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(58) Field of Search:
Other: **WPI, EPODOC, JAPIO, MEDLINE, EMBASE, BIOSIS, CAPLUS, SCISEARCH, BLASTp, tBLASTn**

(54) Abstract Title: **Diagnostic method for brain damage-related disorders**

(57) A method of diagnosis of a brain damage-related disorder comprising the detection in a sample of body fluid of at least one polypeptide listed in Table 1 of the application. Each of the polypeptides is found in cerebrospinal fluid, and include, for example, signal-regulatory protein beta-1. Assay devices for use in the diagnosis of brain damage-related disorders are also disclosed.

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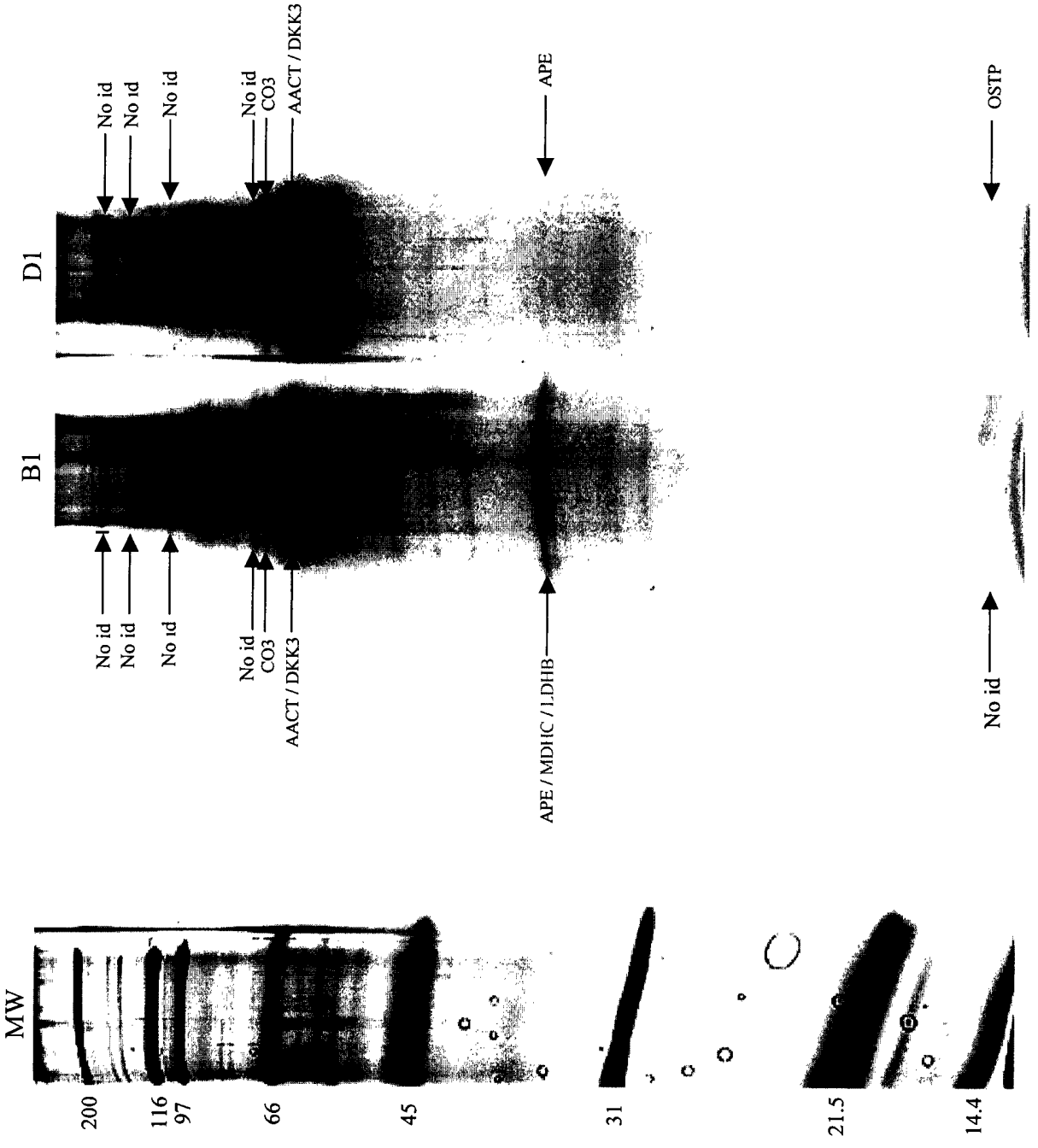
Figure 1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45
31
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14.4
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D1
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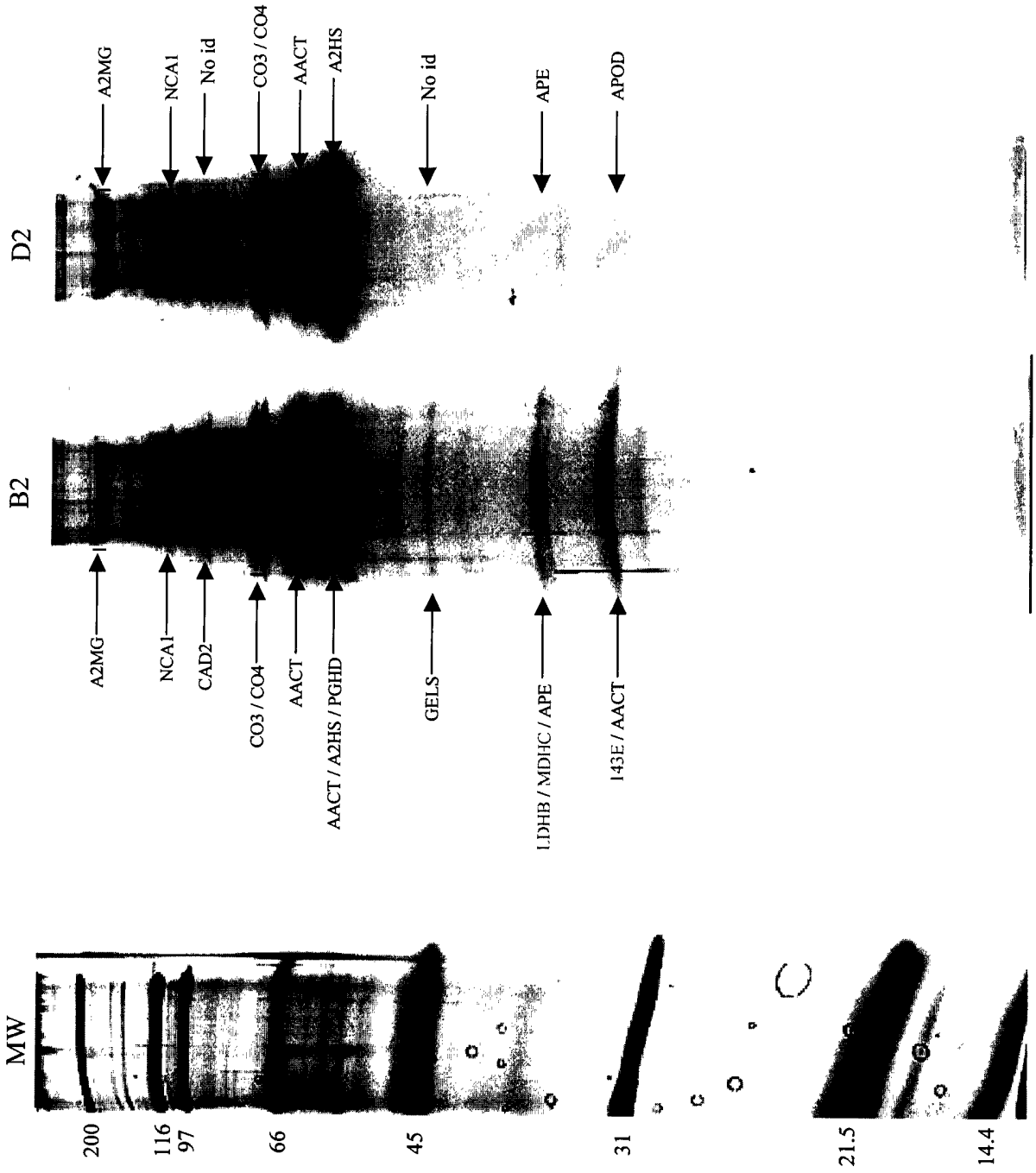
No id
No id
No id
CO3
AACT / DKK3
APE / MDHC / LDHB
APE
No id
OSTP

Figure 2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AACT
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GELS
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AACT
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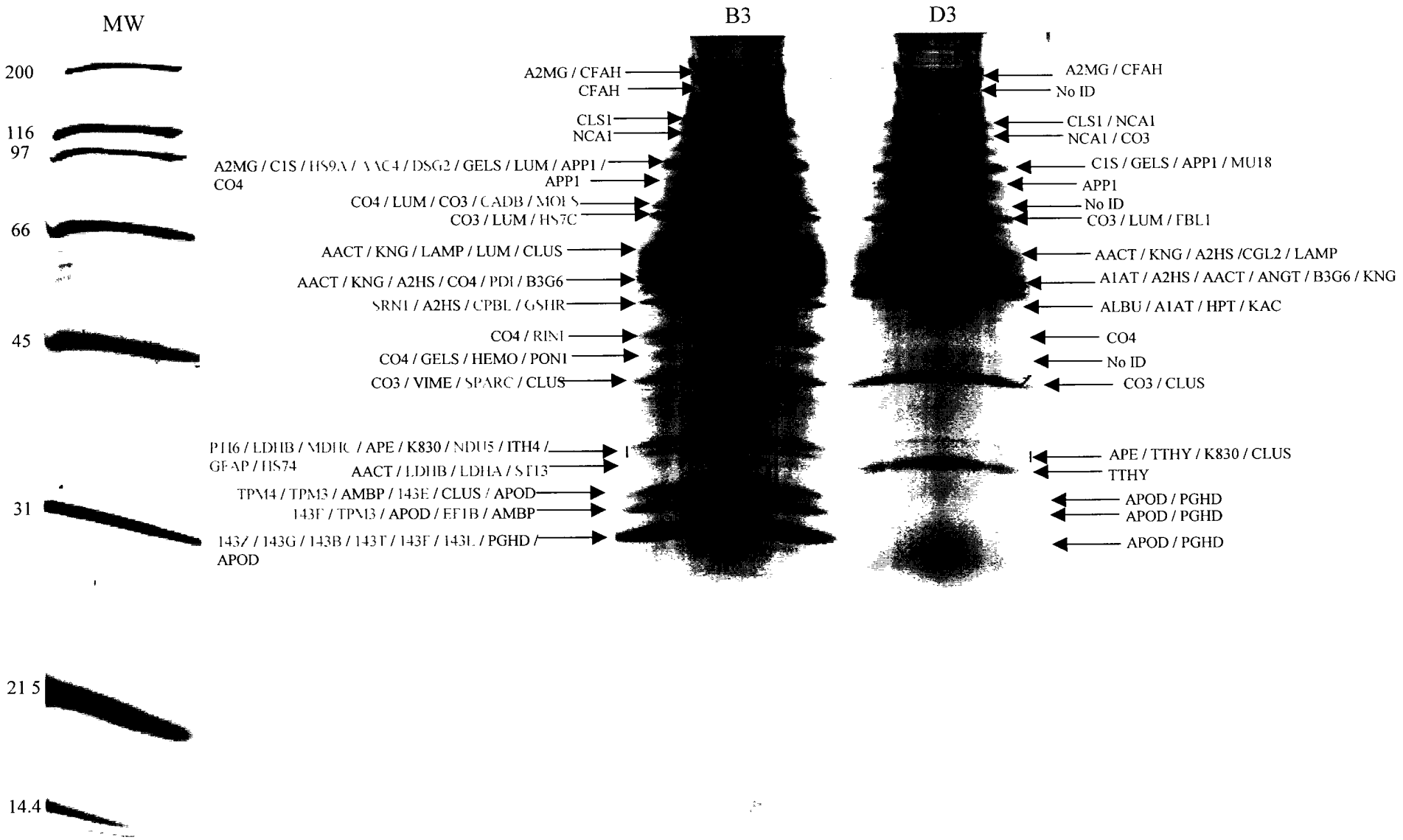
APE
APOD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200
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97
66
45
31
21.5
14.4
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CFAH
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NCA1
A2MG / C1S / HS9A / AAC4 / DSG2 / GELS / LUM / APP1 /
CO4
APP1
CO4 / LUM / CO3 / CADB / MOES
CO3 / LUM / HS7C
AACT / KNG / LAMP / LUM / CLUS
AACT / KNG / A2HS / CO4 / PDI / B3G6
SRN1 / A2HS / CPBL / GSHR
CO4 / RINI
CO4 / GELS / HEMO / PON1
CO3 / VIME / SPARC / CLUS
PTI6 / LDHB / MDHC / APE / K830 / NDU5 / ITH4 /
GFAP / HS74
AACT / LDHB / LDHA / ST13
TPM4 / TPM3 / AMBP / 143E / CLUS / APOD
143F / TPM3 / APOD / EF1B / AMBP
143Z / 143G / 143B / 143T / 143F / 143L / PGHD /
APOD
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NCA1 / CO3
C1S / GELS / APP1 / MU18
APP1
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ALBU / A1AT / HPT / KAC
CO4
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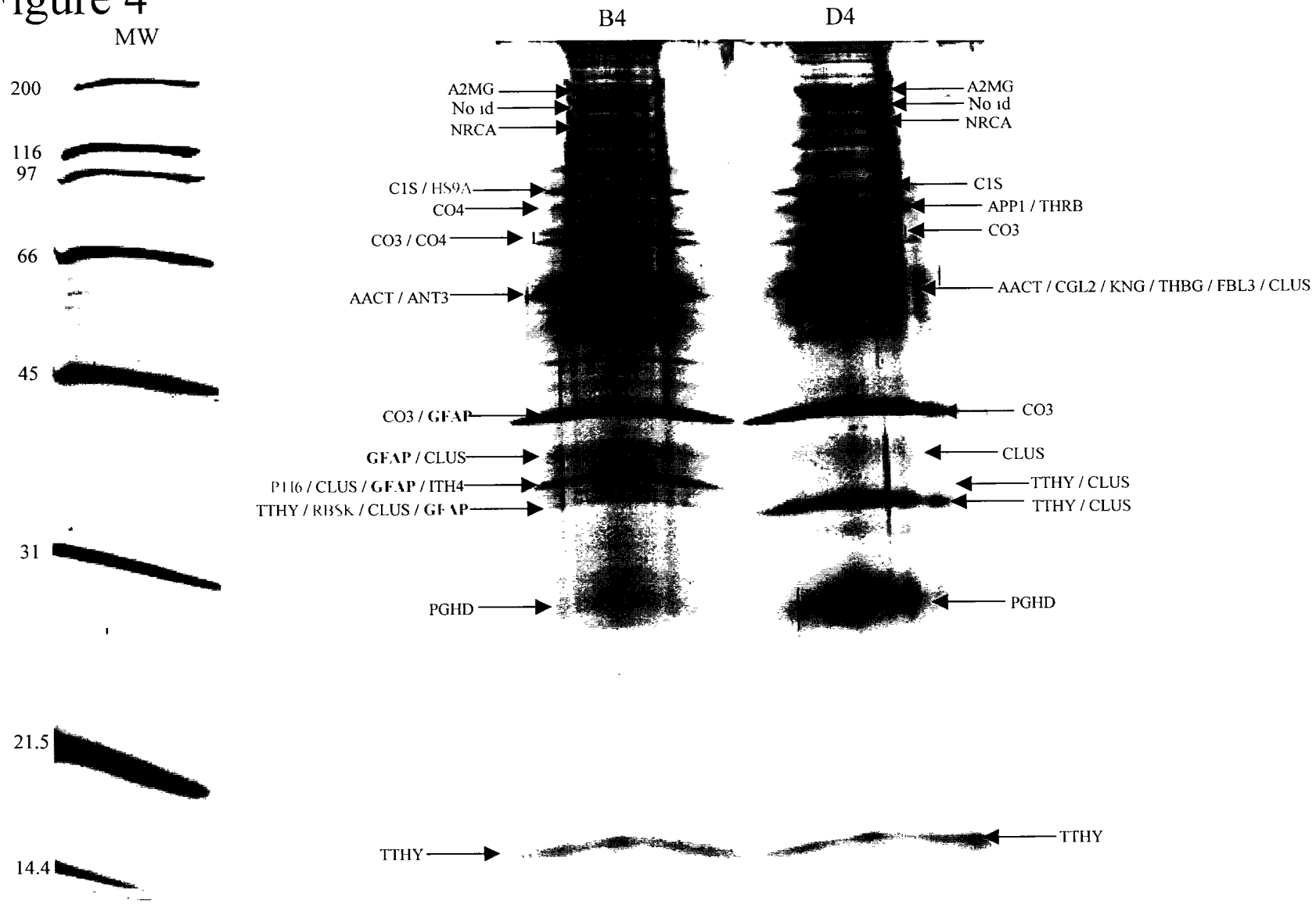
TTHY
APOD / PGHD
APOD / PGHD
APOD / PGHD

Figure 3

Figure 4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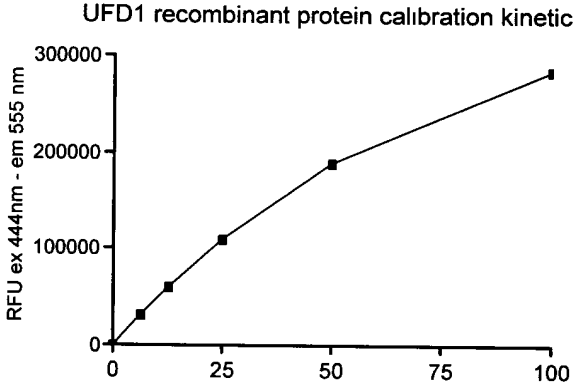
PGHD
TTHY
A2MG
No id
NRCA
C1S
APP1 / THRB
CO3
AACT / CGL2 / KNG / THBG / FBL3 / CLUS
CO3
CLUS
TTHY / CLUS
TTHY / CLUS
PGHD
TTHY

Figure 5. UFD1 detection in new plasma samples 2 fold diluted. Antibodies sandwich immunofluorescent ELISA. Crude values kinetic mode. Controls/stroke matched age/sex



Rec UFD1	RFU
100 ng/mL	284423 5
50 ng/mL	189477 5
25 ng/mL	109317 5
12 5 ng/mL	60429 5
6 25 ng/mL	32226
0	687 015

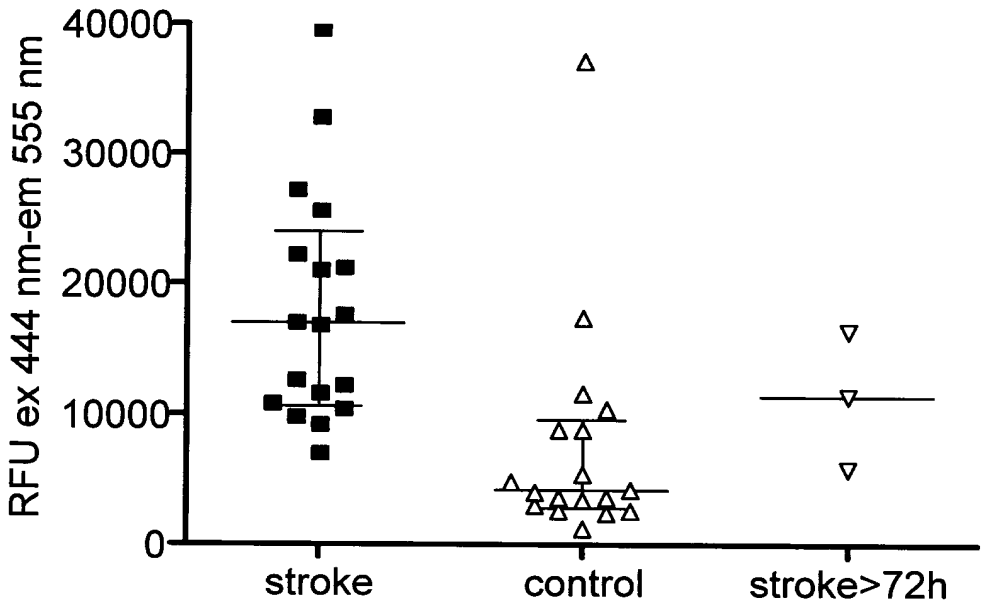
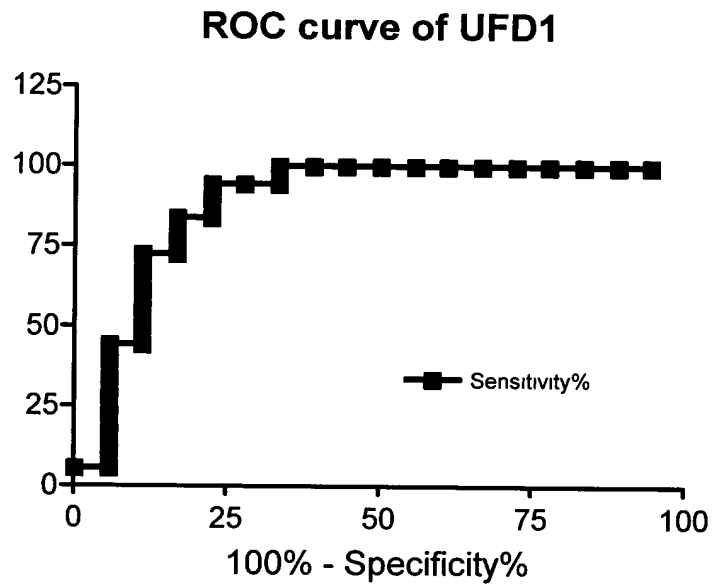


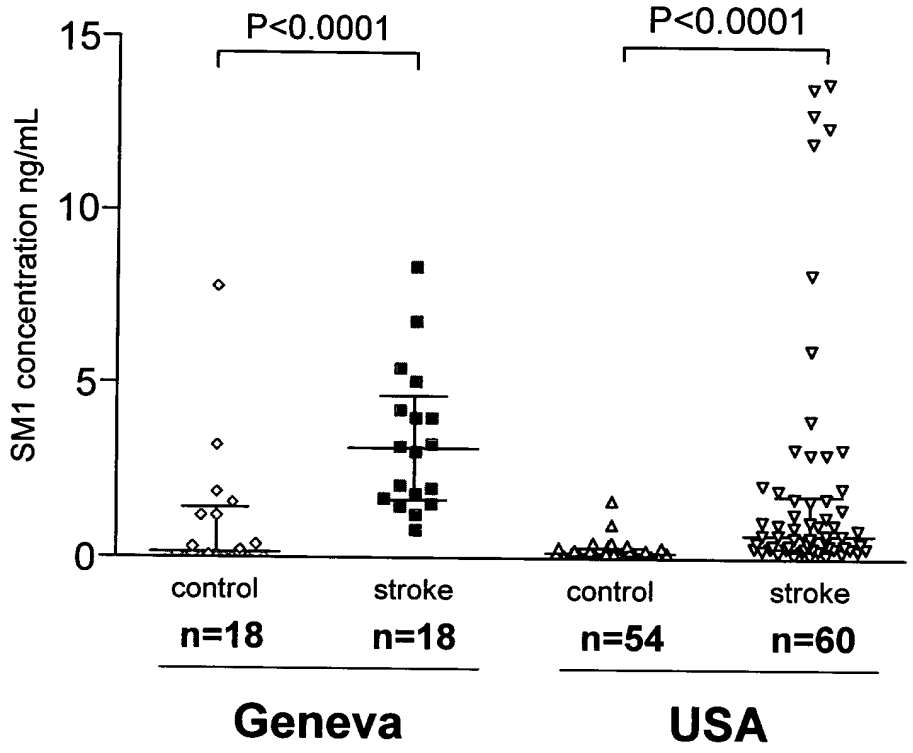
Figure 6. ROC curve of UFD1



UFD1 best cutoff value to differentiate stroke vs control. Determination of sensitivity and specificity

	cutoff	P (Mann et Whitney)	SE	SP
S vs. C	9047	<0.0001	94.4%	77.8%

Figure 7 - UFDP1 plasma concentration: ELISA



	Geneva	USA
sensitivity	94.4%	86.6%
specificity	78%	70%

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DIAGNOSTIC METHOD FOR BRAIN DAMAGE-RELATED DISORDERS

BACKGROUND OF THE INVENTION

Field of the invention

This invention relates to a diagnostic method for brain damage-related disorders.

No biological marker is currently available for the routine diagnosis of brain damage-related disorders including cerebrovascular, dementia and neurodegenerative diseases. This invention relates to the use of cerebrospinal fluid from deceased patients as a model for the discovery of brain damage-related disorder markers, and to the use of such markers in diagnosis.

Description of the related art

Over the last two decades, a number of biological markers (biomarkers) have been studied in the cerebrospinal fluid (CSF) and serum of patients with brain damage-related disorders, including creatine kinase-BB [1], lactate dehydrogenase [2], myelin basic protein [3], S100 protein [4], neuron-specific enolase (NSE) [5], glial fibrillary acidic protein [6] and tau [7]. Most of them have not proved useful indicators of the extent of brain damage and accurate predictors of clinical status and functional outcome. In fact, the diagnostic value of biomarkers for brain damage-related disorders has been hampered by their late appearance and a delayed peak after the damage event, their poor sensitivity and specificity, and the limited understanding of the mechanisms governing the release of these molecules into the CSF and ultimately in the blood. As a result of these limitations, the use of brain damage-related disorder biomarkers is currently limited to research settings and none has been recommended for routine assessment [8].

WO 01/42793 relates to a diagnostic assay for stroke in which the concentration of heart or brain fatty acid binding protein (H-FABP or B-FABP) is determined in a sample of body fluid.

SUMMARY OF THE INVENTION

Ideally, a biomarker for the diagnosis, monitoring and prognosis of brain damage-related disorders should include at least the following characteristics: (1) it should be brain-specific; (2) because of obvious difficulties to obtain CSF samples in patients, detection in serum is highly desirable; (3) it should appear very early; (4) its peak level, alternatively the area under the curve of sequential concentrations, should reflect the extent of brain damage; finally (5) it should be indicative of functional outcome. We demonstrate here new brain damage-related disorder biomarkers.

We describe how proteins have been identified as new diagnostic biomarkers for brain damage-related disorders using a proteomics-based analysis of CSF from deceased patients as a model of massive brain damage. Diagnostic assays for stroke using FABP has been described in WO 01/42793 and using RNA-BP, UFD1 and NDKA have been described in WO2005/029088. Use of the polypeptides according to the present invention can be validated in a similar way.

According to a first object of the invention, compositions are provided which comprise polypeptides for which the level was found either increased or decreased in the cerebrospinal fluid from deceased patients compared to cerebrospinal fluid from healthy donors. According to this same object, compositions are disclosed which comprise antibodies which are derived from the above polypeptides

According to a second object of the invention, methods are provided which utilize the inventive compositions in the diagnosis and prognosis of brain damage-related disorders including cerebrovascular, dementia and neurodegenerative diseases.

The present invention provides the following:

1. A method of diagnosis of a brain damage-related disorder or the possibility thereof in a subject suspected of suffering therefrom, which comprises detecting at least one polypeptide, or a variant, mutant or isoform thereof, selected from proteins listed in Table 1 below in a sample of body fluid taken from the subject.

2. A method according to 1, in which the polypeptide is differentially contained in the body fluid of brain damage-related disorder-affected subjects and non-brain damage-related disorder-affected subjects (control subjects), and the method includes determining whether the concentration of polypeptide in the sample is consistent with a diagnosis of brain damage-related disorder.

3. A method according to 1 or 2, in which an antibody to the polypeptide is used in the detection or the determination of the concentration.

4. A method according to any of 1 to 3, in which the body fluid is cerebrospinal fluid, plasma, serum, blood, tears, urine or saliva.

5. A method according to any of 1 to 4, in which the polypeptide is present in the body fluid of brain damage-related disorder-affected subjects and not present in the body fluid of non-brain damage-related disorder-affected subjects, whereby the presence of the polypeptide in a body fluid sample is indicative of brain damage-related disorder.

6. A method according to any of 1 to 4, in which the polypeptide is not present in the body fluid of brain damage-related disorder-affected subjects and present in the body fluid of non-brain damage-related disorder-affected subjects, whereby the non-presence of the polypeptide in a body fluid sample is indicative of brain damage-related disorder.

7. A method according to any of 1 to 6, in which a plurality of peptides is determined in the sample.

8. A method according to any of 1 to 7, in which one or more specific isoforms of the polypeptide are determined.

9. A method according to 8, in which diagnosis is made on the basis of differing levels of specific isoforms of the polypeptide.

10. A method according to any of 1 to 9, in which the polypeptide is differentially subject to post-translational modification in the body fluid of brain damage-related disorder-affected subjects and non-brain damage-related disorder-affected subjects, and the method includes detecting the post-translational modification of the polypeptide in the sample and determining whether this is consistent with a diagnosis of a brain damage-related disorder.

11. A method according to 10, in which the post-translational modification comprises N-glycosylation.

12. A method according to any of 1 to 11, in which at least one autoantibody to one or more of the polypeptides of Table 1 is determined.

13. A method according to any of to 12, in which two or more markers selected from antibodies to polypeptides of Table 1 are used in a single well of an ELISA microtiter plate.

14. A method according to any of 1 to 13, in which two or more polypeptides selected from Table 1 are separately assayed, and a predictive algorithm is used for diagnosis.

15. Use of a polypeptide, or a variant or mutant thereof, selected from Table 1, or a combination of such polypeptides, for diagnostic, prognostic and therapeutic applications relating to brain damage-related disorders.

16. Use according to 15, in which the polypeptide is differentially contained in a body fluid of brain damage-related disorder-affected subjects and non-brain damage-related disorder-affected subjects.

17. Use according to 15 or 16, in which a vaccine directed against a polypeptide, or a variant or mutant thereof, selected from Table 1, or an antigenic determinant thereof, is administered to a subject.

18. Use for diagnostic, prognostic and therapeutic applications, relating to brain damage-related disorders, of a material which recognises, binds to or has affinity for a polypeptide, or a variant or mutant thereof, selected from Table 1.

19. Use according to 18 of a combination of materials, each of which respectively recognises, binds to or has affinity for a polypeptide, or a variant or mutant thereof, selected from Table 1.

20. Use according to 18 or 19, in which the or each material is an antibody or antibody chip.

21. Use according to 20, in which the material is an antibody to any polypeptide listed in Table 1, or a variant or mutant thereof.

22. An assay device for use in the diagnosis of brain damage-related disorders, which comprises a solid substrate having a location containing a material, which recognizes, binds to or has affinity for a polypeptide, or a variant or mutant thereof, selected from Table 1.

23. An assay device according to 22, in which the solid substrate has a plurality of locations each respectively containing a material which recognizes, binds to or has affinity for a polypeptide, or a variant or mutant thereof, selected from Table 1.

24. An assay device according to 22 or 23, in which the material is an antibody or antibody chip.

25. An assay device according to 24, which has a unique addressable location for each antibody, thereby to permit an assay readout for each individual polypeptide or for any combination of polypeptides.

26. An assay device according to any of 22 to 25, including an antibody to any polypeptide listed in Table 1, or a variant or mutant thereof.

27. An assay device according to any of 22 to 26, further having a location containing a material which recognizes, binds to or has affinity for glutathione S transferase P.

28. An assay device according to 27, in which the material is an antibody or antibody chip.

29. A kit for use in the diagnosis of brain damage-related disorders, comprising an assay device according to any of 22 to 28, and means for detecting the amount of one or more of the polypeptides in a sample of body fluid taken from a subject.

The polypeptides (also referred to as proteins) useful in the present invention are not restricted to the sequences corresponding to the accession numbers in Table 1, and include variants, mutants and isoforms thereof. A variant is defined as a naturally ocurring variation in the sequence of a polypeptide which has a high degree of homology with the given sequence, and which has substantially the same functional and immunological properties. A mutant is defined as an artificially created variant. A high degree of homology is defined as at least 90%, preferably at least 95% and most preferably at least 99% homology. Variants may occur within a single species or between different species. An isoform of a polypeptide has the same function as the polypeptide but is encoded by a different gene and may have small differences in its sequence. The above proteins are of human origin, but the invention encompasses use of the corresponding polypeptides from other mammalian species, e.g. bovine animals.

Brain damage-related disorders in the context of the present invention include the following: head trauma, ischemic stroke, hemorrhagic stroke, subarachnoid hemorrhage, intra cranial hemorrhage, transient ischemic attack, vascular dementia, corticobasal ganglionic degeneration, encephalitis, epilepsy, Landau-Kleffner syndrome, hydrocephalus, pseudotumor cerebri, thalamic diseases, meningitis, myelitis, movement disorders, essential tremor, spinal cord diseases, syringomyelia, Alzheimer's disease (early onset), Alzheimer's disease (late onset), multi-infarct dementia, Pick's disease, Huntingdon's disease, Parkinson, Parkinson syndromes, frontotemporal dementia, corticobasal degeneration,

multiple system atrophy, progressive supranuclear palsy, Lewy body disease, amyotrophic lateral sclerosis, Creutzfeldt-Jakob disease, Dandy-Walker syndrome, Friedreich ataxia, Machado-Joseph disease, migraine, schizophrenia, mood disorders and depression. Corresponding disorders in non-human mammals are also included, such as transmissible spongiform encephalopathies (TSEs), e.g. bovine spongiform encephalopathy (BSE) in cattle or scrapie in sheep.

In one embodiment the brain damage-related disorder is stroke and the polypeptide is a homolog of one of the proteins listed in Table 1.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1-4 show portions of 1-DE maps after Off-gel electrophoresis for ante- and post-mortem CSF, with arrows indicating bands corresponding to proteins listed in Table 1. 5-10 µg of protein was loaded on a SDS PAGE slab gel (12.5%T / 2.6% C). The gel was silver stained.

Figures 5-7 show results of an assay for UFD1 for two groups of patients: a control group and a group with acute stroke.

DESCRIPTION OF PREFERRED EMBODIMENTS

The invention presented here is directed towards compositions and methods for detecting increasing or reducing polypeptides levels in body fluids including blood components (e.g. plasma or serum) or cerebrospinal fluid from subjects affected by a brain damage-related disorder including cerebrovascular, dementia and neurodegenerative diseases, as compared with control (non-affected) subjects. For this purpose, use can be made of antibodies or any specific polypeptide detection method.

The invention also includes embodiments where the polypeptides of Table 1 are determined indirectly. For example, at least one autoantibody to one or more of the polypeptides of Table 1 may be determined.

Antibodies against brain damage protein markers, in particular their protein-binding domains, are suitable as detection tools. Molecular biological and biotechnological methods can be used to alter and optimize the antibody properties of the said molecules in a specific manner. In addition to this, the antibodies can be modified chemically, for example by means of acetylation, carbamoylation, formylation, biotinylation, acylation, or derivatization with polyethylene glycol or hydrophilic polymers, in order to increase their stability.

A specific polypeptide marker selected from any of the proteins listed in Table 1 is determined in a body fluid sample, for example by using an antibody thereto. The marker may simply be detected and/or its concentration may be measured. The marker is preferably measured by an immunoassay, using a specific antibody to the polypeptide and measuring the extent of the antigen (polypeptide)/antibody interaction. The antibody may be a monoclonal antibody or an engineered (chimeric) antibody. Antibodies to the polypeptides are known and are commercially available. Also, the usual Köhler-Milstein method may be used to raise antibodies. Less preferably, the antibody may be polyclonal. In the context of the present invention, the term "antibodies" includes binding fragments of antibodies, such as single chain or Fab fragments.

Any known method of immunoassay may be used. In a sandwich assay an antibody (e.g. polyclonal) to the polypeptide is bound to the solid phase such as a well of a plastics microtitre plate, and incubated with the sample and with a labelled second antibody specific to the polypeptide to be detected. Alternatively, an antibody capture assay (also called "indirect immunoassay") can be used. Here, the test sample is allowed to bind to a solid phase, and the anti-polypeptide antibody (polyclonal or monoclonal) is then added and allowed to bind. If a polyclonal antibody is used in this context, it should desirably be one which exhibits a low cross-reactivity with other forms of polypeptide. After washing away unbound material, the amount of antibody bound to the solid phase is determined using a labeled second antibody, anti- to the first.

A direct assay can be performed by using a labelled anti-polypeptide antibody. The test sample is allowed to bind to the solid phase and the anti-polypeptide

antibody is added. After washing away unbound material, the amount of antibody bound to the solid phase is determined. The antibody can be labeled directly rather than via a second antibody.

In another embodiment, a competition assay can be performed between the sample and a labeled polypeptide or a peptide derived therefrom, these two antigens being in competition for a limited amount of anti-polypeptide antibody bound to a solid support. The labeled polypeptide or peptide can be pre-incubated with the antibody on the solid phase, whereby the polypeptide in the sample displaces part of the polypeptide or peptide thereof bound to the antibody.

In yet another embodiment, the two antigens are allowed to compete in a single co-incubation with the antibody. After removal of unbound antigen from the support by washing, the amount of label attached to the support is determined and the amount of protein in the sample is measured by reference to standard titration curves established previously.

Throughout, the label is preferably an enzyme. The substrate for the enzyme may be color-forming, fluorescent, chemiluminescent or electrochemical, and can be soluble or precipitating. Alternatively, the label may be a radioisotope or fluorescent, e.g. using conjugated fluorescein.

The enzyme may, for example, be alkaline phosphatase or horseradish peroxidase and can conveniently be used colorimetrically, e.g. using p-nitrophenyl phosphate as a yellow-forming substrate with alkaline phosphatase.

For a chemiluminescent assay, the antibody can be labeled with an acridinium ester or horseradish peroxidase. The latter is used in enhanced chemiluminescent (ECL) assay. Here, the antibody, labeled with horseradish peroxidase, participates in a chemiluminescent reaction with luminol, a peroxide substrate and a compound, which enhances the intensity and duration of the emitted light, typically, 4-iodophenol or 4-hydroxycinnamic acid.

An amplified immunoassay such as immuno-PCR can be used. In this technique, the antibody is covalently linked to a molecule of arbitrary DNA comprising PCR primers, whereby the DNA with the antibody attached to it is amplified by the polymerase chain reaction. See E. R. Hendrickson *et al.*, Nucleic Acids Research 1995; 23, 522-529 (1995) or T. Sano et al., in "Molecular Biology and Biotechnology" ed. Robert A. Meyers, VCH Publishers, Inc. (1995), pages 458 - 460. The signal is read out as before.

In one procedure, an enzyme-linked immunosorbent assay (ELISA) can be used to detect the polypeptide.

The full automation in a widely used clinical chemistry analyser such as the COBAS™ MIRA Plus system from Hoffmann-La Roche, described by M.Robers *et al.* Clin Chem. 1998 Jul;44(7):1564-7 or the AxSYM™ system from Abbott Laboratories, is possible and can be applied for routine clinical diagnosis of brain damage-related disorders.

The polypeptide concentrations can be measured by other means than immunoassay. For example, the sample can be subjected to 2D-gel electrophoresis and the amount of the polypeptide estimated by densitometric scanning of the gel or of a blot therefrom. However, it is desirable to carry out the assay in a rapid manner, so that the patient can be treated promptly.

In principle, any body fluid can be used to provide a sample for diagnosis, but preferably the body fluid is cerebrospinal fluid (CSF), plasma, serum, blood, urine, tears or saliva.

According to the invention, a diagnosis of brain damage-related disorders may be made from determination of a single polypeptide or any combination of two or more of the polypeptides.

The invention also relates to the use of one or more of the specified polypeptides which is differentially contained in a body fluid of brain damage-affected subjects and non-brain damage-affected subjects, for diagnostic, prognostic and therapeutic

applications. This may involve the preparation and/or use of a material which recognizes, binds to or has some affinity to the above-mentioned polypeptide. Examples of such materials are antibodies and antibody chips. The term "antibody" as used herein includes polyclonal antiserum, monoclonal antibodies, fragments of antibodies such as Fab, and genetically engineered antibodies. The antibodies may be chimeric or of a single species. The above reference to "prognostic" applications includes making a determination of the likely course of a brain damage-related disorder by, for example, measuring the amount of the above-mentioned polypeptide in a sample of body fluid. The above reference to "therapeutic follow-up" applications includes making a determination of the likely course of a brain damage-related disorder by, for example, measuring the amount of the above-mentioned polypeptide in a sample of body fluid (and evaluating its level as a function of the treatment, the disability recovery or not, the size of the lesions etc.). The above reference to "therapeutic" applications includes, for example, preparing materials which recognize, bind to or have affinity to the above-mentioned polypeptides, and using such materials in therapy. The materials may in this case be modified, for example by combining an antibody with a drug, thereby to target the drug to a specific region of the patient. In a further embodiment, a vaccine directed against a polypeptide, or a variant or mutant thereof, selected from Table 1, or an antigenic determinant (epitope) thereof, is administered to a subject.

The above reference to "presence" or "absence" of a polypeptide, and the equivalent expressions "present" and "not present", should be understood to mean simply that there is a significant difference in the amount of a polypeptide which is detected in the affected and non-affected (or control) sample. Thus, the "absence" of a polypeptide in a test sample may include the possibility that the polypeptide is actually present, but in a significantly lower amount than in a comparative test sample. According to the invention, a diagnosis can be made on the basis of the presence or absence of a polypeptide, and this includes the presence of a polypeptide in a significantly lower or significantly higher amount with reference to a comparative (or control) test sample.

The above references to "detecting" a polypeptide should be understood to include a reference to compositions and methods for detecting post-translational modifications of the polypeptides in addition to quantitative variations. The invention therefore encompasses the detection of post-translational modifications in general, and determining whether such modifications of a polypeptide are consistent with a diagnosis of a brain damage-related disorder. One example of such post-translational modification is N-glycosylation.

Kits and assay devices for use in diagnosis of brain damage-related disorders are also within the scope of the invention. These may include one or more antibodies to a polypeptide selected from any of the proteins listed in Table 1. The antibodies will bind to the appropriate polypeptides in a fluid sample taken from a patient. The antibodies may be immobilised on a solid support. Preferably, each antibody is placed in a unique addressable location, thereby to permit separated assay readout for each individual polypeptide in the sample, as well as readouts for any selected combination of polypeptides. Such kits and assay devices may also include antibodies to other marker polypeptides in addition to one or more of those in Table 1. Such other marker polypeptides include those described in WO01/42793 and WO2005/029088. In one particular embodiment, the other marker polypeptide is glutathione S transferase P.

The following Examples illustrate the invention.

EXAMPLE 1

Using one-dimensional gel electrophoresis (1-DE) separation of cerebrospinal fluid (CSF) proteins and mass spectrometry techniques, 58 polypeptides named in Table 1 were found elevated or decreased in the CSF of deceased patients, used as a model of massive brain damage.

Study population and sample handling

Twenty CSF samples were used for the proteomics-based approach aiming at discovering brain damage-related disorder markers. Five of these samples were obtained at autopsy from deceased patients 6 hours after death with no pathology

of the central nervous system. Fifteen others were collected by lumbar puncture from living patients who had a neurological workup for benign conditions unrelated to brain damage (atypical headache and idiopathic peripheral facial nerve palsy). CSF samples were centrifuged immediately after collection, aliquoted, frozen at -80°C and stored until analysis.

CSF depletion fractionation

Immunodepletion of human serum albumin, transferrin, haptoglobin, IgG, IgA and antitrypsin was performed using a Multiple Affinity Removal System (Agilent Technologies, Wilmington, USA). 3 ml of CSF was concentrated to approximately 300 µl using ultrafiltration (10 kDa MWCO, Vivascience). The CSF was divided into 200 µl aliquots for immunodepletion according to the manufacturer's instructions. Combined fractions following depletion were concentrated using ultrafiltration. Final CSF protein concentrations of between 600 and 900 µg/µl were measured using a Bradford assay. All reagents and apparatus for off-gel electrophoresis (OGE) have been described in detail elsewhere (Ros, A., et al., *Protein purification by Off-Gel electrophoresis.* Proteomics, 2002. **2**(2): p. 151-6). 750 µl of the immunodepleted CSF samples were loaded on the strip for OGE using all-well loading (50 µl per well). The samples were focused for a total of 31.6 kVhrs (1 hr at 100 V, 1 hr at 500 V, 1 hr at 1000 V, 15 hrs at 2000 V). The current was limited to 50 µA and the temperature was controlled at 20°C. Fractions (20-100 µl) were collected from each well and stored at -20°C prior to SDS-PAGE.

1-DE of OGE fractionated CSF proteins

Fractions from OGE were mixed with a 5X concentrated solution of Laemmli's buffer (0.125 M Tris–HCl, 4% SDS, 40% glycerol, 0.1% bromophenol blue, pH 6.8) up to 70µl and heated at 95°C for 5 min. Samples were centrifuged at 14000g and supernatant loaded on the 12.5% SDS-polyacrylamide gel. Migration was performed in a Tris-Glycine-SDS pH 8.3 buffer. The gel was then stained using MS compatible silver staining derived from Blum [Blum, H., Beier, H. and Gross, H. J., *Electrophoresis* 1987, *8*, 93-99]. The gel was first fixed for a minimum of 30 min 50% (v/v) methanol 10% (v/v) acetic acid and then 15 min in

5% (v/v) methanol. The gel was then washed 3 times 5 min in milli-Q H_2O and incubated 2 min in 0.2 g/L (w/v) fresh sodium thiosulfate ($Na_2S_2O_3$, 5 H_2O). The gel was further washed 3 times 30 sec in milli-Q H_2O, and incubated in the staining solution, i.e. 25 min in 2 g/L silver nitrate ($AgNO_3$) solution. The gel was washed 3 times 1 min in milli-Q H_2O, and incubated in the developing solution (sodium carbonate Na_2CO_3 30g/L (w/v), 0.05% of 37% HCOH (v/v), 2% (v/v) of a fresh 0.2 g/L (w/v) sodium thiosulfate ($Na_2S_2O_3$, 5 H_2O)) for 10 min maximum. The gel development was stopped using a 14 g/l (w/v) Na_2-EDTA solution for 10 min before washing in milli-Q H_2O. The apparent molecular masses were determined by running 2 µg of broad range molecular weight standards (Bio-Rad, Hercules, CA, USA). The gel was scanned on a Arcus II Agfa scanner, with Agfa Fotolook version 3.6 software. Bands to be identified were cut, placed in an Eppendorf tubes and destained. Each gel piece was incubated in 30µl destaining solution (30 mM K_3FeCN_6, 100 mM $Na_2S_2O_3$) with occasional vortexing until the gels were completely destained (5-10 min). Gel pieces were then washed twice for 10 min with a minimum of 100µl milli-Q-H_2O for 10 and then stored at 4°C in 10% ethanol (v/v).

Identification of the proteins by nanoLC-ESI-MS/MS

Gel pieces were washed with 200 µl of 50 mM ammonium bicarbonate, for 10 min. Gel pieces were then dehydrated with 100µl of 100% CH_3CN and dried in a vacuum centrifuge (HETO, Allerod, Denmark). Trypsin digestion was performed as described previously [Scherl, A., Coute, Y., Deon, C., Calle, A., Kindbeiter, K., *et al.*, *Mol Biol Cell* 2002, *13*, 4100-9]. NanoLC-ESI-MS/MS was performed on a LCQ DecaXP ion trap (Thermofinnigan, San Jose, CA) coupled to a LC-PAL autosampler (CTC Analytics, Zwingen, Switzerland) and a Rheos 2000 Micro HPLC Pump (Flux Instruments, Basel, Switzerland). For each experiment, 5µl of sample in 5 % CH_3CN, 0.1 % formic acid was injected on a C18 reverse phase column (75 µm inner diameter) packed in house with 5µm Zorbax 300Extend-C18 (Agilent Technologies, Wilmington USA). Peptides were eluted from the column using a CH_3CN gradient in the presence of 0.1 % formic acid. For peptide elution, the acetonitrile concentration was increased from 8 to 47 % in 15 min. A flow splitter was used to decrease the flow rate from 40µl/min to

approximately 0.2µl/min. A 1.8 kV potential was applied on the nano-electrospray capillary (New Objective, Woburn, MA). Helium was used as collision gas. The collision energy was set at 35 % to the maximum. MS/MS spectra were acquired by automatic switching between MS and MS/MS mode. The two highest peaks from each MS scan were chosen for MS/MS. Dynamic exclusion was applied with a repeat count of 2 and a repeat duration of 0.5 mins. Following these two MSMS acquisitions on the same precursor, the precursor was excluded from MSMS analysis for 1.0 min. Spectra were converted to DTA files, regrouped using in house software and the database search was performed with MASCOT 1.8 (http://www.matrixscience.co.uk/). A tolerance of 2.0 Da was chosen for the precursor and 1.0 Da for fragments. ESI-TRAP was selected as the instrument. The UniProt Swiss-Prot database was searched without species restriction. In these conditions, the threshold of significance was given by a score of 42 or higher by Mascot. The data was also searched against the UniProt SwissProt database using the Phenyx program (http://www.phenyx-ms.com/). Protein hits with less than three peptides above the threshold were manually validated. The data was further searched against the Trembl database, resulting in the identification of a further 22 proteins. The results are shown in Table 1.

Table 1:

Post-mortem CSF Accession number	Protein name
O00241	Signal-regulatory protein beta-1
O43396	Thioredoxin-like protein 1
O43488	Aflatoxin B1 aldehyde reductase member 2
O43707	Alpha-actinin 4
O75223	Protein C7orf24
O95336	6-phosphogluconolactonase
O95861	3'(2'),5'-bisphosphate nucleotidase 1
P00352	Retinal dehydrogenase 1
P00390	Glutathione reductase, mitochondrial
P00491	Purine nucleoside phosphorylase
P00915	Carbonic anhydrase I
P01859	Ig gamma-2 chain C region*
P01876, P01877	Ig alpha-1 or -2 chain C region
P02024	Hemoglobin beta chain
P02545	Lamin A/C (70 kDa lamin)
P02741	C-reactive protein

P02760	AMBP protein
P04642	L-Lactate dehydrogenase A chain
P04746, P04745, P19961	Alpha-amylase (pancreatic, salivary or 2B)
P05089	Arginase 1
P05209, Q9BQE3	Tubulin alpha-1 or alpha-6 chain
P05413	Fatty acid-binding protein, heart (H-FABP)
P05976 or P06741	myosin light chain 1 or 3, skeletal muscle isoform
P06576	ATP synthase beta chain, mitochondrial
P06753	Tropomyosin alpha 3 chain
P07148	Fatty acid-binding protein, liver (L-FABP)
P07203	Glutathione peroxidase 1
P07225	Vitamin K-dependent protein S
P07226	Tropomyosin alpha 4 chain
P07237	Protein disulphide-isomerase
P07357	Complement C8 alpha chain
P07738	Bisphosphoglycerate mutase
P07900	Heat shock protein HSP 90-alpha (HSP 86)
P07996	Thrombospondin 1
P08059	Glucose-6-phosphate isomerase
P08133	Annexin A6
P08758	Annexin A5
P09417	Dihydropteridine reductase
P09488	Glutathione S-transferase Mu 1
P09493 or P06753	Tropomyosin 1 alpha chain or alpha 3 chain
P09525	Annexin A4
P09668	Cathepsin H
P10586	Receptor-type tyrosine-protein phosphatase F
P10599	Thioredoxin
P10768	Esterase D
P11021	78 kDa glucose-regulated protein
P12833	Myosin heavy chain, cardiac muscle beta isoform
P12882	Myosin heavy chain, skeletal muscle, adult 1
P13489	Placental ribonuclease inhibitor
P13535	Myosin heavy chain, skeletal muscle, perinatal
P13611	Versican core protein
P13693	Translationally controlled tumor protein (TCTP)
P13716	Delta-aminolevulinic acid dehydratase
P13929	Beta enolase
P14136	Glial fibrillary acidic protein, astrocyte (GFAP)
P14550	Alcohol dehydrogenase [NADP+]
P14923	Junction plakoglobin
P15103	Glutamine synthetase

P15121	Aldose reductase
P15259	Phosphoglycerate mutase 2
P15289	Arylsulfatase A
P15924	Desmoplakin
P16930	Fumarylacetoacetase
P17066	Heat shock 70 kDa protein 6
P18206	Vinculin
P21266	Glutathione S-transferase Mu 3
P21333	Filamin A
P21695	Glycerol-3-phosphate dehydrogenase [NAD+], cytoplasmic
P22061	Protein-L-isoaspartate (D-aspartate) O-methyltransferase
P22314	Ubiquitin-activating enzyme E1
P23141	Liver carboxylesterase 1
P24534	Elongation factor 1-beta
P25788	Proteasome subunit alpha type 3
P26038	Moesin
P26641	Elongation factor 1-gamma
P27169	Serum paraoxonase/arylesterase 1
P27348	14-3-3 protein tau
P28072	Proteasome subunit beta type 6
P28161	Glutathione S-transferase Mu 2
P28827	Receptor-type protein-tyrosine phosphatase mu
P29218	Inositol-1 [or 4] -monophosphate
P29401	Transketolase
P30040	Endoplasmic reticulum protein ERp29
P30041	Peroxiredoxin 6
P30101	Protein disulfide-isomerase A3
P30626	Sorcin (22 kDa protein)
P31946	14-3-3 protein beta/alpha
P31948	Stress-induced-phosphoprotein 1
P34932	Heat shock 70 kDa protein 4
P35080	Profilin-2
P35237	Placental thrombin inhibitor
P36980	Complement factor H-related protein 2
P37837	Transaldolase
P40121	Macrophage capping protein
P42126	3,2-trans-enoyl-CoA isomerase, mitochondrial
P42655	14-3-3 protein epsilon
P45381	Aspartoacylase

P46940	Ras GTPase-activating-like protein IQGAP1
P47756	F-actin capping protein beta subunit
P48637	Glutathione synthetase
P49419	Aldehyde dehydrogenase family 7 member A1
P50135	Histamine N-methyltransferase
P50395	Rab GDP dissociation inhibitor beta
P52565	Rho GDP-dissociation inhibitor 1
P52566	Rho GDP-dissociation inhibitor 2
P52907	F-actin capping protein alpha-1 subunit
P54289	Dihydropyridine-sensitive L-type, calcium channel alpha-2/delta subunits
P54652	Heat shock-related 70 kDa protein 2
P54922	ADP-ribosylarginine hydrolase
P55287	Cadherin-11
P55854, P61956	Ubiquitin-like protein SMT 3A or 3B
P57087	Junctional adhesion molecule 2
P60900	Proteasome subunit alpha type 6
P61088	Ubiquitin-conjugating enzyme E2 N
P62258	14-3-3 protein epsilon
P62993	Growth factor receptor-bound protein 2
P63104	14-3-3 protein zeta/delta
P68133	Actin, alpha skeletal muscle
Q00169	Phosphatidylinositol transfer protein alpha isoform
Q01082	Spectrin beta chain, brain 1
Q01995	Transgelin
Q04917	14-3-3 protein eta
Q06033	Inter-alpha-trypsin inhibitor heavy chain H3
Q12765	Secernin 1
Q13332	Receptor-type tyrosine-protein phosphatase S
Q13509	Tubulin beta-4
Q13740	CD166 antigen
Q13813	Spectrin alpha chain, brain
Q13938	Calcyphosine
Q14126	Desmoglein 2
Q15149	Plectin 1
Q15181	Inorganic pyrophosphatase
Q16620	BDNF/NT-3 growth factors receptor
Q16881	Thioredoxin reductase 1, cytoplasmic

Q86UP2	Kinectin
Q86YZ3	Hornerin
Q8N0Y7	Putative phosphoglycerate mutase 3
Q8TAG5	Immunoglobulin-like domain protein MGC33530
Q8TD26	Chromodomain-helicase-DNA-binding protein 6
Q92598	Heat shock protein 105 kDa
Q92890	Ubiquitin fusion degradation protein 1 homolog
Q969H8	Protein C19 or F10 precursor
Q96IU4	CCG1-interacting factor B
Q9BX68	Histidine triad nucleotide-binding protein 2
Q9H477	Ribokinase
Q9NVS9	Pyridoxine-5'-phosphate oxidase
Q9NZT1	Calmodulin-like protein 5
Q9P0L0	Vesicle-associated membrane protein-associated protein A
Q9P121	Neurotrimin
Q9UBQ7	Glyoxylate reductase/hydroxypyruvate reductase
Q9UKK9	ADP-sugar pyrophosphatase
Q9UKX2	Myosin heavy chain, skeletal muscle, adult 2
Q9UN36	NDRG2 protein
Q9Y617	Phosphoserine aminotransferase
Q9Y623	Myosin heavy chain, skeletal muscle, fetal

Ante-mortem CSF

P00748	Coagulation factor XII
P01833	polymeric-immunoglobulin receptor
P04083	Annexin A1
P04121	Macrophage capping protein
P05109	Calgranulin A (MRP-8)
P12109	Collagen alpha 1(VI) chain
P22352	Plasma glutathione peroxidase
P35247	Pulmonary surfactant-associated protein D
P43121	Cell surface glycoprotein MUC18
P58876 + others	Histone H2B (different forms)
P78509	Reelin

Trembl accession no.	Description
O95784	IgG Fc binding protein (Fragment)

Q07898, Q07899, Q07900, Q07901, Q86VB7	M130 antigen; M130 antigen cytoplasmic variant 1; variant 2; M130 antigen extracellular variant; Similar to CD163 antigen
Q7Z664	Hypothetical protein DKFZp779N0926 (Fragment)
Q7Z623	hypothetical protein
Q8IZY7	Hepatocellular carcinoma associated protein TB6
Q8N240	Hypothetical protein FLJ34957
Q8N466	Hypothetical protein with 1 extra peptide over SP entry (Contactin Q12860)
Q8NCW5	ApoA-I binding protein precursor
Q8NFZ8 or Q9Y4A4	TSLC1-like 2 or F22162_1 (Fragment)
Q969J9	Hypothetical protein (Similar to dystroglycan 1)
Q96AC3, Q96FV2, Q9BU04	Hypothetical protein, Ses2 protein, Similar to KIAA0193 gene product (Fragment)
Q96B89, Q9H3J8, Q9HC37, Q9HC38, Q9Y3E8	Hypothetical protein, My027 protein, Hypothetical protein, Hypothetical protein, CGI-150 protein
Q96B89, Q9H3J8, Q9HC37, Q9HC38, Q9Y3E8	various names
Q96B89, Q9H3J8, Q9HC38, Q9Y3E8	Hypothetical protein, My027 protein, Hypothetical protein, CGI-150 protein
Q96EI3, Q9H0W9	Hypothetical protein
Q96NV4, Q9H0R4	Hypothetical protein FLJ30028, Hypothetical protein
Q9H008	Phospholysine phosphohistidine inorganic pyrophosphate phosphatase
Q9H2Y2, Q9NPH2, Q9NVW7	Inositol 1-phosphate synthase, Myo-inositol 1-phosphate synthase A1, Hypothetical protein FLJ10463
Q9NQ56, Q9NQ48	Leucine zipper transcription factor-like 1
Q9NX46	DJ665N4.2 (Similar to hypothetical protein FLJ20446) (ADP-ribosyl-hydrolase precursor)
Q9Y5Z5, Q9NRV9	Heme-binding protein, Heme-binding protein (Hypothetical protein)
Q9Y6R7	Human Fc gamma BP (Fragment)

EXAMPLE 2

Introduction

A survey of stroke patients was carried out and the results are shown in Figures 5 to 7. An ELISA intensity signal was obtained for Ubiquitin fusion degradation protein 1 homolog (UFD1) in plasma samples of the patients and of negative

control patients. Plasma samples were taken from patients between 0-24 hours and/or after 72 hours of arrival at emergency hospital, and were matched for age/sex with samples from control patients.

ELISA was performed using 96-well Reacti-Bind™ NeutrAvidin™ coated Black Plates (Pierce, Rockford, IL). Plates were first rinsed in Borate Buffer Saline pH 8.4 (BBS) (100 mM H_3BO_3, 25 mM $Na_2B_4O_7$ (Sigma, St Louis, MO, USA), 75 mM NaCl (Merck, Darmastadt, Germany)) on a NOVAPATH™ washer (Bio-Rad, Hercules, CA). Then, 50µl of biotin-conjugated antibody (2 µg/ml) prepared in the dilution buffer A at pH 7 (DB, Polyvinyl Alcohol, 80% hydrolyzed, Mol. Wt. 9000-10,000 (Aldrich, Milwaukee, WI, USA), MOPS (3-[N-Morpholino] propane sulfonic acid) (Sigma), NaCl, $MgCl_2$ (Sigma), $ZnCl_2$ (Aldrich), pH6.90, BSA 30% Solution, Manufacturing Grade (Serological Proteins Inc., Kankakee, IL)), was added and incubated for one hour at 37°C. Plates were then washed 3 times in BBS in the plate washer. 50 µl of antigen was then added and incubated for one hour at 37°C. Recombinant proteins were diluted at 100, 50, 25, 12.5, 6.25 ng/ml in the dilution buffer A to establish a calibration curve. Plasma samples were diluted to the appropriate concentration in the dilution buffer A. After the washing step, 50µl of alkaline phosphatase-conjugated antibody was added at the appropriate dilution in buffer A and incubated for one hour at 37°C. The 96-well plate was then washed 3 times with BBS in the plate washer and 50 µl of Attophos® AP Fluorescent substrate (Promega, Madison, WI) was added. Plates were read immediately on a SpectraMax GEMINI-XS fluorometer microtiter plate reader, (Molecular Devices Corporation, Sunnyvale, CA, U.S.A.) ($\lambda_{excitation}$=444 nm and $\lambda_{emission}$=555 nm). Results are expressed in RFU and can be obtained in endpoint mode (only one reading) or in kinetic mode for 10 minutes. In kinetic mode, the plate reader was set to record using 6 flashes (per well) which were then integrated into an average. In this manner each well was analysed 6 times using a minimal interval time between each reading. This translated to a 2 minutes delay between readings. The slope was calculated and used to determine the final value for each well. The best cut-off value to discriminate between the control and the stroke (Ischemic plus hemorrhagic or

Ischemic vs. Hemorrhagic) groups was determined using ROC curves generated in GraphPad Prism 4 software.

Conclusion

It is clear from Figure 5 that UFD1 is overexpressed in stroke patients compared to control patients. Statistical analysis was performed and ROC curves (GraphPad Prism 4 software) indicating sensitivity of the test as a function of 1-specificity (Figures 6) were drawn. Best cutoff values to distinguish between stroke and control patients were deduced from these ROC curves. A sensitivity and specificity of 94.4% and 77.8%, respectively, was obtained using the best cutoff values. A non-parametric Mann-Whitney test was performed to compare stroke and control groups. Very low p values (<0.0001) were obtained, indicating that the difference between stroke and controls was highly significant.

This result demonstrates that Ubiquitin fusion degradation protein 1 homolog (UFD1) is a useful marker for early diagnosis of stroke, alone, or in combination with other biomarkers.

As UFD1 has been found in deceased CSF, it is a reasonable prediction that other polypeptides and proteins found in deceased CSF will also be useful as markers for brain damage-related disorders.

EXAMPLE 3

This Example provides additional data showing plasma levels of UFDP1 in stroke and control patients. Additional data has been obtained from two cohorts of patients and controls, the smaller from Geneva, and a more comprehensive panel from the US.

ELISA was performed using 96-well Reacti-Bind™ NeutrAvidin™ coated Black Plates (Pierce, Rockford, IL). Plates were first rinsed in Borate Buffer Saline pH 8.4 (BBS) (100 mM H3BO3, 25 mM Na2B4O7 (Sigma, St Louis, MO, USA), 75 mM NaCl (Merck, Darmastadt, Germany)) on a NOVAPATH™ washer (Bio-Rad, Hercules, CA). Then, 50µl of relevant

biomarker specific biotin-conjugated antibody (2 μg/mL) prepared in the dilution buffer A at pH 7 was added and incubated for one hour at 37°C. Plates were then washed 3 times in BBS in the plate washer. 50 μl of antigen or plasma was then added and incubated for one hour at 37°C. Recombinant protein antigens were diluted at 100, 50, 25, 12.5, 6.25, 3.125, 1.56 ng/ml in dilution buffer A to generate a calibration curve. Plasma samples were diluted to the appropriate concentration in dilution buffer A. After a further washing step, 50μl of relevant biomarker specific alkaline phosphatase-conjugated antibodies was added at the appropriate concentration in dilution buffer A and incubated for one hour at 37°C. The 96-well plate was then washed 3 times with BBS in the plate washer and 50 μl of Attophos® AP Fluorescent substrate (Promega, Madison, WI) was added. Plates were read immediately on a SpectraMax GEMINI-XS fluorometer microtiter plate reader (Molecular Devices Corporation, Sunnyvale, CA, U.S.A.) ($\lambda_{excitation}$ = 444 nm and $\lambda_{emission}$ = 555 nm).

Results are expressed in RFU and can be obtained in endpoint mode (only one reading) or in kinetic mode for 10 minutes. In kinetic mode, for each well 6 flashes were averaged and each well was analysed 6 times using a minimal interval time between each reading (2 minutes). The slope was calculated and used to determine the final value for each well. The best cut-off value to discriminate between the control and the stroke (Ischemic plus hemorrhagic or Ischemic vs. Hemorrhagic) groups was determined using ROC curves generated in GraphPad Prism 4 software.

The results are shown in Figure 7. This result further demonstrates that Ubiquitin fusion degradation protein 1 homolog (UFD1) is a useful marker for early diagnosis of stroke, alone, or in combination with other biomarkers.

As UFD1 has been found in deceased CSF, it is a reasonable prediction that other polypeptides and proteins found in deceased CSF will also be useful as markers for brain damage-related disorders.

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CLAIMS

1. A method of diagnosis of a brain damage-related disorder or the possibility thereof in a subject suspected of suffering therefrom, which comprises detecting at least one polypeptide, or a variant, mutant or isoform thereof, selected from Table 1 below in a sample of body fluid taken from the subject:

Table 1:

Post-mortem CSF Accession number	Protein name
O00241	Signal-regulatory protein beta-1
O43396	Thioredoxin-like protein 1
O43488	Aflatoxin B1 aldehyde reductase member 2
O43707	Alpha-actinin 4
O75223	Protein C7orf24
O95336	6-phosphogluconolactonase
O95861	3'(2'),5'-bisphosphate nucleotidase 1
P00352	Retinal dehydrogenase 1
P00390	Glutathione reductase, mitochondrial
P00491	Purine nucleoside phosphorylase
P00915	Carbonic anhydrase I
P01859	Ig gamma-2 chain C region*
P01876, P01877	Ig alpha-1 or -2 chain C region
P02024	Hemoglobin beta chain
P02545	Lamin A/C (70 kDa lamin)
P02741	C-reactive protein
P02760	AMBP protein
P04642	L-Lactate dehydrogenase A chain
P04746, P04745, P19961	Alpha-amylase (pancreatic, salivary or 2B)
P05089	Arginase 1
P05209, Q9BQE3	Tubulin alpha-1 or alpha-6 chain
P05413	Fatty acid-binding protein, heart (H-FABP)
P05976 or P06741	myosin light chain 1 or 3, skeletal muscle isoform
P06576	ATP synthase beta chain, mitochondrial
P06753	Tropomyosin alpha 3 chain
P07148	Fatty acid-binding protein, liver (L-FABP)
P07203	Glutathione peroxidase 1
P07225	Vitamin K-dependent protein S
P07226	Tropomyosin alpha 4 chain
P07237	Protein disulphide-isomerase
P07357	Complement C8 alpha chain
P07738	Bisphosphoglycerate mutase
P07900	Heat shock protein HSP 90-alpha (HSP 86)

P07996	Thrombospondin 1
P08059	Glucose-6-phosphate isomerase
P08133	Annexin A6
P08758	Annexin A5
P09417	Dihydropteridine reductase
P09488	Glutathione S-transferase Mu 1
P09493 or P06753	Tropomyosin 1 alpha chain or alpha 3 chain
P09525	Annexin A4
P09668	Cathepsin H
P10586	Receptor-type tyrosine-protein phosphatase F
P10599	Thioredoxin
P10768	Esterase D
P11021	78 kDa glucose-regulated protein
P12833	Myosin heavy chain, cardiac muscle beta isoform
P12882	Myosin heavy chain, skeletal muscle, adult 1
P13489	Placental ribonuclease inhibitor
P13535	Myosin heavy chain, skeletal muscle, perinatal
P13611	Versican core protein
P13693	Translationally controlled tumor protein (TCTP)
P13716	Delta-aminolevulinic acid dehydratase
P13929	Beta enolase
P14136	Glial fibrillary acidic protein, astrocyte (GFAP)
P14550	Alcohol dehydrogenase [NADP+]
P14923	Junction plakoglobin
P15103	Glutamine synthetase
P15121	Aldose reductase
P15259	Phosphoglycerate mutase 2
P15289	Arylsulfatase A
P15924	Desmoplakin
P16930	Fumarylacetoacetase
P17066	Heat shock 70 kDa protein 6
P18206	Vinculin
P21266	Glutathione S-transferase Mu 3
P21333	Filamin A
P21695	Glycerol-3-phosphate dehydrogenase [NAD+], cytoplasmic
P22061	Protein-L-isoaspartate (D-aspartate) O-methyltransferase
P22314	Ubiquitin-activating enzyme E1
P23141	Liver carboxylesterase 1
P24534	Elongation factor 1-beta
P25788	Proteasome subunit alpha type 3

P26038	Moesin
P26641	Elongation factor 1-gamma
P27169	Serum paraoxonase/arylesterase 1
P27348	14-3-3 protein tau
P28072	Proteasome subunit beta type 6
P28161	Glutathione S-transferase Mu 2
P28827	Receptor-type protein-tyrosine phosphatase mu
P29218	Inositol-1 [or 4] -monophosphate
P29401	Transketolase
P30040	Endoplasmic reticulum protein ERp29
P30041	Peroxiredoxin 6
P30101	Protein disulfide-isomerase A3
P30626	Sorcin (22 kDa protein)
P31946	14-3-3 protein beta/alpha
P31948	Stress-induced-phosphoprotein 1
P34932	Heat shock 70 kDa protein 4
P35080	Profilin-2
P35237	Placental thrombin inhibitor
P36980	Complement factor H-related protein 2
P37837	Transaldolase
P40121	Macrophage capping protein
P42126	3,2-trans-enoyl-CoA isomerase, mitochondrial
P42655	14-3-3 protein epsilon
P45381	Aspartoacylase
P46940	Ras GTPase-activating-like protein IQGAP1
P47756	F-actin capping protein beta subunit
P48637	Glutathione synthetase
P49419	Aldehyde dehydrogenase family 7 member A1
P50135	Histamine N-methyltransferase
P50395	Rab GDP dissociation inhibitor beta
P52565	Rho GDP-dissociation inhibitor 1
P52566	Rho GDP-dissociation inhibitor 2
P52907	F-actin capping protein alpha-1 subunit
P54289	Dihydropyridine-sensitive L-type, calcium channel alpha-2/delta subunits
P54652	Heat shock-related 70 kDa protein 2
P54922	ADP-ribosylarginine hydrolase
P55287	Cadherin-11
P55854, P61956	Ubiquitin-like protein SMT 3A or 3B

P57087	Junctional adhesion molecule 2
P60900	Proteasome subunit alpha type 6
P61088	Ubiquitin-conjugating enzyme E2 N
P62258	14-3-3 protein epsilon
P62993	Growth factor receptor-bound protein 2
P63104	14-3-3 protein zeta/delta
P68133	Actin, alpha skeletal muscle
Q00169	Phosphatidylinositol transfer protein alpha isoform
Q01082	Spectrin beta chain, brain 1
Q01995	Transgelin
Q04917	14-3-3 protein eta
Q06033	Inter-alpha-trypsin inhibitor heavy chain H3
Q12765	Secernin 1
Q13332	Receptor-type tyrosine-protein phosphatase S
Q13509	Tubulin beta-4
Q13740	CD166 antigen
Q13813	Spectrin alpha chain, brain
Q13938	Calcyphosine
Q14126	Desmoglein 2
Q15149	Plectin 1
Q15181	Inorganic pyrophosphatase
Q16620	BDNF/NT-3 growth factors receptor
Q16881	Thioredoxin reductase 1, cytoplasmic
Q86UP2	Kinectin
Q86YZ3	Hornerin
Q8N0Y7	Putative phosphoglycerate mutase 3
Q8TAG5	Immunoglobulin-like domain protein MGC33530
Q8TD26	Chromodomain-helicase-DNA-binding protein 6
Q92598	Heat shock protein 105 kDa
Q92890	Ubiquitin fusion degradation protein 1 homolog
Q969H8	Protein C19 or F10 precursor
Q96IU4	CCG1-interacting factor B
Q9BX68	Histidine triad nucleotide-binding protein 2
Q9H477	Ribokinase
Q9NVS9	Pyridoxine-5'-phosphate oxidase

Q9NZT1	Calmodulin-like protein 5
Q9P0L0	Vesicle-associated membrane protein-associated protein A
Q9P121	Neurotrimin
Q9UBQ7	Glyoxylate reductase/hydroxypyruvate reductase
Q9UKK9	ADP-sugar pyrophosphatase
Q9UKX2	Myosin heavy chain, skeletal muscle, adult 2
Q9UN36	NDRG2 protein
Q9Y617	Phosphoserine aminotransferase
Q9Y623	Myosin heavy chain, skeletal muscle, fetal

Ante-mortem CSF

P00748	Coagulation factor XII
P01833	polymeric-immunoglobulin receptor
P04083	Annexin A1
P04121	Macrophage capping protein
P05109	Calgranulin A (MRP-8)
P12109	Collagen alpha 1(VI) chain
P22352	Plasma glutathione peroxidase
P35247	Pulmonary surfactant-associated protein D
P43121	Cell surface glycoprotein MUC18
P58876 + others	Histone H2B (different forms)
P78509	Reelin

Trembl accession no.	Description
O95784	IgG Fc binding protein (Fragment)
Q07898, Q07899, Q07900, Q07901, Q86VB7	M130 antigen; M130 antigen cytoplasmic variant 1; variant 2; M130 antigen extracellular variant; Similar to CD163 antigen
Q7Z664	Hypothetical protein DKFZp779N0926 (Fragment)
Q7Z623	hypothetical protein
Q8IZY7	Hepatocellular carcinoma associated protein TB6
Q8N240	Hypothetical protein FLJ34957
Q8N466	Hypothetical protein with 1 extra peptide over SP entry (Contactin Q12860)
Q8NCW5	ApoA-I binding protein precursor
Q8NFZ8 or Q9Y4A4	TSLC1-like 2 or F22162_1 (Fragment)
Q969J9	Hypothetical protein (Similar to dystroglycan 1)
Q96AC3, Q96FV2, Q9BU04	Hypothetical protein, Ses2 protein, Similar to KIAA0193 gene product (Fragment)

Q96B89, Q9H3J8, Q9HC37, Q9HC38, Q9Y3E8	Hypothetical protein, My027 protein, Hypothetical protein, Hypothetical protein, CGI-150 protein
Q96B89, Q9H3J8, Q9HC37, Q9HC38, Q9Y3E8	various names
Q96B89, Q9H3J8, Q9HC38, Q9Y3E8	Hypothetical protein, My027 protein, Hypothetical protein, CGI-150 protein
Q96EI3, Q9H0W9	Hypothetical protein
Q96NV4, Q9H0R4	Hypothetical protein FLJ30028, Hypothetical protein
Q9H008	Phospholysine phosphohistidine inorganic pyrophosphate phosphatase
Q9H2Y2, Q9NPH2, Q9NVW7	Inositol 1-phosphate synthase, Myo-inositol 1-phosphate synthase A1, Hypothetical protein FLJ10463
Q9NQ56, Q9NQ48	Leucine zipper transcription factor-like 1
Q9NX46	DJ665N4.2 (Similar to hypothetical protein FLJ20446) (ADP-ribosyl-hydrolase precursor)
Q9Y5Z5, Q9NRV9	Heme-binding protein, Heme-binding protein (Hypothetical protein)
Q9Y6R7	Human Fc gamma BP (Fragment)

2. A method according to Claim 1, in which the polypeptide is differentially contained in the body fluid of brain damage-related disorder-affected subjects and non-brain damage-related disorder-affected subjects, and the method includes determining whether the concentration of polypeptide in the sample is consistent with a diagnosis of brain damage-related disorder.

3. A method according to Claim 1 or 2, in which an antibody to the polypeptide is used in the detection or the determination of the concentration.

4. A method according to any of Claims 1 to 3, in which the body fluid is cerebrospinal fluid, plasma, serum, blood, tears, urine or saliva.

5. A method according to any of Claims 1 to 4, in which the polypeptide is present in the body fluid of brain damage-related disorder-affected subjects and not present in the body fluid of non-brain damage-related disorder-affected

subjects, whereby the presence of the polypeptide in a body fluid sample is indicative of brain damage-related disorder.

6. A method according to any of Claims 1 to 4, in which the polypeptide is not present in the body fluid of brain damage-related disorder-affected subjects and present in the body fluid of non-brain damage-related disorder-affected subjects, whereby the non-presence of the polypeptide in a body fluid sample is indicative of brain damage-related disorder.

7. A method according to any of Claims 1 to 6, in which a plurality of peptides is determined in the sample.

8. A method according to any of Claims 1 to 7, in which one or more specific isoforms of the polypeptide are determined.

9. A method according to Claim 8, in which diagnosis is made on the basis of differing levels of specific isoforms of the polypeptide.

10. A method according to any of Claims 1 to 9, in which the polypeptide is differentially subject to post-translational modification in the body fluid of brain damage-related disorder-affected subjects and non-brain damage-related disorder-affected subjects, and the method includes detecting the post-translational modification of the polypeptide in the sample and determining whether this is consistent with a diagnosis of a brain damage-related disorder.

11. A method according to Claim 10, in which the post-translational modification comprises N-glycosylation.

12. A method according to any of Claims 1 to 11, in which at least one autoantibody to one or more of the polypeptides of Table 1 is determined.

13. A method according to any of Claims 1 to 12, in which two or more markers selected from antibodies to polypeptides of Table 1 are used in a single well of an ELISA microtiter plate.

14. A method according to any of Claims 1 to 13, in which two or more polypeptides selected from Table 1 are separately assayed, and a predictive algorithm is used for diagnosis.

15. Use of a polypeptide, or a variant or mutant thereof, selected from Table 1, or a combination of such polypeptides, for diagnostic, prognostic and therapeutic applications relating to brain damage-related disorders.

16. Use according to Claim 15, in which the polypeptide is differentially contained in a body fluid of brain damage-related disorder-affected subjects and non-brain damage-related disorder-affected subjects.

17. Use according to Claim 15 or 16, in which a vaccine directed against a polypeptide, or a variant or mutant thereof, selected from Table 1, or an antigenic determinant thereof, is administered to a subject.

18. Use for diagnostic, prognostic and therapeutic applications, relating to brain damage-related disorders, of a material which recognises, binds to or has affinity for a polypeptide, or a variant or mutant thereof, selected from Table 1.

19. Use according to Claim 18 of a combination of materials, each of which respectively recognises, binds to or has affinity for a polypeptide, or a variant or mutant thereof, selected from Table 1.

20. Use according to Claim 18 or 19, in which the or each material is an antibody or antibody chip.

21. Use according to Claim 20, in which the material is an antibody to any polypeptide listed in Table 1, or a variant or mutant thereof.

22. An assay device for use in the diagnosis of brain damage-related disorders, which comprises a solid substrate having a location containing a material, which

recognizes, binds to or has affinity for a polypeptide, or a variant or mutant thereof, selected from Table 1.

23. An assay device according to Claim 22, in which the solid substrate has a plurality of locations each respectively containing a material which recognizes, binds to or has affinity for a polypeptide, or a variant or mutant thereof, selected from Table 1.

24. An assay device according to Claim 22 or 23, in which the material is an antibody or antibody chip.

25. An assay device according to Claim 24, which has a unique addressable location for each antibody, thereby to permit an assay readout for each individual polypeptide or for any combination of polypeptides.

26. An assay device according to any of Claims 22 to 25, including an antibody to any polypeptide listed in Table 1, or a variant or mutant thereof.

27. An assay device according to any of Claims 22 to 26, further having a location containing a material which recognizes, binds to or has affinity for glutathione S transferase P.

28. An assay device according to Claim 27, in which the material is an antibody or antibody chip.

29. A kit for use in the diagnosis of brain damage-related disorders, comprising an assay device according to any of Claims 22 to 28, and means for detecting the amount of one or more of the polypeptides in a sample of body fluid taken from a subject.

Application No:	GB0514435.7	**Examiner:**	Dr Rowena Dinham
Claims searched:	1-29 (in part)	**Date of search:**	28 November 2005

Patents Act 1977: Search Report under Section 17

Documents considered to be relevant:

Category	Relevant to claims	Identity of document and passage or figure of particular relevance
A	-	US 2003/0109002 A1 (ULLRICH) See especially para [0009]-[0011, Figure 1 and Examples
A	-	Nature; Vol 386, pp 181-186 (1997). Kharitonenkov et al. "A family of proteins that inhibit signalling through tyrosine kinase receptors" See entire document, especially Figure 1
A	-	US 2003/0054415 A1 (BUHRING) See entire document, especially para [0017]-[0024] and Examples
A	-	WO 97/48723 A2 (MAX-PLANCK-GESELLSCHAFT) see especially page 7 line 33-page 8 line 15, Examples 13-17 and Figure 5
A	-	J Neurovirol; Vol 9 (suppl 3), pg 82 (2003). Sulkowski et al. "SIRP signalling modulates neuronal Akt" See Abstract
A	-	J Immunol; Vol 161, pp 1853-1859 (1998). Adams et al. "Signal-regulatory protein is selectively expressed by myeloid and neuronal cells" See entire document, especially Results and Discussion

Categories:

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category	P	Document published on or after the declared priority date but before the filing date of this invention
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.

Field of Search:

Search of GB, EP, WO & US patent documents classified in the following areas of the UKC[X] :

Worldwide search of patent documents classified in the following areas of the IPC[07]

The following online and other databases have been used in the preparation of this search report

WPI, EPODOC, JAPIO, MEDLINE, EMBASE, BIOSIS, CAPLUS, SCISEARCH, BLASTp, tBLASTn