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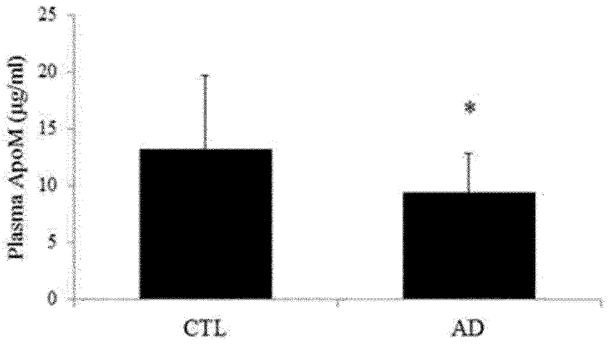
(54) DIAGNOSTIC MARKER COMPOSITION COMPRISING APOLIPOPROTEIN M FOR ALZHEIMER'S DISEASE

(57) The present invention relates to a bio-marker comprising apolipoprotein M for diagnosing Alzheimer's disease, an Alzheimer's disease diagnostic composition comprising material for measuring expression levels of apolipoprotein M or mRNA of genes coding same, an Alzheimer's disease diagnostic kit comprising the composition, and a method for providing information for diagnosing Alzheimer's disease by means of the composition. The apolipoprotein M according to the present invention is expressed at a notably reduced level in an Alzheimer's disease patient in comparison to a normal person, and thus can be effectively used to accurately and rapidly diagnose whether Alzheimer's disease is present.

【Fig 1】



$p < 0.05$
CTL: healthy control group members (n = 10)
AD: Alzheimer's disease patients (Aβ accumulation) (n = 24)

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Description

Technical Field

[0001] It is an object of the present invention to provide a bio-marker composition for diagnosing Alzheimer's disease comprising apolipoprotein M, a composition for diagnosing Alzheimer's disease comprising a formulation measuring a protein expression level of apolipoprotein M or an mRNA expression level of a gene coding apolipoprotein M, a kit for diagnosing Alzheimer's disease comprising the composition, and a method for providing information for diagnosing Alzheimer's disease using the composition.

Background Art

[0002] Alzheimer's disease is known as caused by death of neurons by extracellular ß-amyloid deposits, which are senile plaques, and intercellular hyperphosphorylated tau proteins throughout the brain. Alzheimer's disease causes gradual memory impairment, cognitive deficit, changes in individual personality, etc. Once Alzheimer's disease occurs, the disease continuously proceeds. Also, methods for diagnosing Alzheimer's disease are restrictive, and there is no fundamental therapy for the disease. Furthermore, it is known that a group with the disease is known as having reduced average life expectancy per age group compared to a normal group.

[0003] Alzheimer's disease causes dementia which is the most common disease among the elderly. In Korea, as the age increases by 5 in the age range of 65 to 85, the incidence rate of Alzheimer's disease increases twice each. Additionally, the number of the elderly with dementia is estimated to be about 0.47 million in 2010, which accounts for 8.8% of the elderly population over 65. Considering the rapid aging rate in Korea, the number of patients with dementia will also rapidly increase. In future, the number of patients with dementia is estimated to be 0.75 million in 2020, 1.14 million in 2030, and 2.13 million in 2050.

[0004] Currently, cholinesterase inhibitors (Aricept, Exelon, Reminyl, etc.) targeting for cholinergic neuron, and Memantine, which is a glutamate antagonist, are drugs for preventing or treating Alzheimer's disease. These drugs were made to slow down the progress of Alzheimer's disease or to treat symptoms of Alzheimer's disease, and are prescribed after an exact confirmation thereon is made. Most Alzheimer's disease could be diagnosed when the disease proceeds to some degree and symptoms thereof are shown. Accordingly, an early diagnosis from which a disease could be confirmed before a symptom thereof is shown has been difficult to be made as to Alzheimer's disease. Thus, new means for early diagnosis related with early diagnosis methods for Alzheimer's disease are keenly required.

[0005] Accordingly, the present inventors have continuously conducted a research on the development of a marker for diagnosing Alzheimer's disease, confirmed that patients with Alzheimer's diseases have a significantly reduced expression of apolipoprotein M compared to normal people, and completed the present invention.

Summary of invention

[0006] It is an object of the present invention to provide a bio-marker composition for diagnosing Alzheimer's disease comprising apolipoprotein M.

[0007] It is another object of the present invention to provide a composition for diagnosing Alzheimer's disease comprising a formulation measuring a protein expression level of apolipoprotein M or an mRNA expression level of a gene coding apolipoprotein M.

[0008] It is another object of the present invention to provide a kit for diagnosing Alzheimer's disease comprising the composition.

[0009] It is another object of the present invention to provide a method for providing information for diagnosing Alzheimer's disease using the composition.

[0010] In order to achieve the above-mentioned objects, the present invention provides a bio-marker composition for diagnosing Alzheimer's disease comprising apolipoprotein M.

[0011] Also, the present invention provides a composition for diagnosing Alzheimer's disease comprising a formulation measuring a protein expression level of apolipoprotein M or an mRNA expression level of a gene coding apolipoprotein M.

[0012] Also, the present invention provides a kit for diagnosing Alzheimer's disease comprising the composition.

[0013] Also, the present invention provides a method for providing information for diagnosing Alzheimer's disease using the composition.

[0014] Since an expression of apolipoprotein M according to the present invention is significantly reduced in patients with Alzheimer's disease compared to normal people, apolipoprotein M may be useful for accurate and fast diagnosis on the incidence of Alzheimer's disease.

Brief description of drawings

[0015]

Fig. 1 is a view illustrating a numerical value of apolipoprotein M in plasma obtained from patients with Alzheimer's disease and from a normal control group;

Fig. 2 is a view illustrating a numerical value of apolipoprotein M in HDL fractionated from plasma of patients with Alzheimer's disease and of a normal control group;

Fig. 3 is a view illustrating a numerical value of apolipoprotein M in plasma of model mice with Alzheimer's disease and of a normal control group mice; and

Fig. 4 is a view illustrating a numerical value of apolipoprotein M in HDL fractionated from plasma of model mice with Alzheimer's disease and of a normal control group mice.

Best mode for carrying out the invention

[0016] Hereinafter, the present invention will be explained in detail.

[0017] The present invention provides a bio-marker composition for diagnosing Alzheimer's disease comprising apolipoprotein M.

[0018] Also, the present invention provides a composition for diagnosing Alzheimer's disease comprising a formulation measuring a protein expression level of apolipoprotein M or an mRNA expression level of a gene coding apolipoprotein M.

[0019] The term "diagnosis" used herein means confirmation of the presence or characteristics of pathological conditions. With regard to the purposes of the present invention, the diagnosis means confirmation on whether Alzheimer's disease occurs or whether Alzheimer's disease is likely to occur.

[0020] The term "marker" used herein means a material capable of diagnosing Alzheimer's disease, and includes organic biomolecules, such as polypeptide, nucleic acids (e.g., mRNA, etc.), lipid, glycolipid, glycoprotein, sugar (monosaccharides, disaccharides, oligosaccharides, etc.) related with Alzheimer's disease. With regard to the purposes of the present invention, the marker is apolipoprotein M which is a protein whose expression is reduced in patients with Alzheimer's disease.

[0021] The term "measurement on a protein expression level" used herein is a process of confirming the presence or expression level of apolipoprotein M, which is a protein related with Alzheimer's disease in a biological sample for diagnosing Alzheimer's disease, and is made by measuring an amount of protein. The analysis method includes western blot, enzyme linked immunosorbent assay (ELISA), radioimmunoassay (RIA), radioimmunodiffusion, Ouchterlony immunodiffusion, rocket immunoelectrophoresis, immunohistostaining, immunoprecipitation assay, complement fixation assay, fluorescence activated cell sorter (FACS), protein chip, etc., but is not limited thereto. A formulation measuring a protein expression level of the present invention is preferably an antibody.

[0022] The term "antibody" used herein means a specific protein molecule instructed for an antigenic site. With regard to the purposes of the present invention, the antibody means an antibody which is specifically binds to apolipoprotein M, and includes all of a polyclonal antibody, a monoclonal antibody, and a recombinant antibody.

[0023] The term "measurement on an mRNA expression level" used herein means measurement on the presence and expression level of mRNA of a gene coding apolipoprotein M, which is a protein related with Alzheimer's disease, in a biological sample for diagnosing Alzheimer's disease. The analysis method includes, for example, reverse transcription polymerase chain reaction (RT-PCR), competitive RT-PCR, realtime RT-PCR, RNase protection assay (RPA), Northern blotting, DNA chip, etc., but is not limited thereto. A formulation measuring an mRNA level of a gene according to the present invention is preferably an antisense oligonucleotide, a primer, or a probe.

[0024] The term "antisense" used herein refers to an oligomer with a sequence of nucleotide bases and a backbone between sub-units, where an antisense oligomer is hybridized with a target sequence in RNA by the formation of Watson-Crick base pair, typically allowing the formation of mRNA and RNA: hetero-oligomer duplex. The oligomer may have an exact sequence complementarity to the target sequence or near complementarity. The antisense oligomer may block or inhibit the translation of mRNA, and change the processing procedure of mRNA to produce a splice variant of the mRNA.

[0025] The term "primer" means a short nucleic acid sequence with short free 3' terminal hydroxyl group capable of forming a base pair with a complementary template and functioning as a starting point for template strand duplication. A primer may initiate DNA synthesis in the presence of a reagent for polymerization (i.e., DNA polymerase or reverse transcriptase) in a proper buffer solution at a proper temperature and 4 different nucleoside triphosphates.

[0026] The term "probe" means a fragment of nucleic acid such as RNA or DNA, etc., which is ones to hundreds of bases pairs capable of specifically binding to mRNA. Since a probe is labeled, the presence of specific mRNA may be confirmed. A probe may be prepared in the form of an oligo nucleotide probe, a single-stranded DNA probe, a double stranded DNA probe, an RNA probe, etc. Selection of proper probes and hybridization conditions may be modified based on those disclosed in the related art.

[0027] Since nucleic acid sequences of genes expressing apolipoprotein M, which is a protein related with Alzheimer's disease, are registered at a gene bank, a person skilled in the art may design an antisense oligonucleotide, a primer, or a probe which specifically amplifies a specific region of the genes based on the sequences.

[0028] The antisense oligonucleotide, primer, or probe of the present invention may be chemically synthesized using a phosphoramidite solid substrate method, or other methods widely known in the art. These nucleic acid sequences may be modified using various means disclosed in the related field. As non-restrictive examples of the modification, there are methylation, capsulation, substitution into at least one homologue of natural nucleotide, and modification between nucleotides, for example, modification to uncharged linkages (e.g., methylphosphonate, phosphotriester, phosphoroamidate,carbamate, etc.) or charged linkages (e.g., phosphorothio-

ate, phosphorodithioate, etc.).

[0029] Since the expression of apolipoprotein M according to the present invention is significantly reduced in patients with Alzheimer's disease compared to normal people, apolipoprotein M may be useful for exact and rapid diagnosis on the incidence of Alzheimer's disease.

[0030] Also, the present invention provides a kit for diagnosing Alzheimer's disease comprising the composition.

[0031] The kit of the present invention may diagnose Alzheimer's disease by confirming a protein expression level of apolipoprotein M or an mRNA expression level of a gene coding apolipoprotein M. The kit of the present invention may further include one or more types of other component compositions, solutions or devices, as well as primers, probes, antibodies, etc. for diagnosing Alzheimer's disease. The kit may be preferably in the form of a RT-PCR kit, a microarray chip kit, a DNA chip kit, and a protein chip kit, but is not limited thereto.

[0032] As a specific example, the kit for measuring the mRNA expression level of the gene coding apolipoprotein M may be a kit comprising essential elements necessary for performing RT-PCR. The RT-PCR kit may include test tubes or other suitable containers, reaction buffer solutions, deoxynucleotide (dNTPs), enzyme such as Taq-polymerase and reverse transcriptase, DNase and RNase inhibitors, DEPC-water, sterilized water, etc., other than each primer which is specific for marker genes.

[0033] The kit of the present invention may also be a kit including essential elements necessary for performing a microarray. The microarray kit may include a substrate where a gene or cDNA, which is a fragment of the gene, is attached as a probe, and the substrate may include a gene of a quantitative control group or cDNA, which is a fragment of the gene. The microarray kit may be prepared using a marker of the present invention according to a manufacturing method commonly used in the related field. In order to prepare the microarray, it is preferable to use a micropipetting method using a piezoelectric manner in order to fix a DNA chip on the substrate using the searched marker as DNA molecule, or a method using a spotter in the shape of pin, etc., but is not limited thereto. The substrate of the microarray chip is preferably a chip coated with an active group selected from the group consisting of amino-silane, poly-L-lysine and aldehyde, but is not limited thereto. Also, the substrate is preferably selected from the group consisting of slide glass, plastic, metal, silicone, nylon membrane, and nitrocellulose membrane, but is not limited thereto.

[0034] The kit of the present invention may also be a kit including essential elements necessary for performing a DNA chip. The DNA chip kit may include a substrate where a gene or cDNA, which is a fragment of the gene, or oligonucleotide is attached, a reagent, a formulation, an enzyme, etc. for preparing fluorescent probes. Also, the substrate may include a control group gene or cDNA, which is a fragment of the gene, or oligonucleotide.

[0035] Also, the present invention provides a method for providing information for diagnosing Alzheimer's disease, which includes the following steps:

(a) measuring a protein expression level of apolipoprotein M or an mRNA expression level of a gene coding apolipoprotein M from a biological sample;
(b) comparing the protein expression level of apolipoprotein M or the mRNA expression level of the gene coding apolipoprotein M of the biological sample with the protein expression level of apolipoprotein M or the mRNA expression level of the gene coding apolipoprotein M of a normal sample; and
(c) predicting or diagnosing that Alzheimer's disease occurs, when the protein expression level of apolipoprotein M or the mRNA expression level of the gene coding apolipoprotein M of the biological sample is more reduced than the protein expression level of apolipoprotein M or the mRNA expression level of the gene coding apolipoprotein M of the normal sample.

[0036] The term "biological sample" used herein includes whole blood, serum, plasma, saliva, urine, sputum, lymph, cell, etc., which are separated from an individual, but is not limited thereto.

[0037] A method for measuring a protein expression level according to the present invention includes western blot, enzyme linked immunosorbent assay (ELISA), radioimmunoassay (RIA), radioimmunodiffusion, Ouchterlony immunodiffusion, rocket immunoelectrophoresis, immunohistostaining, immunoprecipitation assay, complement fixation assay, fluorescence activated cell sorter (FACS), protein chip, etc., but is not limited thereto.

[0038] A method for measuring an mRNA expression level according to the present invention includes reverse transcription polymerase chain reaction (RT-PCR), competitive RT-PCR, realtime RT-PCR, RNase protection assay (RPA), Northern blotting, DNA chip, etc., but is not limited thereto.

[0039] Since the expression of apolipoprotein M is significantly reduced in patients with Alzheimer's disease compared to normal people, diagnosis on whether Alzheimer's disease occurs may be made exactly and rapidly.

[0040] Hereinafter, the present invention will be explained in detail with reference to embodiments. The embodiments are simply to explain the present invention more specifically. Thus, according to the gist of invention, it would be obvious to a skilled person in the art in the related field that the scope of invention is not limited by the embodiments.

Example 1. Experimental materials and experimental methods

1-1. Blood collection and plasma separation from patients with Alzheimer's disease

[0041] Blood was collected from 24 patients diagnosed

with Alzheimer's disease and a normal control 10 people. Each blood sample was centrifuged at 3,000 rpm for 15 minutes to divide the blood sample into blood corpuscle and plasma, and the plasma of a supernatant was separated. A plasma sample before being used for analysis was kept at -80°C.

1-2. Blood collection and plasma separation of model mice with Alzheimer's disease

[0042] As model mice with Alzheimer's disease, transgenic mice overexpressing hAPP695swe (APPswe) and presenilin-1M146V (PS1) mutants were used. They are mouse lines produced by GlaxoSmithKline (Harlow, UK) with a standard technology for C57BL/6 background (Charles River, UK), and APPswe mice were backcrossed with purebred C57BL/6 background mice, and crossed with PS1 mice, to produce double heterozygote mutant mice (APP/PS1). The mice used in the experiment were 3-, 6-, 9-, 12- and 15-month-old mice. Blood was collected from model mice with Alzheimer's disease (AD) and normal control mice (WT). More specifically, mice were anesthetized, and 500 μl to 700 μl of blood was collected from the heart and was put in a heparin tube (BD Falcon). Then, each blood sample was centrifuged at 1,200 rpm for 5 minutes to separate plasma of the supernatant. A plasma sample before being used for analysis was kept at -80°C.

1-3. HDL fraction in plasma

[0043] 60 μl of plasma sample was put in a tube for ultracentrifugation, the same amount of PBS (Gibco) solution was added thereto to form a layer onto the plasma, and was centrifuged at 4°C, 70,000 rpm for 3 hours by using an ultracentrifuge (HITACHI cp100wx Centrifuge P70AT rotor). In the sample separated into two layers, 60 μl of the bottom layer was transferred to a new tube for ultracentrifugation, the same amount of NaBr (Sigma-Aldrich) solution (density = 1.12 g/ml) was added thereto, was mixed about five times using a pipette, and was centrifuged at 4 °C, 70,000 rpm for 18 hours by using the ultracentrifuge again. In the sample separated into two layers, HDL 60 μl of the bottom layer was transferred to a new tube, and the sample before being used for analysis was kept at -80°C.

1-4. Apo M ELISA (Enzyme-linked immunosorbent assay)

[0044] The amount of apolipoprotein M included in the plasma and fractionated HDL of human beings or mice and was quantified using a commercial ELISA kit (CUSABIO Human ApoM ELISA Kit and Mouse ApoM ELISA Kit). As a standard curve, a refined apolipoprotein M standard was used.

Example 2. Numerical value change of apolipoprotein M in plasma and HDL fraction in patients with Alzheimer's disease

[0045] In order to find out a correlation between apolipoprotein M in blood and Alzheimer's disease, an experiment was carried out for comparing a numerical value of apolipoprotein M in plasma and HDL fraction of patients with Alzheimer's disease obtained from Example 1 with a numerical value of a normal control group.

2-1. Confirmation on numerical value of apolipoprotein M in plasma of patients with Alzheimer's disease

[0046] Blood was collected from patients with Alzheimer's disease and from people of the normal control group, the plasma was separated by the method mentioned in Example 1-1, and then the numeral value of apolipoprotein M in the plasma was quantitatively analyzed by the method mentioned in Example 1-4. A result thereof is shown in Fig. 1.

[0047] As shown in Fig. 1, it can be confirmed that the content of apolipoprotein M in the plasma of patients with Alzheimer's disease is significantly reduced compared to that in the plasma of people of the normal control group ($p < 0.05$, normal control group $n = 10$, patients with Alzheimer's disease $n = 24$).

2-2. Confirmation on numerical value of apolipoprotein M in HDL fraction of patients with Alzheimer's disease

[0048] In the plasma separated from patients with Alzheimer's disease and people from a normal control group, HDL was fractionated and separated by the method mentioned in Example 1-3, and the numerical value of apolipoprotein M in the plasma was quantitatively analyzed by the method mentioned in Example 1-4. A result thereof is shown in Fig. 2.

[0049] As shown in Fig. 2, it can be confirmed that the numerical value of apolipoprotein M in HDL fraction of patients with Alzheimer's disease is significantly reduced compared to that in HDL of people of the normal control group ($p = 0.052$, $n = 10$).

Example 3. Numerical change of apolipoprotein M in plasma and HDL fraction of model mice with Alzheimer's disease

[0050] In order to re-verify a correlation between apolipoprotein M in blood and Alzheimer's disease in model mice with Alzheimer's disease according to time, an experiment was carried out for comparing the numerical value of apolipoprotein M in the plasma and HDL fraction of model mice with Alzheimer's disease with that of mice of the normal control group.

3-1. Confirmation on numerical value of apolipoprotein M in plasma of model mice with Alzheimer's disease

[0051] Blood was collected from 3-, 6-, 9-, 12- and 15-month-old model mice with Alzheimer's disease (AD) and mice from a normal control group (WT), plasma was separated by the method mentioned in Example 1-3, and then the numerical value of apolipoprotein M in plasma was quantitatively analyzed by the method mentioned in Example 1-4. A result thereof is shown in Fig. 3.

[0052] As shown in Fig. 3, it can be confirmed that the numerical value of apolipoprotein M in the plasma of model mice with Alzheimer's disease according to time is significantly reduced compared to that in the plasma of mice of the normal control group (* $p < 0.05$, $n = 3$).

3-2. Confirmation on numerical value of apolipoprotein M in HDL fraction of model mice with Alzheimer's disease

[0053] In the plasma of 9-month-old model mice with Alzheimer's disease and mice of the normal control group, HDL was fractionated and separated by the method mentioned in Example 1-3, and the numerical value of apolipoprotein M in the plasma was quantitatively analyzed by the method mentioned in Example 1-4. A result thereof is shown in Fig. 4.

[0054] As shown in Fig. 4, it can be confirmed that the content of apolipoprotein M in HDL fraction of model mice with Alzheimer's disease is significantly reduced compared to that in HDL of mice of the normal control group ($p < 0.05$, mice of the normal control group $n = 5$, model mice with Alzheimer's disease $n = 7$).

Claims

1. A bio-marker composition for diagnosing Alzheimer's disease comprising apolipoprotein M.

2. The composition of claim 1, wherein an expression level of the apolipoprotein M in a sample with Alzheimer's disease is more reduced than an expression levelof the apolipoprotein M in a normal sample.

3. A composition for diagnosing Alzheimer's disease, comprising a formulation measuring a protein expression level of apolipoprotein M or an mRNA expression level of a gene coding apolipoprotein M.

4. The composition of claim 3, wherein the formulation measuring the protein expression level is an antibody specific for apolipoprotein M.

5. The composition of claim 3, wherein the formulation measuring the mRNA expression level is at least one selected from the group consisting of an antisense oligonucleotide, a primer, and a probe which specifically bind to a gene of apolipoprotein M.

6. A kit for diagnosing Alzheimer's disease comprising the composition of any one of claims 3 to 5.

7. The kit of claim 6, wherein the kit is at least one selected from the group consisting of a reverse transcription polymerase chain reaction (RT-PCR) kit, a microarray chip kit, a DNA kit, and a protein chip kit.

8. A method for providing information for diagnosing Alzheimer's disease, the method comprising:

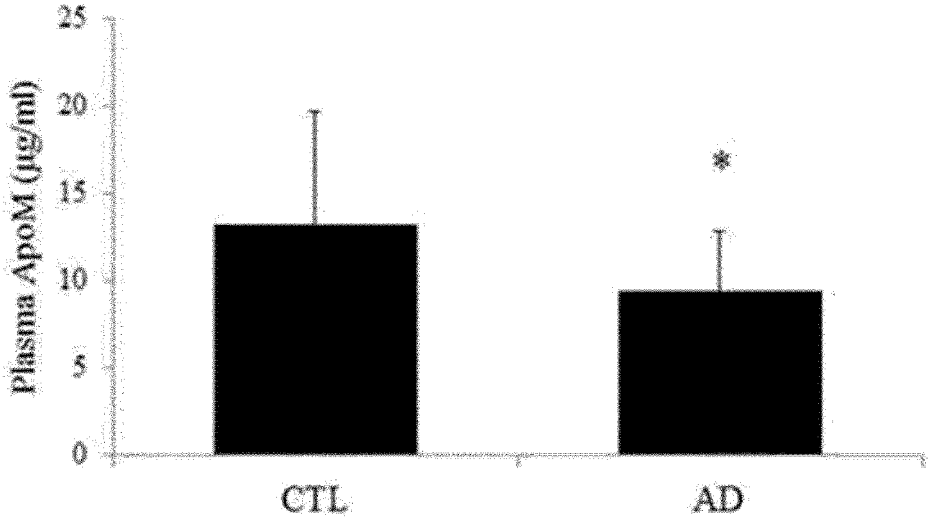
   (a) measuring a protein expression level of apolipoprotein M or an mRNA expression level of a gene coding apolipoprotein M from a biological sample;
   (b) comparing the protein expression level of apolipoprotein M or the mRNA expression level of the gene coding apolipoprotein M of the biological sample with the protein expression level of apolipoprotein M or the mRNA expression level of the gene coding apolipoprotein M of a normal sample; and
   (c) predicting or diagnosing that Alzheimer's disease occurs, when the protein expression level of apolipoprotein M or the mRNA expression level of the gene coding apolipoprotein M of the biological sample is more reduced than the protein expression level of apolipoprotein M or the mRNA expression level of the gene coding apolipoprotein M of the normal sample.

9. The method of claim 8, wherein the biological sample in the step (a) is at least one selected from the group consisting of whole blood, serum, plasma, saliva, urine, sputum, lymph, and cell, which are separated from an individual.

10. The method of claim 8, wherein the method for measuring the protein expression level in the step (a) is at least one selected from the group consisting of western blot, enzyme linked immunosorbent assay (ELISA), radioimmunoassay (RIA), radioimmunodiffusion, Ouchterlony immunodiffusion, rocket immunoelectrophoresis, immunohistostaining, immunoprecipitation assay, complement fixation assay, fluorescence activated cell sorter (FACS), and protein chip.

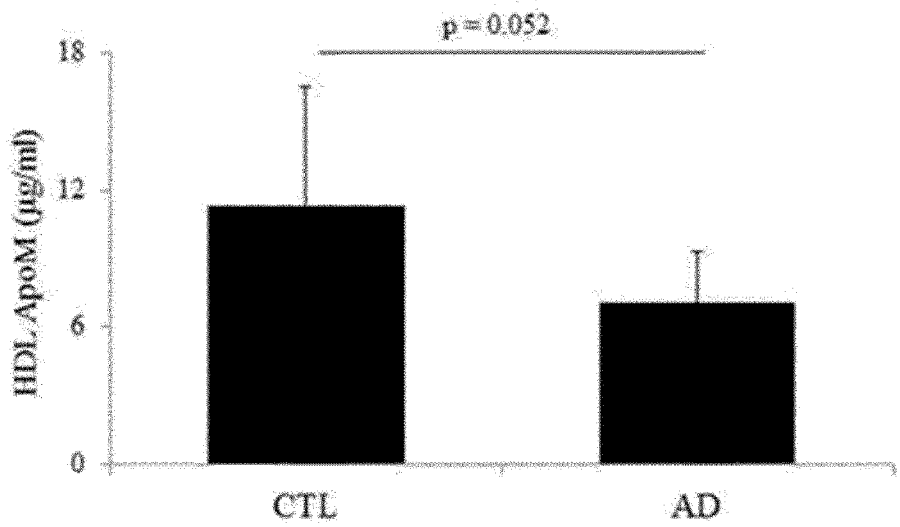
11. The method of claim 8, wherein the method for measuring the mRNA expression level in the step (a) is at least one selected from the group consisting of reverse transcription polymerase chain reaction (RT-PCR), competitive RT-PCR, realtime RT-PCR, RNase protection assay (RPA), Northern blotting, and DNA chip.

【Fig 1】



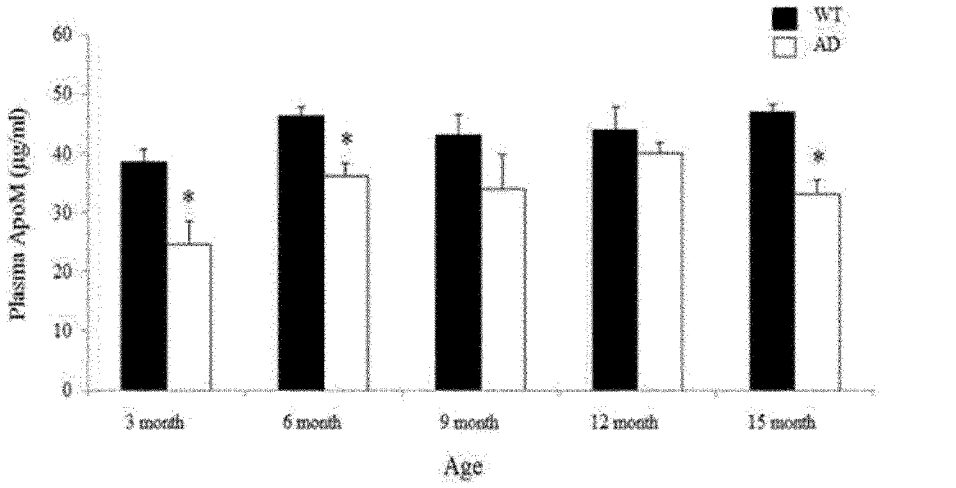
$p < 0.05$
CTL: healthy control group members (n = 10)
AD: Alzheimer's disease patients (Aβ accumulation) (n = 24)

【Fig 2】



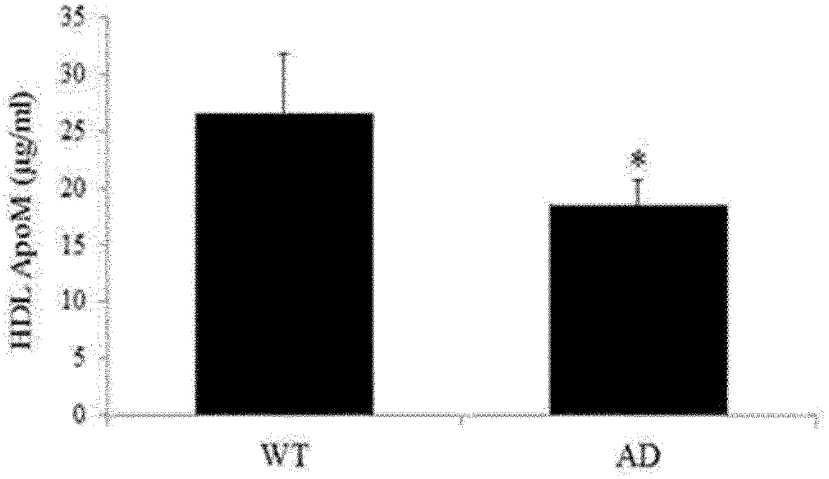
CTL: healthy control group members (n = 10)
AD: Alzheimer's disease patients (Aβ accumulation) (n = 10)

【Fig 3】



WT: Control mice (n = 3)
AD: APP/PS1 double transgenic Alzheimer's disease model mice (n = 3)

【Fig 4】



$p < 0.05$
WT: Control mice (9 month; n = 5)
AD: APP/PS1 double transgenic Alzheimer's disease model mice (9 month; n = 7)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2014/004058

A. CLASSIFICATION OF SUBJECT MATTER

G01N 33/68(2006.01)i, G01N 33/53(2006.01)i, C12Q 1/68(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

G01N 33/68; C40B 40/10; G01N 33/53; C07K 16/20; C40B 30/04; C12Q 1/68

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean Utility models and applications for Utility models: IPC as above
Japanese Utility models and applications for Utility models: IPC as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

eKOMPASS (KIPO internal) & Keywords: apolipoprotein M(apolipoprotein M), Alzheimer, kit, biomarker

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 8389222 B2 (BREYER et al.) 05 March 2013 See abstract, claims 1, 4, 6-8, 17 and 18, and columns 8-9	1,3-7
A		2,8-11
A	US 2003-0113808 A1 (JACKOWSKI et al.) 19 June 2003 See abstract and claims 1 and 2	1-11
A	US 2013-0023428 A1 (MUELLER et al.) 24 January 2013 See abstract and claim 1	1-11
A	ORDONEZ et al., "Gender differences in apolipoprotein D expression during aging and in Alzheimer disease" Neurobiology of Aging, vol. 33, no. 2, pp. 433.e11-433.e20 (2012) See abstract	1-11
A	VERGHESE et al., "Apolipoprotein E in Alzheimer's disease and other neurological disorders" The Lancet Neurology, vol. 10, no. 3, pp. 241-252 (2011) See abstract	1-11

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
05 AUGUST 2014 (05.08.2014)	**11 AUGUST 2014 (11.08.2014)**
Name and mailing address of the ISA/KR Korean Intellectual Property Office Government Complex-Daejeon, 189 Seonsa-ro, Daejeon 302-701, Republic of Korea Facsimile No. 82-42-472-7140	Authorized officer Telephone No.

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/KR2014/004058

Patent document cited in search report	Publication date	Patent family member	Publication date
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专利名称(译)	包含载脂蛋白m的阿尔茨海默氏病的诊断标志物组合物		
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摘要(译)

本发明涉及包含用于诊断阿尔茨海默病的载脂蛋白M的生物标志物，包含用于测量载脂蛋白M或编码其的基因的mRNA的表达水平的材料的阿尔茨海默氏病诊断组合物，包含所述组合物的阿尔茨海默氏病诊断试剂盒，用于通过所述组合物提供用于诊断阿尔茨海默病的信息。与正常人相比，根据本发明的载脂蛋白M在阿尔茨海默病患者中以显着降低的水平表达，因此可以有效地用于准确和快速诊断是否存在阿尔茨海默氏病。