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(71) Applicant *(for all designated States except US)*: OXFORD VIROLOGY PLC [GB/GB]; 10 Storey's Gate, Westminster, London SW1P 3AY (GB).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)* : HAJIMOHAMMADREZA, Iradj [IR/GB]; Department of Neuroscience, Institute of Psychiatry, London SE5 8F (GB). BRAMMER, Michael [GB/GB]; 29 Rodenhurst Road, London SW4 8AE (GB).

(74) Agent: BANNERMAN, David, Gardner; Withers & Rogers, 4 Dyer's Buildings, Holborn, London EC1N 2JT (GB).

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(54) Title: A DIAGNOSTIC TEST METHOD

(57) Abstract

The invention provides a method for diagnosing Alzheimer's Disease comprising detecting the amount of one or more polypeptides in a solubilised blood platelet polypeptide sample taken from a suspected Alzheimer's Disease sufferer and, if present, comparing the amount thereof with the amount of the polypeptide or polypeptides present in a corresponding sample taken from disease-free individuals. Significantly lowered amounts of said polypeptide or polypeptides in the sample, when compared to the amount in samples from disease-free individuals, being indicative of Alzheimer's Disease.

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A DIAGNOSTIC TEST METHOD

This invention relates to a method of diagnosis for Alzheimer's Disease.

Alzheimer's Disease is a degenerative brain disorder producing a range of symptoms including dementia. Elevated aluminium levels, virus-like agents and predisposing genetic factors have all been put forward as casual agents. However, the absence of a specific diagnostic test for Alzheimer's Disease has greatly hampered further research and the development of therapeutic agents for the treatment of afflicted individuals.

This invention exploits the finding that certain polypeptides normally found in blood platelets are present in significantly lowered amounts or are absent from the platelets of Alzheimer's Disease sufferers.

According to one aspect, the invention provides a method of diagnosing Alzheimer's Disease comprising detecting the presence of one or more polypeptides in a solubilised blood platelet polypeptide sample taken from a suspected Alzheimer's Disease sufferer, and if such a polypeptide or polypeptides are present, comparing the amount thereof with the amount present in the platelets of disease-free individuals.

In a further aspect, the invention provides a method of diagnosing Alzheimer's Disease comprising separating the components of a solubilised blood platelet polypeptide sample taken from a suspected Alzheimer's Disease sufferer, detecting the presence of the separated polypeptides and comparing the amount thereof with the amount of the corresponding polypeptide observed in a corresponding sample taken from a disease-free individual.

As will be apparent to the skilled worker, many separation techniques can be employed to separate particular polypeptides on the basis of such characteristics as size, solubility, charge and specific binding affinity,n using methods such as dialysis through a semi-permeable membrane; gel-filtration chromatography; ion-exchange chromatography and affinity chromatography (see Stryer L. Biochemistry 2nd Edition, [1981] Pg. 18 to 20, W.H. Freeman and Co.).

The separation of the components may, for example, be carried out by chromatography or by gel electrophoresis such as polyacrylamide gel electrophoresis (PAGE). Preferably two dimensional PAGE is used to separate the polypeptide components in the samples.

Detection techniques such as immunoassay and colourimetry may be employed in the above methods to determine the presence and/or amount of particular polypeptides in a test sample.

In another aspect the invention provides a diagnostic test method for Alzheimer's disease comprising

detecting or determining the level of one or more particular polypeptides in a solubilised blood platelet polypeptide sample taken from a test subject, wherein the or each particular polypeptide is one which, if the components of the said sample are subjected to two-dimensional polyacrylamide gel electrophoresis are present in the 110 kDa Mwt region at an isoelectric point of 5.4 and/or the 50 kDa Mwt region at an isoelectric point of 5.5 and/or the 40 kDa Mwt region at an isoelectric point in the range of from 5.6 to 6.2.

The invention further provides a method of diagnosing Alzheimer's Disease comprising separating the components of a solubilised blood platelet polypeptide sample taken from a suspected Alzheimer's Disease sufferer by two dimensional PAGE, detecting the presence of the separated polypeptides by staining the gel, and comparing the degree of band staining, in one or more regions selected from the 100 Mwt region at an isoelectric point of approximately 5.4, the 50 kDa Mwt region at an isoelectric point of approximately 5.5, and the 40 kDa Mwt region at an isoelectric point in the range of from 5.6 to 6.2, with the degree of staining in the corresponding region or regions observed in a sample taken from a disease-free individual.

Advantageously, the gels are run in pairs, that is one sample from a suspected sufferer with one from a disease-free individual to account for any variability in running conditions.

Advantageously, the samples taken from suspected sufferers and disease-free individuals are matched as closely as possible for age and sex so that samples are more strictly comparable.

The presence of the separated polypeptide bands on the gel may be detected using a polypeptide stain such as "Coomassie blue", or by using immunoassay techniques.

Preferably, the or each particular polypeptide is extracted from the sample or gel using conventional techniques and is used to immunise a mammal such as a rabbit in order to raise antisera specific for said polypeptide. Such antisera can be used as an immunoassay reagent in further methods of the invention.

In a further aspect the invention provides a method for separating from a solubilised blood platelet sample a polypeptide which, if the components of the sample are subjected to two-dimensional polyacrylamide gel electrophoresis, is present in the 110 kDa Mwt

region at an isoelectric point of 5.4 and/or the 50 kDa Mwt region at an isoelectric point of 5.5 and/or the 40 kDa Mwt region at an isoelectric point in the range of from 5.6 to 6.2 which method comprises:

(i) applying said sample to an affinity chromatography column including antibodies specific for said polypeptide, which antibodies are bound to a solid support;

(ii) washing the support to remove unbound components of the sample to which the antibodies do not bind specifically;

(iii) eluting said polypeptide from the support so that it is obtained in substantially purified form.

Preferably the solid support to which said antibodies bind comprises plastic beads.

The invention also provides said polypeptide in an isolated and substantially purified form, which polypeptide is present in a reduced concentration in a solubilised blood platelet sample from a sufferer from Alzheimer's disease and which, if the components of such a sample are subjected to two-dimensional polyacrylamide gel electrophoresis are present in the 110 kDa Mwt region at an isoelectric point of 5.4 and/or the 50 kDa Mwt region at an isoelectric point

of 5.5 and/or the 40 kDa Mwt region at an isoelectric point in the range of from 5.6 to 6.2.

In a still further aspect the invention provides an immunoassay method for determining the amount of a particular polypeptide as described previously which method comprises

(i) coating a solid support surface with antibodies specific for the particular polypeptide;

(ii) washing the support to remove unbound antibodies;

(iii) applying a solubilised blood platelet polypeptide sample from a test sample;

(iv) washing the support to remove unbound components of the sample;

(v) applying labelled antibodies specific for the particular polypeptide;

(vi) washing the support to remove any unbound labelled antibodies;

(vii) determining the amount of labelled antibody present, which amount is proportional to the amount of the particular polypeptide present in the sample.

Preferably the labelled antibodies are labelled with enzymes (such as peroxidase and phosphatase) which

leave a coloured deposit on reaction with substrate. The coloured deposit being measurable using colourmetric techniques.

In another aspect the invention provides an immunoassay kit for diagnosing Alzheimer's Disease in a solubilised blood platelet polypeptide sample from a test subject comprising

(i) an antibody preparation containing antibodies specific for a particular polypeptide as defined previously;

(ii) An antibody preparation containing labelled antibodies specific for the said polypeptide for determining the amount of said polypeptide in the sample from the test subject; and

(iii) a comparative blood platelet polypeptide sample from an Alzheimer's disease-free individual to allow comparison of the amount of said polypeptide therein with the amount present in the sample from the test subject.

Preferably the above kit further comprises a multiwell plastic plate for using the kit according to the methods of the invention. Advantageously the sample from the test subject is assayed alongside the comparative sample from the disease-free individual so

that the amount of the particular polypeptide in the respective samples can be readily compared.

As will be apparent to those skilled in the art examples of the label of the labelled antibodies include an enzyme as mentioned previously, a fluorescent dye which can be seen using UV microscopy, or a radioactive substance which can be detected by deposition of silver grains from a photographic emulsion or by using counting equipment.

A preferred embodiment of the invention will now be described by way of example only, with reference to Fig. 1.

Fig. 1 shows a diagnostic test according to the invention.

A total of 13 2-D SDS PAGE were carried out to separate the components of solubilised platelet polypeptide samples.

Eight Alzheimer's Disease (ALZ) patients and 5 disease-free control (CON) individuals were tested. These were matched as closely as possible for age (63 * 4 years for ALZ group and 62 * 5 years for CON group) and sex (4 males/4 females for ALZ group and 3 males/2

females for CON group).

Gels were run in paris, that is, one ALZ with one CON, to account for the variability in running conditions.

Coomassie blue staining was used to detect the presence of the separated polypeptide bands.

In 5 out of 8 SDS-gels from the ALZ group, there was a consistent decrease in the degree of Coomassie blue staining of a spot around the 110 kDa Mwt region with an isoelectric point of approximately 5.4, as shown in Fig. 1, B=ALZ, marked 1. However, all 5 SDS-gels from the control group showed a mich stronger staining as shown in Fig. 1, A=CON, marked 1.

Further differential staining was observed in the spot around the 50 kDa Mwt region with an isoelectric point of approximately 5.5, marked 2 in Fig. 1 and the spot marked 3 around the 40 kDa Mwt region with an isoelectric point in the range 5.6 to 6.2.

Thus, the differential staining of certain blood platelet polypeptide bands between Alzheimer's Disease patients and controls provides a specific marker or markers for the diagnosis of the disease in suspected sufferers.

CLAIMS

1. A diagnostic test method for Alzheimer's Disease characterised in that at least one polypeptide of a solubilised blood platelet polypeptide sample taken from a suspected Alzheimer's Disease suffer is detected and, if present, the amount thereof is compared with the amount of the same polypeptide in a corresponding sample taken from a disease-free individual.

2. A diagnostic test method for Alzheimer's disease comprising detecting or determining the level of one or more particular polypeptides in a solubilised blood platelet polypeptide sample taken from a test subject, wherein the or each particular polypeptide is one which, if the components of the said sample are subjected to two-dimensional polyacrylamide gel electrophoresis are present in the 110 kDa Mwt region at an isoelectric point of 5.4 and/or the 50 kDa Mwt region at an isoelectric point of 5.5 and/or the 40 kDa Mwt region at an isoelectric point in the range of from 5.6 to 6.2.

3. A method as claimed in claim 1 wherein the components of the solubilised blood platelet polypeptide sample are separated prior to the

detection of the polypeptide.

4. A method as claimed in claim 3 wherein the separation of the components is achieved by two dimensional polyacrylamide gel electrophoresis.

5. A method as claimed in claim 2 wherein the presence of the separated polypeptides in the gel is detected by staining the gel and comparing the degree of band staining in one or more regions selected from the 110 kDa Mwt region at an isoelectric point of 5.4, the 50 kDa Mwt region at an isoelectric point of 5.5 and the 40 kDa Mwt region at an isoelectric point in the range of from 5.6 to 6.2, with the degree of staining in the corresponding region or regions in a corresponding sample taken from a disease-free individual.

6. A method for separating from a solubilised blood platelet sample a polypeptide which, if the components of the sample are subjected to two-dimensional polyacrylamide gel electrophoresis are present in the 110 kDa Mwt region at an isoelectric point of 5.4 and/or the 50 kDa Mwt region at an isoelectric point of 5.5 and/or the 40 kDa Mwt region at an isoelectric point in the range of from 5.6 to 6.2 which method comprises:

(i) applying said sample to an affinity chromatography column including antibodies specific for said polypeptide, which antibodies are bound to a solid support;

(ii) washing the support to remove unbound components of the sample to which the antibodies do not bind specifically;

(iii) eluting said polypeptide from the support so that it is obtained in substantially purified form.

7. A polypeptide in isolated and purified form, which polypeptide is present in a reduced concentration in a solubilised blood platelet sample from a sufferer from Alzheimer's disease and which, if the components of such a sample are subjected to two-dimensional polyacrylamide gel electrophoresis are present in the 110 kDa Mwt region at an isoelectric point of 5.4 and/or the 50 kDa Mwt region at an isoelectric point of 5.5 and/or the 40 kDa Mwt region at an isoelectric point in the range of from 5.6 to 6.2.

8. A method as claimed in claim 1 wherein the particular polypeptide or polypeptides are detected by immunoassay.

9. An immunoassay method for determining the amount

of a particular polypeptide as claimed in claim 1 or 2 comprises:

(i) coating a solid support surface with antibodies specific for the particular polypeptide;

(ii) washing the support to remove unbound antibodies;

(iii) applying a solubilised blood platelet polypeptide sample from a test sample;

(iv) washing the support to remove unbound components of the sample;

(v) applying labelled antibodies specific for the particular polypeptide;

(vi) washing the support to remove any unbound labelled antibodies;

(vii) determining the amount of labelled antibody present, which amount is proportional to the amount of the particular polypeptide present in the sample.

10. An immunoassay kit for diagnosing Alzheimer's disease in a solubilised blood platelet polypeptide sample from a test subject comprises:

(i) an antibody preparation containing antibodies specific for a particular polypeptide as defined in claim 1 or 2;

(ii) An antibody preparation containing labelled

antibodies specific for the said polypeptide for determining the amount of said polypeptide in the sample from the test subject; and

(iii) a comparative blood platelet polypeptide sample from an Alzheimer's disease-free individual to allow comparison of the amount of said polypeptide therein with the amount present in the sample from the test subject.

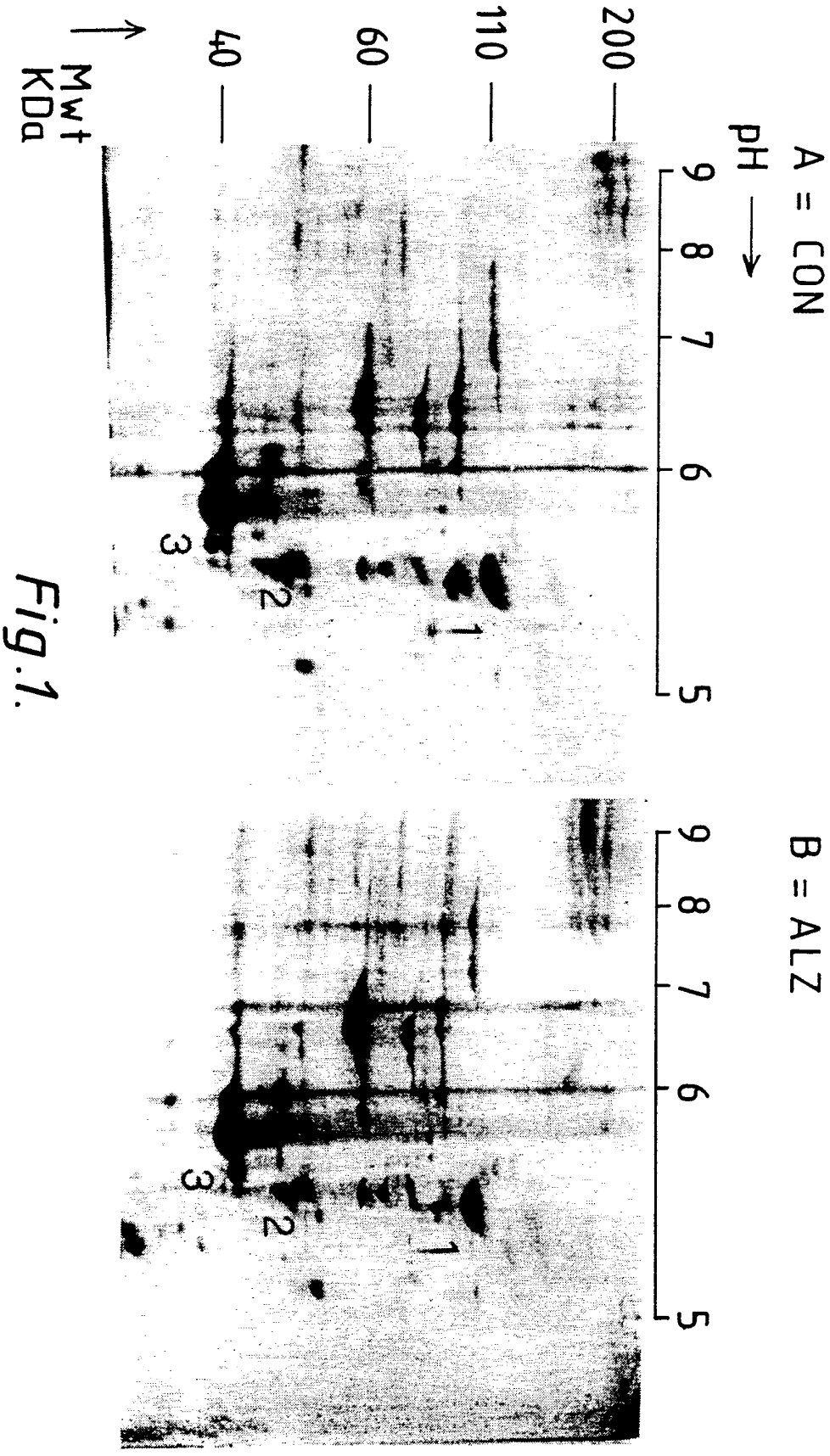

Fig.1.

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 91/00545

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[5]: G 01 N 33/68 // C 07 K 15/06

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[5]	G 01 N, G 07 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [9]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y,P	Science, volume 248, no. 4956, May 1990, W.E. Van Nostrand et al.: "Protease nexin-II (amyloid β-protein precursor): A platelet α-granule protein", pages 745-748 see the whole document	1,3,4,8
A	--	2,5-7,9-10
Y	US, A, 4666829 (G.G. GLENNER et al.) 19 May 1987 see the whole document	1,3,4,8
A	--	
A	Biotechnology, volume 7, February 1989, C.R. Abraham et al.: "Alzheimer's disease: Recent advances in understanding the brain amyloid deposits", pages 147-152 see pages 147-148; table 1	1
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	./.	

* Special categories of cited documents: [10]

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
19th June 1991	31 JUL 1991
International Searching Authority	**Signature of Authorized Officer**
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III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	WO, A, 8907657 (THE CHILDREN'S MEDICAL CENTER CORP.) 24 August 1989 see abstract; pages 4-5, pages 15-16; claims 10-20	1-10
P,A	The Journal of Biological Chemistry, volume 265, no. 26, 15 September 1990, The American Society for Biochemistry and Molecular Biology, Inc., (US), A.I. Bush et al.: "The amyloid precursor protein of Alzheimer's disease is released by human platelets", pages 15977-15983 see the whole document	1-10
P,A	Science, volume 248, no. 4959, 1 June 1990, R.P. Smith et al.: "Platelet coagulation factor XIa-inhibitor, a form of Alzheimer myloid precursor protein", see the whole document	1-10

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. GB 9100545 SA 46702

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 22/07/91
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US-A- 4666829	19-05-87	CA-A- 1268131	24-04-90
WO-A- 8907657	24-08-89	AU-A- 3204689	06-09-89