In one embodiment, there is provided a method for detecting Alzheimer's disease or mild cognitive impairment comprising measuring biomarkers (a), (b), and (c) in a biological sample simultaneously or separately.

In one aspect, there is provided a method for detecting Alzheimer's disease comprising measuring a biomarker set consisting of the following biomarkers (b) and (c) for detecting cognitive dysfunction diseases in a biological sample from a subject, and analyzing significant differences between the measured values of the biomarkers (b) and (c) and those in a biological sample from a non-cognitive impairment subject:

(b) an intact protein of transthyretin comprising the amino acid sequence represented by SEQ ID NO.:2, a protein consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence of the intact protein, a polypeptide consisting of the amino acid sequence represented by SEQ ID NO.: 2, or a polypeptide consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence represented by SEQ ID NO.: 2, and

(c) an intact protein of Complement C3 comprising the amino acid sequence represented by SEQ ID NO.: 3, a protein consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence of the intact protein, a polypeptide consisting of the amino acid sequence represented by SEQ ID NO.: 3, or a polypeptide consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence represented by SEQ ID NO.: 3.

In another aspect, there is provided a method for detecting mild cognitive impairment comprising measuring a biomarker set consisting of the following biomarkers (a) and (c) for detecting cognitive dysfunction diseases in a biological sample from a subject, and analyzing significant differences between the measured values of the biomarkers (a) and (c) and those in a biological sample from a non-cognitive impairment subject:

(a) an intact protein of Apolipoprotein A1 comprising the amino acid sequence represented by SEQ ID NO.:1, a protein consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence of the intact protein, a polypeptide consisting of the amino acid sequence represented by SEQ ID NO.: 1, or a polypeptide consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence represented by SEQ ID NO.: 1, and

(c) an intact protein of Complement C3 comprising the amino acid sequence represented by SEQ ID NO.: 3, a protein consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence of the intact protein, a polypeptide consisting of the amino acid sequence represented by SEQ ID NO.: 3, or a polypeptide consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence represented by SEQ ID NO.: 3.

In another aspect, there is provided a method for detecting cognitive dysfunction diseases comprising; measuring a biomarker set consisting of the following biomarkers (a), (b) and (c) for detecting cognitive dysfunction diseases in a biological sample from a subject, and analyzing significant differences between the measured values of the biomarkers (a), (b) and (c) and those of the biomarkers (a), (b) and (c) in a biological sample from non-cognitive dysfunction disease control subjects:

(a) an intact protein of Apolipoprotein A1 comprising the amino acid sequence represented by SEQ ID NO.:1, a protein consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence of the intact protein, a polypeptide consisting of the amino acid sequence represented by SEQ ID NO.: 1, or a polypeptide consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence represented by SEQ ID NO.: 1, and

(b) an intact protein of transthyretin comprising the amino acid sequence represented by SEQ ID NO.:2, a protein consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence of the intact protein, a polypeptide consisting of the amino acid sequence represented by SEQ ID NO.: 2, or a polypeptide consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence represented by SEQ ID NO.: 2, and

(c) an intact protein of Complement C3 comprising the amino acid sequence represented by SEQ ID NO.: 3, a protein consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence in the intact protein, a polypeptide consisting of the amino acid sequence presented by SEQ ID NO.: 3 or a polypeptide consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence represented by SEQ ID NO.: 3.

In another embodiment, there is provided an Alzheimer's disease or mild cognitive impairment detection kit for measuring biomarkers (a), (b), and (c).

In a further aspect, there is provided an Alzheimer's disease detection kit comprising antibodies or aptamers to the following biomarkers (b) and (c); (b) an intact protein of transthyretin comprising the amino acid sequence represented by SEQ ID NO.:2, a protein consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence in the intact protein, a polypeptide consisting of the amino acid sequence represented by SEQ ID NO.: 2, or a polypeptide consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence represented by SEQ ID NO.: 2, and

(c) an intact protein of Complement C3 comprising the amino acid sequence represented by SEQ ID NO.: 3, a protein consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence in the intact protein, a polypeptide consisting of the amino acid sequence represented by SEQ ID NO.: 3, or a polypeptide consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence represented by SEQ ID NO.: 3..

In yet another aspect, there is provided a mild cognitive impairment detection kit comprising antibodies or aptamers to the following biomarkers (a) and (c) for detecting cognitive dysfunction diseases:

(a) an intact protein of Apolipoprotein A1 comprising the amino acid sequence represented by SEQ ID NO.:1, a protein consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence of the intact protein, a polypeptide consisting of the amino acid sequence represented by SEQ ID NO.: 1 or, a polypeptide consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence represented by SEQ ID NO.: 1, and

(c) an intact protein of Complement C3 comprising the amino acid sequence represented by SEQ ID NO.: 3, a protein consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence in the intact protein, a polypeptide consisting of the amino acid sequence represented by SEQ ID NO.: 3, or a polypeptide consisting of the amino acid sequence resulting from deletion, substitution, or addition of

one to three amino acids in the amino acid sequence represented by SEQ ID NO.: 3.

In another aspect, there is provided a cognitive dysfunction diseases detection kit comprising antibodies or aptamers to the following biomarkers (a), (b) and (c);

(a) an intact protein of Apolipoprotein A1 comprising the amino acid sequence represented by SEQ ID NO.:1, a protein consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence in the intact protein, a polypeptide consisting of the amino acid sequence represented by SEQ ID NO.: 1, or a polypeptide consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence represented by SEQ ID NO.: 1,

(b) an intact protein of transthyretin comprising the amino acid sequence represented by SEQ ID NO.:2, a protein consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence in the intact protein, a polypeptide consisting of the amino acid sequence represented by SEQ ID NO.: 2, or a polypeptide consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence represented by SEQ ID NO.: 2, and

(c) an intact protein of Complement C3 comprising the amino acid sequence represented by SEQ ID NO.: 3, a protein consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence in the intact protein, a polypeptide consisting of the

amino acid sequence represented by SEQ ID NO.: 3, or a polypeptide consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence represented by SEQ ID NO.: 3.

In another aspect, there is provided a method for improving accuracy of detection of cognitive dysfunction diseases by the followings steps (A) to (E);

Step (A): measuring a biomarker set consisting of the following biomarkers (a), (b) and (c) for detecting cognitive dysfunction diseases in biological samples:

(a) an intact protein of Apolipoprotein A1 comprising the amino acid sequence represented by SEQ ID NO.:1, a protein consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence in the intact protein, a polypeptide consisting of the amino acid sequence represented by SEQ ID NO.: 1, or a polypeptide consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence represented by SEQ ID NO.: 1,

(b) an intact protein of transthyretin comprising the amino acid sequence represented by SEQ ID NO.:2, a protein consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence in the intact protein, a polypeptide consisting of the amino acid sequence represented by SEQ ID NO.: 2, or a polypeptide consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence represented by SEQ ID NO.: 2, and

(c) an intact protein of Complement C3 comprising the amino acid sequence represented by SEQ ID NO.: 3, a protein consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence in the intact protein, a polypeptide consisting of the amino acid sequence represented by SEQ ID NO.: 3, or a polypeptide consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence represented by SEQ ID NO.: 3,

Step (B): analyzing the measured value of the biomarkers (a), (b) and (c) by logistic regression analysis;

Step (C): selecting a combination of the biomarkers and a regression equation for detecting cognitive dysfunction diseases which gives a high percentage of correct answers based on the results of the analysis of the biological samples;

Step (D): measuring the biomarker (a), (b) and (c) in a biological sample from a subject, and

Step (E): distinguishing the subject to mild cognitive impairment, Alzheimer's disease or non-cognitive impairment healthy person based on a result obtained by inserting the measured value of the subject obtained by Step (D) to the regression equation of the combination of the biomarkers .

Advantageous Effects of Invention

CLAIMS:

1. A method for detecting mild cognitive impairment comprising;

measuring a biomarker set consisting of the following biomarkers (a) and (c) for detecting cognitive dysfunction diseases in a biological sample from a subject, and

analyzing significant differences between the measured values of the biomarkers (a) and (c) and those in a biological sample from a non-cognitive impairment subject:

(a) an intact protein of Apolipoprotein A1 comprising the amino acid sequence represented by SEQ ID NO.:1,

a protein consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence of the intact protein,

a polypeptide consisting of the amino acid sequence represented by SEQ ID NO.: 1, or

a polypeptide consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence represented by SEQ ID NO.: 1, and

(c) an intact protein of Complement C3 comprising the amino acid sequence represented by SEQ ID NO.: 3,

a protein consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence of the intact protein,

a polypeptide consisting of the amino acid sequence represented by SEQ ID NO.: 3, or

a polypeptide consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence represented by SEQ ID NO.: 3.

2. A method for detecting Alzheimer's disease comprising;

measuring a biomarker set consisting of the following biomarkers (b) and (c) for detecting cognitive dysfunction diseases in a biological sample from a subject, and

analyzing significant differences between the measured values of the biomarkers (b) and (c) and those in a biological sample from a non-cognitive impairment subject:

(b) an intact protein of transthyretin comprising the amino acid sequence represented by SEQ ID NO.:2,

a protein consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence of the intact protein,

a polypeptide consisting of the amino acid sequence represented by SEQ ID NO.: 2, or

a polypeptide consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence represented by SEQ ID NO.: 2, and

(c) an intact protein of Complement C3 comprising the amino acid sequence represented by SEQ ID NO.: 3,

a protein consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence of the intact protein,

a polypeptide consisting of the amino acid sequence represented by SEQ ID NO.: 3, or

a polypeptide consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence represented by SEQ ID NO.: 3.

3. A method for detecting cognitive dysfunction diseases comprising;

measuring a biomarker set consisting of the following biomarkers (a), (b) and (c) for detecting cognitive dysfunction diseases in a biological sample from a subject, and

analyzing significant differences between the measured values of the biomarkers (a), (b) and (c) and those of the biomarkers (a), (b) and (c) in a biological sample from non-cognitive dysfunction disease control subjects:

(a) an intact protein of Apolipoprotein A1 comprising the amino acid sequence represented by SEQ ID NO.:1,

a protein consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence of the intact protein,

a polypeptide consisting of the amino acid sequence represented by SEQ ID NO.: 1, or

a polypeptide consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence represented by SEQ ID NO.: 1, and

(b) an intact protein of transthyretin comprising the amino acid sequence represented by SEQ ID NO.:2,

a protein consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence of the intact protein,

a polypeptide consisting of the amino acid sequence represented by SEQ ID NO.: 2, or

a polypeptide consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence represented by SEQ ID NO.: 2, and

(c) an intact protein of Complement C3 comprising the amino acid sequence represented by SEQ ID NO.: 3,

a protein consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence in the intact protein,

a polypeptide consisting of the amino acid sequence presented by SEQ ID NO.: 3 or

a polypeptide consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence represented by SEQ ID NO.: 3.

4. A mild cognitive impairment detection kit comprising antibodies or aptamers to the following biomarkers (a) and (c) for detecting cognitive dysfunction diseases;

(a) an intact protein of Apolipoprotein A1 comprising the amino acid sequence represented by SEQ ID NO.:1,

a protein consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence of the intact protein,

a polypeptide consisting of the amino acid sequence represented by SEQ ID NO.: 1 or,

a polypeptide consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence represented by SEQ ID NO.: 1, and

(c) an intact protein of Complement C3 comprising the amino acid sequence represented by SEQ ID NO.: 3,

a protein consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence in the intact protein,

a polypeptide consisting of the amino acid sequence represented by SEQ ID NO.: 3, or

a polypeptide consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence represented by SEQ ID NO.: 3.

5. An Alzheimer's disease detection kit comprising antibodies or aptamers to the following biomarkers (b) and (c);

(b) an intact protein of transthyretin comprising the amino acid sequence represented by SEQ ID NO.:2,

a protein consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence in the intact protein,

a polypeptide consisting of the amino acid sequence represented by SEQ ID NO.: 2, or

a polypeptide consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence represented by SEQ ID NO.: 2, and

(c) an intact protein of Complement C3comprising the amino acid sequence represented by SEQ ID NO.: 3,

a protein consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence in the intact protein,

a polypeptide consisting of the amino acid sequence represented by SEQ ID NO.: 3, or

a polypeptide consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence represented by SEQ ID NO.: 3.

6. A cognitive dysfunction diseases detection kit comprising antibodies or aptamers to the following biomarkers (a), (b) and (c);

(a) an intact protein of Apolipoprotein A1 comprising the amino acid sequence represented by SEQ ID NO.:1,

a protein consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence in the intact protein,

a polypeptide consisting of the amino acid sequence represented by SEQ ID NO.: 1, or

a polypeptide consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence represented by SEQ ID NO.: 1,

(b) an intact protein of transthyretin comprising the amino acid sequence represented by SEQ ID NO.:2,

a protein consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence in the intact protein,

a polypeptide consisting of the amino acid sequence represented by SEQ ID NO.: 2, or

a polypeptide consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence represented by SEQ ID NO.: 2, and

(c) an intact protein of Complement C3comprising the amino acid sequence represented by SEQ ID NO.: 3,

a protein consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence in the intact protein,

a polypeptide consisting of the amino acid sequence represented by SEQ ID NO.: 3, or

a polypeptide consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence represented by SEQ ID NO.: 3.

7. A method for improving accuracy of detection of cognitive dysfunction diseases by the followings steps (A) to (E);

Step (A): measuring a biomarker set consisting of the following biomarkers (a), (b) and (c) for detecting cognitive dysfunction diseases in biological samples:

(a) an intact protein of Apolipoprotein A1 comprising the amino acid sequence represented by SEQ ID NO.:1,

a protein consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence in the intact protein,

a polypeptide consisting of the amino acid sequence represented by SEQ ID NO.: 1, or

a polypeptide consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence represented by SEQ ID NO.: 1,

(b) an intact protein of transthyretin comprising the amino acid sequence represented by SEQ ID NO.:2,

a protein consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence in the intact protein,

a polypeptide consisting of the amino acid sequence represented by SEQ ID NO.: 2, or

a polypeptide consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence represented by SEQ ID NO.: 2, and

(c) an intact protein of Complement C3comprising the amino acid sequence represented by SEQ ID NO.: 3,

a protein consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence in the intact protein,

a polypeptide consisting of the amino acid sequence represented by SEQ ID NO.: 3, or

a polypeptide consisting of the amino acid sequence resulting from deletion, substitution, or addition of one to three amino acids in the amino acid sequence represented by SEQ ID NO.: 3,

Step (B): analyzing the measured value of the biomarkers (a), (b) and (c) by logistic regression analysis;

Step (C): selecting a combination of the biomarkers and a regression equation for detecting cognitive dysfunction diseases which gives a high percentage of correct answers based on the results of the analysis of the biological samples;

Step (D): measuring the biomarker (a), (b) and (c) in a biological sample from a subject, and

Step (E): distinguishing the subject to mild cognitive impairment, Alzheimer's disease or non-cognitive impairment healthy person based on a result obtained by inserting the measured value of the subject obtained by Step (D) to the regression equation of the combination of the biomarkers .