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(54) Title: DIFFERENTIAL DIAGNOSIS OF NEUROLOGICAL DISEASES

(57) Abstract: The present invention provides a method for the differential diagnosis of an individual suffering from Alzheimer's disease versus an individual suffering from another neurological disease. More specifically, the present invention provides a method for the differential diagnosis of an individual suffering from Alzheimer's disease versus an individual suffering from dementia with Lewy bodies, versus an individual suffering from Parkinson's disease without dementia, versus an individual suffering from multi-system atrophy and/or versus an individual suffering from progressive supranuclear palsy, said method characterized that phospho-tau is used as a neurological marker.



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DIFFERENTIAL DIAGNOSIS OF NEUROLOGICAL DISEASES

FIELD OF THE INVENTION

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The present invention relates to the field of the diagnosis of neurological diseases. The present invention provides a new method for the differential diagnosis of Alzheimer's disease versus other neurological diseases. More particular, the present invention provides a method for the differential diagnosis of Alzheimer's disease versus dementia with Lewy bodies, versus Parkinson's disease without dementia, versus multi-system atrophy and/or versus progressive supranuclear palsy.

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BACKGROUND OF THE INVENTION

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The use of tau and phospho-tau as neurological markers in the diagnosis of neurological diseases has been postulated (Blennow et al., 1995; Vigo-Pelfrey et al., 1995; Andreasen et al., 1998; Andreasen et al., 1999a; Ishiguro et al., 1999). The microtubule-associated protein tau is a major protein component of paired helical filaments (PHF) and neurofibrillar tangles (NFT), associated with Alzheimer's disease (Brion et al., 1985; Delacourte and Defossez, 1986; Grundke-Iqbal et al., 1986; Kosik et al., 1986; Wood et al., 1986; Kondo et al., 1988). Tau protein exists in different isoforms, of which 4 to 6 are found in adult brain but only 1 isoform is detected in fetal brain. The diversity of the isoforms is generated from a single gene on human chromosome 17 by alternative mRNA splicing (Himmler, 1989; Goedert et al., 1989; Andreadis et al., 1992). The most striking feature of tau protein, as deduced from molecular cloning, is a stretch of 31 or 32 amino acids, occurring in the carboxy-terminal part of the molecule, which can be repeated either 3 or 4 times. Additional diversity is generated through 29 or 58 amino acid-long insertions in the NH₂-terminal part of tau molecules (Goedert et al., 1989). *In vivo* tau promotes microtubule assembly and stability in the axonal compartment of neurons by interactions involving its microtubule binding domain which is localized in the repeat

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region of tau (255-381) (Lewis et al., 1988). In normal circumstances adult brain contains 2-3 mole phosphate per mole of tau (Selden and Pollard, 1983; Ksiezak-Reding et al., 1992). Phosphorylation of different sites in normal tau as studied in rat and humans is dependent on the developmental state (Lee et al., 1991; Bramblett et al., 1993; Goedert et al., 1993). Tau variants of 60, 64 and 68 kDa arising as a consequence of phosphorylation have been detected in brain areas showing neurofibrillary tangles (Delacourte et al., 1990; Goedert et al., 1992; Flament et al., 1990, Greenberg and Davies, 1990). These brains contain 6-8 mole phosphate per mole tau (Ksiezak-Reding et al., 1992). In tau isolated from PHF (PHF-tau), phosphorylation occurs at several positions (Iqbal et al., 1989; Lee et al., 1991; Hasegawa et al., 1992; Hanger et al., 1998; Buee et al., 1999).

Alzheimer's disease (AD) and frontotemporal dementia (FTD) are the most common types of primary degenerative dementia associated with a tau pathology, having a prevalence of respectively 42-75% and 8-10% (Brun, 1993; Gustafson, 1993; Ebly et al., 1994). Filamentous tau pathology i.e. neurofibrillary tangles (NFT), are consistently found in AD (Tomlinson and Corsellis, 1984) but may also be found in FTD (Spillantini and Goedert, 1998). Pathological tau proteins are found both in AD and FTD (Vermersch et al., 1995; Delacourte et al., 1996). Studies on brain tissue, however, have suggested that the tau pathology differs between AD and FTD, possibly being related to the degree of phosphorylation (Delacourte et al., 1996). Other forms of dementia associated with a tau pathology include familial FTD, progressive supranuclear palsy (PSP), corticobasal degeneration (CBD) and subacute sclerosing panencephalitis. The role of hyperphosphorylation in the pathology of these tauopathies is at present not well understood.

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Dementia with Lewy bodies (DLB) is an illness that presents with progressive dementia or psychosis. Parkinsonian signs, which may be absent or mild at the onset, eventually become common and rigidity is usually severe. Lewy bodies are found profusely in the brainstem, basal forebrain, hypothalamic nuclei and neocortex. Dementia with Lewy bodies is characterized by the relative absence of tangles and hyperphosphorylated tau in the brain. Parkinson's disease (PD) is a type of Lewy Body disease occurring in the

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middle or late life, with very gradual progression and a prolonged course. It can be considered as an example of neuronal system disease, involving mainly the nigrostriatal dopaminergic system. Dementia with Lewy bodies was recently defined as a special form of dementia requiring differential patient management (Lebert et al., 1998; McKeith et al., 1999). Dementia with Lewy bodies, which is sensitive to neuroleptics, is clinically very difficult to differentiate from Alzheimer's disease (McKeith et al., 1996; Ballard et al., 1998). Most patients (more than 75%) are neuropathologically defined as Alzheimer's disease patients while it is estimated that 15 to 25 % of the clinically diagnosed Alzheimer's disease patients have dementia with Lewy bodies (Hooten et al., 1998). As dementia with Lewy bodies is more susceptible to acetylcholinesterase treatment, differentiation of dementia with Lewy bodies from Alzheimer's disease is essential for optimization of treatment (Levy et al., 1994; Perry et al., 1994; Wilcock et al., 1994).

Cerebrospinal fluid (CSF) β -amyloid and CSF-tau have been validated to discriminate Alzheimer's disease from normal aging, depression and Parkinson's disease (Galasko et al., 1998; Kanai et al., 1998; Hulstaert et al., 1999) and these markers are well suited for the differential diagnosis of these disorders (Andreasen et al., 1999b). More controversial, however, is their role in the discrimination of Alzheimer's disease from closely related conditions such as dementia with Lewy bodies and from other dementia associated with tau pathology such as frontotemporal dementia, multi-system atrophy (MSA) and/or progressive supranuclear palsy. At present, no accurate methods exist for the differential diagnosis of these neurological diseases.

AIMS OF THE INVENTION

It is an aim of the present invention to provide a method for the differential diagnosis of an individual suffering from Alzheimer's disease versus an individual suffering from another neurological disease.

It is an aim of the present invention to provide a method for the differential diagnosis of an individual suffering from Alzheimer's disease versus an individual suffering from dementia with Lewy bodies.

It is an aim of the present invention to provide a method for the differential diagnosis of an individual suffering from Alzheimer's disease versus an individual suffering from Parkinson's disease without dementia.

It is an aim of the present invention to provide a method for the differential diagnosis of an individual suffering from Alzheimer's disease versus an individual suffering from multi-system atrophy.

It is an aim of the present invention to provide a method for the differential diagnosis of an individual suffering from Alzheimer's disease versus an individual suffering from progressive supranuclear palsy.

It is an aim of the present invention to provide an *in vitro* method for the differential diagnosis of an individual suffering from Alzheimer's disease versus an individual suffering from another neurological disease.

It is an aim of the present invention to provide an *in vitro* method for the differential diagnosis of an individual suffering from Alzheimer's disease versus an individual suffering from dementia with Lewy bodies.

It is an aim of the present invention to provide an *in vitro* method for the differential diagnosis of an individual suffering from Alzheimer's disease versus an individual suffering from Parkinson's disease without dementia.

It is an aim of the present invention to provide an *in vitro* method for the differential diagnosis of an individual suffering from Alzheimer's disease versus an individual suffering from multi-system atrophy.

It is an aim of the present invention to provide an *in vitro* method for the differential diagnosis of an individual suffering from Alzheimer's disease versus an individual suffering from progressive supranuclear palsy.

5 It is an aim of the present invention to provide a diagnostic kit for the differential diagnosis of an individual suffering from Alzheimer's disease versus an individual suffering from another neurological disease.

It is an aim of the present invention to provide a diagnostic kit for the differential diagnosis of an individual suffering from Alzheimer's disease versus an individual suffering from dementia with Lewy bodies.

10 It is an aim of the present invention to provide a diagnostic kit for the differential diagnosis of an individual suffering from Alzheimer's disease versus an individual suffering from Parkinson's disease without dementia.

15 It is an aim of the present invention to provide a diagnostic kit for the differential diagnosis of an individual suffering from Alzheimer's disease versus an individual suffering from multi-system atrophy.

It is an aim of the present invention to provide a diagnostic kit for the differential diagnosis of an individual suffering from Alzheimer's disease versus an individual suffering from progressive supranuclear palsy.

20 It is an aim of the present invention to provide a method to screen or monitor the effect of compounds which prevent dementia with Lewy bodies, Parkinson's disease without dementia, multi-system atrophy and/or progressive supranuclear palsy or which treat an individual suffering from dementia with Lewy bodies, an individual suffering from Parkinson's disease without dementia, an individual suffering from multi-system atrophy and/or an individual suffering from progressive supranuclear palsy.

TABLES

Table 1: Demographic data and CSF results by different diagnostic groups.

Group	Subjects, n (M/F)	Age, y, median, (range)	MMSE	Aβ42, pM,		Tau, pM,		Ptau(181), pM,	
				median (p25-p75)	median (p25-p75)	median (p25-p75)	median (p25-p75)	median (p25-p75)	median (p25-p75)
			n	Median					
				(p25-p75)					
AD	80 (35/45)	72 (53-86)	78	22 (14-24)	69.2 (47.1-96.0)*	13.2 (9.4-17.0)*	14.7 (11.6-19.1)*		
Controls	40 (20/20)	70 (56-84)	20	30 (29-30)	99.3 (75.5-145.3)\$	3.0 (2.1-4.0)\$	7.8 (6.4-8.9)\$		
FTD	69 (42/27)	67 (40-94)	61	22 (16-25)	90.3 (67.0-132.5)\$	7.5 (5.2-10.8)£,\$	9.4 (8.2-12.3)£,\$		
LBD	43 (35/8)	72 (61-87)	37	19 (14-24)	72.2 (53-103)£	5.7 (1.6-9.0)\$	8.1 (6.1-10.0)\$		
PD	15 (8/7)	70 (51-79)	1	23	72.3 (58.1-99.1)	4.2 (2.2-7.6)\$	7.4 (6.9-8.8)\$		
MSA	16 (11/5)	64 (42-77)	3	20 (20-22)	91.5 (41.9-113.4)	5.3 (3.8-8.3)\$	7.6 (6.2-10.9)\$		
PSP	15 (11/4)	67 (64-76)	4	26 (21-27)	96.6 (82.2-101.1)	2.8 (2.0-4.5)\$	6.9 (6.1-7.5)\$		
CBD	5 (0/5)	70 (57-75)	4	13 (12-15)	70.2 (43.2-71.2)	12.9 (9.8-15.4)	12.7 (9.1-13.1)		

AD = Alzheimer's disease, FTD = frontotemporal dementia, DLB = dementia with Lewy bodies, PD = Parkinson's disease,

MSA = multi-system atrophy, PSP = progressive supranuclear palsy, CBD = corticobasal degeneration

*Significantly different from controls (p<0.001)

£Significantly different from controls (p<0.05)

\$\$Significantly different from AD (p<0.001)

Table 2: Comparison of the discriminative power of CSF-tau and CSF-phospho-tau using ROC analysis.

Groups	CSF-tau (AUC \pm SE)	CSF-phospho-tau (AUC \pm SE)	p-value
AD vs controls (n=40)	0.862 \pm 0.038	0.897 \pm 0.032	0.191
AD vs FTD (n=69)	0.711 \pm 0.045	0.754 \pm 0.044	0.049
AD vs DLB (n=43)	0.782 \pm 0.048	0.839 \pm 0.042	0.039
AD vs Parkinson related conditions (n=46)	0.873 \pm 0.035	0.864 \pm 0.037	0.319

Receiver operating curve (ROC) analysis with area under the curve (AUC) and standard error (SE) calculated according to Hanley and McNeil.

- 5 AD = Alzheimer's disease, FTD = frontotemporal dementia, DLB = dementia with Lewy bodies, Parkinson-related conditions include Parkinson's disease without dementia (n=15), multiple system atrophy (n=16) and progressive supranuclear palsy (n=15).

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a method for the differential diagnosis of an individual suffering from Alzheimer's disease (AD) versus an individual suffering from another neurological disease characterized that phospho-tau is used as a neurological marker. More specifically, the present invention relates to a method as described above comprising the steps of:

- determining the level of phospho-tau in said individual;
- inference that said individual is suffering from a neurological disease other than Alzheimer's disease by comparing the obtained level of phospho-tau in said individual with the level of phospho-tau in individuals suffering from Alzheimer's disease, whereby a decreased level of phospho-tau being an indication that said individual is suffering from a neurological disease other than Alzheimer's disease.

'Differential diagnosis of an individual suffering from a neurological disease versus an individual suffering from another neurological disease' as used in the present invention refers to the discrimination between said first neurological disease and other neurological diseases in this way that a certain neurological disease or a certain cause of neurological disorder in an individual is associated with a certain neurodegenerative condition of said individual. The method of the present invention allows the differential diagnosis of an individual suffering from Alzheimer's disease versus an individual suffering from a neurological disease other than Alzheimer's disease. In a specific embodiment, the present invention allows the differential diagnosis of an individual suffering from Alzheimer's disease (AD) versus an individual suffering from dementia with Lewy bodies (DLB). In another specific embodiment, the present invention allows the differential diagnosis of an individual suffering from Alzheimer's disease (AD) versus an individual suffering from Parkinson's disease (PD) without dementia. In another specific embodiment, the present invention allows the differential diagnosis of an individual suffering from Alzheimer's disease (AD) versus an individual suffering from multi-system atrophy (MSA). In another specific embodiment, the present invention allows the differential diagnosis of an individual suffering from Alzheimer's disease (AD) versus an

individual suffering from progressive supranuclear palsy (PSP). Alzheimer's disease, dementia with Lewy bodies, Parkinson's disease without dementia, multi-system atrophy and progressive supranuclear palsy as well as other neurological diseases have been described in detail by Wilson et al. (1991) and McKeith et al. (1999).

5 The present invention is based on the finding that the level of phospho-tau in CSF from individuals suffering from dementia with Lewy bodies, from PD without dementia, from MSA or from PSP is significantly decreased compared to the level of phospho-tau in CSF from individuals suffering from Alzheimer's disease. The indication that the level of phospho-tau differs between said neurological diseases, forms the basis for the
10 development of a diagnostic test for the differential diagnosis of said neurological diseases in an individual. Accordingly, the present invention relates to a method for the differential diagnosis of an individual suffering from Alzheimer's disease versus an individual suffering from dementia with Lewy bodies, versus an individual suffering from Parkinson's disease without dementia, versus an individual suffering from multi-
15 system atrophy and/or versus an individual suffering from progressive supranuclear palsy, said method characterized that phospho-tau is used as a neurological marker.

The method of the invention thus comprises the step of determining the level of phospho-tau in said individual suspected of suffering from AD, from DLB, from PD without dementia, from MSA or from PSP and comparing it with a previously defined phospho-tau level range characteristic for AD, DLB, PD without dementia, MSA or PSP. A level
20 of phospho-tau falling within the previously defined phospho-tau level range for Alzheimer's disease is an indication that said individual is suffering from AD. A level of phospho-tau falling within the previously defined phospho-tau level range for DLB is an indication that said individual is suffering from DLB. A level of phospho-tau falling
25 within the previously defined phospho-tau level range for PD without dementia is an indication that said individual is suffering from PD without dementia. A level of phospho-tau falling within the previously defined phospho-tau level range for MSA is an indication that said individual is suffering from MSA. A level of phospho-tau falling
30 within the previously defined phospho-tau level range for PSP is an indication that said individual is suffering from PSP.

Accordingly, the present invention relates to a method for the differential diagnosis of an individual suffering from Alzheimer's disease versus an individual suffering from dementia with Lewy bodies, versus an individual suffering from Parkinson's disease without dementia, versus an individual suffering from multi-system atrophy and/or versus an individual suffering from progressive supranuclear palsy, comprising the steps of:

- determining the level of phospho-tau in said individual;
- inference that said individual is suffering from DLB, from PD without dementia, from MSA and/or from PSP by comparing the obtained level of phospho-tau in said individual with the level of phospho-tau in individuals suffering from AD, whereby a decreased level of phospho-tau being an indication that said individual is suffering from DLB, from PD without dementia, from MSA and/or from PSP.

The level of phospho-tau can be detected *in vitro* as well as *in vivo*. The method for the *in vitro* detection of the level of phospho-tau in an individual comprises the steps of obtaining a sample from said individual, determining the level of phospho-tau in said sample and comparing it with a previously defined phospho-tau level range for said neurological disease.

The term 'sample' refers to any source of biological material, for instance body fluids, brain extract, peripheral blood or any other sample comprising phospho-tau protein. In a preferred embodiment, the level of phospho-tau is determined *in vitro* by analysis of the level of phospho-tau in a body fluid sample of the patient. The term 'body fluid' refers to all fluids that are present in the human body including but not limited to blood, lymph, urine and cerebrospinal fluid (CSF) comprising phospho-tau protein. The blood sample may include a plasma sample or a serum sample.

In a preferred embodiment of the present invention the level of phospho-tau is determined in a cerebrospinal fluid sample taken from the patient. In accordance, the present invention relates to a method as described above, comprising the steps of:

- obtaining a cerebrospinal fluid sample from said individual;
- determining the level of phospho-tau in said cerebrospinal fluid sample;

- inference that said individual is suffering from a neurological disease other than AD such as DLB, PD without dementia, MSA and/or PSP by comparing the obtained level of phospho-tau in said cerebrospinal fluid sample with the level of phospho-tau in a cerebrospinal fluid sample from individuals suffering from AD, whereby a decreased level of phospho-tau being an indication that said individual is suffering from another neurological disease such as DLB, PD without dementia, MSA and/or PSP.

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10 The level of phospho-tau as measured in the CSF of the individual suspected to suffer from DLB, from PD without dementia, from MSA and/or from PSP is compared with the level of phospho-tau in the CSF of an individual suffering from AD. A decreased level of CSF-phospho-tau is interpreted as an indication of the individual suffering from DLB, from PD without dementia, from MSA and/or from PSP.

15 Phospho-tau include all forms of tau that have a phosphorylation on any position of the tau protein sequence, but more specifically it refers to phosphorylations on amino acid positions that are not phosphorylated in human normal tau isolated from adult individuals not suffering from any neurological disorder.

‘A decreased level of phospho-tau’ means that the level of phospho-tau measured in the patient is lower than the level of phospho-tau measured in patients suffering form AD.

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Phospho-tau can be quantified by any method known in the art, including but not limited to the use of antibodies. In a preferred embodiment, phospho-tau is quantified by an immunoassay comprising at least the following steps:

- obtaining a sample from the patient;
- 25 - bringing said sample into contact with a monoclonal antibody specifically recognizing phospho-tau, under conditions being suitable for producing an antigen-antibody complex;
- detecting the immunological binding of said antibody to said sample.

In another embodiment, phospho-tau can be quantified by a sandwich ELISA comprising the following steps:

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- obtaining a sample from the patient;

- bringing said sample into contact with an antibody (primary antibody or capturing antibody) recognizing phospho-tau, under conditions being suitable for producing an antigen-antibody complex;
- bringing said sample into contact with a monoclonal antibody (secondary antibody or detector antibody) specifically recognizing phospho-tau, under conditions being suitable for producing an antigen-antibody complex;
- bringing the antigen-antibody complex into contact with a marker either for specific tagging or coupling with said secondary antibody, with said marker being any possible marker known to the person skilled in the art;
- possibly also, for standardization purposes, bringing the antibodies in contact with a purified phospho-tau protein or phospho-peptide reactive with both antibodies.

Advantageously, the secondary antibody itself carries a marker or a group for direct or indirect coupling with a marker.

The expression 'recognizing', 'reacting with', 'immunological binding' or 'producing an antigen-antibody complex' as used in the present invention is to be interpreted that binding between the antigen and antibody occurs under all conditions that respect the immunological properties of the antibody and the antigen.

The expression 'specifically recognizing' as used in the present invention is to be interpreted that said antibody is capable of forming an immunological complex with phospho-tau but not with human normal tau.

Any monoclonal antibody that specifically recognizes phospho-tau can be used in said method for the quantification of phospho-tau. Monoclonal antibodies for use in the quantification of phospho-tau include AT8 (WO 93/08302), AT180 and AT270 (WO 95/17429) and AT100 (WO 96/04309). Other antibodies known in the art that specifically recognize phospho-tau can be used as well.

Also fragments derived from these monoclonal antibodies such as Fab, F(ab)₂, ssFv ('single chain variable fragment') and other antibody-like constructs that retain the variable region of the antibody, providing they have retained the original binding properties, can be used in a method of the present invention. Such fragments are commonly generated by, for instance, enzymatic digestion of the antibodies with papain, pepsin, or other proteases. It is well

known to the person skilled in the art that monoclonal antibodies, or fragments thereof, can be modified for various uses. Also mini-antibodies and multivalent antibodies such as diabodies, triabodies, tetravalent antibodies and peptabodies can be used in a method of the invention. The preparation and use of these fragments and multivalent antibodies has been
5 described extensively in International Patent Application WO 98/29442.

The monoclonal antibodies used in a method of the invention may be humanized versions of the mouse monoclonal antibodies made by means of recombinant DNA technology, departing from the mouse and/or human genomic DNA sequences coding for H and L chains or from cDNA clones coding for H and L chains. Alternatively the monoclonal
10 antibodies used in a method of the invention may be human monoclonal antibodies. The term 'humanized antibody' means that at least a portion of the framework regions of an immunoglobulin is derived from human immunoglobulin sequences.

The antibodies used in a method of the present invention may be labeled by an appropriate label of the enzymatic, fluorescent, or radioactive type.

15 The method for the *in vivo* detection of the level of phospho-tau in an individual comprises the steps of determining the level of phospho-tau in said individual and comparing it with a previously defined phospho-tau level range characteristic for AD, DLB, PD without dementia, MSA or PSP. In an embodiment of the invention, phospho-tau can be quantified by *in vivo* imaging. Phospho-tau can be quantified *in situ* by non-
20 invasive methods including but not limited to brain imaging methods described by Arbit et al. (1995), Tamada et al. (1995), Wakabayashi et al. (1995), Huang et al. (1996), Sandroock et al. (1996), Mariani et al. (1997). These *in vivo* imaging methods may allow the localization and quantification of phospho-tau, for example, by use of labeled antibodies specifically recognizing phospho-tau.

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The present invention thus relates to the use of phospho-tau as a neurological marker for the differential diagnosis of an individual suffering from AD versus an individual suffering another neurological disease. The present invention specifically relates to the use of phospho-tau as a neurological marker for the differential diagnosis of an individual
30 suffering from AD versus an individual suffering from DLB, versus an individual

suffering from PD without dementia, versus an individual suffering from MSA and/or versus an individual suffering from PSP.

The present invention also relates to the use of phospho-tau as a neurological marker for the manufacture of a diagnostic kit for the differential diagnosis of an individual suffering
5 from Alzheimer's disease versus an individual suffering from another neurological disease. The present invention specifically relates to the use of phospho-tau as a neurological marker for the manufacture of a diagnostic kit for the differential diagnosis of an individual suffering from Alzheimer's disease versus an individual suffering from DLB, versus an individual suffering from PD without dementia, versus an individual
10 suffering from MSA and/or versus an individual suffering from PSP.

The present invention also relates to the use of an antibody that specifically recognizes phospho-tau for the manufacture of a diagnostic kit for the differential diagnosis of an individual suffering from Alzheimer's disease versus an individual suffering from another neurological disease. The present invention specifically relates to the use of an
15 antibody that specifically recognizes phospho-tau for the manufacture of a diagnostic kit for the differential diagnosis of an individual suffering from Alzheimer's disease versus an individual suffering from DLB, versus an individual suffering from PD without dementia, versus an individual suffering from MSA and/or versus an individual suffering from PSP.

In accordance, the present invention also relates to a diagnostic kit for use in the
20 differential diagnosis of an individual suffering from Alzheimer's disease versus an individual suffering from another neurological disease, comprising an antibody that specifically recognizes phospho-tau. The present invention specifically relates to a diagnostic kit for use in the differential diagnosis of an individual suffering from
25 Alzheimer's disease versus an individual suffering from DLB, versus an individual suffering from PD without dementia, versus an individual suffering from MSA and/or versus an individual suffering from PSP, comprising an antibody that specifically recognizes phospho-tau.

A possible kit for carrying out the method of the invention is based on an immunoassay
30 and comprises:

- an antibody (primary antibody) which forms an immunological complex with an epitope of phospho-tau;
- a monoclonal antibody (secondary antibody) which specifically recognizes phospho-tau;
- 5 - a marker either for specific tagging or coupling with said secondary antibody;
- appropriate buffer solutions for carrying out the immunological reaction between the primary antibody and the test sample, between the secondary antibody and the test sample and/or between the bound
- 10 secondary antibody and the marker;
- possibly, a purified phospho-tau protein or a phospho-peptide for standardization purposes.

The present invention further relates to the use of a diagnostic kit as described above for the differential diagnosis of an individual suffering from Alzheimer's disease versus an individual suffering from another neurological disease. The present invention specifically

15 relates to the use of a diagnostic kit as described above for the differential diagnosis of an individual suffering from Alzheimer's disease versus an individual suffering from DLB, versus an individual suffering from PD without dementia, versus an individual suffering from MSA and/or versus an individual suffering from PSP.

20 The invention also relates to a method to screen or monitor the effect on an individual of compounds which prevent or treat Alzheimer's disease, dementia with Lewy bodies, Parkinson's disease without dementia, multi-system atrophy and/or progressive supranuclear paly.

25 Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of stated integers or steps but not to the exclusion of any other integer or step or group of integers or steps.

The present invention will now be illustrated by reference to the following examples that set forth particularly advantageous embodiments. However, it should be noted that these examples are illustrative and can not be construed as to restrict the invention in any way.

EXAMPLES

Subjects and methods

5 14 university centers involved in CSF research were contacted to participate in a multicenter study. Each center was requested to include 500 μ l CSF of exactly 10 AD patients (McKhann et al., 1984) and a minimum of 6 samples from patients with DLB (McKeith et al., 1996) or FTD (Anonymous, 1994). Eight centers complied with these conditions. If CSF was available from patients with PSP (Golbe et al, 1993), CBD (Rinne
10 et al., 1994) and multiple system atrophy (MSA) (Colosimo et al., 1995) they were also included in the study. Age-matched controls without neurological and cognitive problems and Parkinson's disease (PD) without dementia (Langston et al., 1992) were included if 10 samples were available per center.

15 The study was performed on CSF samples available for research purposes and if required the protocol was reviewed and approved by the local Independent Ethics Committee/Institutional Review Board (IEC/IRB) prior to the study start.

20 Samples were collected in polypropylene tubes using lumbar puncture. Because it has been demonstrated that freeze-thawing seriously affects β -amyloid₄₂ (A β 42) levels (Andreasen et al., 1999b; Vanderstichele et al., 1998) the number of freeze-thaw cycles was documented. CSF samples that contained > 500 red blood cells per microliter were not included.

25 All determinations were performed at Innogenetics (Gent, Belgium) using standard kits for CSF-A β 42 (K-1080, Innogenetics) (Andreasen et al., 1999b; Vanderstichele et al., 1998), hTau (K-1032, Innogenetics)(Vandermeeren et al., 1993; Blennow et al., 1995; Van de Voorde et al., 1995) and a research version of the INNOTEST PHOSPHO-TAU. The phospho-tau kit is developed using a human-specific antibody, HT7, for capturing
30 and a phospho-threonine-181-specific antibody, AT270, for detection (Goedert et al., 1994). As a standard, a synthetic peptide (Ac-

P₁₅₄RGAAPPGQKGGQANATRIPAKTPPAPKT(p)PPSSGE₁₈₇-NH₂) with the corresponding threonine 181 phosphorylated was used. The reliability and performance of the phospho-tau assay was monitored using 5 pooled CSF samples, covering the performance range of the assay.

5

Statistical methods

Normal distribution of CSF-tau, A β 42 and phospho-tau were tested using the Shapiro-Wilk test and if normality was rejected, non-parametric (Kruskal-Wallis) tests were used for comparison. For comparisons with the AD or control group, p-values were adjusted using Dunn's multiple comparison test. Receiver operating curve (ROC) analysis was used to examine the discriminatory power of tau and phospho-tau between AD and controls, DLB, FTD or Parkinson-related conditions, respectively (Hanley and McNeil, 1983). Possible correlations were determined with the Spearman rank correlation coefficient.

15

CSF-phospho-tau levels in the different neurological disease groups

Overall 294 CSF samples were collected from 8 centers. Transportation of CSF samples from one center took longer than 5 days and samples were thawed during transportation. Consequently, new aliquots of these CSF samples were reshipped, apart from one CSF sample (FTD). Five samples from center 2 (5 FTD) and 5 from center 8 (5 PD) were also not assayed because the amount of CSF that was shipped was not sufficient to perform all tests. In summary, 283 CSF samples from the different diagnostic groups (40 controls, 80 AD, 43 DLB, 69 FTD, 15 PD, 15 PSP, 16 MSA and 5 CBD) were assayed for at least one of the biochemical markers (see Table 1). CSF samples were collected between 1985 and 1999. The percentage of males in the group of patients with DLB is high, as expected.

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The levels of phospho-tau and their confidence intervals were determined on five QC samples which were used to monitor the performance of the phospho-tau tests. Values for all QC samples fell within the established criteria (results not shown).

30

Center effects were observed for A β 42 and tau, but not for phospho-tau in the AD group. An effect of freeze-thawing was observed ($p < 0.0001$) for A β 42, but not for tau and phospho-tau and was particularly pronounced in the AD and DLB groups. The effect was further confirmed in the analysis of CSF from center 03. These samples have been assayed previously on site for both tau and A β 42 and were re-assayed at Innogenetics (Gent, Belgium) after two freeze-thaw cycles. An overall 20% reduction of CSF-A β 42 was observed in 8 of the 10 control samples.

10 No effect of age on the different markers was observed in any of the diagnostic groups. No significant correlation was found between a biomarker and MMSE in the male or female AD group or the total AD group, except for tau and MMSE in the total group (Spearman, $r = -0.275$ [-0.491, -0.059], $p = 0.01$). ApoE genotype did not correlate with CSF-tau or CSF-phospho-tau levels, while a significant correlation was observed for CSF-A β 42 (Spearman, $r = -0.263$ [-0.434, -0.093], $p = 0.003$).

Normality was rejected for A β 42, tau and phospho-tau in all groups and thus analyses were performed using non-parametric tests (Table 1). Significantly increased CSF-tau levels were present in the AD ($p < 0.001$) and FTD group ($p < 0.05$) compared to the control group. Although CSF-tau levels were increased in FTD, levels were significantly higher in the AD group ($p < 0.01$). Using PSP as a reference group, in addition to AD and FTD, CBD had significantly higher CSF-tau levels ($p < 0.05$). For phospho-tau a similar pattern was observed when AD and controls were used to compare. Significantly decreased A β 42 levels are only observed in the AD ($p < 0.001$) and DLB group ($p < 0.05$) compared to the control group and comparing A β 42 levels of all groups with the AD group shows a clearly significant difference between AD and FTD ($p < 0.001$). Since in all the Parkinson-related conditions, except CBD, mean levels of biomarkers were within the normal range, these groups were treated as one group in the subsequent analysis.

30 The strong correlation between tau and phospho-tau for all patients, independent of the diagnostic group ($y = 0.75x + 4.6$, $r = 0.904$, $p < 0.001$) suggests that the discrimination

between AD and other groups obtained with tau can also be obtained with phospho-tau. Using ROC analysis, the discriminative power of total tau with that of phospho-tau was compared. A significant difference between tau and phospho-tau was observed for discriminating AD versus FTD and AD versus DLB, while no difference was observed for AD compared to controls or to the Parkinson-related conditions (Table 2). Since males are overrepresented in the DLB group, the ROC analysis was also performed for male AD versus male DLB with an AUC of 0.800 ± 0.057 , comparable to females and males together.

- 10 Using the previously established discrimination line that combines CSF-tau and CSF- β -amyloid₄₂ ($A\beta_{42} = 240 + 1.18 \text{ tau}$) (Hulstaert et al., 1999), the sensitivity (98% (CI 91-99%)) and specificity for the control population (73% (CI 56-85%)) was comparable to the previous studies. The specificity of this discrimination line for FTD is 77% (CI 66-85%), for DLB 67% (CI 52-80%) and for the Parkinson-related conditions 68% (CI 50-15 81%).

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CLAIMS

- 1) A method for the differential diagnosis of an individual suffering from Alzheimer's disease versus an individual suffering from another neurological disease characterized that phospho-tau is used as a neurological marker.
- 2) A method according to claim 1 comprising the steps of:
- determining the level of phospho-tau in said individual;
 - inference that said individual is suffering from a neurological disease other than Alzheimer's disease by comparing the obtained level of phospho-tau in said individual with the level of phospho-tau in individuals suffering from Alzheimer's disease, whereby a decreased level of phospho-tau being an indication that said individual is suffering from a neurological disease other than Alzheimer's disease.
- 3) A method according to any of claims 1 to 2 further characterized that an individual suffering from Alzheimer's disease is differentially diagnosed versus an individual suffering from dementia with Lewy bodies, versus an individual suffering from Parkinson's disease without dementia, versus an individual suffering from multi-system atrophy and/or versus an individual suffering from progressive supranuclear palsy.
- 4) A method according to claim 3 comprising the steps of:
- determining the level of phospho-tau in said individual;
 - inference that said individual is suffering from dementia with Lewy bodies, from Parkinson's disease without dementia, from multi-system atrophy and/or from progressive supranuclear palsy by comparing the obtained level of phospho-tau in said individual with the level of phospho-tau in individuals suffering from Alzheimer's disease, whereby a decreased level of phospho-tau being an indication that said individual is suffering from dementia with Lewy bodies, from Parkinson's disease

without dementia, from multi-system atrophy and/or from progressive supranuclear palsy.

- 5) A method according to any of claims 1 to 4 further characterized that said method is carried out *in vitro*, on a sample obtained from said individual.
- 6) A method according to claim 5 further characterized that said sample is taken from the cerebrospinal fluid or from blood derivatives of said individual.
- 7) A method according to claim 6 further characterized that said method comprises the steps of:
- obtaining a cerebrospinal fluid sample from said individual;
 - determining the level of phospho-tau in said cerebrospinal fluid sample;
 - inference that said individual is suffering from a neurological disease other than Alzheimer's disease such as dementia with Lewy bodies, Parkinson's disease without dementia, multi-system atrophy and/or progressive supranuclear palsy by comparing the obtained level of phospho-tau in the cerebrospinal fluid sample of said individual with the level of phospho-tau in a cerebrospinal fluid sample of individuals suffering from Alzheimer's disease, whereby a decreased level of phospho-tau being an indication that said individual is suffering from a neurological disease other than Alzheimer's disease such as dementia with Lewy bodies, Parkinson's disease without dementia, multi-system atrophy and/or progressive supranuclear palsy.
- 8) Method according to any of claims 1 to 7 further characterized that the level of phospho-tau is determined immunologically, making use of an antibody that specifically recognizes phospho-tau.

- 9) Use of phospho-tau as a neurological marker for the manufacture of a diagnostic kit for the differential diagnosis of an individual suffering from Alzheimer's disease versus an individual suffering from another neurological disease.
- 5 10) Use of phospho-tau as a neurological marker for the manufacture of a diagnostic kit for the differential diagnosis of an individual suffering from Alzheimer's disease versus an individual suffering from dementia with Lewy bodies, versus an individual suffering from Parkinson's disease without dementia, versus an individual suffering from multi-system atrophy and/or versus an individual suffering from progressive
10 supranuclear palsy.
- 11) Use of an antibody that specifically recognizes phospho-tau for the manufacture of a diagnostic kit for the differential diagnosis of an individual suffering from Alzheimer's disease versus an individual suffering from another neurological disease.
15
- 12) Use of an antibody that specifically recognizes phospho-tau for the manufacture of a diagnostic kit for the differential diagnosis of an individual suffering from Alzheimer's disease versus an individual suffering from dementia with Lewy bodies, versus an individual suffering from Parkinson's disease without dementia, versus an
20 individual suffering from multi-system atrophy and/or versus an individual suffering from progressive supranuclear palsy.
- 13) A diagnostic kit for use in the differential diagnosis of an individual suffering from Alzheimer's disease versus an individual suffering from another neurological disease.
25
- 14) A diagnostic kit for use in the differential diagnosis of an individual suffering from Alzheimer's disease versus an individual suffering from dementia with Lewy bodies, versus an individual suffering from Parkinson's disease without dementia, versus an individual suffering from multi-system atrophy and/or versus an individual suffering
30 progressive supranuclear palsy.

- 15) Method to screen or monitor the effect on an individual of compounds which prevent or treat Alzheimer's disease, dementia with Lewy bodies, Parkinson's disease without dementia, multi-system atrophy and/or progressive supranuclear palsy, said method comprising the step of determining the level of phospho-tau in said individual.

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INTERNATIONAL SEARCH REPORT

International Application No

CT/EP 01/07029

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/68

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 7 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)

MEDLINE, EPO-Internal, CHEM ABS Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CHEMICAL ABSTRACTS, vol. 133, no. 9, 28 August 2000 (2000-08-28) Columbus, Ohio, US; abstract no. 117039, E. VANMECHELEN ET AL.: "Quantification of tau phosphorylated at threonine 181 in human cerebrospinal fluid: a sandwich ELISA with a synthetic phosphopeptide for standardization." page 328; column 1; XP002154344 abstract & NEUROSCI. LETT., vol. 285, no. 1, 2000, pages 49-52, ----- -/--	1-15

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

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O document referring to an oral disclosure, use, exhibition or other means

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Date of the actual completion of the international search

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International Application No

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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.
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INTERNATIONAL SEARCH REPORT

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专利名称(译)	鉴别诊断神经系统疾病		
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优先权	2000870151 2000-06-30 EP 60/218907 2000-07-18 US		
其他公开文献	EP1295129B1		
外部链接	Espacenet		

摘要(译)

本发明提供了一种鉴别诊断患有阿尔茨海默病的个体与患有另一种神经疾病的个体的方法。更具体地说，本发明提供了一种鉴别诊断患有阿尔茨海默病的个体与患有路易体痴呆的个体相比，患有帕金森病而没有痴呆的个体与患有多系统萎缩的个体的鉴别诊断方法。/或与患有进行性核上性麻痹的个体相比，所述方法的特征在于磷酸化tau用作神经学标记物。