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(54) **PROTEIN ANTIGEN COMBINATION FOR  
DETECTION OF ALZHEIMER'S DISEASE  
AND APPLICATION THEREOF**

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(57) **ABSTRACT**

The present disclosure belongs to the field of biological detection, and particularly relates to a protein antigen combination for detection of Alzheimer's disease (AD) and application thereof. The specific technical solution is as follows: An antigen combination is provided, wherein the antigen combination at least simultaneously includes the following proteins: DOC2A, LGALS1, KDM4D, and ADARB1. The present disclosure provides several new protein antigens and the combination thereof, which can be used for early-screening detection or diagnosis of AD, and are particularly suitable for risk assessment and prediction prior to the onset of AD. Meanwhile, the protein antigens and the combination thereof can distinguish AD from other types of dementia, and can be further prepared into related reagents or kits according to needs.

**Specification includes a Sequence Listing.**

**PROTEIN ANTIGEN COMBINATION FOR  
DETECTION OF ALZHEIMER'S DISEASE  
AND APPLICATION THEREOF**

**INCORPORATION-BY-REFERENCE OF  
SEQUENCE LISTING**

[0001] The application contains a sequence listing which has been submitted electronically in XML format and is hereby incorporated by reference in its entirety. Said XML copy, created on Feb. 12, 2025, is named 11540\_013019-USO\_SL-v2.xml and is 13615 bytes in size.

**TECHNICAL FIELD**

[0002] The present disclosure belongs to the field of biological detection, and particularly relates to a protein antigen combination for early detection of Alzheimer's disease and application thereof.

**BACKGROUND ART**

[0003] Alzheimer's disease (AD) is one of the most prevalent forms of dementia diseases, accounting for 60% to 80% of all dementia cases. AD is generally classified into pre-clinical, mild cognitive impairment (MCI), mild (early), moderate (mid), and severe (late) depending on patients' conditions. Preclinical (including an AD-derived subjective cognitive decline stage) and MCI stages are identified as extremely early stages of AD. At the preclinical and MCI stages, patients often exhibit less obvious symptoms, while abnormalities in biomarker indexes have already emerged. Should the timely and accurate diagnosis be feasible during the early stage without (obvious) symptoms, the therapeutic intervention for patients could be initiated a decade or two decades prior to the onset.

[0004] In 2023, the Alzheimer's Association International Conference (AAIC) announced National Institute on Aging-Alzheimer's Association (NIA-AA) Revised Clinical Criteria for Alzheimer's Disease (draft). The draft has classified biomarkers into three major categories, respectively: blood based biomarkers (BBBM), cerebrospinal fluid (CSF) biomarkers and "Golden standard" imaging (positron emission tomography/magnetic resonance imaging (PET/MRI)) biomarkers.

[0005] The CSF biomarkers can be used for detection during the asymptomatic stage, with changes appearing earlier than those observed in PET imaging. However, this diagnostic approach necessitates an invasive lumbar puncture, a procedure that is met with low clinical acceptance. Brain PET/MRI imaging can be used for detecting MCI and asymptomatic AD, however, changes tested by the brain PET/MRI imaging occurred later than those observed in the CSF biomarkers. Additionally, such imaging techniques are costly, and the availability of detection devices is low, with only a few hospitals in China capable of performing such detections. Furthermore, during brain PET/MRI imaging, the injection of contrast agents is required, which involves radiation, leading to relatively low patient acceptance.

[0006] Compared with detections of CSF and brain PET/MRI imaging and other biomarkers, BBBM detection has the advantages of being minimally invasive, low in cost, easy to operate and popularize and the like. Compared with CSF and PET imaging, the blood detection method is characterized by lower cost, expedited detection, and more simplified operation, and merely requires the collection of

venous blood. It is non-invasive, free of radiation risks, and applicable to a broader population, thus providing significant guidance for the early detection and prevention of AD.

[0007] The BBBMs are used for the early detection of AD, as indicated by an important tool in an AD referral path, such that diagnosis and treatment time can be shortened. That is, BBBM (+) would further aid in cognitive function evaluation and pathological diagnosis of AD (CSF examination and PET imaging), while BBBM (-) would contribute to the pathological diagnosis of AD, eliminate other examinations of etiology leading to cognitive decline, and avoid unnecessary CSF and PET imaging examinations.

[0008] SCD refers to a condition where patients perceive a decline in memory or cognitive function over their previous normal states, while objective neuropsychological tests remain within the normal range. SCD is a stage between normal cognitive aging and MCI. SCD is caused by a number of reasons, including preclinical AD, depression, anxiety, personality disorders, sleep disorders, and the like.

[0009] SCD is quite common in the elderly population, and population-based investigations have shown that 50% to 80% of elderly with normal cognition over 65 years old reported a decline in self-consciousness. However, there is not a high clinical interest in SCD. Factors such as cognitive decline forms, characteristics of individual personality, cultural backgrounds, and false positives in statistics would lead to great difficulty in the classification and quantification of SCD. Since SCD is a subjective cognitive perception lacking objective judgment criteria, investigators have proposed additional self-rating scales specific to SCD. Currently, common scales include subjective cognitive decline questionnaires (SCD-Qs), measurement of everyday cognition (Ecog), and the like.

[0010] Many clinical epidemiological investigations have shown that individuals with SCD are at an elevated risk of progressing to AD compared to those without SCD, and meanwhile, not all patients with SCD will progress to the stage of AD or dementia.

[0011] MCI is a heterogeneous clinical syndrome characterized by mild cognitive deficits that have no significant impact on daily life. It represents a transitional stage between normal aging and mild dementia. MCI can be either caused by neurodegenerative diseases such as AD, Parkinson's disease, and frontotemporal lobar degeneration, or by other systemic, neuro-systemic and psychiatric diseases. If targeted treatment can be administered based on the etiology of MCI, it is expected to reverse the cognitive impairment or prevent its further progression.

[0012] Therefore, if AD can be screened from the pre-clinical stage to SCD and MCI stages, and can be effectively distinguished from other types of dementia, it would enable early intervention and targeted treatment to be available, thus preventing further disease progression.

**SUMMARY**

[0013] The present disclosure aims to provide a protein antigen combination for early detection of AD and application thereof.

[0014] In order to fulfill the objective of the present disclosure, the technical solution adopted in the present disclosure is as follows: An antigen combination is provided, wherein the antigen combination at least simultaneously includes any two of the following proteins: DOC2A, LGALS1, KDM4D, and ADARB1.

**[0015]** Preferably, an amino acid sequence of the DOC2A protein is shown in SEQ ID NO: 1, and/or an amino acid sequence of the LGALS1 protein is shown in SEQ ID NO: 2, and/or an amino acid sequence of the KDM4D protein is shown in SEQ ID NO: 7, and/or an amino acid sequence of the ADARB1 protein is shown in SEQ ID NO: 9.

**[0016]** Preferably, the antigen combination further includes an SERF2 protein.

**[0017]** Preferably, an amino acid sequence of the SERF2 protein is shown in SEQ ID NO: 6.

**[0018]** Preferably, the antigen combination further includes any one or more of the following proteins: HIST1H2BD, ICAM1, RIOK2, and DNAJC8.

**[0019]** Preferably, an amino acid sequence of the HIST1H2BD protein is shown in SEQ ID NO: 3, and/or an amino acid sequence of the ICAM1 protein is shown in SEQ ID NO: 4, and/or an amino acid sequence of the RIOK2 protein is shown in SEQ ID NO: 5, and/or an amino acid sequence of the DNAJC8 protein is shown in SEQ ID NO: 8.

**[0020]** Accordingly, the antigen combination can be applied in preparation of products for detecting/identifying AD.

**[0021]** Preferably, the antigen combination can be applied in preparation of products for detecting/identifying AD at a middle stage or earlier, and particularly, has outstanding advantages in preparation of products for detecting/identifying AD at an early stage or earlier.

**[0022]** Accordingly, reagents or kits for detecting/identifying AD are prepared by using the antigen combination.

**[0023]** Accordingly, the LGALS1 protein and/or the HIST1H2BD protein and/or the RIOK2 protein can be applied in preparation of products for detecting/identifying AD, wherein the amino acid sequence of the LGALS1 protein is shown in SEQ ID NO: 2, and/or the amino acid sequence of the HIST1H2BD protein is shown in SEQ ID NO: 3, and/or the amino acid sequence of the RIOK2 protein is shown in SEQ ID NO: 5.

**[0024]** Accordingly, products, such as the reagents or the kits, for detecting/identifying AD are prepared using the proteins. The antigen combination has outstanding advantages in preparation of products for detecting/identifying AD at the middle stage or earlier, and particularly, the early stage or earlier.

**[0025]** The present disclosure has the following beneficial effects:

**[0026]** The present disclosure provides several protein antigens for effectively differentiating AD through autoantibody detection from the preclinical stage to the subjective cognitive decline (SCD) and mild cognitive impairment (MCI) stages. The detection method is simple and convenient and free from radiation, and does not cause trauma that is difficult for subjects to accept. Detection results demonstrate a high degree of consistency with those obtained through detection by CSF or “golden standard”. The protein antigens involved herein can be prepared by artificial synthesis, gene recombination and other methods.

**[0027]** The protein antigens or the combination thereof according to the present disclosure can be used to test biological samples obtained from the subjects for the presence or levels of autoantibodies against the antigen combination, thus determining whether the subjects are at the early stage of AD or at risk of developing AD, and meanwhile, facilitate the identification of whether the type of dementia

affecting the subjects is AD. Wherein the biological samples may be serum, plasma, whole blood, saliva, an oral mucosal swab, urine, lymph fluid, cerebrospinal fluid, and the like. Depending on specific conditions, the biological samples can be pretreated through methods such as extraction, dilution, and enrichment. The methods are versatile, simple, and easy to implement. When the protein antigens and the combination thereof according to the present disclosure are applied in the above tests, the presence or levels of the autoantibodies are tested by allowing the proteins or fragments thereof in the protein antigens and the combination thereof to bind to or interact with the corresponding autoantibodies, if present.

**[0028]** The protein antigens or the combination thereof according to the present disclosure can also be used for preparing AD-associated autoantibody early-screening reagents or AD-associated early-screening diagnostic reagents. It should be understood that the protein antigen combination can also be used to prepare kits for early-screening of AD-associated autoantibodies, and the kit can be prepared by referring to the method and the reagents for detecting the early-screening AD-associated autoantibodies by using the protein antigen combination in the examples of the present disclosure, with corresponding adjustments made as needed. The protein antigens or the combination thereof according to the present disclosure can be used for the early screening of AD and also exhibits excellent performance in the identification of AD at the early and middle stages.

**[0029]** In summary, the present disclosure provides several new protein antigens and the combination thereof, which can be used for early-screening detection or diagnosis of AD, and are particularly suitable for risk evaluation and prediction prior to the onset of AD. Meanwhile, the protein antigens and the combination thereof can distinguish AD from other types of dementia, and can be further prepared into the related reagents or kits according to needs.

#### DETAILED DESCRIPTION OF THE EMBODIMENTS

**[0030]** The present disclosure discovers that DOC2A, LGALS1, HIST1H2BD, ICAM1, RIOK2, SERF2, DNAJC8, KDM4D (also known as JMJD 2D) and ADARB1 proteins are closely related to the extremely early diagnosis of AD for the first time, and particularly, three proteins: LGALS1, HIST1H2BD, and RIOK2, are found to be related to the diagnosis of AD for the first time. An amino acid sequence of the DOC2A protein is shown in SEQ ID NO: 1. An amino acid sequence of the LGALS1 protein is shown in SEQ ID NO: 2. An amino acid sequence of the HIST1H2BD protein is shown in SEQ ID NO: 3. An amino acid sequence of the ICAM1 protein is shown in SEQ ID NO: 4. An amino acid sequence of the RIOK2 protein is shown in SEQ ID NO: 5. An amino acid sequence of the SERF2 protein is shown in SEQ ID NO: 6. An amino acid sequence of the KDM4D protein is shown in SEQ ID NO: 7. An amino acid sequence of the DNAJC8 protein is shown in SEQ ID NO: 8. An amino acid sequence of the ADARB1 protein is shown in SEQ ID NO: 9.

**[0031]** Based on the new findings, the present disclosure provides application of the proteins in preparation of related products (such as kits) for detecting and diagnosing AD. The proteins may be used alone or in combination to form a protein composition.

**[0032]** The technical solutions in the examples of the present disclosure will be clearly and completely described below, and apparently, the described examples are merely part, rather than all of the examples of the present disclosure. Unless otherwise specified, technical means used in the examples are conventional means that are well-known to a person skilled in the art. The data obtained is average values obtained after at least three repetitions, and the data obtained from each repetition is valid data.

Example 1: Construction, Expression and  
Purification of Recombinant Vector of Protein  
Antigen

**[0033]** A method for obtaining a protein antigen is as follows: coding DNA of the protein antigen was synthesized, primers were designed by taking the synthetic DNA as a template, a gene fragment of the protein antigen or a fragment thereof was cloned into an expression plasmid through polymerase chain reaction (PCR), enzyme digestion, ligation and other molecular cloning methods, the gene fragment in the expression plasmid was then expressed by means of *Escherichia coli* (*E. coli*), yeast or cells, and finally, a target protein was obtained through chromatographic purification. Meanwhile, Trx, GST, AVI, HIS, and c-myc tags could also be selectively added to the protein antigen or the fragment thereof. The addition of these tags could facilitate the purification or labeling of the protein antigen without substantially altering the binding properties of the antigen to autoantibodies thereof.

**[0034]** DOC2A, LGALS1, HIST1H2BD, ICAM1, RIOK2, SERF2, DNAJC8, KDM4D and ADARB1 proteins were selected, and respective database IDs of the above proteins are specifically shown in Table 1.

TABLE 1

Comparison table of database ID of each candidate protein		
Protein name	Database ID (uniprot)	Sequence
DOC2A	Q14183	SEQ ID NO: 1
LGALS1	P09382	SEQ ID NO: 2
HIST1H2BD	P58876	SEQ ID NO: 3
ICAM1	P05362	SEQ ID NO: 4
RIOK2	Q9BVS4	SEQ ID NO: 5
SERF2	P84101-3	SEQ ID NO: 6
KDM4D	Q6B0I6	SEQ ID NO: 7
DNAJC8	O75937	SEQ ID NO: 8
ADARB1	P78563	SEQ ID NO: 9

**[0035]** Using a human cDNA library (purchased from Invitrogen Company) or whole gene synthetic DNA as a template, primers were designed respectively, and a gene fragment of the protein was cloned into a pET28 plasmid through PCR, enzyme digestion, ligation and other molecular cloning methods. Meanwhile, HIS, FLAG and other tags were added to an N-terminal of the protein to form a fusion protein. A recombinant expression vector was identified by DNA sequencing and confirmed to contain the correct protein gene fragment. It should be noted that the added tag(s) is merely convenient for identification and extraction of the protein, and does not have a decisive impact on the function of the protein as the antigen. When in use, the tag may not be added necessarily, or other tags may be added as needed, or alternative labeling/identification methods may be employed.

**[0036]** The recombinant plasmid containing the protein gene fragment was transformed into competent cells of *E. coli* BL21 (DE3), and the clones were selected and inoculated into LB media, and then shaking-cultured at 37° C. When the *E. coli* BL21 (DE3) density OD600 reached approximately 0.8, temperature was reduced to 16° C., and 0.1 mM isopropylthio-beta-D-galactoside (IPTG) was added to each LB medium to induce expression overnight, and bacterial cells were obtained.

**[0037]** The expression-induced bacterial cells were collected by centrifugation and rinsed twice with phosphate buffer saline (PBS). The bacterial cells were resuspended and dispersed in a lysis buffer (5-10 mL of lysis buffer per gram of bacterial cells), and were then subjected to ultrasonic disruption (ultrasound power: 200 W, ultrasonication for 5 seconds with a 5-second interval) under an ice bath. After cell disruption, the bacterial cells were centrifuged at 13000 rpm and at 10° C. for 20 minutes, and a supernatant was collected, and then purified via two steps: Ni column affinity chromatography and molecular sieve chromatography. The purified target protein was analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) to confirm the molecular weight and purity of the protein, and after the concentration of the protein was determined via a Bradford method, the protein was stored at -80° C. for later use. The purified candidate proteins were obtained.

Example 2: Display of Effect of Each Candidate  
Protein Alone in Detection of AD-Derived SCD  
and AD-Derived MCI Samples

**[0038]** 1. Solutions and reagents used in this example were as follows:

**[0039]** (1) A coating buffer was a PBS buffer (pH=7.4). A preparation method is as follows: 3.58 g of  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ , 0.23 g of  $\text{KH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ , 0.2 g of KCl and 8.0 g of NaCl were accurately weighed and dissolved in water, and then diluted with the water to 1 L.

**[0040]** (2) Blocking buffer/sample diluent/antibody diluent: 10 g of BSA (bovine serum albumin) was dissolved in 800 mL of coating buffer, and diluted with the coating buffer to 1 L.

**[0041]** (3) Washing buffer: A washing buffer should be prepared when using. 0.5% Tween 20 (V/V) was added to the coating buffer before use, and pH is 7.4.

**[0042]** (4) A TMB chromogenic agent was purchased from KPL Company.

**[0043]** (5) Stopping solution: 1M hydrochloric acid.

**[0044]** 2. A solid phase was coated with the protein. Each purified candidate protein obtained in Example 1 was diluted to a concentration of 5  $\mu\text{g/mL}$  using the coating buffer, and the diluted protein was then added to a 96-well plate at 50  $\mu\text{L}$ /well and coated overnight at 4° C. for incubation. On the following day, the solution was discarded, and the plate was dried, and washed with the washing buffer three times, with each well receiving 200  $\mu\text{L}$  per time. 200  $\mu\text{L}$  of blocking buffer was added for incubation at room temperature for 1 hour, and then the plate was dried after the blocking buffer was discarded, washed with the washing buffer three times, with each well receiving 200  $\mu\text{L}$  per time, and dried again. The solid phase-coated protein antigen in the 96-well plate was obtained.

**[0045]** 3. The sample to be tested was added. Human serum to be tested was diluted by a factor of 100 with the sample diluent and then added to the 96-well plate containing the protein to be tested, and 50  $\mu$ L of the diluted sample to be tested was added to each well. The 96-well plate was then placed on a microplate shaker, where it was incubated at room temperature for 1 hour; and the plate was dried, washed with the washing buffer three times, with each well receiving 200  $\mu$ L per time, and then dried again.

**[0046]** 4. An enzyme-labeled second antibody was added. 1.0 mg/mL horseradish peroxidase-labeled recombinant goat anti-human immunoglobulin G antibody (purchased from Jackson ImmunoResearch Inc.) was diluted by a factor of 20000 with the antibody diluent, and then added to the 96-well plate treated in step 3 at 50  $\mu$ L/well. The 96-well plate was then placed on a microplate shaker, where it was incubated at room temperature for 0.5 hour; and the plate was dried, washed with the washing buffer three times, with each well receiving 200  $\mu$ L per time, and then dried again.

**[0047]** 5. Color reaction and optical density reading. In the 96-well plate treated in step 4, a TMB chromogenic agent was added to the plate at 50  $\mu$ L/well, followed by shaking for 15 seconds. The reaction was kept away from light for 15 minutes at room temperature, and then 50  $\mu$ L of stopping solution was added. Then an absorbance value at 450 nm was read by a microplate reader to obtain a detection signal(S) of each sample to be tested.

**[0048]** 6. Analysis of sensitivity and specificity. 300 positive samples (identified as positive samples for enrollment) and 300 negative samples (serum from healthy subjects) were collected, respectively.

**[0049]** The inclusion criteria for defining the positive samples are as follows: subjects simultaneously meet the following conditions: subjective sensory memory decline rather than other cognitive impairment diseases, onset duration of less than 5 years, age at onset greater than 55 years, concern about cognitive deterioration, and self-sensory memory being inferior compared to peers of the same age. No recent (within the past 7 days) or long-term ( $\geq 5$  years) administration of the following drugs does not happen, including immunomodulators, antidepressants or neuroleptics containing anticholinergic ingredients, anti-Parkinsonian drugs, or other clinical trial drugs; and no history of autoimmune diseases, primarily including rheumatoid arthritis, hyperthyroidism, systemic lupus erythematosus, primary biliary cirrhosis, and the like. Scale evaluation: Global Deterioration Scale (GDs) scores of 2-3, Clinical Dementia Rating Scale (CDR) score  $\leq 0.5$ , memory score below 1.5 standard deviations from the age- and education-matched control group, Mini-Mental State Examination (MMSE) scores  $> 24$ , and Mattis Dementia Rating Scale (DRs) score  $> 123$ .

**[0050]** It should be noted that the existing universal diagnosis methods of AD are not completely accurate, and particularly, are difficult in precisely diagnosing patients with extremely early (potential) AD. Therefore, samples, as identified as positive herein, do not necessarily represent all true cases of (extremely early) AD, where there may be normal individuals who have been misdiagnosed or misjudged, as well as individuals with cognitive decline, cognitive impairment, or deterioration of memory caused by conditions other than AD.

**[0051]** The inclusion criteria for defining negative samples (healthy individuals) are as follows: subjects simultaneously meet the following conditions: (1) subjects are conscious and stable in vital signs, and have no abnormal neurological symptoms or signs, and no history of nervous system diseases and mental system diseases; (2) no subjective memory function decline or history of other neurological or psychiatric disorders, and no insomnia or chronic insomnia recently (insomnia symptoms occurring  $\geq 3$  times per week and persisting for  $\geq 3$  months); (3) Mini-Mental State Examination (MMSE) score  $> 26$ ; (4) Montreal Cognitive Assessment (MoCA) score  $> 26$ ; (5) Hamilton Depression Scale (HAMD) score  $< 7$ ; (6) Hamilton Anxiety Scale (HAMA) (14 items) score  $< 7$ ; (7) Clinical Dementia Rating (CDR) score of ( ) (8) no recent (within the past 7 days) or long-term ( $\geq 5$  years) administration of the following drugs, including the immunomodulators, the antidepressants or neuroleptics containing anticholinergic ingredients, the anti-Parkinsonian drugs, or other clinical trial drugs; and (9) no history of autoimmune diseases, primarily including rheumatoid arthritis, hyperthyroidism, systemic lupus erythematosus, primary biliary cirrhosis, and the like.

**[0052]** A detection signal(S) of each sample was determined according to the method (absorption value at 450 nm wavelength) in step 4. The negative samples were taken as negative reference samples. The mean (M, average value) and standard deviation (SD) of detection signals(S) of all negative reference samples were calculated, and  $M+3$  SD was taken as a cut-off value.

**[0053]** The samples ( $S \geq M+3$  SD) with the detection signal (S)  $\geq$  cut off value were regarded as positive; and the samples ( $S < M+3$  SD) with the detection signal(S)  $<$  cut off value were regarded as negative.

**[0054]** The specificity and sensitivity were calculated based on the positive and negative results of the samples. Wherein, specificity refers to the proportion of healthy subjects whose samples are correctly determined as negative, that is, the number of the negative samples which are correctly determined as negative divided by the total number of the negative samples. Sensitivity refers to the proportion of positive samples of enrolled patients that are determined as positive, that is, the number of the positive samples which are correctly determined as positive divided by the total number of the positive samples. The sensitivity and specificity of each protein to be tested as an antigen for sample detection are obtained through calculation.

**[0055]** Meanwhile, the positive samples were respectively detected in hospitals through the following methods: beta-Amyloid Accumulation (CSF/PET), FDG-PET/fMRI or Tau-mediated neuronal injury (CSF). The sensitivity was calculated using the detection results from the hospitals as a control. Additionally, due to the presence of radiation and/or invasive procedures associated with tests such as beta-Amyloid Accumulation and the like, normal individuals (negative samples) did not perform the corresponding detection, resulting in the absence of specificity data for these cases. The results are shown in Table 2.

TABLE 2

Comparison table of detection result of each candidate protein				
Candidate protein/detection method	Specificity		Sensitivity	
	The number of samples detected as negative	Ratio	The number of samples detected as positive	Ratio
LGALS1	294	98%	66	22%
HIST1H2BD	285	95%	57	19%
RIOK2	288	96%	72	24%
KDM4D	285	95%	81	27%
SERF2	288	96%	93	31%
ADARB1	282	94%	99	33%
ICAM1	282	94%	66	22%
DOC2A	282	94%	72	24%
DNAJC8	294	98%	60	20%
$\beta$ -Amyloid Accumulation	\	\	117	39%
FDG-PET/fMRI	\	\	72	24%
Tau-mediated neuronal injury	\	\	54	18%

**[0056]** Results indicate that the existing commonly used convenient methods (self-evaluation and scale evaluation) had low accuracy in detecting the extremely early AD, and samples as identified as positive contained a significant proportion of misdiagnosed patients.

Example 3: Display of Effect of Protein  
Compositions in Combined Detection of  
AD-Derived SCD and AD-Derived MCI Samples

1. Antigen Compositions are Used for Detection Based on a “Single-Index” Method.

**[0057]** The “single-index” method refers to an approach where detection is based on each antigen, that is, one antigen corresponding to a piece of data. When a certain sample to be tested is detected using a certain antigen combination, the result for the detection of the sample based on the antigen combination is regarded as positive if any antigen from the antigen combination yields a positive detection signal; and otherwise, the result is regarded as negative.

**[0058]** Different antigen combinations are formed by using candidate protein antigens in Example 2, as specifically shown in Table 3. The antigen combinations were used to detect the specificity and sensitivity of the positive and negative samples from Example 1 with reference to the method as described in Example 2. The detection results are shown in Table 4. It should be noted that the inventor did not limit experiments to the combinations as shown in Table 3; rather, the inventor obtained the antigen combinations shown in Table 3 after a large number of preliminary tests.

TABLE 3

Comparison table of each antigen combination	
Combination	Candidate protein antigens contained in combination
Combination 1	SERF2, LGALS1, KDM4D
Combination 2	SERF2, DOC2A, ADARB1
Combination 3	SERF2, HIST1H2BD, RIOK2
Combination 4	SERF2, DNAJC8, ADARB1
Combination 5	LGALS1, KDM4D, ADARB1

TABLE 3-continued

Comparison table of each antigen combination	
Combination	Candidate protein antigens contained in combination
Combination 6	SERF2, LGALS1, KDM4D, ADARB1
Combination 7	DOC2A, LGALS1, KDM4D, ADARB1
Combination 8	SERF2, LGALS1, KDM4D, ADARB1, DOC2A
Combination 9	SERF2, DOC2A, LGALS1, HIST1H2BD, ICAM1, RIOK2, ADARB1, KDM4D, DNAJC8

TABLE 4

Comparison table of detection result of each antigen combination based on “single-index” method				
Combination	Sensitivity		Specificity	
	The number of samples detected as positive	Ratio	The number of samples detected as negative	Ratio
Combination 1	171	57%	267	89%
Combination 2	198	66%	255	85%
Combination 3	159	53%	261	87%
Combination 4	192	64%	267	89%
Combination 5	171	57%	264	88%
Combination 6	207	69%	255	85%
Combination 7	201	67%	246	82%
Combination 8	225	75%	237	79%
Combination 9	231	77%	198	66%
$\beta$ -Amyloid Accumulation	117	39%	\	\
FDG-PET/fMRI	72	24%	\	\
Tau-mediated neuronal injury	54	18%	\	\

**[0059]** Results indicate that in an event of judgment based on the “single-index” method, sensitivity was higher compared to three commonly used existing methods. However, specificity was relatively lower compared to the detection based on individual protein antigens, suggesting that the detection accuracy could be further improved.

2. Antigen Compositions are Used for Detection Based on a “Double-Index” Method.

**[0060]** The “double-index” method refers to an approach where when a certain sample to be tested is detected using a certain antigen combination, the detection result of the sample is regarded as positive if two or more antigens from the antigen combination yield positive detection signals; and otherwise, the result is regarded as negative. The rest of operations are the same as the “single-index” method. Meanwhile, in an event of the detection based on multiple existing detection methods, the “double-index” method is also employed for judgment, that is, the detection result is identified as positive only if both detection methods yield positive results simultaneously; and otherwise, the result is regarded as negative. For example, in an event of the combination of beta-Amyloid Accumulation+FDG-PET/fMRI (CSF/PET), only when the results from detections of both beta-Amyloid Accumulation and FDG-PET/fMRI (CSF/PET) are positive can the detection result be regarded as positive. The results are shown in Table 5.

TABLE 5

Comparison table of detection result of each antigen combination based on “double-index” method				
Combination	Sensitivity		Specificity	
	The number of samples detected as positive	Ratio	The number of samples detected as negative	Ratio
Combination 1	60	20%	300	100%
Combination 2	60	20%	297	99%
Combination 3	57	19%	300	100%
Combination 4	57	19%	300	100%
Combination 5	66	22%	297	99%
Combination 6	93	31%	294	98%
Combination 7	102	34%	297	99%
Combination 8	135	45%	294	98%
Combination 9	177	59%	282	94%
β-Amyloid Accumulation	117	39%	\	\
FDG-PET/fMRI (CSF/PET)	72	24%	\	\
Tau-mediated neuronal injury(CSF)	54	18%	\	\
β-Amyloid Accumulation + FDG-PET/fMRI (CSF/PET)	72	24%	\	\
β-Amyloid Accumulation + Tau-mediated neuronal injury(CSF)	42	14%	\	\

TABLE 5-continued

Comparison table of detection result of each antigen combination based on “double-index” method				
Combination	Sensitivity		Specificity	
	The number of samples detected as positive	Ratio	The number of samples detected as negative	Ratio
FDG-PET/fMRI + Tau-mediated neuronal injury(CSF)	33	11%		

[0061] Results indicate that the detection result of the “double-index” method was closer to the detection results of beta-Amyloid Accumulation and the like, and the specificity was obviously higher, suggesting that the detection accuracy of the “double-index” method was higher.

3. Samples Identified as Positive by Detection of Beta-Amyloid and the Like were Detected by Using Each Individual Protein Antigen or by Using Antigen Compositions Based on the “Single-Index” Method or “Double-Index” Method.

[0062] To further confirm the overlap between the positive results detected by the individual antigens or each antigen combination and the positive results detected by the existing methods, the samples identified as positive through the following methods in Table 5: beta-Amyloid Accumulation, FDG-PET/fMRI (CSF/PET), Tau-mediated neuronal injury (CSF), beta-Amyloid Accumulation+FDG-PET/fMRI (CSF/PET),beta-Amyloid Accumulation+Tau-mediated neuronal injury (CSF), and FDG-PET/fMRI+Tau-mediated neuronal injury (CSF), were detected again by using each antigen combination. The results are shown in Table 6. The total number of the samples identified as positive in Table 6 corresponds to the number of the samples identified as positive through detection by each existing method in Table 5.

TABLE 6

Comparison table of detection result of each antigen or antigen combination						
Antigen or antigen Combination	β-Amyloid Accumulation (CSF/PET) Total number 117	FDG-PET/fMRI Total number 72	Tau-mediated neuronal injury (CSF) Total number 54	β-Amyloid Accumulation + FDG-PET/fMRI (CSF/PET) Total number 72	β-Amyloid Accumulation + Tau-mediated neuronal injury (CSF) Total number 42	FDG-PET/fMRI + Tau-mediated neuronal injury (CSF) Total number 33
LGALS1	48	36	36	36	30	21
HIST1H2BD	48	36	33	36	30	24
RIOK2	57	45	18	45	18	18
KDM4D	60	45	18	45	18	18
SERF2	63	33	24	33	21	18
ADARB1	66	42	33	42	27	18
ICAM1	60	36	27	36	27	18
DOC2A	42	30	18	30	12	12
DNAJC8	36	21	15	21	9	9
Single-index combination 1	111	69	48	69	42	33
Single index Combination 2	114	69	54	69	42	33
Single-index combination 3	111	69	45	69	42	33
Single-index combination 4	108	63	54	63	42	33

TABLE 6-continued

Comparison table of detection result of each antigen or antigen combination						
Antigen or antigen Combination	$\beta$ -Amyloid Accumulation (CSF/PET) Total number 117	FDG-PET/fMRI Total number 72	Tau-mediated neuronal injury (CSF) Total number 54	$\beta$ -Amyloid Accumulation + FDG-PET/fM (CSF/PET) Total number 72	$\beta$ -Amyloid Accumulation + Tau-mediated neuronal injury (CSF) Total number 42	FDG-PET/fMRI + Tau-mediated neuronal injury (CSF) Total number 33
Single-index combination 5	102	66	48	66	39	30
Single-index combination 6	117	72	51	72	42	33
Single-index combination 7	117	72	54	72	42	33
Single-index combination 8	117	72	54	72	42	33
Single-index combination 9	117	72	54	72	42	33
Double-index combination 1	51	36	24	36	21	18
Double-index combination 2	51	30	18	30	15	12
Double-index combination 3	51	39	24	39	21	21
Double-index combination 4	54	30	18	30	15	12
Double-index combination 5	63	48	36	48	33	24
Double-index combination 6	81	54	45	54	39	30
Double-index combination 7	117	72	54	72	42	33
Double-index combination 8	117	72	54	72	42	33
Double-index combination 9	117	72	54	72	42	33

[0063] Results indicate that the detection results of the combinations 7, 8 and 9 had a high matching degree with those from the existing detection methods which were recognized to have higher accuracy, and patients who were regarded as positive through detection by the existing methods could be detected completely and accurately.

Example 4: Display of Effect of Protein Compositions in Combined Detection of Patients with Early AD and Middle AD

[0064] 100 clinical samples were taken as positive samples with early AD, 100 clinical samples were taken as positive samples with middle AD, and a detection signal(S) of each sample was determined using the candidate antigens and combinations thereof as shown in Table 3 based on the method in Example 2. The samples ( $S \geq M+3$  SD) with the detection signal(S)  $\geq$  cut off value were regarded as positive; and the samples ( $S < M+3$  SD) with the detection signal(S)  $<$  cut off value were regarded as negative.

[0065] The specificity and sensitivity were calculated based on the positive and negative results of the samples. Wherein, specificity refers to the proportion of healthy subjects whose samples are correctly determined as negative, that is, the number of the negative samples which are correctly determined as negative divided by the total number of the negative samples. Sensitivity refers to the proportion of positive samples of enrolled patients that are determined as positive, that is, the number of the positive samples which are correctly determined as positive divided by the total

number of the positive samples. The sensitivity and specificity of each protein to be tested as an antigen for sample detection are obtained through calculation. The results are shown in Table 7.

TABLE 7

Comparison table of detection result of each antigen or antigen combination				
Antigen or antigen Combination	Early sensitivity	Medium sensitivity	Medium and early sensitivity	Specificity
LGALS1	46%	28%	37%	98%
HIST1H2BD	36%	24%	30%	95%
RIOK2	28%	20%	24%	96%
KDM4D	44%	24%	34%	95%
SERF2	34%	28%	31%	96%
ADARB1	42%	38%	40%	94%
ICAM1	20%	16%	18%	94%
DOC2A	18%	10%	14%	94%
DNAJC8	36%	34%	35%	98%
Single-index combination 1	68%	62%	65%	89%
Single-index combination 2	68%	62%	65%	85%
Single-index combination 3	70%	54%	62%	87%
Single-index combination 4	74%	72%	73%	89%
Single-index combination 5	72%	64%	68%	88%



TABLE 7-continued

Comparison table of detection result of each antigen or antigen combination				
Antigen or antigen Combination	Early sensitivity	Medium sensitivity	Medium and early sensitivity	Specificity
Single-index combination 6	74%	76%	75%	85%
Single-index combination 7	80%	66%	73%	82%
Single-index combination 8	80%	78%	79%	79%
Single-index combination 9	94%	86%	90%	66%
Double-index combination 1	36%	18%	27%	100%
Double-index combination 2	24%	14%	19%	99%
Double-index combination 3	24%	18%	21%	100%
Double-index combination 4	32%	26%	29%	100%
Double-index combination 5	46%	22%	34%	99%
Double-index combination 6	52%	34%	43%	98%

TABLE 7-continued

Comparison table of detection result of each antigen or antigen combination				
Antigen or antigen Combination	Early sensitivity	Medium sensitivity	Medium and early sensitivity	Specificity
Double-index combination 7	50%	30%	40%	99%
Double-index combination 8	58%	42%	50%	98%
Double-index combination 9	78%	66%	72%	94%

[0066] Results indicate that the detection results of the combinations 5, 6, 7, 8, and 9 had higher specificity and better sensitivity at early and middle stages of AD.

[0067] The above-described examples are merely the description of preferred implementations of the present disclosure, and do not limit the scope of the present disclosure. Various modifications, variations, alterations, and substitutions made by those of ordinary skill in the art to the technical solutions of the present disclosure are intended to fall within the scope of protection identified by the claims of the present disclosure without departing from the design spirit of the present disclosure.

SEQUENCE LISTING

Sequence total quantity: 9

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FEATURE                   Location/Qualifiers  
source                   1..400  
                          mol\_type = protein  
                          note = DOC2A  
                          organism = Homo sapiens

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LAPPAALLGA TTPEDGAEVD SYDSDDATAL GTLEFDLLYD RASCTLHCSI LRAGLKPMD   120  
FNLGADPYVK LHLPLGACKA NKLLTKTQRN TLNPVWNEDL TYSGITDDDI THKVLRIAVC   180  
DEDKLSHNEF IGEIRVPLRR LKPSQKKHFN ICLERQVPLA SPSSMSAALR GISCYLKELE   240  
QAEQGQGLLE ERGRILLSLS YSSRRRGLLV GILRCAHLAA MDVNGYSDPY VKTYLRPDVD   300  
KSKXHKTCVK KKTLPNPEFNE EFFEYIELST LATKTLEVTV WDYDIGKSND FIGGVSLGPG   360  
ARGEARKHWS DCLQQPDAAL ERWHTLTSEL PPAAGALSSA                   400

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FEATURE                   Location/Qualifiers  
source                   1..135  
                          mol\_type = protein  
                          note = LGALS1  
                          organism = Homo sapiens

SEQUENCE: 2  
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CNSKDGGAWG TEQREAVFPF QPGSVAEVC I TFDQANLTVK LPDGYEFKFP NRLNLEAINY   120  
MAADGDFKIK CVAFD   135

SEQ ID NO: 3                   moltype = AA   length = 126  
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source                   1..126  
                          mol\_type = protein  
                          note = HIST1H2BD  
                          organism = Homo sapiens

SEQUENCE: 3  
MPEPTKSAPA PKKGSKKAVT KAQKKGKKR KRSRKESYSV YVYKVLQVH PDTGISSKAM   60  
GIMNSFVNDI FERIAGEASR LAHYNKRSTI TSREIQTAVR LLLPGELAKH AVSEGTKAVT   120  
KYTSSK   126

SEQ ID NO: 4                   moltype = AA   length = 532  
FEATURE                   Location/Qualifiers  
source                   1..532  
                          mol\_type = protein  
                          note = ICAM1

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organism = Homo sapiens

SEQUENCE: 4

MAPSSRPAL	PALLVLLGAL	FPGPGNAQTS	VSPSKVILPR	GGSVLVTCST	SCDQPKLLGI	60
ETPLPKKELL	LPGNMRKVYE	LSNVQEDSQP	MCYSNCPDGQ	STAKTFLTVY	WTPERVELAP	120
LPSWQPVGKN	LTLRCQVEGG	APRANLTVVL	LRGEKELKRE	PAVGEPAEVT	TTVLVRRDHH	180
GANFSCRTLE	DLRPQGLELF	ENTSAPYQLQ	TFVLPATPPQ	LVSPRVLEVD	TQGTVVCSLD	240
GLFPVSEAQV	HLALGDQRLN	PTVTYGNDSE	SAKASVSVTA	EDEGTQRLTC	AVILGNQSQE	300
TLQTVTIYSF	PAPNVILTKP	EVSEGTEVTV	KCEAHPRAKV	TLNGVPAQPL	GPRAQLLLKA	360
TPEDNGRSFS	CSATLEVAGQ	LIHKNQTRLE	RVLYGPRLDE	RDCPGNWTWP	ENSQQTPMCQ	420
AWGNPLPELK	CLKDGTPLP	IGESVTVTRD	LEGTYLCLAR	STQGEVTRKV	TVNVLSPRYE	480
IVIITVVAAG	VIMGTAGLST	YLYNRQRKIK	KYRLQQAQKG	TPMKPNTQAT	PP	532

SEQ ID NO: 5

FEATURE

source

moltype = AA length = 552

Location/Qualifiers

1..552

mol\_type = protein

note = RIOK2

organism = Homo sapiens

SEQUENCE: 5

MGKVNVAKLK	YMSRDDFRVL	TAVEMGMKNH	EIVPGSLIAS	IASLKHGGCN	KVLRELVKHK	60
LIAWERTKT	QGYRLTNAGY	DYLALKTLSS	RQVVEVGNQ	MGVGKESDIY	IVANEEGQQF	120
ALKLHRLGRT	SFRNLKNKRD	YHKHRHNVSW	LYLSRLSANK	EFAYMKALYE	RKFPVPKPID	180
YNRHAVVMEL	INGYPLCQIH	HVEDPASVYD	EAMELIVKLA	NHGLIHGDFN	EFNLILDESD	240
HITMIDFPQM	VSTSHPNAEW	YFDRDVKCIK	DFFMKRFSYE	SELPPTFKDI	RREDTLDVEV	300
SASGYTKEMQ	ADDELLHPLG	PDDKNIETKE	GSEFSFSDGE	VAEKAENVYGS	ENESERNCLE	360
ESEGCYCRSS	GDPEQIKEDS	LSEESADARS	FEMTEFNQAL	EEIKGQVVEN	NSVTEFSEEK	420
NRTENYNRQD	QQRVQGGVPA	GSDEYEDECP	HLIALSSSLNR	EFRPFRDEEN	VGAMNQYRTR	480
TLISITSSGSA	VSCSTIPPEL	VKQKVKRQLT	KQQKSAVRRR	LQKGANIFT	KQRRENMQNI	540
KSSLEAASFW	GE					552

SEQ ID NO: 6

FEATURE

source

moltype = AA length = 61

Location/Qualifiers

1..61

mol\_type = protein

note = SERF2

organism = Homo sapiens

SEQUENCE: 6

MTRGNQRELA	RQKNMKQSD	SVKGKRRDDG	LSAAARKQRD	SEIMQQQKKK	ANEKKEPKS	60
E						61

SEQ ID NO: 7

FEATURE

source

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Location/Qualifiers

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mol\_type = protein

note = KDM4D

organism = Homo sapiens

SEQUENCE: 7

METMKSAMC	AQNPNCNIMI	FHPTKEEFND	FDKYIAYMES	QGAHRAGLAK	IIPPKEWKAR	60
ETYDNISEIL	IATPLQQVAS	GRAGVFTQYH	KKKAMTVGE	YRHLANSKKY	QTPPHQNFED	120
LERKYWKMRI	YNSPIYGADI	SGSLFDENTK	QWNLGHGLTI	QDLLEKECGV	VIEGVNTPYL	180
YFGMWKTTFA	WHTEDMDLYS	INYLHLGEPK	TWYVVPPEHG	QRLERLAREL	FPGSSRGCGA	240
FLRHKVALIS	PTVLKENGIP	FNIRITQAGE	FMVTFPYGYH	AGFNHGFNCA	EAINFATPRW	300
IDYGKMASQC	SCGEARVTF	MDAFVRILQP	ERYDLWKRQ	DRAVVDHMEP	RVPASQELST	360
QKEVQLPRRA	ALGLRQLPSH	WARHSPWMA	ARSGTRCHTL	VCSSLPRRSA	VSGTATQPPA	420
AAVHSSKKPS	STPSSTPGPS	AQIIHPSNGR	RGRGRPPQKL	RAQELTLQTP	AKRPLLAGTT	480
CTASGPEPEP	LPEDGALMDK	PVPLSPGLQH	PVKASGCSWA	PVP		523

SEQ ID NO: 8

FEATURE

source

moltype = AA length = 253

Location/Qualifiers

1..253

mol\_type = protein

note = DNAJC8

organism = Homo sapiens

SEQUENCE: 8

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VLQIDPEVTD	EEIKKRFRQL	SILVHPDKNQ	DDADRAQKAF	EAVDKAYKLL	LDQEQKKRAL	120
DVIQAGKEYV	EHTVKERKKQ	LKKEGKPTIV	EEDDPFLFKQ	AVYKQTMKLF	AELEIKRKER	180
EAKEMHERKR	QREEEIEAQE	KAKREREWQK	NFEESRDGRV	DSWRNFQANT	KGKKEKKNRT	240
FLRPPKVKME	QRE					253

SEQ ID NO: 9

FEATURE

source

moltype = AA length = 741

Location/Qualifiers

1..741

mol\_type = protein

note = ADARB1

organism = Homo sapiens

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EGSGPTKKKA KLHAAEKALR SFVQFPNASE AHLAMGRTLS VNTDFTSDQA DFPDTLFNGF 180
ETPDKAEPFF YVGSNGDDSF SSSGDLSLSA SPVPASLAQP PLPVLPPFPF PSGKNPVMIL 240
NELRPGLKYD FLSESGESHA KSFVMSVVVD GQFFEGSGRN KKLAKARAAQ SALAAIFNLH 300
LDQTPSRQPI PSEGLQLHLP QVLADAVSRL VLGKFGDLTD NFSSPHARRK VLAGVVMTTG 360
TDVKDAKVIS VSTGKTCING EYMSDRGLAL NDCHAEIISR RSLLRFLYTQ LELYLNNKDD 420
QKRSIFQKSE RGGFRLKENV QPHLYISTSP CGDARIFSPH EPILEGSRSY TQAGVQWCNH 480
GSLQPRPPGL LSDPSTSTFQ GAGTTEPADR HPNRKARGQL RTKIESGEGT IPVRSNASIQ 540
TWDGVLQGER LLTMSCSDKI ARWNVVGIIQG SLLSIFVEPI YFSSIILGSL YHGDHLSTRAM 600
YQRISNIEDL PPLYTLNKPL LSGISNAEAR QPGKAPNFSV NWTVGDSAIE VINATTGKDE 660
LGRASRLCKH ALYCRWMRVH GKVPSHLLRS KITKPNVYHE SKLAAKEYQA AKARLFTAFI 720
KAGLGAWVEK PTEQDQFSLT P 741

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**1-10.** (canceled)

**11.** An antigen combination for diagnosis of early Alzheimer's disease, wherein the antigen combination comprises (a) SERF2, LGALS1, KDM4D, and ADARB1, or (b) DOC2A, LGALS1, KDM4D, and ADARB1, wherein SERF2 comprises an amino acid sequence set forth in SEQ ID NO: 6, LGALS1 comprises an amino acid sequence set forth in SEQ ID NO: 2, KDM4D comprises an amino acid sequence set forth in SEQ ID NO: 7, ADARB1 comprises an amino acid sequence set forth in SEQ ID NO: 9, DOC2A comprises an amino acid sequence set forth in SEQ ID NO: 1, and wherein symptoms of the early Alzheimer's disease comprise subjective cognitive decline and mild cognitive impairment.

**12.** The antigen combination according to claim 11, wherein the antigen combination comprises SERF2, LGALS1, KDM4D, ADARB1, and DOC2A.

**13.** The antigen combination according to claim 12, wherein the antigen combination further comprises HIST1H2BD, ICAM1, RIOK2, and DNAJC8, wherein HIST1H2BD comprises an amino acid sequence set forth in SEQ ID NO: 3, ICAM1 comprises an amino acid sequence set forth in SEQ ID NO: 4, RIOK2 comprises an amino acid sequence set forth in SEQ ID NO: 5, and DNAJC8 comprises an amino acid sequence set forth in SEQ ID NO: 8.

**14.** Use of the antigen combination according to claim 11 in preparation of a product for diagnosing/detecting early Alzheimer's disease.

**15.** A reagent or kit for diagnosing/detecting early Alzheimer's disease, comprising the antigen combination according to claim 11.

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