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(54) Title: ANTEMORTEM DIAGNOSTIC TEST FOR ALZHEIMER'S DISEASE

(57) Abstract

An antemortem diagnostic test for neurodegenerative disease, particularly Alzheimer's disease, is disclosed. The method of the invention involves the collection of a sample of bodily fluid; the analysis of the concentration of neuronal membrane phospholipids and phospholipid metabolites, or the ratio of a phospholipid to its metabolite, in the sample; and the correlation of the concentration or the ratio to the corresponding value from control samples. The method can be used to determine the presence or the possibility that an individual can develop a neurodegenerative disease, such as Alzheimer's disease; to follow the course of neurodegenerative disease in an individual; and to validate the efficacy of a drug used to treat a neurodegenerative disease.

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ANTEMORTEM DIAGNOSTIC TEST FOR ALZHEIMER'S DISEASE

Description

Background

Alzheimer's disease (AD) is a chronic progressive neurodegenerative disease. Clinically, it is characterized by progressive deficits in memory and other cognitive functions that occur in the face of an otherwise normal neurological examination. Postmortem examination reveals a variety of typical AD brain lesions, including deposition of amyloid plaques, formation of neurofibrillary tangles, and neuronal degeneration. The etiology and pathophysiology of neuronal death are unknown.

Despite the fact that Alzheimer's disease is the most common neurological disease in the United States, there are no reliable antemortem biological tests for its diagnosis. Currently diagnosis rests on clinical grounds, and thus accuracy depends on the degree of physician expertise. An objective method for antemortem diagnosis of AD would be extremely useful: it would distinguish AD from other forms of dementia which may be curable; it would enable the patient to enter a treatment program early in the development of the disease, thus enhancing the opportunity for beneficial therapy; and it would allow patients in the early stages of the disease to plan for future nursing care, as well as to address other issues concerning the future needs of the patient's family.

Summary of Invention

This invention is based in the discovery that the presence of abnormalities in phospholipid metabolism in the brain can be identified by measuring levels of

neuronal phospholipids and their metabolites in other tissues or in bodily fluids, such as cerebrospinal fluid (CSF), blood, or urine; and in the discovery that there is a relationship between these levels and neurodegenerative disease, particularly AD. The phospholipids and their metabolites are separated by known methods, such as HPLC and fractionation. When compared with standardized controls, altered levels of two of the most common phospholipids -- phosphatidylcholine and phosphatidylethanolamine -- and of their respective metabolites can be found. Certain changes, such as an elevated level of the phosphatidylcholine metabolite glycerophosphocholine (GPC), or an increased ratio of phosphatidylcholine to GPC, or of phosphatidylethanolamine to glycerophosphoethanolamine (GPE), indicate the presence of AD.

The present invention includes a method of diagnosing AD, and of assessing or detecting the presence of abnormalities which may exist before the clinical manifestations of the disease become evident and, thus, can be predictive of AD. The potential for effective treatment is greatest when commenced early in the progression of the disease, and, therefore, the present method is particularly useful because earlier diagnosis is now possible. Furthermore, the invention makes available a biological marker useful as an indicator of the effectiveness of treatments in altering or correcting abnormal neuronal metabolism.

Brief Description of the Figure

The Figure is a graphic representation CSF levels of GPC in several neurodegenerative diseases. Key: PD - Parkinson's Disease; AD - Alzheimer's Disease; PSP - Progressive Supranuclear Palsy; PICK'S - Pick's Disease.

Detailed Description of the Invention

The present invention relates to a method of measuring the levels, in a bodily fluid, or in a sample of a tissue other than brain, of certain phospholipids and their metabolites whose presence within a range is known to be indicative of the occurrence of a neurodegenerative disease such as Alzheimer's Disease. It has been demonstrated that cell membrane lipid abnormalities contribute to the amyloid deposition and neuronal dysfunction of AD (Dyrks et al., EMBO J. 7, 949 (1988); Nitsch et al., Neurology 41 (Suppl. 1), 269 (abstr.) (1991); Pettegrew et al., Arch. Neurol. 45, 1093 (1988)). The current invention pertains to the discovery that it is possible to detect and quantify amounts of certain neuronal phospholipids and their metabolites in the bodily fluid of a human, such as cerebrospinal fluid (CSF), blood, and urine, and to correlate the levels to indicators of neurodegenerative disease, such as AD. Levels of phospholipids and phospholipid metabolites in samples of fluid from humans who do not currently demonstrate clinical manifestations of neurodegenerative disease are compared to the levels in samples from humans displaying neurodegenerative disease characteristics.

Differences in amounts of certain phospholipids or their metabolites lipids and their metabolites are then linked to particular diseases, such as AD.

As described in the Exemplification, Applicants have shown that a high level of the phosphatidylcholine metabolite GPC corresponds to the presence of AD, and not to the presence of other neurodegenerative diseases. As described, levels of GPC were measured in samples of CSF from individuals (humans) with Parkinson's Disease, Progressive Supranuclear Palsy, Pick's Disease, Alzheimer's Disease (AD), and other miscellaneous (nonspecified) diseases. The results, as shown in the Figure, indicated that the levels of GPC in the AD samples were higher than the levels in the other samples.

Samples obtained from three groups of individuals -- individuals diagnosed with AD by presently available diagnostic methods (the AD-positive group); individuals diagnosed with a neurodegenerative disease other than AD (the neurodegenerative disease-positive, AD-negative group); and individuals who are not currently clinically presenting symptoms of any neurodegenerative disease (the neurodegenerative disease-negative group) -- provide data concerning the ranges of concentrations and the ratios of phospholipids to phospholipid metabolites which are typically present in such individuals. Corresponding samples of bodily fluid, such as CSF, blood, and/or urine, are taken from individuals from each of the three groups. Samples from each control group are analyzed using known methods to determine the levels of neuronal phospholipids,

such as phosphatidylcholine, phosphatidylethanolamine, and phosphatidylserine, and/or their metabolites, such as choline, GPC, ethanolamine and GPE; ratios of phospholipids and metabolites can also be calculated. The results from these groups provide control levels and control ratios to which other samples can be compared. After controls are established, samples can be taken from individuals with unknown disease status. The levels of phospholipids and their metabolites are quantified and compared to the controls. A significant variation from the controls indicates the presence of an abnormality in phospholipid metabolism indicative of neurodegenerative disease such as AD.

Although the method described results in quantification of the concentrations of phospholipids such as phosphatidylcholine and phosphatidylethanolamine, and phospholipid metabolites such as GPC and GPE, any other neuronal component for which a relationship can be shown can serve as a basis for the current method. Other bases for the current invention can be identified using the method described. The current method can also be used to identify the presence of other diseases, in addition to AD, in which a relationship between phospholipids or phospholipid metabolites and the occurrence of disease can be shown.

In addition, the current method can be used to follow the course of a neurodegenerative disease in an individual. Levels or ratios of phospholipids and phospholipid metabolites are taken from the individual in

a series of correlating samples over a period of time, in order to monitor changes in the values. The values from these samples are compared to the control samples described above, to relate the values to those of other individuals with the disease, and thereby determine the severity of disease.

The method can also be used to assess the efficacy of a drug for the treatment of neurodegenerative disease. Levels or ratios of phospholipids and phospholipid metabolites in samples taken from an individual before the administration of the drug are compared to levels or ratios in a corresponding samples taken from the individual after administration of the drug. These values are further compared to those of the control samples described above. Efficacy of the drug is demonstrated if the levels or ratios after drug administration are closer to neurodegenerative-disease negative values than the levels or ratios before drug administration.

The invention will now be illustrated by the following Exemplification.

Exemplification: Comparison of Levels of Glycerophosphocholine in Cerebrospinal Fluid of Patients with Neurodegenerative Disease

Protocol for Collection of Cerebrospinal Fluid Sample

Before samples are obtained from an individual, a medical history is taken and a physical examination performed. The individual may take any usual medication,

except for antidepressants, sedatives, hypnotics, or other agents which may affect brain function. The individual must lie flat from midnight until the time the sample is collected in the morning, and refrain from smoking, from taking a morning dosage of any usual medication, from eating, and from drinking any substance other than water during the same time period. A sample of cerebrospinal fluid is then collected using known methods.

Comparison of Samples

Cerebrospinal fluid samples were obtained from the Alzheimer's Disease Research Center at Massachusetts General Hospital. The specimens had been collected previously and had been stored at -70°C until biochemical analysis. Initial experiments showed that GPC is stable over long periods of time (data not shown); thus, samples collected over a time period of years can easily be used for comparisons.

GPC levels in the CSF of five patients with clinically diagnosed probable Alzheimer's disease were compared to those of ten patients with Parkinson's disease; one Pick's disease patient, one patient with progressive supranuclear palsy, and two patients with miscellaneous diseases which were not further specified. among those 19 samples, which were measured in a blind study, the five values which ranked highest were from the five AD patients. There was no overlap of the GPC values in the CSF of the five AD patients and the values from those patients with other diagnoses.

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described specifically herein. Such equivalents are intended to be encompassed in the scope of the following claims.

CLAIMS

1. A method of diagnosing the presence of neurodegenerative disease in a human, e.g., Alzheimer's disease, comprising determining the concentration of a neuronal membrane phospholipid or phospholipid metabolite through analysis of a sample of bodily fluid obtained from the human, and correlating the concentration to control concentration values, wherein the deviation of the sample concentration from the control concentration values is indicative of the presence of neurodegenerative disease.

2. A method of diagnosing the presence of neurodegenerative disease in a human, e.g. Alzheimer's disease, comprising determining the ratio of a neuronal membrane phospholipid to its metabolite through analysis of a sample of bodily fluid obtained from the human, and correlating the ratio to control ratio values, wherein the deviation of the sample ratio from the control ratio values is indicative of the presence of neurodegenerative disease.

3. A method of diagnosing the presence of neurodegenerative disease in a human, comprising the steps of:

a. collecting a sample of bodily fluid from the human;

b. determining the concentration of a phospholipid, a phospholipid metabolite, or the ratio of a phospholipid to its metabolite in the sample, thereby producing a test value; and

c. comparing the test value with a control value wherein the control comprises the concentration of the phospholipid or phospholipid metabolite, or the ratio of the phospholipid to the phospholipid metabolite, in a sample of the corresponding bodily fluid from an individual previously diagnosed with Alzheimer's disease, by presently available diagnostic methods,

wherein deviation of the test value from the control value is indicative of the presence of neurodegenerative disease.

4. A method of predicting the likelihood that a human will develop a neurodegenerative disease, e.g. Alzheimer's disease, comprising the steps of:

a. collecting a sample of bodily fluid from the human;

b. determining the concentration of a phospholipid, a phospholipid metabolite, or the ratio of a phospholipid to its metabolite in the sample, thereby producing a test value; and

c. comparing the test value with a control value wherein the control comprises the concentration of the phospholipid or phospholipid metabolite, or the ratio of the phospholipid to the

phospholipid metabolite, in a sample of the corresponding bodily fluid from a human previously diagnosed with Alzheimer's disease by presently available diagnostic methods, wherein the deviation of the test value from the control value is indicative of the likelihood of development of neurodegenerative disease.

5. The method of Claim 3 or Claim 4, wherein the control value further comprises the concentration of the phospholipid or phospholipid metabolite, or the ratio of the phospholipid to the phospholipid metabolite, in a sample of the corresponding bodily fluid from a human previously diagnosed with a neurodegenerative disease other than Alzheimer's disease.

6. The method of Claim 5, wherein the control further comprises the concentration of the phospholipid or phospholipid metabolite, or the ratio of the phospholipid to the phospholipid metabolite, in a sample of the corresponding bodily fluid from a human who is not clinically presenting symptoms of any neurodegenerative disease.

7. The method of Claim 3 or Claim 4, wherein the bodily fluid is cerebrospinal fluid, blood, or urine.

8. A method of following the course of neurodegenerative disease in a human, e.g. Alzheimer's disease,

comprising comparing the concentration of a neuronal membrane phospholipid or phospholipid metabolite in a series of correlating samples of bodily fluids taken from the individual at regular intervals after diagnosis of the neurodegenerative disease, and comparing the concentration to control concentration values, wherein an increase in the deviation of the concentration in one sample and in the sample from the subsequent interval from the control concentration values is indicative of progression of the neurodegenerative disease.

9. A method of following the course of neurodegenerative disease in a human, e.g. Alzheimer's disease, comprising comparing the ratio of a neuronal membrane phospholipid to its metabolite in a series of correlating samples of bodily fluids taken from the human periodically after diagnosis of the neurodegenerative disease, and comparing the ratio to control ratio values, wherein an increase in the deviation of the sample ratios from the control ratio values is indicative of progression of the neurodegenerative disease.

10. A method of assessing the efficacy of a drug for the treatment of a neurodegenerative disease, such as Alzheimer's disease, comprising determining the concentration of a neuronal membrane phospholipid or phospholipid metabolite in a sample of bodily fluid

from a human who has the neurodegenerative disease and has not yet been subjected to the drug, and correlating the concentration phospholipid or phospholipid metabolite to the concentration in corresponding samples taken after the administration of the drug and to control concentration values.

11. A method of assessing the efficacy of a drug for the treatment of neurodegenerative disease, such as Alzheimer's disease, comprising determining the ratio of a neuronal membrane phospholipid to its metabolite in a sample of bodily fluid from a human who has the neurodegenerative disease and has not yet been subjected to the drug, and correlating the ratio to the ratio from corresponding samples taken after the administration of the drug to the human and to control ratio values.

12. The method of any of Claims 1 to 4 or 8 to 11, wherein the phospholipid is selected from the group consisting of phosphatidylcholine and phosphatidylethanolamine, and the phospholipid metabolite is selected from the group consisting of choline, glycerophosphocholine, ethanolamine, and glycerophosphoethanolamine.

13. A method of diagnosing the presence of Alzheimer's disease in a human, comprising determining the concentration of glycerophosphocholine through

analysis of a sample of bodily fluid obtained from the human, and correlating the concentration to control concentration values, wherein deviation of the sample concentration upwards from the control concentration values is indicative of the presence of Alzheimer's disease.

14. A method of diagnosing the presence of Alzheimer's disease in a human, comprising determining the ratio of phosphatidylcholine to glycerophosphocholine through analysis of a sample of bodily fluid obtained from the human, and correlating the ratio to control ratio values, wherein deviation of the sample ratio upwards from the control ratio values is indicative of the presence of Alzheimer's disease.

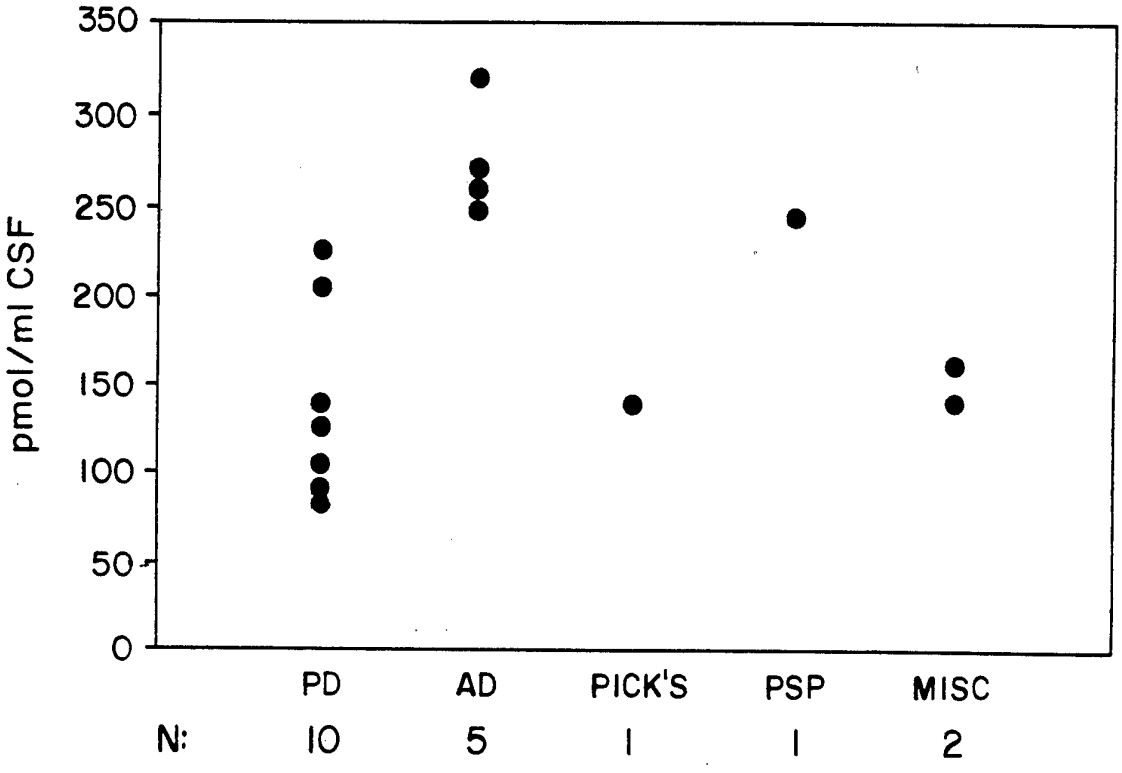
15. A method of diagnosing the presence of Alzheimer's disease in a human, comprising the steps of:

a. collecting a sample of bodily fluid from the human;

b. determining the concentration of glycerophosphocholine or the ratio of phosphatidylcholine to glycerophosphocholine in the sample, thereby producing a test value; and

c. comparing the test value with a control value wherein the control comprises the concentration of glycerophosphocholine or the ratio of phosphatidylcholine to glycerophosphocholine, in a sample of the corresponding bodily fluid from an

individual previously diagnosed with Alzheimer's disease by presently available diagnostic methods, wherein deviation of the test value upwards from the control value is indicative of the presence of Alzheimer's disease.


350
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pmol/ml CSF
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FIGURE

INTERNATIONAL SEARCH REPORT

Inter ional Application No
PCT/US 93/09465

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 G01N33/92

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA vol. 89, no. 3 , 1 March 1992 , WASHINGTON US pages 1671 - 1675 ROGER M. NITSCH ET AL. 'Evidence for a membrane defect in Alzheimer disease brain' see the whole document --- -/--	1-15

[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ARCHIVES OF NEUROLOGY vol. 45, no. 10 , October 1988 , CHICAGO, IL, US pages 1093 - 1096 JAY W. PETTEGREW ET AL. 'Correlation of Phosphorus-31 Magnetic Resonance Spectroscopy and Morphological Findings in Alzheimer's Disease' cited in the application see the whole document	1-15
A	CHEMICAL ABSTRACTS, vol. 116, no. 19, 11 May 1992, Columbus, Ohio, US; abstract no. 192004, KWEE, INGRID L. ET AL. 'Elevation in relative levels of membrane unsaturated fatty acids in Alzheimer's disease: high resolution proton spectroscopic studies of membrane lipid extracts' page 510 ; see abstract & MAGN. RESON. MED. vol. 21, no. 1 , 1991 pages 49 - 54	
A	EMBO JOURNAL vol. 7, no. 4 , April 1988 , EYNSHAM, OXFORD GB pages 949 - 957 THOMAS DYRKS ET AL. 'Identification, transmembrane orientiation and biogenesis of the amyloid A4 precursor of Alzheimer's disease' cited in the application	