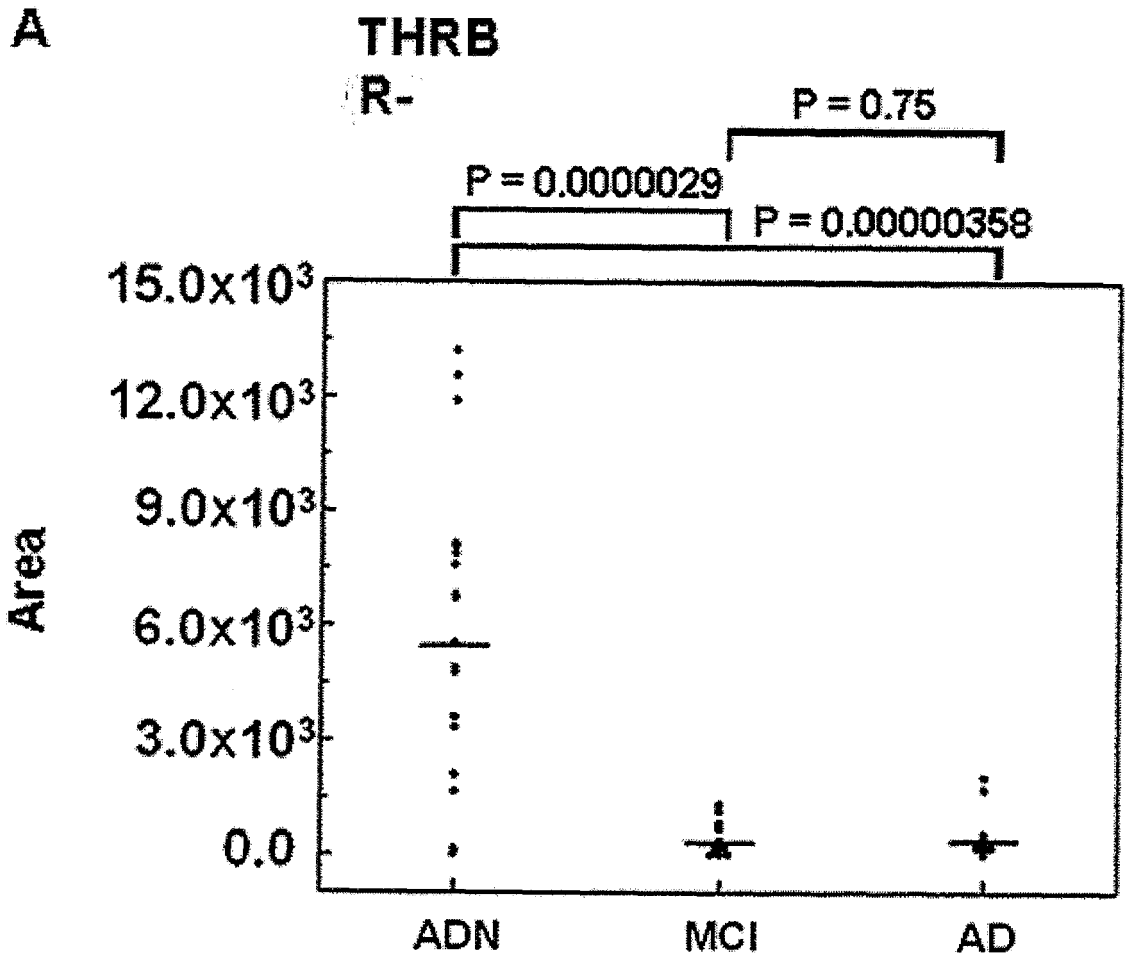
E

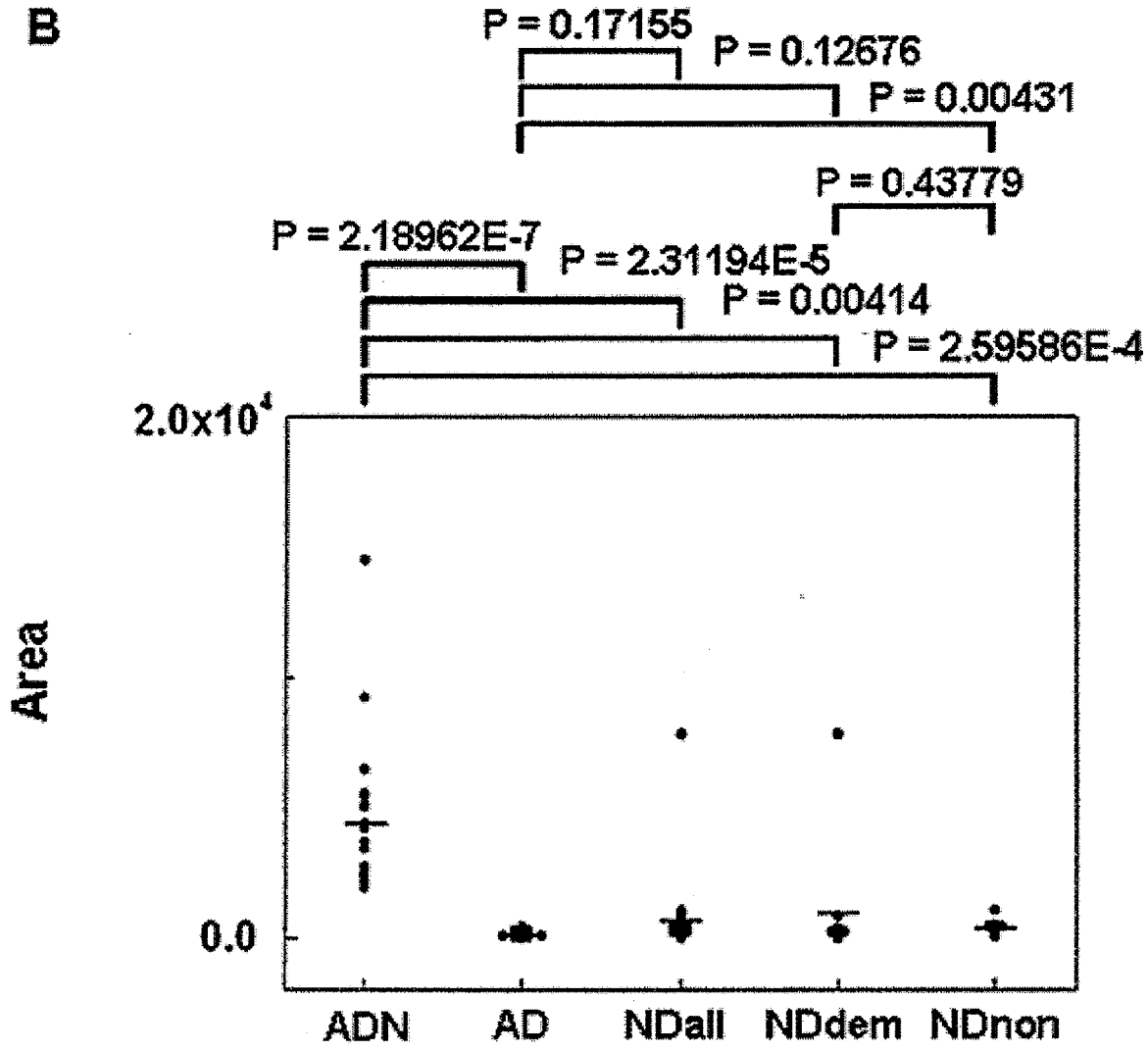

100
80
60
40
20
0
Sensitivity
0
20
40
60
80
100
100-Specificity
AD vs NDnon

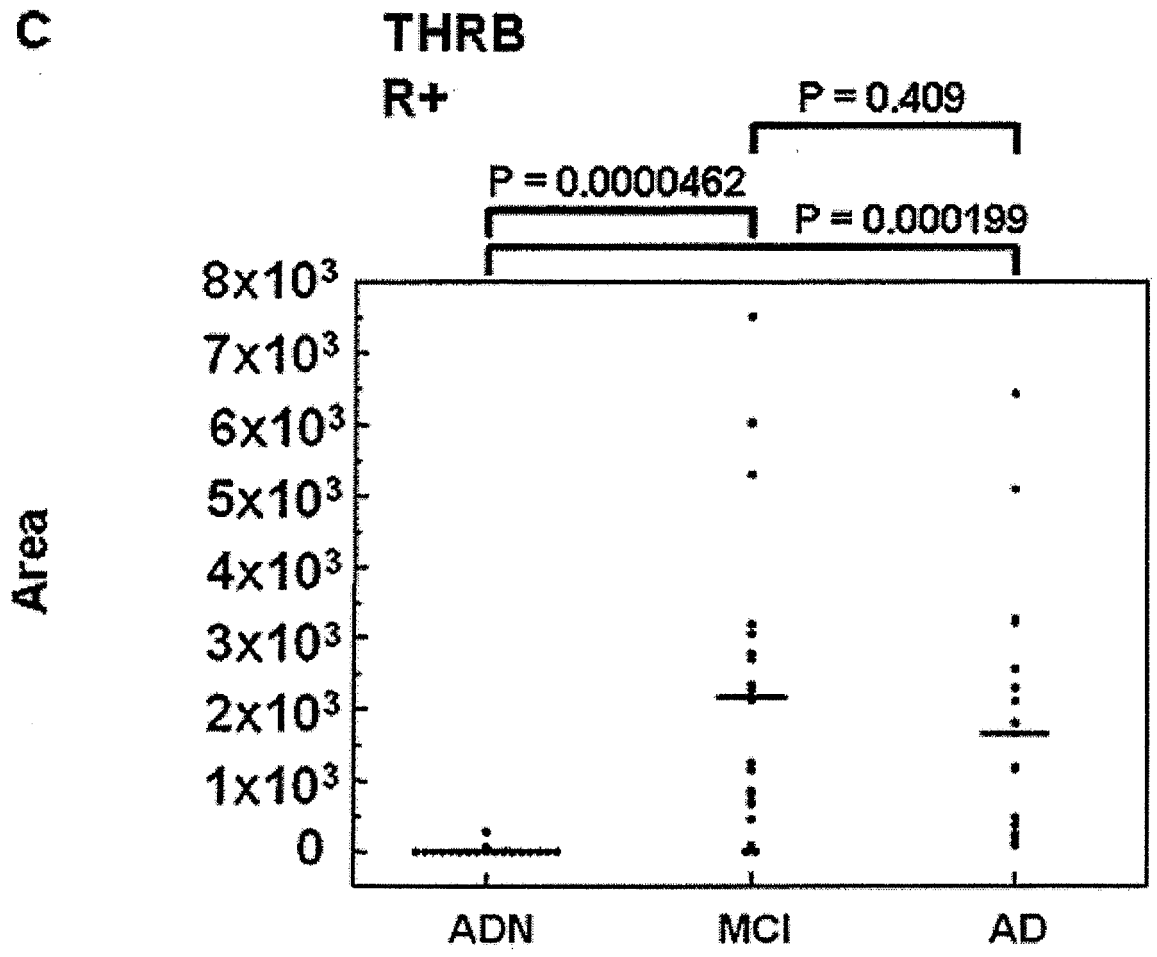
[Fig. 3]


Abs. Int. *1000
m/z
a
b
y
R R P W S G S N D A V T Q V D L T A V D S G G N
1487.661
y 14
1244.361
y12
1186.710
b12
1187.113
y11
1159.063
a12
1130.477
y10
1099.870
b11
1016.614
y9
984.711
b10
945.463
y8
885.349
b9
844.174
y7
731.007
y6
644.019
b7
615.955
a7
602.976
y5
542.920
b6
503.981
y4
472.004
b5
444.023
a5
389.001
y3
329.951
a4
301.975
y2
300.969
b3
243.964
b2
156.976
b1
128.998
a1
115.998
y1

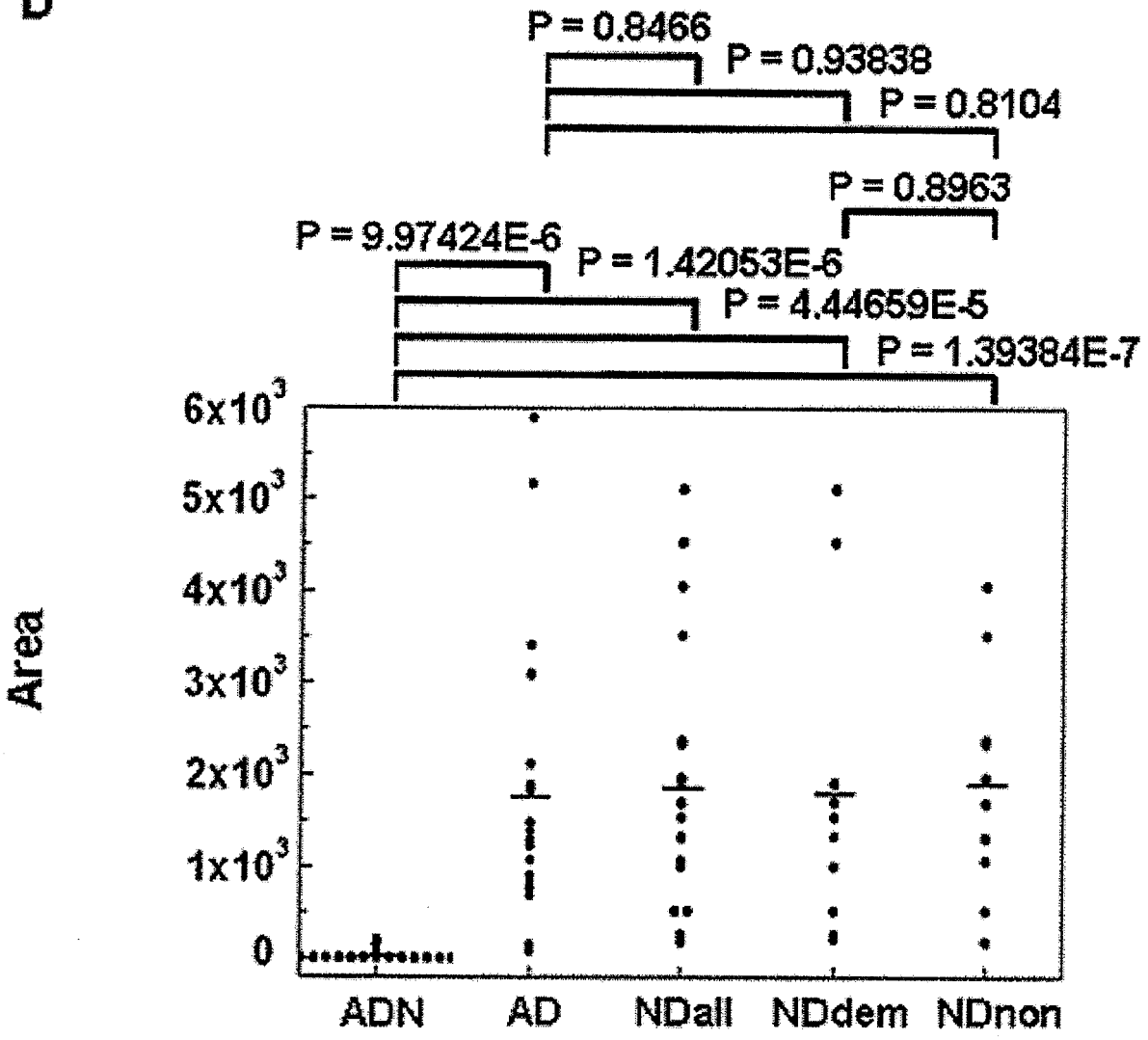
[Fig. 4]


A
THRB
R-
P = 0.75
P = 0.0000029
P = 0.00000358
15.0x10³
12.0x10³
9.0x10³
6.0x10³
3.0x10³
0.0
Area
ADN
MCI
AD

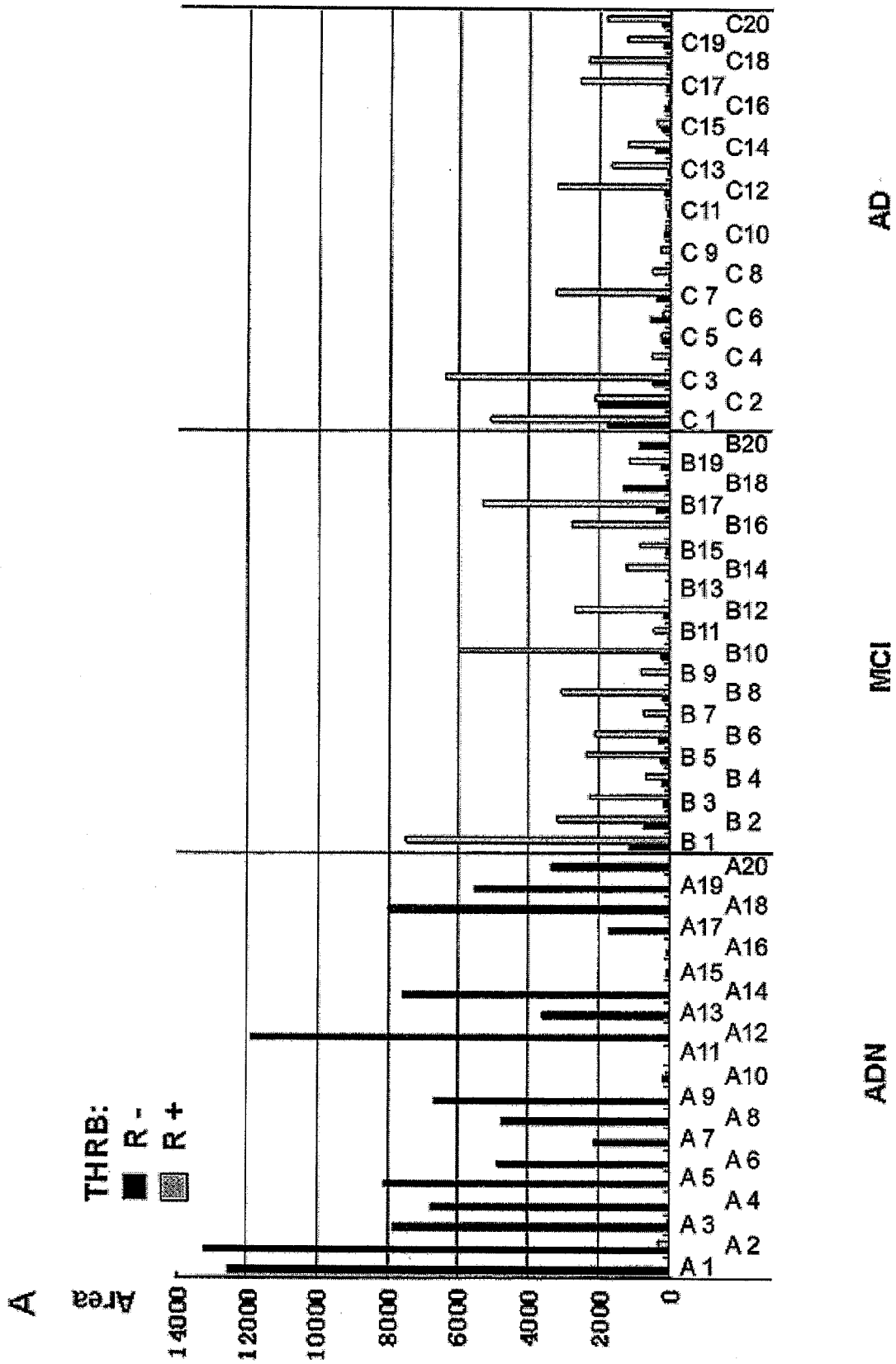

B
P = 0.17155
P = 0.12676
P = 0.00431
P = 0.43779
P = 2.18962E-7
P = 2.31194E-5
P = 0.00414
P = 2.59586E-4
2.0x10⁴
Area
0.0
ADN
AD
NDall
NDdem
NDnon

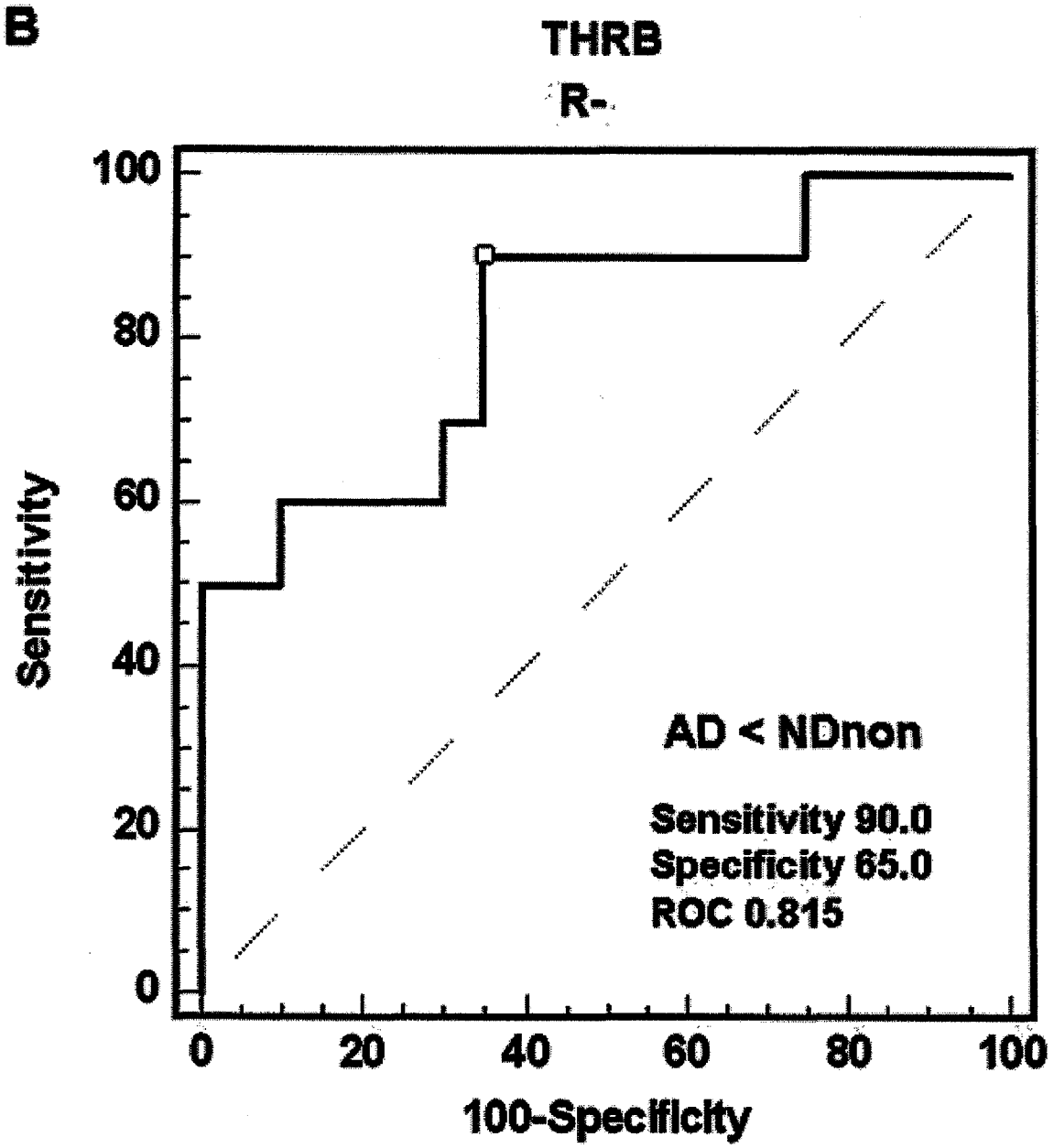

C
THRB
R+
P = 0.409
P = 0.0000462
P = 0.000199
Area
8x10³
7x10³
6x10³
5x10³
4x10³
3x10³
2x10³
1x10³
0
ADN
MCI
AD

D


P = 0.8466
P = 0.93838
P = 0.8104
P = 0.8963
P = 9.97424E-6
P = 1.42053E-6
P = 4.44659E-5
P = 1.39384E-7
Area
6x10^3
5x10^3
4x10^3
3x10^3
2x10^3
1x10^3
0
ADN
AD
NDall
NDdem
NDnon

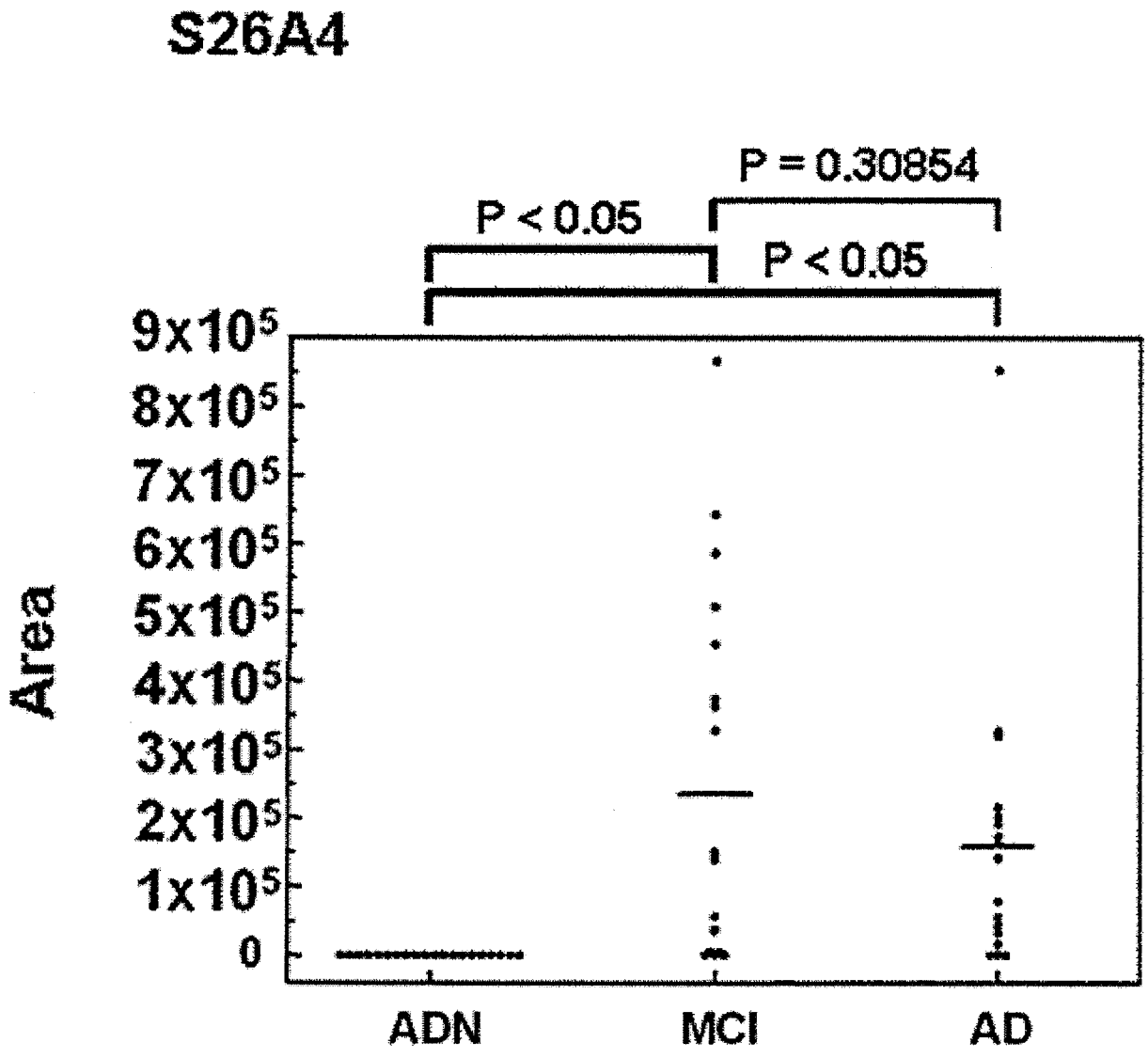
[Fig. 5]


A
Area
14000
12000
10000
8000
6000
4000
2000
0
THRB:
R -
R +
A1 A2 A3 A4 A5 A6 A7 A8 A9 A10 A11 A12 A13 A14 A15 A16 A17 A18 A19 A20
B1 B2 B3 B4 B5 B6 B7 B8 B9 B10 B11 B12 B13 B14 B15 B16 B17 B18 B19 B20
C1 C2 C3 C4 C5 C6 C7 C8 C9 C10 C11 C12 C13 C14 C15 C16 C17 C18 C19 C20
ADN
MCI
AD


B
THRB
R-
Sensitivity
100
80
60
40
20
0
0
20
40
60
80
100
100-Specificity
AD < NDnon
Sensitivity 90.0
Specificity 65.0
ROC 0.815

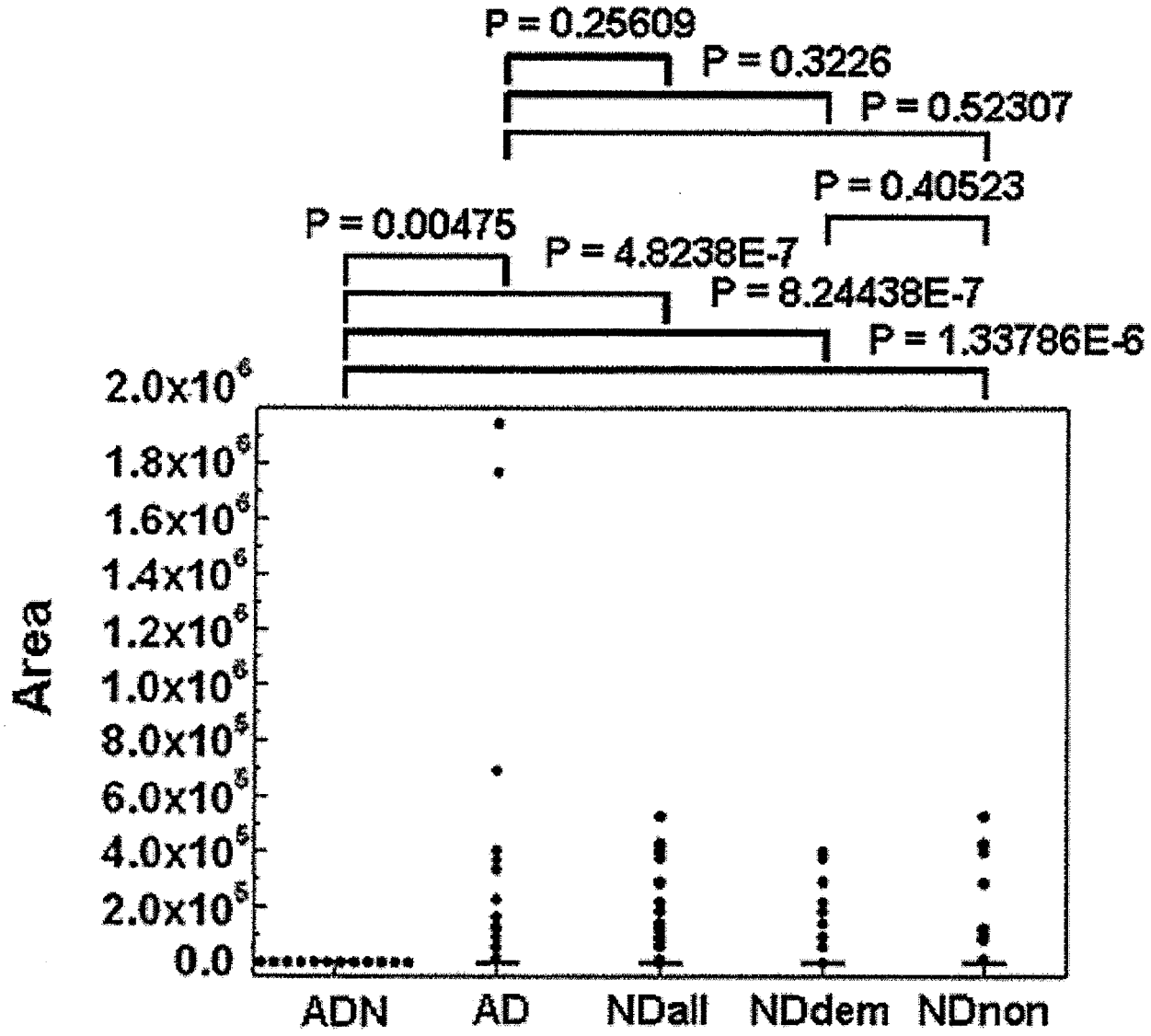
[Fig. 6]

A

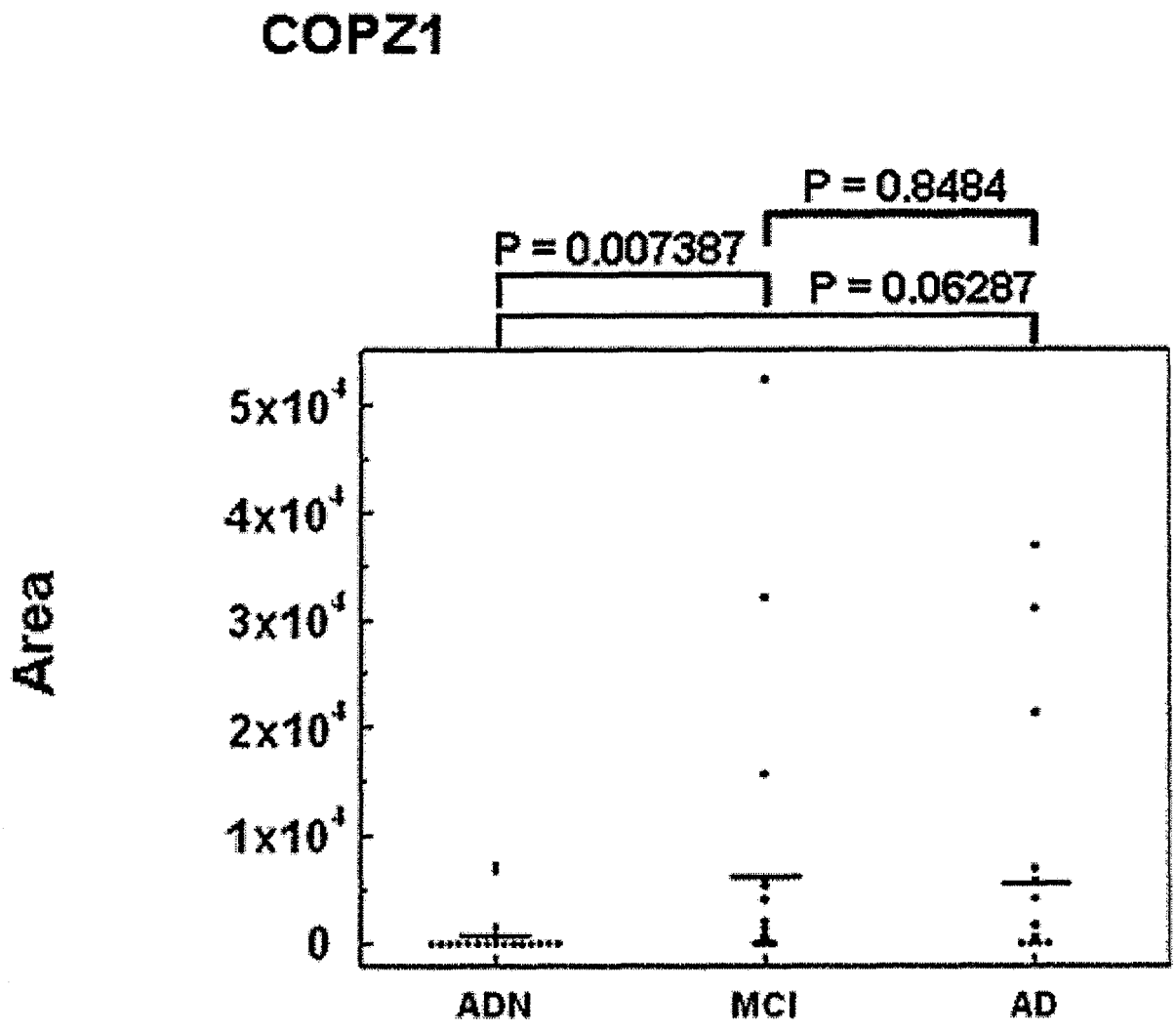

S26A4
P = 0.30854
P < 0.05
P < 0.05
Area
9x10^5
8x10^5
7x10^5
6x10^5
5x10^5
4x10^5
3x10^5
2x10^5
1x10^5
0
ADN
MCI
AD

B

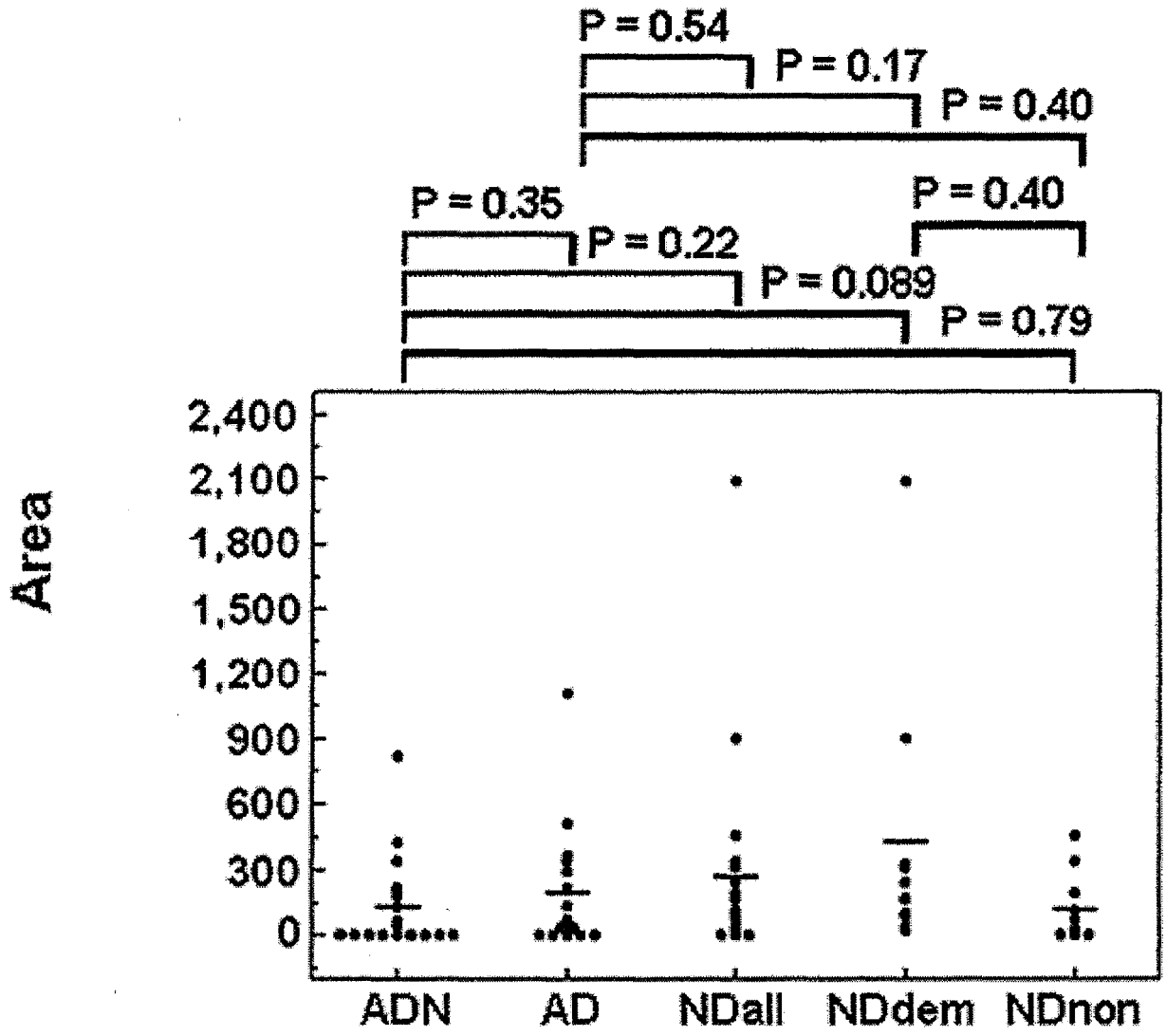
S26A4


P = 0.25609
P = 0.3226
P = 0.52307
P = 0.40523
P = 0.00475
P = 4.8238E-7
P = 8.24438E-7
P = 1.33786E-6
Area
2.0x10^6
1.8x10^6
1.6x10^6
1.4x10^6
1.2x10^6
1.0x10^6
8.0x10^5
6.0x10^5
4.0x10^5
2.0x10^5
0.0
ADN
AD
NDall
NDdem
NDnon

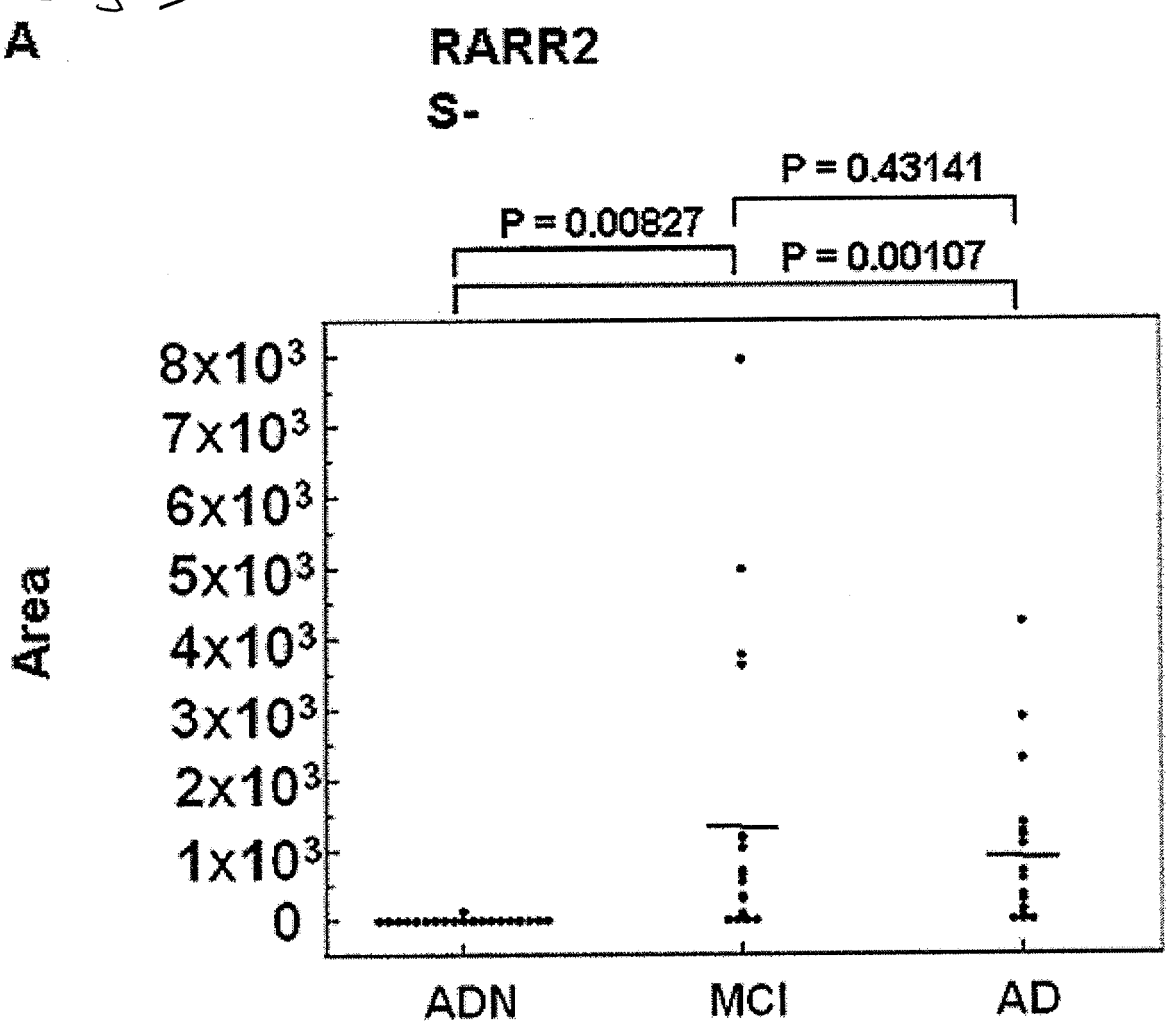
C


COPZ1
P = 0.8484
P = 0.007387
P = 0.06287
Area
5x10⁴
4x10⁴
3x10⁴
2x10⁴
1x10⁴
0
ADN
MCI
AD

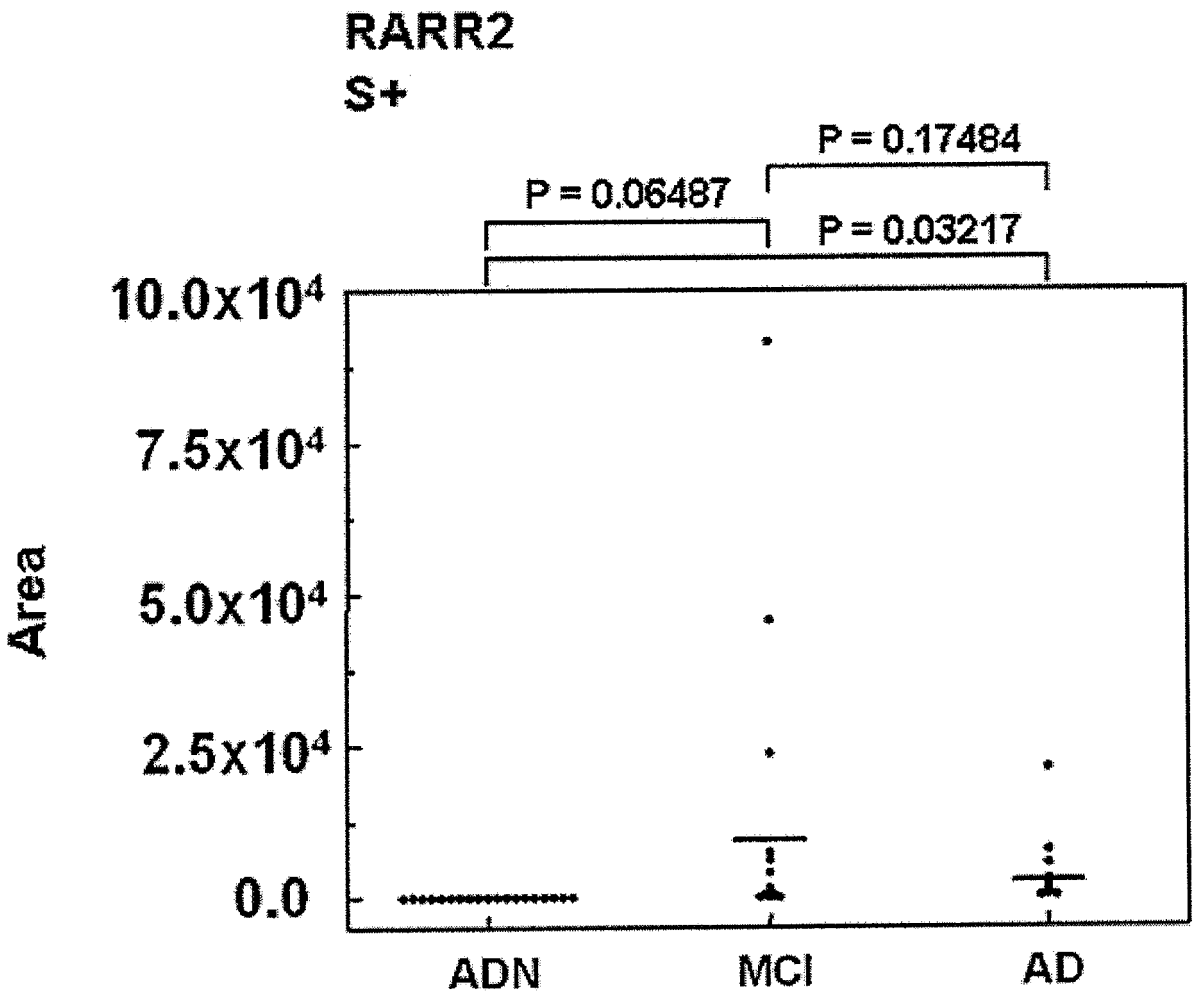
D


COPZ1
P = 0.54
P = 0.17
P = 0.40
P = 0.35
P = 0.22
P = 0.40
P = 0.089
P = 0.79
Area
2,400
2,100
1,800
1,500
1,200
900
600
300
0
ADN
AD
NDall
NDdem
NDnon

[Fig. 7]


A
RARR2
S-
P = 0.43141
P = 0.00827
P = 0.00107
Area
8x10³
7x10³
6x10³
5x10³
4x10³
3x10³
2x10³
1x10³
0
ADN
MCI
AD

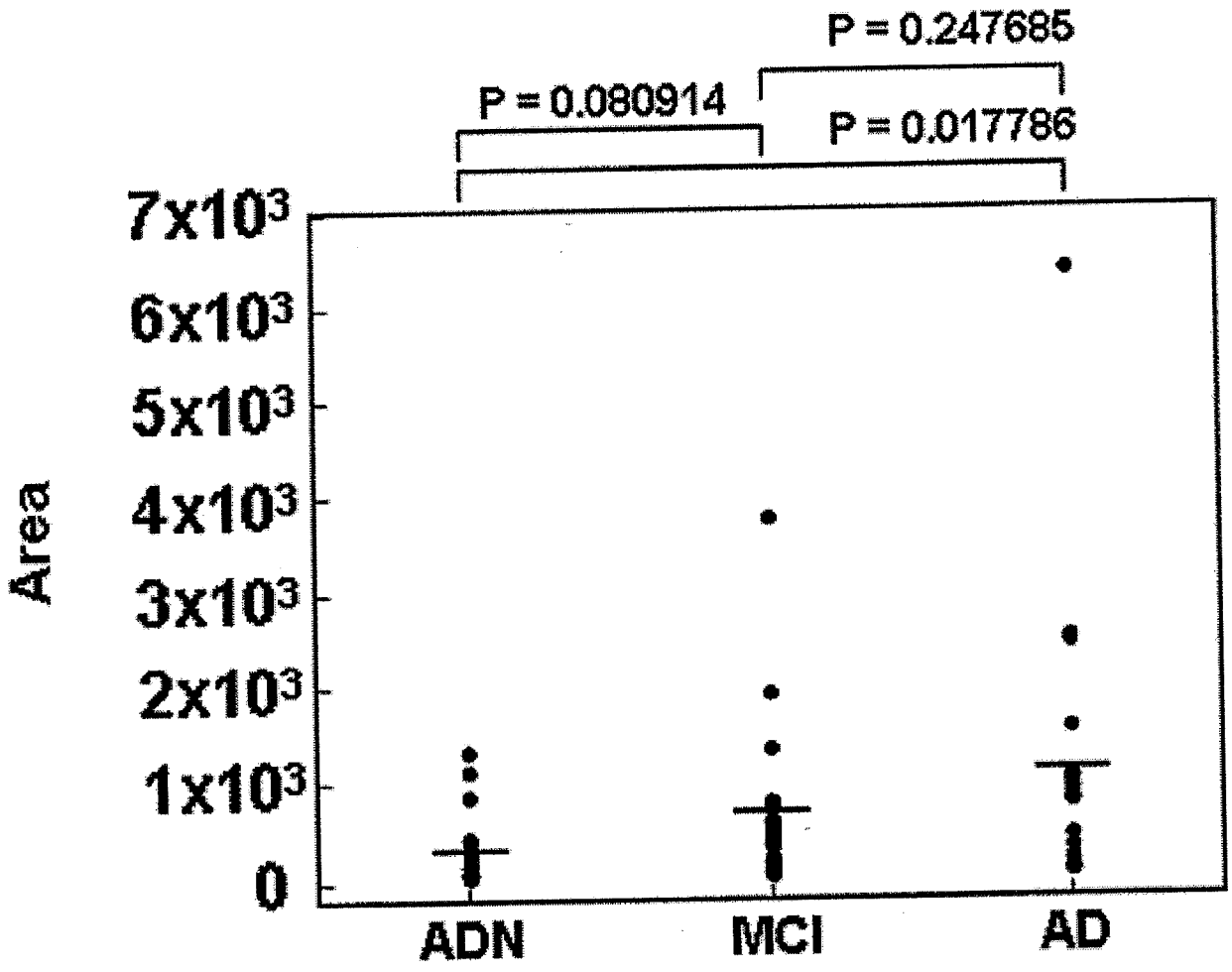
B


RARR2
S+
P = 0.17484
P = 0.06487
P = 0.03217
10.0x10⁴
7.5x10⁴
5.0x10⁴
2.5x10⁴
0.0
Area
ADN
MCI
AD

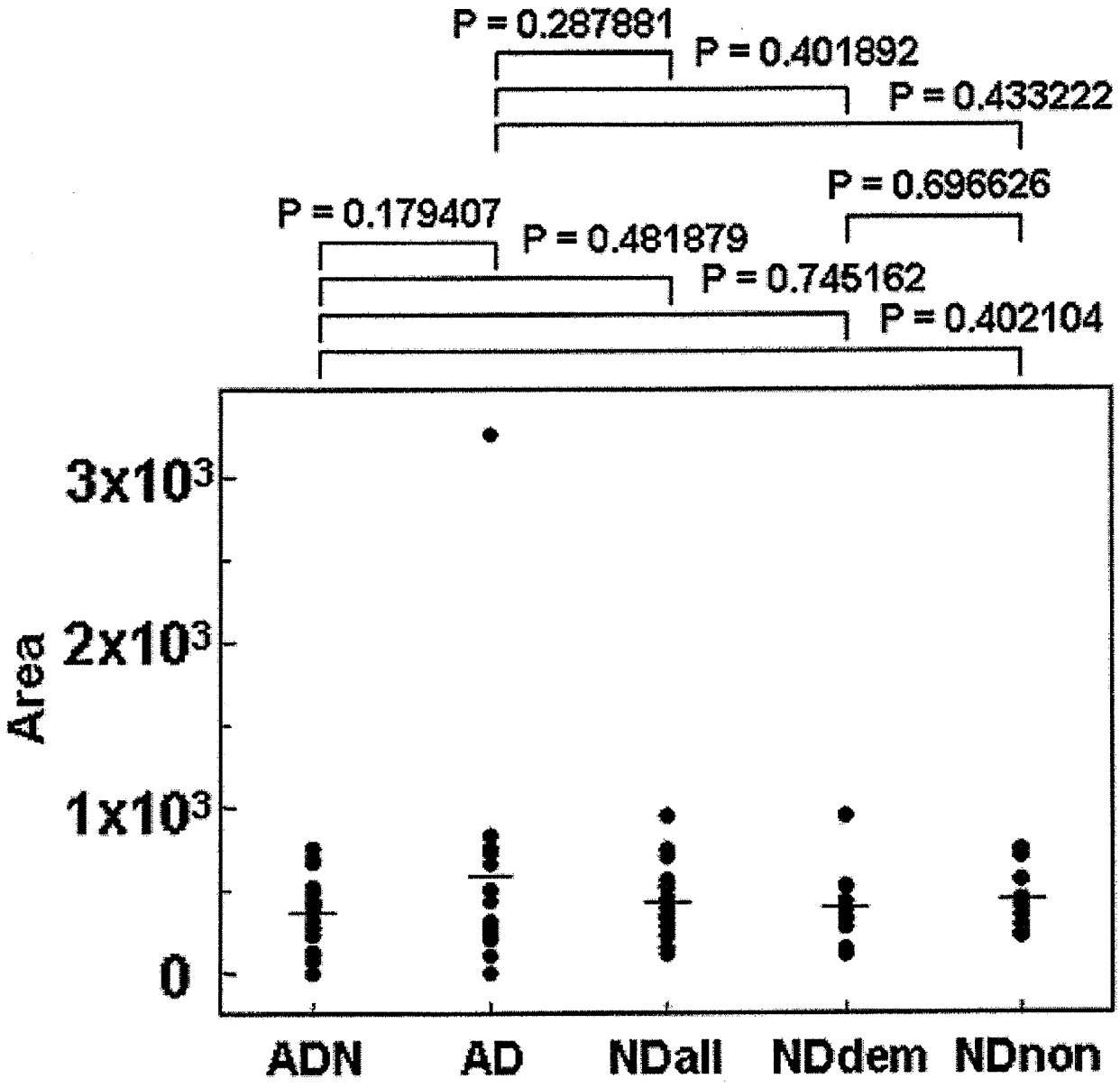
[Fig. 8]

A

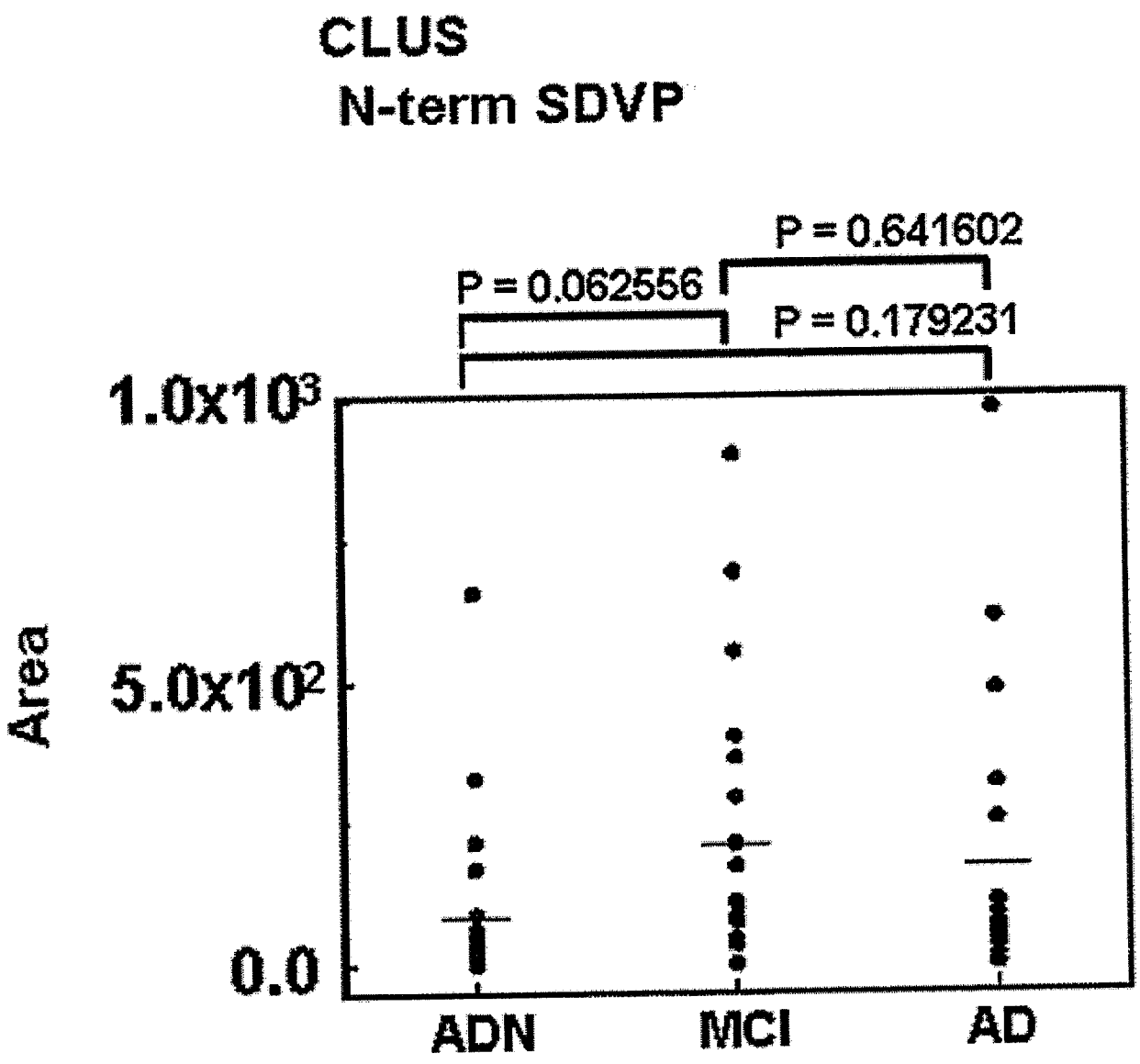
GELS


P = 0.247685
P = 0.080914
P = 0.017786
Area
7x10³
6x10³
5x10³
4x10³
3x10³
2x10³
1x10³
0
ADN
MCI
AD

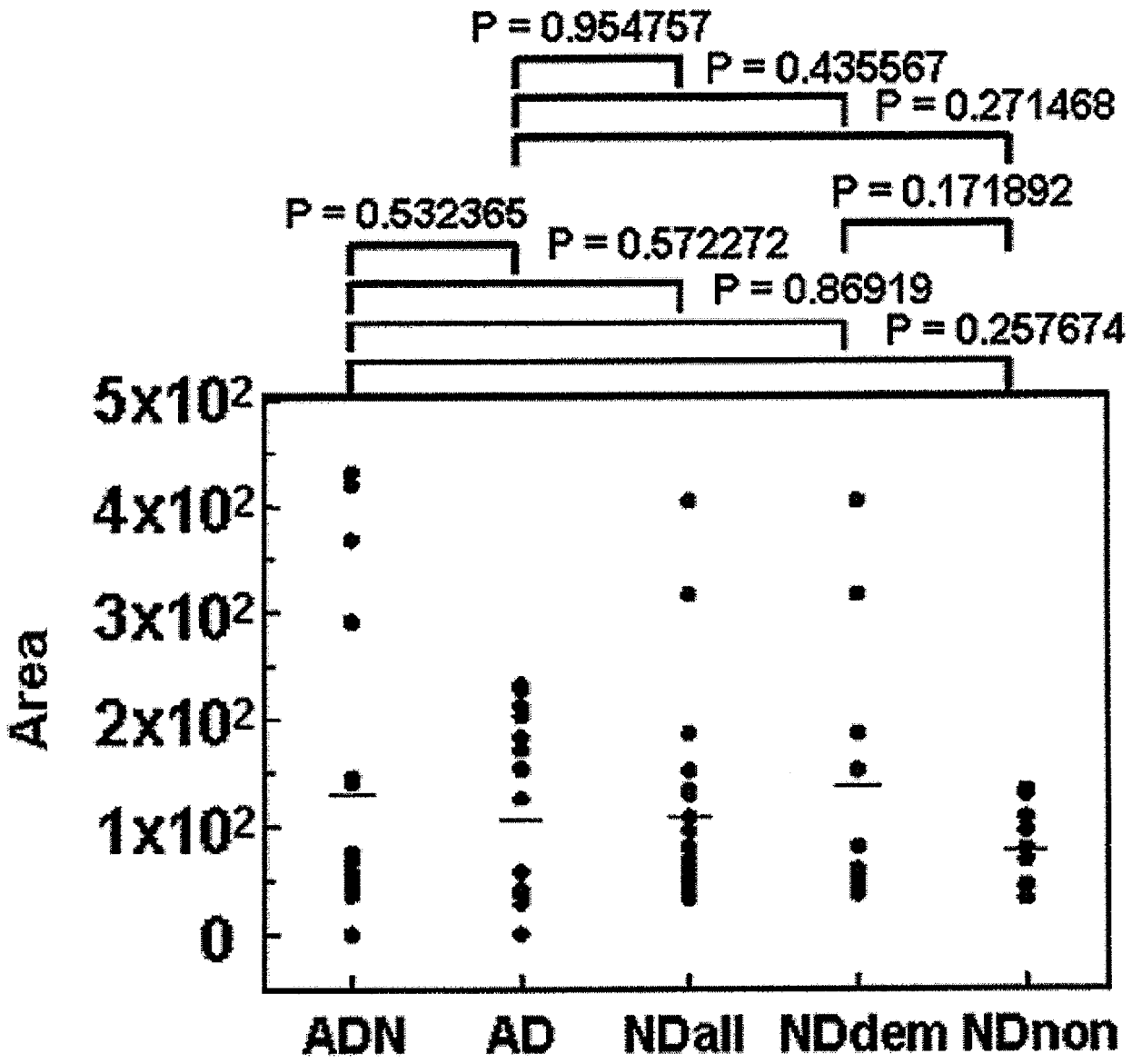
B


GELS
P = 0.287881
P = 0.401892
P = 0.433222
P = 0.696626
P = 0.179407
P = 0.481879
P = 0.745162
P = 0.402104
Area
3x10³
2x10³
1x10³
0
ADN
AD
NDall
NDdem
NDnon

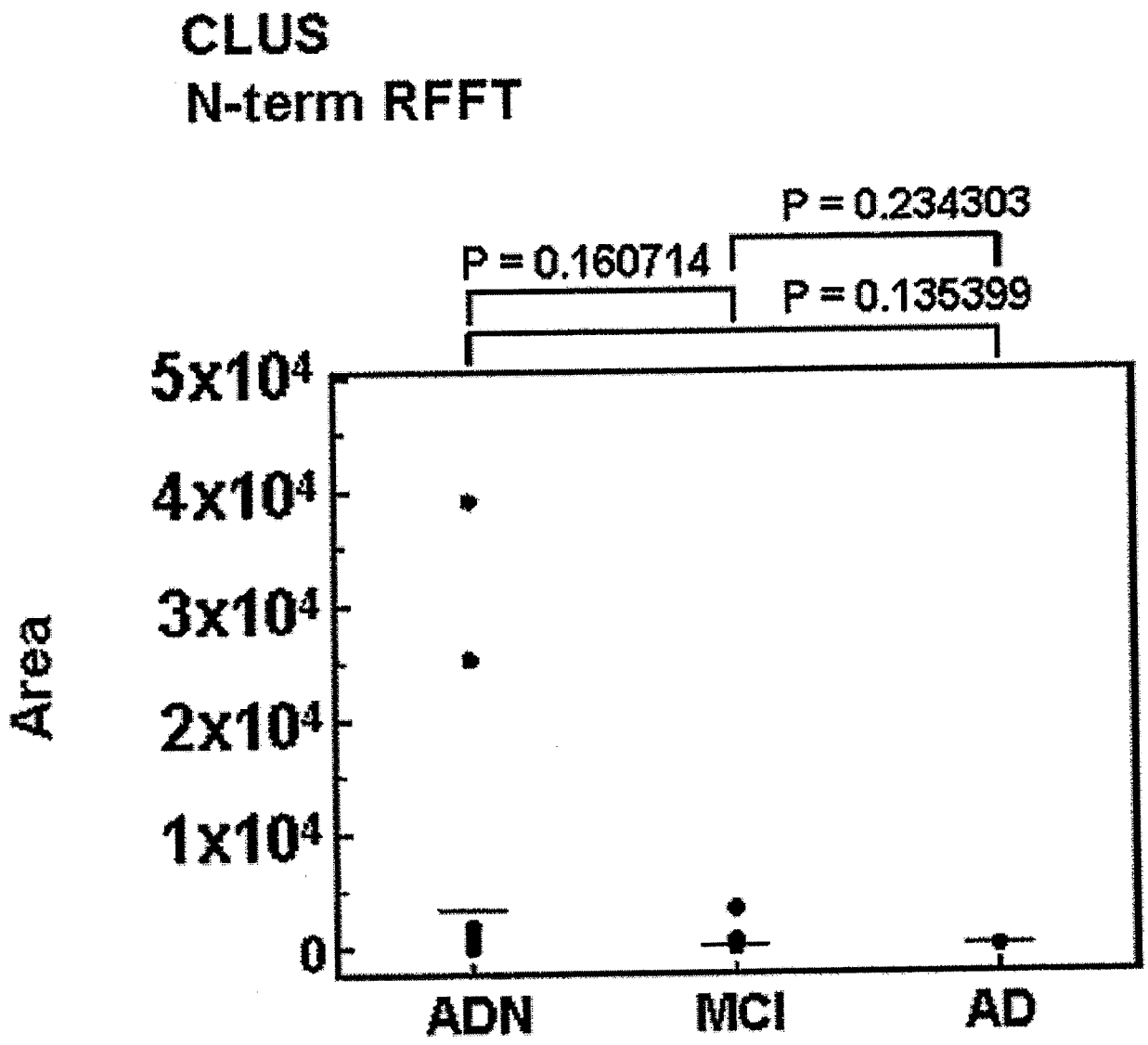
A [Fig. 9]


CLUS
N-term SDVP
P = 0.641602
P = 0.062556
P = 0.179231
1.0x10³
5.0x10²
0.0
Area
ADN
MCI
AD

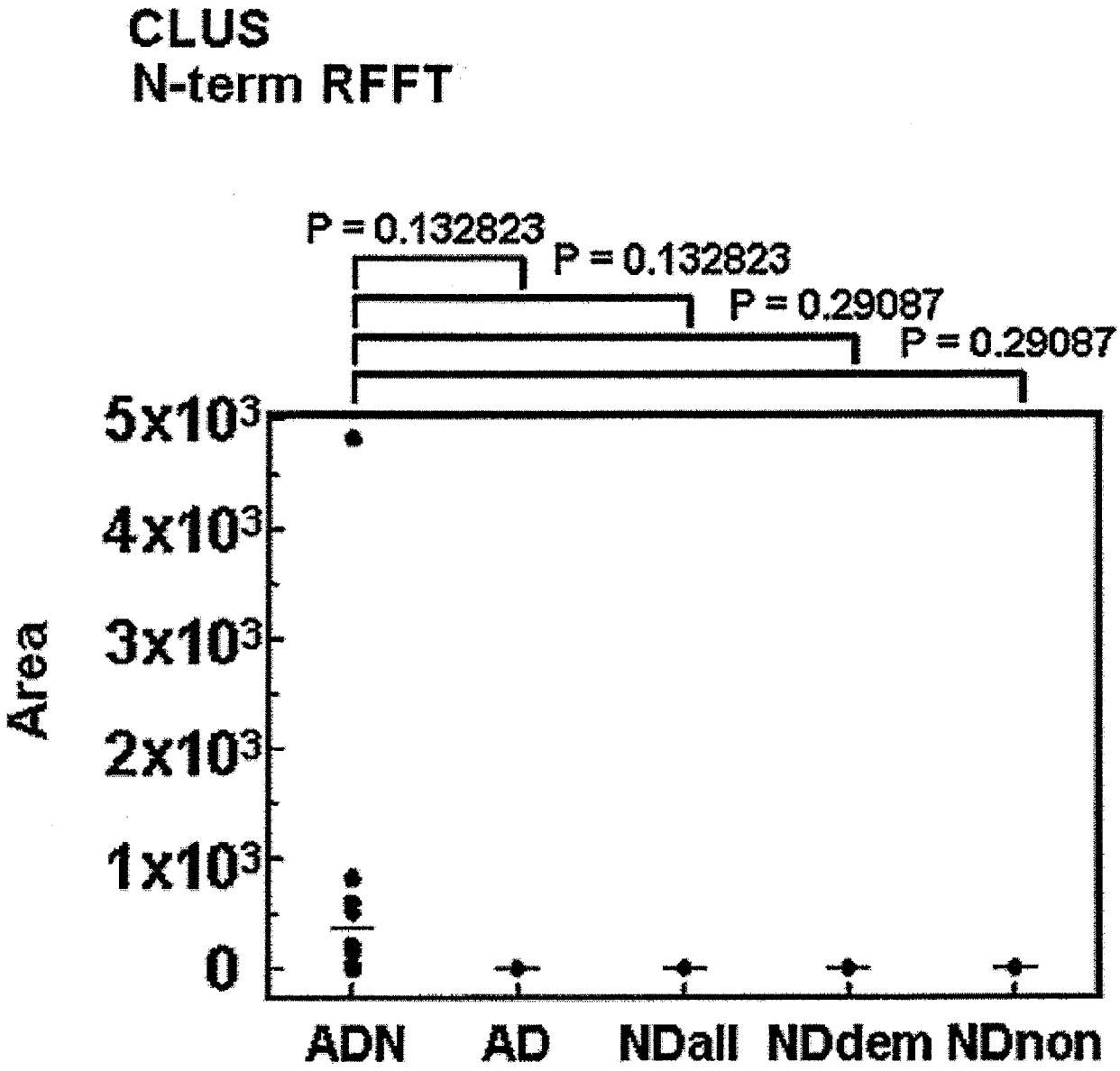
B


CLUS
N-term SDVP
P = 0.954757
P = 0.435567
P = 0.271468
P = 0.171892
P = 0.532365
P = 0.572272
P = 0.86919
P = 0.257674
5x10²
4x10²
3x10²
2x10²
1x10²
0
Area
ADN
AD
NDall
NDdem
NDnon

C

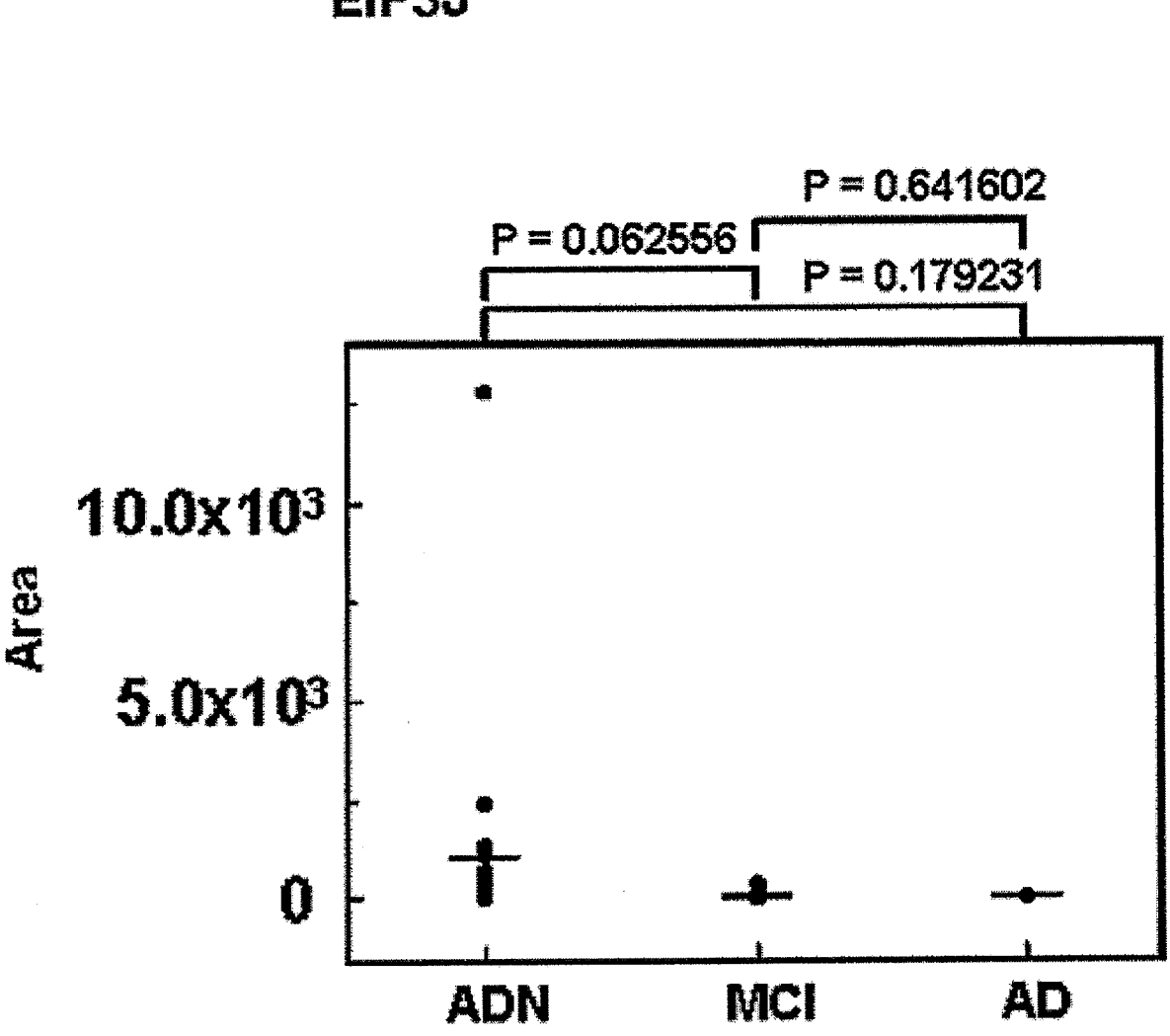

CLUS
N-term RFFT
P = 0.234303
P = 0.160714
P = 0.135399
5x10⁴
4x10⁴
3x10⁴
2x10⁴
1x10⁴
0
Area
ADN
MCI
AD

D

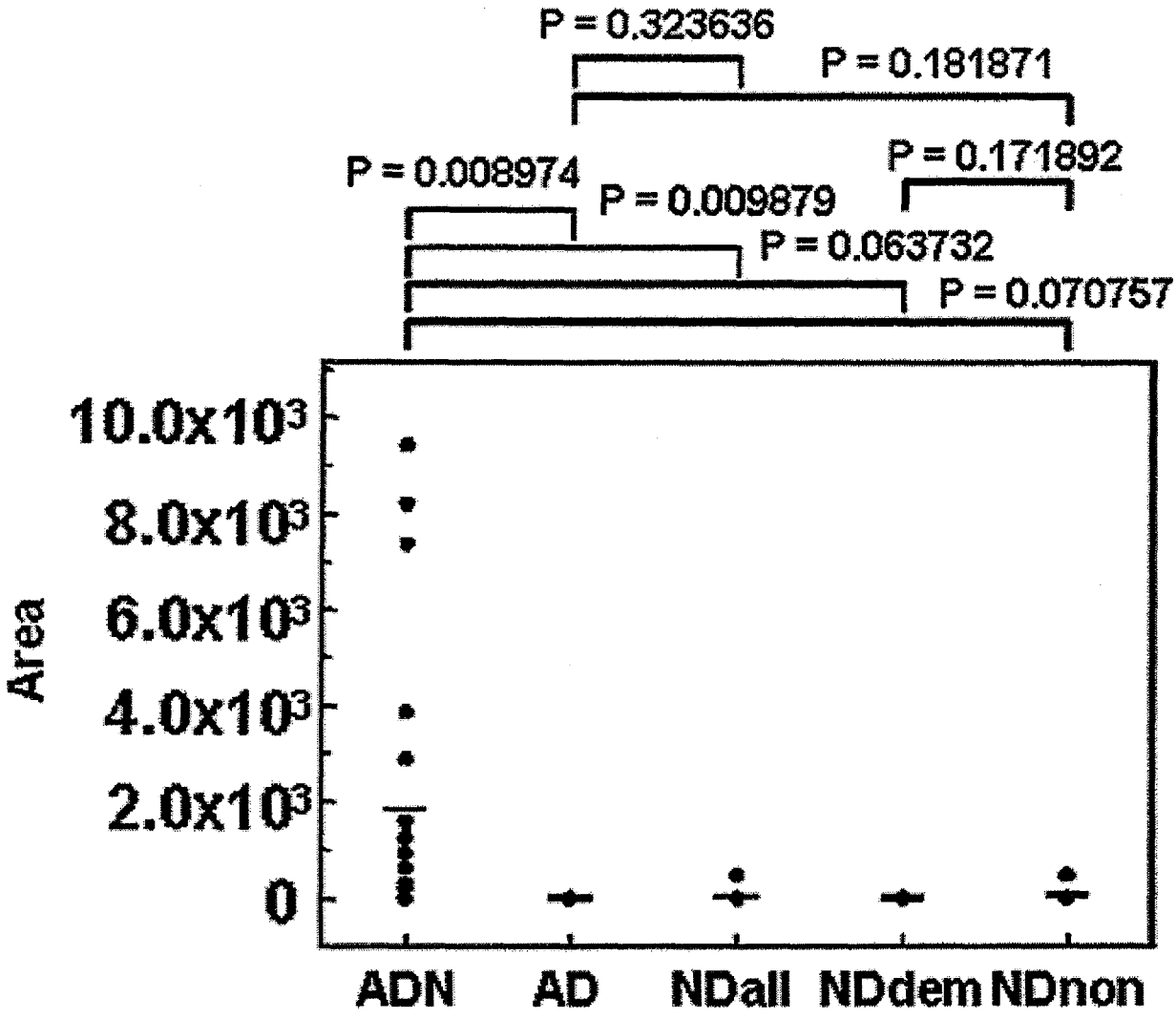

CLUS
N-term RFFT
P = 0.132823
P = 0.132823
P = 0.29087
P = 0.29087
5x10³
4x10³
3x10³
2x10³
1x10³
0
Area
ADN
AD
NDall
NDdem
NDnon

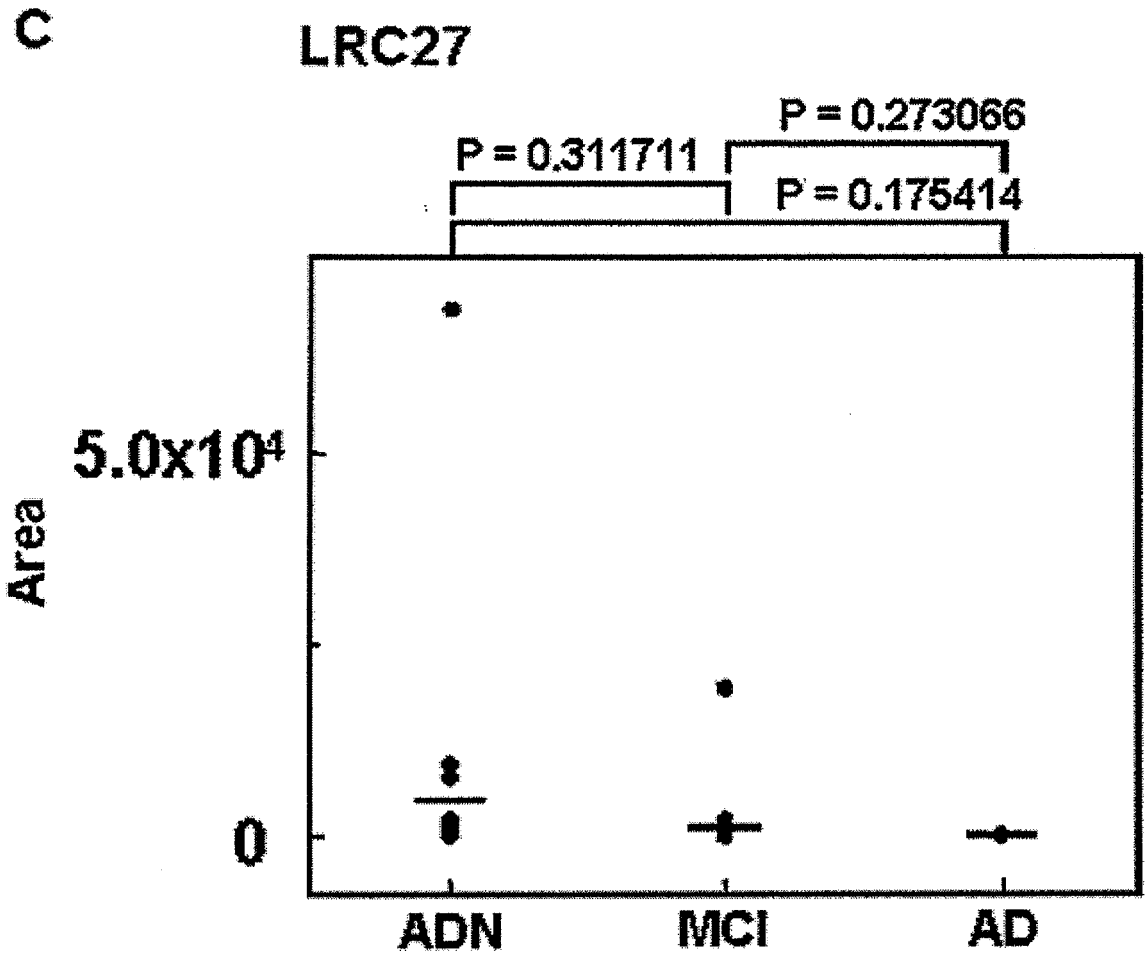
[Fig. 10]

A

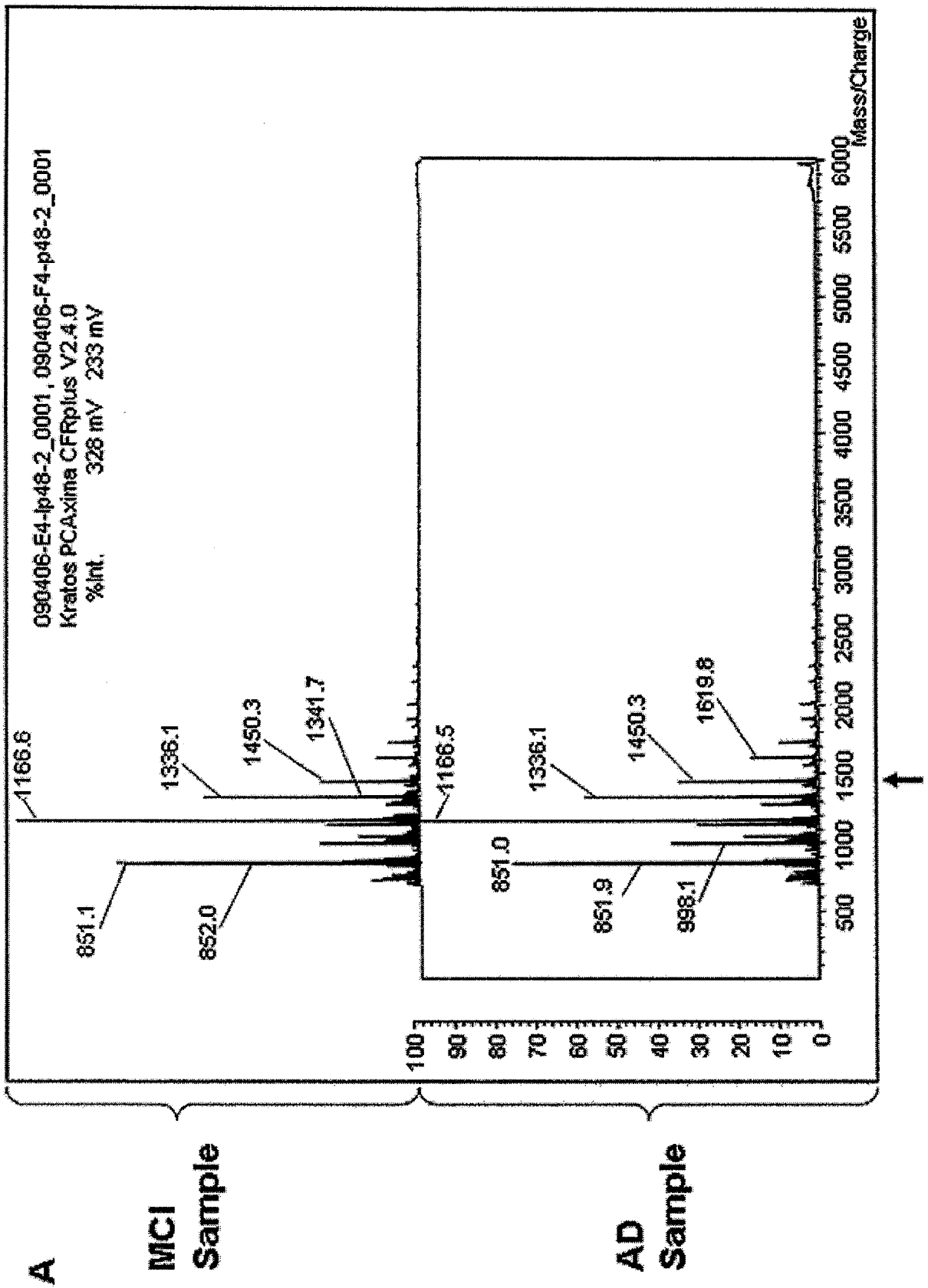

EIF3J
P = 0.641602
P = 0.062556
P = 0.179231
Area
10.0x10³
5.0x10³
0
ADN
MCI
AD

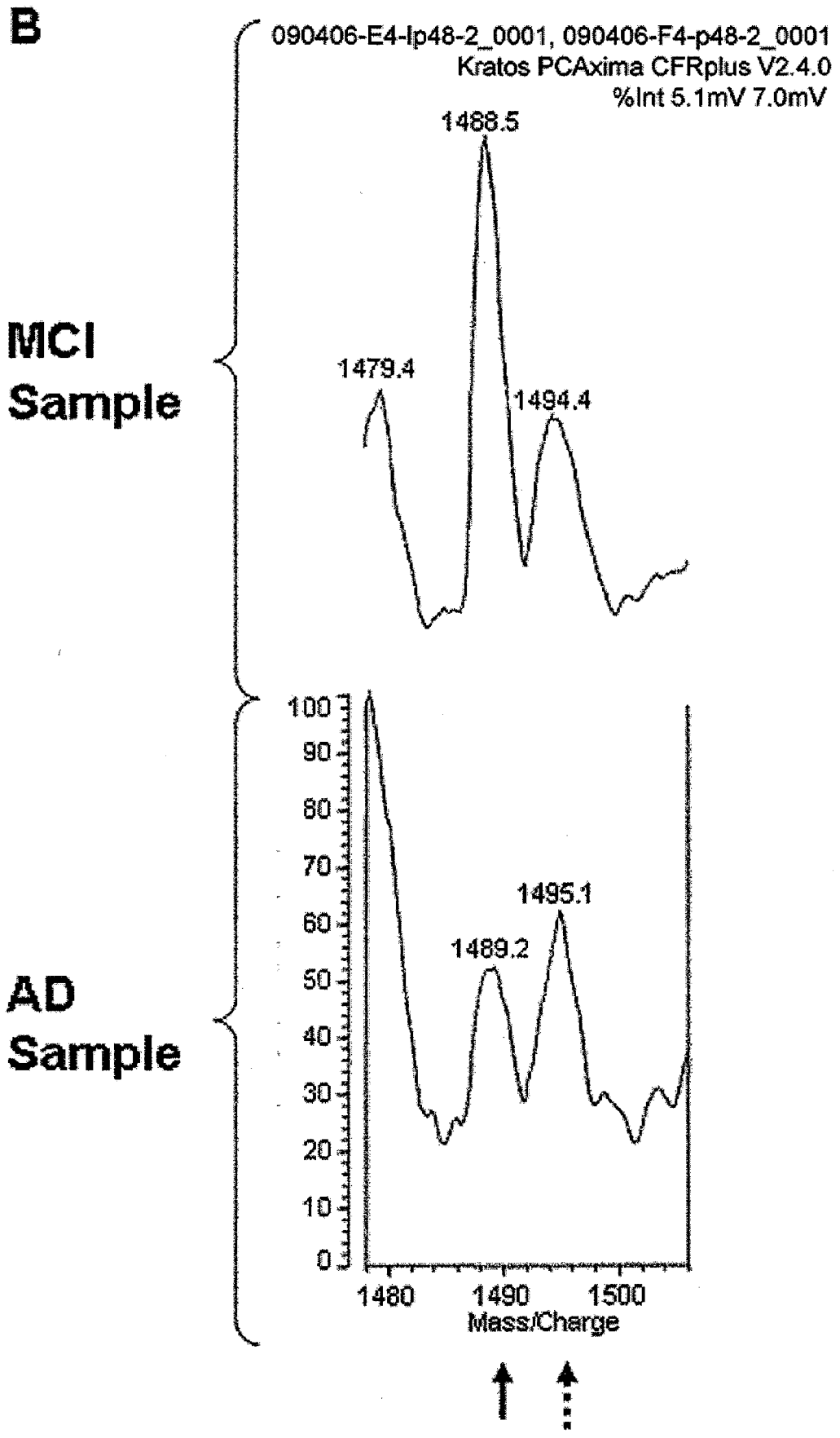
B


EIF3J
P = 0.323636
P = 0.181871
P = 0.171892
P = 0.008974
P = 0.009879
P = 0.063732
P = 0.070757
10.0x10³
8.0x10³
6.0x10³
4.0x10³
2.0x10³
0
Area
ADN
AD
NDall
NDdem
NDnon


C
LRC27
P = 0.273066
P = 0.311711
P = 0.175414
5.0x10⁴
Area
0
ADN
MCI
AD

[Fig. 11]


A
MCI Sample
AD Sample
090406-E4-lp48-2_0001, 090406-F4-p48-2_0001
Kratos PCAxima CFRplus V2.4.0
%Int. 328 mV 233 mV
1166.6
1336.1
1450.3
1341.7
851.1
852.0
1166.5
1336.1
1450.3
1619.8
851.0
851.9
998.1
100
90
80
70
60
50
40
30
20
10
0
500
1000
1500
2000
2500
3000
3500
4000
4500
5000
5500
6000
Mass/Charge


B
090406-E4-Ip48-2_0001, 090406-F4-p48-2_0001
Kratos PCAxima CFRplus V2.4.0
%Int 5.1mV 7.0mV
1488.5
1479.4
1494.4
MCI Sample
AD Sample
100
90
80
70
60
50
40
30
20
10
0
1495.1
1489.2
1480
1490
1500
Mass/Charge

REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

- JP 2004333274 A **[0011]**
- JP 2006308533 A **[0011]**
- WO 03087768 A2 **[0013]**

Non-patent literature cited in the description

- The better understanding of Alzheimer's disease. Nagai Shoten Co., Ltd, 2004 **[0012]**
- **BENKIRANE, N. et al.** *J. Biol. Chem.,* 1993, vol. 268, 26279-26285 **[0012]**