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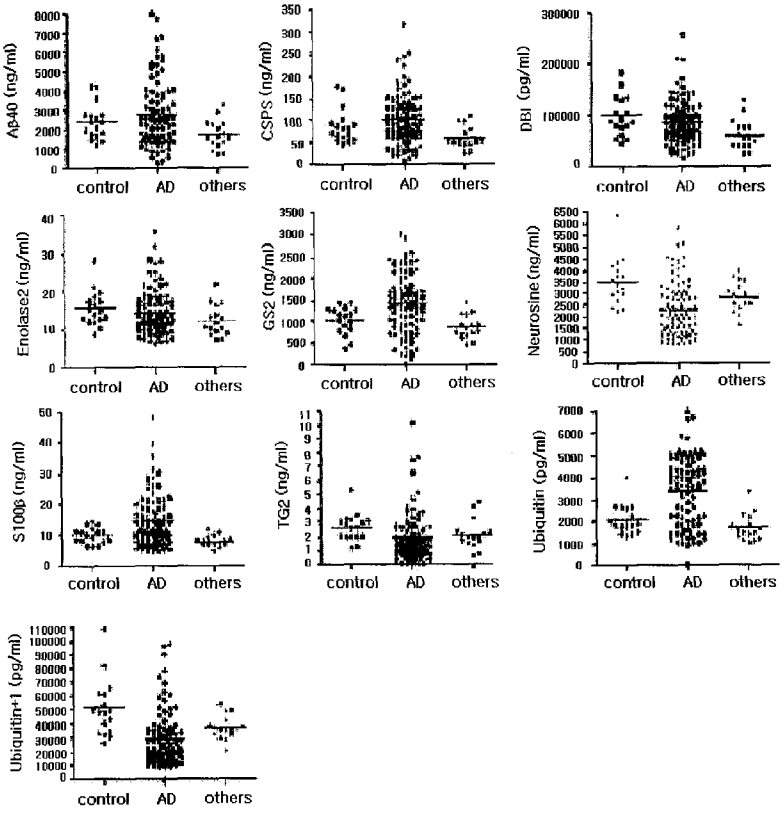
(54) Title: MARKERS AND KIT FOR DIAGNOSIS OF ALZHEIMER'S DISEASE

FIG.2



(57) Abstract: Disclosed are a protein marker for a diagnosis of Alzheimer's disease, a composition for detecting a diagnostic marker for Alzheimer's disease, comprising an agent for measuring the level of the protein marker, a kit for detecting a diagnostic marker for Alzheimer' s disease, comprising an antibody specific to the protein marker, and a method of diagnosing Alzheimer's disease. Since the diagnostic protein marker exists in blood, there is no pain or side effect in collecting a sample, and sample collection is easy and useful.

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MARKERS AND KIT FOR DIAGNOSIS OF ALZHEIMER'S DISEASE

Technical Field

The present invention relates to a diagnostic protein marker specific to Alzheimer's disease, a composition for detecting a diagnostic marker for Alzheimer's disease, comprising an agent for measuring the level of the protein marker, a kit for detecting a diagnostic marker for Alzheimer's disease, comprising an antibody specific to the protein marker, and a method of diagnosing Alzheimer's disease.

Background Art

Alzheimer's disease (AD) is a neurodegenerative disease characterized by memory impairment and other cognitive deficits (Mckhann et al., Neurology 34; 939 (1984)), and is the most major cause of dementia. Dementia, a degenerative brain disorder causing rapid loss of memory, intelligence, and the like, is a typical senile disease that 290,000 old citizens corresponding to 9.5 percent of the population over the age of 65 suffer from, and 73 percent of those, that is, 180,000, correspond to severe patents who show the habitual behavior of wandering the streets and have other serious symptoms. As the population rapidly ages, it is expected that the number of dementia patients will continuously increase and finally reach 700,000 in 2020, 2.4 times as many as now. According to the types of dementia, 51 percent of dementia patients suffer from Alzheimer type dementia, and 34 percent of dementia patients suffer from vascular dementia, that is, a total of 85 percent of dementia patients are attacked by these two types of dementia. The etiological factors of the remaining 15

percent are infectious diseases, metabolic diseases, etc.

Alzheimer's disease and vascular dementia are the most common causes of dementia, and hold a majority of dementia-causing diseases. However, the reliability of their diagnoses is still controversial because an accurate differential diagnosis before death is not applicable. Particularly, in Korea, it is difficult to obtain cerebral cortices due to the oriental way of thinking about death, and thus it is almost impossible to get a final pathological diagnosis. A clinical diagnosis of Alzheimer's disease mainly depends on history taking and neuropsychological examinations, and imaging examinations, such as magnetic resonance imaging (MRI) and positron emission tomography (PET), are performed as secondary examinations. However, a final diagnosis of dementia is determined by pathologic findings. According to foreign pathological research, the accuracy of a clinical diagnosis of Alzheimer's disease is 50 to 82%, and the accuracy of a clinical diagnosis of vascular dementia is 40 to 80%, which suggests that there are large variations between researchers.

In this regard, an attempt has recently been made to use a target for an early diagnosis or differential diagnosis of dementia by measuring the level of the target specifically increasing or decreasing in the cerebrospinal fluid of a dementia patient.

The cerebrospinal fluid has a problem in that its sample collection brings on pain, only a specialized medical institution can collect a sample, and medical side effects are feared in the course of sample collection.

Disclosure of the Invention

Therefore, the present invention has been made in view of the above-mentioned problems. The present inventors have made an effort to diagnosing Alzheimer's disease by using a sample other than the cerebrospinal fluid, and as a result, could find a plurality of blood protein markers for a diagnosis of Alzheimer's disease. Also, the inventors have confirmed that when these proteins are applied solely or in combination to a diagnosis of Alzheimer's disease, Alzheimer's disease can be exactly diagnosed early, thereby completing the present invention.

The present invention provides a blood protein marker for a diagnosis of Alzheimer's disease, comprising one or two or more kinds of proteins selected from the group consisting of ubiquitin, CSPS (catecholamine sulfating phenol sulfotransferase), GS2 (glutamine synthetase 2), S100β, ubiquitin+ 1, DBI (diazepam binding inhibitor), neurosine, ENO2 (enolase 2), Aβ40 (amyloid beta 40), and TG2 (transglutaminase 2), as effective components.

Hereinafter, the present invention will be described in more detail.

As used herein, the term "Alzheimer's disease" means a degenerative brain disease that kills nerve cells in the cerebral cortex to atrophy or reduce gyri in the frontal and temporal lobes of the cerebrum, and may be used in the same sense as "degenerative brain diseases".

As used herein, the term "diagnosis" means identifying the presence or feathers of pathological states. With respect to the objects the present

invention, the diagnosis is to identify whether or not Alzheimer's disease occurs.

As used herein, the term "marker for a diagnosis, marker for diagnosing, or diagnostic marker" means a substance capable of diagnosing Alzheimer's disease by distinguishing between normal cells and cells attacked by Alzheimer's disease, and includes organic biomolecules, such as polypeptides or glycoproteins, the quantities of which increase or decrease in cells attacked by Alzheimer's disease as compared with normal cells. This marker or these markers may consist of a single polypeptide or a combination of polypeptides.

As used herein, the term "blood" includes serum or plasma.

Proteins overexpressed in tissues or the cerebrospinal fluid have difficulty in their diagnoses because they can only be diagnosed by biopsy or collection of the cerebrospinal fluid. However, if it is possible to detect disease markers existing in humors, such as blood, then they may be very effectively utilized. Therefore, in order to verify whether or not protein markers that are expected to exist in blood of Alzheimer's patients can be actually used as markers for a diagnosis of Alzheimer's disease, the present inventors collected serum samples of patients and normal persons as analytes, and analyzed them by the Luminex multi-analyte assay system using antibody conjugated color-coded beads. As a result of this, among the expected proteins, ubiquitin, CSPS (catecholamine sulfating phenol sulfotransferase), GS2 (glutamine synthetase 2), and S100β proteins were highly expressed in blood of the patient group, and DBI (diazepam binding inhibitor), neurosine, ENO2 (enolase 2), Aβ40 (amyloid

beta 40), and TG2 (transglutaminase 2) proteins were little expressed in blood of the patient group. From this, it could be noted that the above proteins may be used as a significant marker for a diagnosis of Alzheimer's disease (see FIGS. 1 and 2).

Accordingly, the present invention provides a protein marker for a diagnosis of Alzheimer's disease, comprising one or two or more kinds of the above proteins as effective components.

Further, the present invention provides a composition for detecting a blood diagnostic marker for Alzheimer's disease, comprising an agent for measuring levels of one or two or more kinds of proteins selected from the group consisting of ubiquitin, CSPS (catecholamine sulfating phenol sulfotransferase), GS2 (glutamine synthetase 2), S100β, ubiquitin+ 1, DBI (diazepam binding inhibitor), neurosine, ENO2 (enolase 2), Aβ40 (amyloid Beta 40), and TG2 (transglutaminase 2).

Typical examples of the agent for measuring protein levels include protein-specific antibodies.

As used herein, the term "antibody" means a protein molecule specific to an antigenic region. With respect to the objects of the present invention, the antibody refers to an antibody specifically binding to a marker protein, and includes all of a polyclonal antibody, a monoclonal antibody, and a recombinant antibody.

Since blood marker proteins for Alzheimer's disease have been identified as described above, antibody production using them may be easily carried out using techniques well known in the art.

A polyclonal antibody may be produced by a method

well known in the art, which includes injecting the Alzheimer's disease marker protein antigen into an animal, and blood samples are collected from the animal to obtain serum containing antibodies. Such a polyclonal antibody may be prepared from any animal host, such as goats, rabbits, sheep, monkeys, horses, pigs, cows and dogs. A monoclonal antibody may be prepared by a method well known in the art, such as a hybridoma method (see Kohler and Milstein (1976) European Journal of Immunology 6: 511-519) or a phage antibody library technique (Clackson et al., Nature, 352: 624-628, 1991; Marks et al., J. Mol. Biol., 222: 58, 1-597, 1991). The hybridoma method employs cells extracted from an immunologically compatible host animal, such as mice, which is injected with a marker protein antigen for a diagnosis of Alzheimer's disease, as one group, and a cancer or myeloma cell line as another group. Cells of these two groups are fused with each other by a method well known in the art, such as a method using polyethylene glycol, and antibody-producing cells are proliferated by a standard tissue culture method. After a uniform cell colony is obtained by subcloning using a limited dilution technique, a hybridoma capable of producing an antibody specific to the diagnostic marker protein for Alzheimer's disease is cultivated in large quantities in vitro or in vivo according to a standard technique. A monoclonal antibody produced by the hybridoma may be used without purification, but is preferably used after being highly purified by a method well known in the art so as to obtain the best outcome. The phage antibody library method is a method in which a phage antibody library is constructed in vitro by obtaining antibody genes (single-chain fragment variable

(scFv) type) for a variety of intracellular Alzheimer's disease markers and expressing them in the form of a fusion protein on the surfaces of phages, and a monoclonal antibody binding to an Alzheimer's disease-specific protein is isolated from the library.

An antibody prepared by the above methods may be isolated using gel electrophoresis, dialysis, salting out, ion exchange chromatography, affinity chromatography, etc.

In addition, the antibody of the present invention include functional fragments of antibody molecules, as well as a complete form having two full-length light chains and two full-length heavy chains. The functional fragment of antibody molecules means a fragment retaining at least an antigen-binding function, and include Fab, F(ab')2, Fv, and the like.

As described above, in order to measure levels of one or two or more kinds of proteins selected from the group consisting of ubiquitin and the like, the present invention provides a composition comprising an agent specific to the target proteins, such as an antibody, as a composition for detecting a diagnostic marker for Alzheimer's disease.

Further, the present invention provides a kit for detecting a blood diagnostic marker for Alzheimer's disease, comprising an antibody specific to one or two or more kinds of proteins selected from the group consisting of ubiquitin, CSPS (catecholamine sulfating phenol sulfotransferase), GS2 (glutamine synthetase 2), S100β, ubiquitin+ 1, DBI (diazepam binding inhibitor), neurosine, ENO2 (enolase 2), Aβ40 (amyloid beta 40), and TG2 (transglutaminase 2).

The inventive kit for detecting a diagnostic

marker may comprise an antibody specific to the above proteins; a secondary antibody conjugate to which a label conducting a chromogenic reaction with a substrate; a chromogenic substrate solution to induce the chromogenic reaction with the label; a washing solution; and an enzyme reaction stop solution.

The inventive kit for detecting a diagnostic marker can diagnose Alzheimer's disease by quantitatively or qualitatively analyzing an antigen for an antibody protein through an antigen-antibody binding reaction, and the antigen-antibody binding reaction may be measured by a conventional method, such as ELISA (enzyme-linked immunosorbent assay), RIA (radioimmunoassay), sandwich assay, western blotting on polyacrylamide gel, immunoblotting, and immunohistochemical staining. For example, the kit for detecting a diagnostic marker may be provided in such a manner as to conduct ELISA for reacting with a recombinant monoclonal antibody protein by using a 96-well microtiter plate surface-coated with an analyte and a control.

As a reactor for the antigen-antibody binding reaction, a nitrocellulose membrane, a PVDF (polyvinylidene fluoride) membrane, a well plate made of a polyvinyl or polystyrene resin, and a slide glass may be used.

A conventional label conducting a chromogenic reaction, such as HRP (horseradish peroxidase), alkaline phosphatase, colloidal gold, fluorescein, such as FITC (poly L-lysine-fluorescein isothiocyanate) and RITC (rhodamine-B-isothiocyanate), and dye, may be preferably used as the label of the secondary antibody conjugate. It is preferred that the substrate inducing the

chromogenic reaction is determined depending on the label, and particularly TMB (3,3',5,5'-tetramethyl bezidine), ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), or OPD (o-phenylenediamine (OPD) may be used. More preferably, the chromogenic substrate is prepared in a dissolved state in a buffer solution (0.1M NaAc, pH 5.5). HRP used as the label of the secondary antibody conjugate decomposes the chromogenic substrate, such as TMB, to produce a chromogenic precipitate. By checking the degree of precipitation of the chromogenic precipitate with naked eye, the presence or absence of a peroxiredoxin protein antigen is detected.

The washing solution preferably comprises a phosphate buffer solution, NaCl, and Tween 20, and more preferably, is a buffer solution comprising 0.02M phosphate buffer solution, 0.13M NaCl, and 0.05% Tween 20. After the antigen-antibody binding reaction is performed to form an antigen-antibody complex, the antigen-antibody complex reacts with the secondary antibody conjugate, and then is washed 3 to 6 times by adding an appropriate amount of the washing solution to the reactor. A sulfuric acid solution (H_2SO_4) may be preferably used as the enzyme reaction stop solution.

Further, the present invention provides a method of diagnosing Alzheimer's disease, comprising the steps of measuring protein levels by contacting an antibody specific to one or two or more kinds of proteins selected from the group consisting of ubiquitin, CSPS (catecholamine sulfating phenol sulfotransferase), GS2 (glutamine synthetase 2), S100β, ubiquitin+ 1, DBI (diazepam binding inhibitor), neurosine, ENO2 (enolase 2), Aβ40 (amyloid beta 40), and TG2 (transglutaminase 2) with analyte blood of a patient with suspected

Alzheimer's disease to form antigen-antibody complexes; and comparing the measured protein levels with protein levels of a normal control sample to determine an increase or decrease in quantities of the formed antigen-antibody complexes.

In the present invention, serum or plasma may be used as the analyte blood. In a preferred embodiment of the present invention, serum is used as the analyte. When serum or plasma is used as a biological sample according to the present invention, analyte collection is much easier than in the case of using the cerebrospinal fluid as a sample, a patient feels no pain during sample collection, and there is no fear of side effects due to the use of medical tools.

The isolation of proteins from an analyte may be carried out using a method well known in the art.

Measuring protein levels is to measure the quantity of proteins by using an agent specially binding to the proteins, such as an antibody, and protein levels may be measured by a variety of methods including, but not limited to, Western blotting, ELISA (enzyme-linked immunosorbent assay), RIA (radioimmunoassay), radioimmunodiffusion, ouchterlony immunodiffusion, rocket immunoelectrophoresis, immunohistostaining, immunoprecipitation assay, complement fixation assay, FACS, and protein chip assay. Through these measurement methods, a patient with suspected Alzheimer's disease is compared with a normal control for the quantity of antigen-antibody complex formation, and the actual incidence of Alzheimer's disease may be diagnosed by evaluating a significant increase or decrease in expression levels of the marker proteins. In a preferred embodiment of the present invention, expression levels

of the proteins are measured by means of the Luminex multi-analyte assay system using antibody conjugated color-coded beads.

As used herein, the term "antigen-antibody complex", means a binding product of an Alzheimer's disease marker protein to an antibody specific thereto. The quantity of antigen-antibody complex formation may be quantitatively determined by measuring the signal size of a detection label or the expression level of a marker protein.

Such a detection label may be selected from, but not limited to, the group consisting of an enzyme, a fluorescein, a ligand, a luminant, a microparticle, a redox molecule. Examples of the enzyme available as the detection label include, but not limited to, D-glucosidase, D-galactosidase, urase, peroxidase or alkaline phosphatase, acetylcholinesterase, glucose oxidase, hexokinase and GDPase, RNase, glucose oxidase and luciferase, phosphofructokinase, phosphoenolpyruvate carboxylase, aspartate aminotransferase, and phosphenolpyruvate decarboxylase. Examples of the fluorescein include, but not limited to, fluorescin, isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, and fluorescamin. Examples of the ligand include, but not limited to, biotin derivatives. Examples of the luminant include, but not limited to, acridinium esters, luciferin, and luciferase. Examples of the microparticle include, but not limited to, colloidal gold, and colored latex. Examples of the redox molecule include, but not limited to, ferrocene, ruthenium complexes, viologen, quinone, Ti ions, Cs ions, diimide, 1,4-benzoquinone, and hydroquinone. Examples of the radioactive isotope include, but not

limited to, ^{3}H, ^{14}C, ^{35}S, ^{36}Cl, ^{51}Cr, ^{57}Co, ^{58}Co, ^{59}Fe, ^{90}Y, ^{125}I, ^{131}I, and ^{186}Re.

Brief Description of the Drawings

The foregoing and other objects, features and advantages of the present invention will become more apparent from the following detailed description when taken in conjunction with the accompanying drawings in which:

FIG. 1 illustrates the Luminex multi-analyte assay system using antibody conjugated color-coded beads; and

FIG. 2 illustrates differences between expression levels of ubiquitin, CSPS (catecholamine sulfating phenol sulfotransferase), GS2 (glutamine synthetase 2), S100β, ubiquitin+ 1, DBI (diazepam binding inhibitor), neurosine, ENO2 (enolase 2), Aβ40 (amyloid beta 40), and TG2 (transglutaminase 2) proteins in a sample of an Alzheimer's disease patient and those in a control sample.

Best Mode for Carrying Out the Invention

Reference will now be made in detail to the preferred embodiments of the present invention. It should be understood that the following examples are illustrative only, and the scope of the present invention is not limited thereto.

<Example 1>

Quantitative Protein Analysis of Blood Collected from Patient with Degenerative Brain Disease and Normal Person

<1> Collection of Analytes (Serum Collection)

The team directed by Professors Jee-Hyang

Jeong/Kyung-Joo Choi (Department of Neurology, Ewha Woman's University) and the team directed by Professors Yong Jeong/Duk-Ryel Na (Samsung Medical Center) collected serums from 86 patients with degenerative brain diseases and 17 normal controls with the consent of the patients and their guardians according to a protocol approved by IRB.

<2> Preparation of Antibody

In order to find significant protein markers from among proteins that were expected to exist in the collected analytes from patients with Alzheimer's disease, an antibody for expected protein markers ubiquitin, CSPS (catecholamine sulfating phenol sulfotransferase), GS2 (glutamine synthetase 2), S100β, ubiquitin+ 1, DBI (diazepam binding inhibitor), neurosine, ENO2 (enolase 2), Aβ40 (amyloid beta 40), and TG2 (transglutaminase 2) was prepared.

A polyclonal antibody was prepared by a method well known in the art, in which a recombinant protein antigen for each of the above protein markers ubiquitin, CSPS (catecholamine sulfating phenol sulfotransferase), GS2 (glutamine synthetase 2), S100β, ubiquitin+ 1, DBI (diazepam binding inhibitor), neurosine, ENO2 (enolase 2), Aβ40 (amyloid beta 40), and TG2 (transglutaminase 2) was injected into an animal and collecting blood samples from the animal to obtain serum containing antibodies.

A monoclonal antibody was prepared using a hybridoma method well known in the art (see Kohler and Milstein (1976) European Journal of Immunology 6: 511-519). A recombinant protein antigen for each of the protein markers ubiquitin, CSPS (catecholamine sulfating phenol sulfotransferase), GS2 (glutamine synthetase 2), S100β, ubiquitin+ 1, DBI (diazepam binding inhibitor),

neurosine, ENO2 (enolase 2), Aβ40 (amyloid beta 40), and TG2 (transglutaminase 2) was mixed with complete Freund's adjuvant, and then a six-week-old female BALB/c mouse was injected with 50 to 100㎍/0.2㎖ of each mixture by intraperitoneal injection. After 4 weeks, each antigen was mixed with incomplete Freund's adjuvant, and then the mouse was intraperitoneally injected with 50 to 100㎍/0.2㎖ of each mixture in the same manner. After two weeks, blood samples were collected from the tail of the mouse and were subjected to ELISA assay, and then blood samples having an antibody titer of 1.0 at 1/1000 were used for cell fusion. Four weeks after secondary immunization for cell fusion, the same quantity of antigens was diluted in PBS, and the mouse was injected with 50 to 100㎍/0.2㎖ of each antigen. After three days, the mouse was sacrificed to conduct a cell fusion process. First, myeloma cells (Sp2/o-Ag14) purchased from ATCC were diluted to 1×10^5cells/㎖ in 10% FBS medium, and were cultured in a CO_2 incubator at 37℃ all night long. This was repeated every day to subculture the cells. One day before cell fusion, cells were subcultured in three 150T flasks in a quantity of 50㎖ respectively, and the subcultured cells were used for cell fusion. Next, the above-prepared immunized mouse was sacrificed to extract its spleen, and the extracted spleen was washed in a medium in a prepared 100㎜ dish (DMEM 10㎖). Subsequently, a mesh was put in a 100㎜ dish (DMEM 5㎖), and the spleen was placed on the mesh and was cut into several pieces. The cut spleen was finely crushed by the plunger part of a 5㎖ injector to isolate spleen cells. The spleen cells were suspended several times by using a disposable pipette, were layered in a prepared tube containing FBS, and then were

left for about 10 minutes to settle large sediments. Cells in the FBS upper layer were thoroughly unbound, and 10㎖ of preheated RBC lysis buffer was added thereto. The mixture was suspended and cultured at 37℃ for 10 minutes, and then 3㎖ of FBS was added thereto. The mixture was centrifuged at 1,000rpm. After the centrifugation, the supernatant was discarded, and only pellets were diluted in basal medium and were centrifuged at 1,000rpm. The centrifuged cells were diluted in 10㎖ of the basal medium, and then were counted. The myeloma cells (Sp2/o-Ag14, ATCC) were grown in three 150T flasks, and the grown myeloma cells were transfused into conical tubes and were centrifuged. Pellets of each tube were gathered and diluted in 20㎖ of the basal medium, and cell counting was conducted. The so-prepared spleen cells and myeloma cells were mixed in a ratio of 5:1, and then the mixture was centrifuged at 1,000rpm for 10 minutes. Pellets were uniformly spread at the end of the tube such that they were not conglomerated, and 1㎖ (based on 1×10^8cells) of preheated PEG (1,500) was drop in such a manner as to uniformly infiltrate into the cells while the tube was slowly revolved for 1 minute. The cells were covered with the PEG while the tube was revolved again for one minute. With regard to this, care was taken not to exceed 2 minutes in total, and temperature was maintained at 37℃. 4㎖ of the basal medium was added to the tube while the tube was slowly tapped for 4 minutes. 10㎖ of the basal medium was added to the tube again while the tube was slowly tapped for 4 minutes. 3㎖ of FBS was put into the tube, and the mixture was centrifuged at 1,000rpm for 10 minutes. The pellets were carefully suspended in 1× HAT medium, and 200㎕ of the

suspension was dispensed into each well of a 96 well-plate. Cell lines that had a positive reaction only to the above proteins (cell lines of positive wells) were transferred to a 24 well plate, and then cell counting was conducted. The cells were diluted to 100 to 200cells/20㎖ in 20㎖ HT medium, and 200㎕ of the cells was dispensed into each well of a 96 well-plate. Next, the cells were cultured in a constant temperature incubator at 37℃ for 7 to 120 days, and feeding was conducted once on the fifth to seventh day. ELISA search and cloning were repeated to obtain final clones, and the obtained final clones were stored in a liquid nitrogen tank. As a result of this, hybridoma cells for producing monoclonal antibodies specific to the above protein antigens were obtained.

<3> Analysis of Analyte

In order to see whether or not the above expected proteins may be significant markers, analysis was conducted by the Luminex multi-analyte assay system using antibody conjugated color-coded beads.

An antibody for the expected protein marker was bound as a capture antibody to each of carboxylated luminex beads (P-33997-10101~19901) that were purchased from Upstate company, America. Each bead was recognized by its unique color, and simultaneously the capture antibody acted as a probe (see FIG. 1). The marker protein binds to the capture antibody by reacting the bead with an analyte, and additionally a bead recognizing a different epitope of the marker protein reacts with and binds to a detection antibody to which a different kind of chromophore was bound. Also, two lasers, that is, red fluorescence and green fluorescence, were irradiated to each bead by using the

Luminex multi-analyte assay system to determine the kind of the bead, that is, the kind of the marker protein, by the red fluorescence and to determine the quantity of the bound protein (see FIG. 1). From the measurement results, the kind of bead was identified and the analyte material was quantified using Bio-Plex Manager software. The monoclonal antibodies prepared in the above <2> step were used as the capture antibody and the detection antibody.

<4> Verification of Degenerative Brain Disease Marker and Diagnosis of Degenerative Brain Disease

As a result of quantitative protein analyses using the antibody conjugated color-coded beads, marker proteins having significance in diagnosing a degenerative brain disease were discovered in blood of the patient group in comparison to blood of the control group. Ubiquitin, CSPS (catecholamine sulfating phenol sulfotransferase), GS2 (glutamine synthetase 2), and S100β proteins showed high expression in blood of the patient group, and ubiquitin+ 1, DBI (diazepam binding inhibitor), neurosine, ENO2 (enolase 2), Aβ40 (amyloid beta 40), and TG2 (transglutaminase 2) proteins showed low expression in blood of the patient group (see FIG. 2). In the case of the marker proteins showing high expression in blood of the patient group, as compared to blood of the control group, they were determined as positive when having a value above the average of normal control group concentrations plus 1/2 times the standard deviation, and were determined as negative when having a value below the average of patient group concentrations minus 1/2 times the standard deviation. Also, in the case of the marker proteins showing low expression in blood of the patient group, as compared to blood of the

control group, they were determined as positive when having a value below the average of normal control group concentrations minus 1/2 times the standard deviation, and were determined as negative when having a value below the average of patient group concentrations plus 1/2 times the standard deviation (see Table 1).

Table 1

marker protein	normal control group concentration (ng/㎖)	patient group concentration (ng/㎖)	sensitivity (%)	specificity (%)
Aβ40	2373.2±906.2	2811.1±1699.9	50.8	66.7
CSPS	86.0±39.2	102.3±58.6	55.9	75.0
DBI	96.8±34.8	87.9±43.5	66.1	50.0
Enolase2	14.8±5.3	14.7±6.0	63.6	33.3
GS2	1050.0±342.7	1417.5±666.8	67.1	50.0
Neurosine	3226.0±771.9	2337.9±1168.3	73.8	72.7
S100β	9.42±2.27	14.48±8.41	62.1	63.6
TG2	2.35±0.78	1.78±1.50	77.1	71.4
Ub	1.98±0.73	3.43±1.54	72.4	87.5
Ub+ 1	49.3±23.5	29.7±20.2	80.1	63.6

From the above results, it can be noted that one or more kinds of proteins selected from the group consisting of ubiquitin, CSPS, GS2, S100β, ubiquitin+ 1, DBI, neurosine, ENO2, Aβ40, and TG2 existing in blood (serum) can be effectively utilized as protein markers for a diagnosis of Alzheimer's disease.

Industrial Applicability

As can be seen from the foregoing, a diagnostic protein marker, a composition comprising an agent for measuring the level of the protein marker, and a kit comprising an antibody specific to the protein marker according to the present invention can be used to accurately and simply diagnose Alzheimer's disease.

While this invention has been described in connection with what is presently considered to be the most practical and preferred embodiment, it is to be understood that the invention is not limited to the disclosed embodiment and the drawings. On the contrary, it is intended to cover various modifications and variations within the spirit and scope of the appended claims.

Claims

1. A blood protein marker for a diagnosis of Alzheimer's disease, comprising one or two or more kinds of proteins selected from the group consisting of ubiquitin, CSPS (catecholamine sulfating phenol sulfotransferase), GS2 (glutamine synthetase 2), S100β, ubiquitin+ 1, DBI (diazepam binding inhibitor), neurosine, ENO2 (enolase 2), Aβ40 (amyloid beta 40), and TG2 (transglutaminase 2), as effective components.

2. The blood protein marker as claimed in claim 1, wherein the blood comprises serum or plasma.

3. A composition for detecting a blood diagnostic marker for Alzheimer's disease, comprising an agent for measuring levels of one or two or more kinds of proteins selected from the group consisting of ubiquitin, CSPS (catecholamine sulfating phenol sulfotransferase), GS2 (glutamine synthetase 2), S100β, ubiquitin+ 1, DBI (diazepam binding inhibitor), neurosine, ENO2 (enolase 2), Aβ40 (amyloid Beta 40), and TG2 (transglutaminase 2).

4. The composition as claimed in claim 3, wherein the agent comprises an antibody specific to one or two or more kinds of proteins selected from the group consisting of ubiquitin, CSPS (catecholamine sulfating phenol sulfotransferase), GS2 (glutamine synthetase 2), S100β, ubiquitin+ 1, DBI (diazepam binding inhibitor), neurosine, ENO2 (enolase 2), Aβ40 (amyloid Beta 40), and TG2 (transglutaminase 2).

5. The composition as claimed in claim 3,

wherein the blood comprises serum or plasma.

6. A kit for detecting a blood diagnostic marker for Alzheimer's disease, comprising an antibody specific to one or two or more kinds of proteins selected from the group consisting of ubiquitin, CSPS (catecholamine sulfating phenol sulfotransferase), GS2 (glutamine synthetase 2), S100β, ubiquitin+ 1, DBI (diazepam binding inhibitor), neurosine, ENO2 (enolase 2), Aβ40 (amyloid beta 40), and TG2 (transglutaminase 2).

7. The kit as claimed in claim 6, wherein the blood comprises serum or plasma.

8. A method of detecting a protein marker for a diagnosis of Alzheimer's disease from analyte blood by using an antigen-antibody reaction.

9. The method as claimed in claim 8, wherein the blood comprises serum or plasma.

10. A method of diagnosing Alzheimer's disease, comprising the steps of:

measuring protein levels by contacting an antibody specific to one or two or more kinds of proteins selected from the group consisting of ubiquitin, CSPS (catecholamine sulfating phenol sulfotransferase), GS2 (glutamine synthetase 2), S100β, ubiquitin+ 1, DBI (diazepam binding inhibitor), neurosine, ENO2 (enolase 2), Aβ40 (amyloid beta 40), and TG2 (transglutaminase 2) with analyte blood of a patient with suspected Alzheimer's disease to form antigen-antibody complexes;

and

comparing the measured protein levels with protein levels of a normal control sample to determine an increase or decrease in quantities of the formed antigen-antibody complexes.

11. The method as claimed in claim 10, wherein the blood comprises serum or plasma.

FIG.1

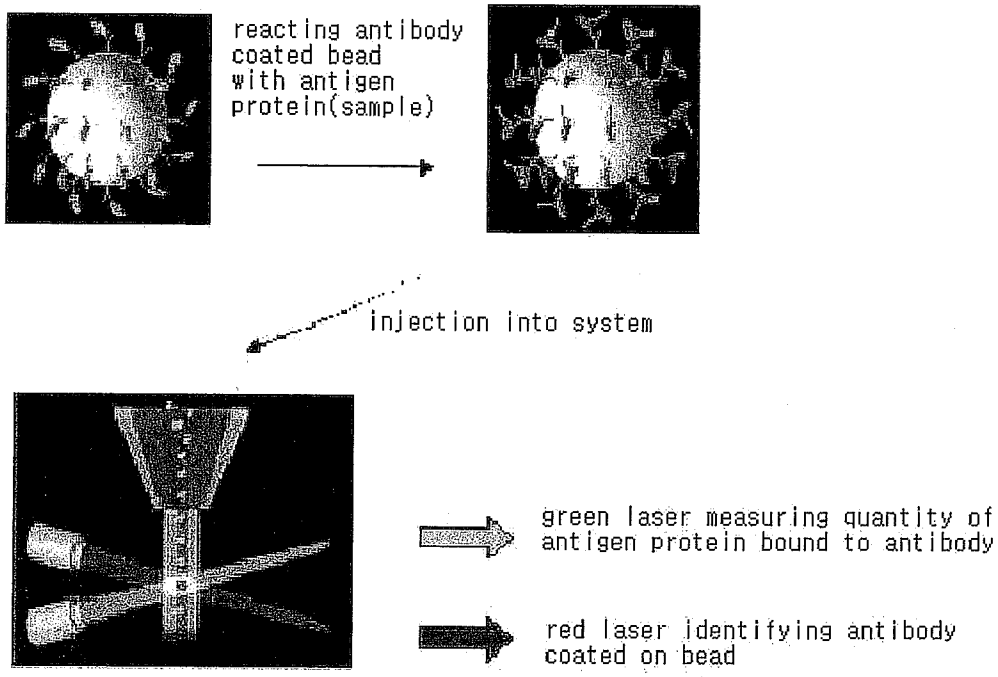
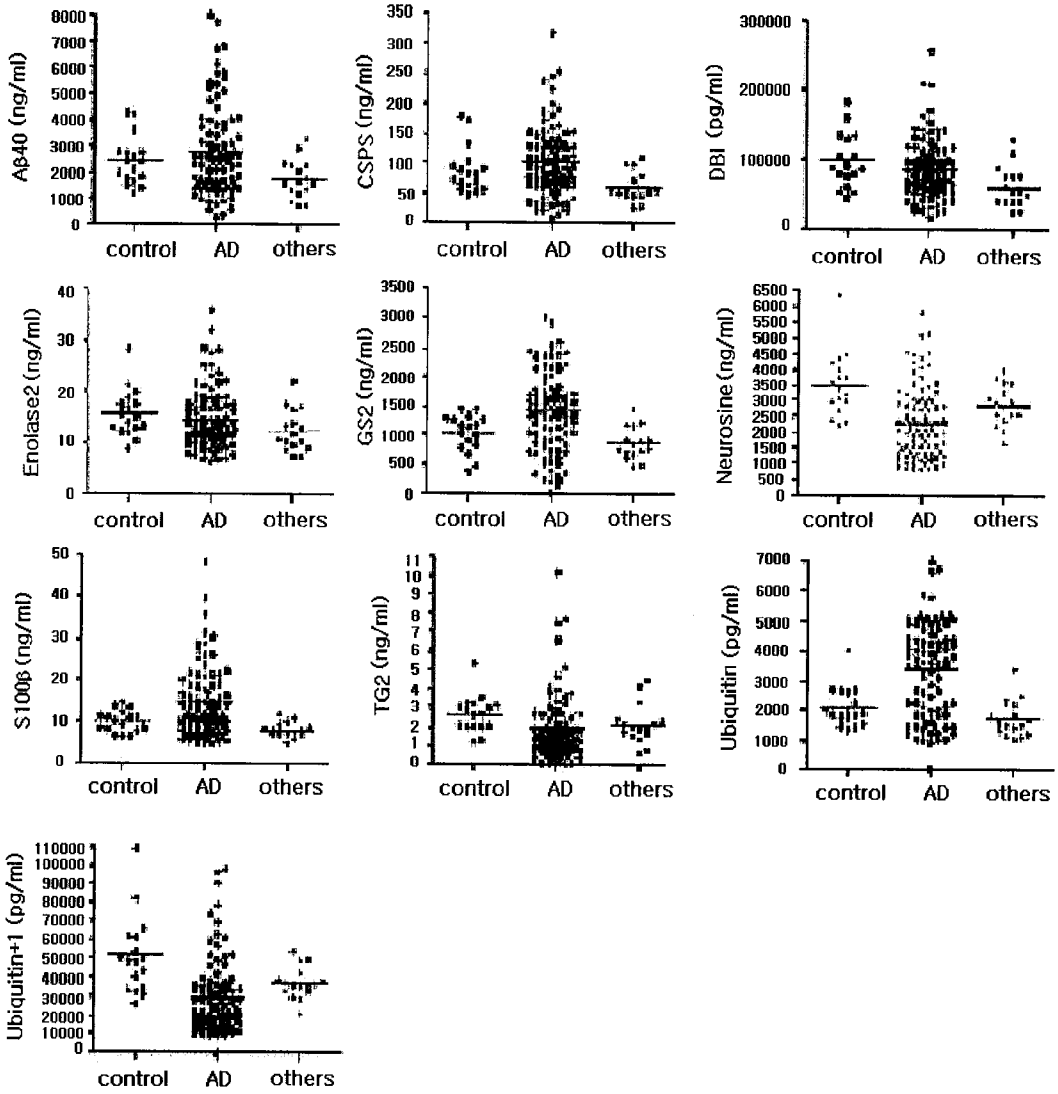

reacting antibody
coated bead
with antigen
protein(sample)
injection into system
green laser measuring quantity of
antigen protein bound to antibody
red laser identifying antibody
coated on bead

FIG.2


Aβ40 (ng/ml)
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6000
5000
4000
3000
2000
1000
0
control
AD
others
CSPS (ng/ml)
350
300
250
200
150
100
50
0
control
AD
others
DBI (pg/ml)
300000
200000
100000
0
control
AD
others
Enolase2 (ng/ml)
40
30
20
10
0
control
AD
others
GS2 (ng/ml)
3500
3000
2500
2000
1500
1000
500
0
control
AD
others
Neurosine (ng/ml)
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6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
control
AD
others
S100β (ng/ml)
50
40
30
20
10
0
control
AD
others
TG2 (ng/ml)
11
10
9
8
7
6
5
4
3
2
1
0
control
AD
others
Ubiquitin (pg/ml)
7000
6000
5000
4000
3000
2000
1000
0
control
AD
others
Ubiquitin+1 (pg/ml)
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90000
80000
70000
60000
50000
40000
30000
20000
10000
0
control
AD
others