



Tau as a biomarker of neurodegenerative diseases

Susanna Schraen-Maschke^{1,2,3},
Nicolas Sergeant^{1,2},
Claire-Marie Dhaenens^{1,2,3},
Stéphanie Bombois^{2,5},
Vincent Deramecourt^{2,3,5},
Marie-Laure Caillet-Boudin^{1,2},
Florence Pasquier^{2,5},
Claude-Alain Maurage^{1,2,3},
Bernard Sablonnière^{1,2,3},
Eugeen Vanmechelen⁴ &
Luc Buée^{1,2†}

[†]Author for correspondence

¹Inserm-U837, Jean-Pierre Aubert Research Centre, Place de Verdun, 59045 Lille, France

Tel.: +33 320 298 866;
Fax: +33 320 622 079;
E-mail: luc.buee@inserm.fr

²Université de Lille 2, Faculté de Médecine, Institut de Médecine prédictive et de Recherche Thérapeutique, Centre de Recherches Jean-Pierre Aubert, Place de Verdun, 59045 Lille, France

³Centre de Biologie-Pathologie, Centre Hospitalier Universitaire, Lille, France

⁴Innogenetics NV, Industriepark Zwijnaarde 7, 9052 Gent, Belgium

⁵Département de Neurologie, Centre de la Mémoire, Centre Hospitalier Universitaire, EA2691, Lille, France

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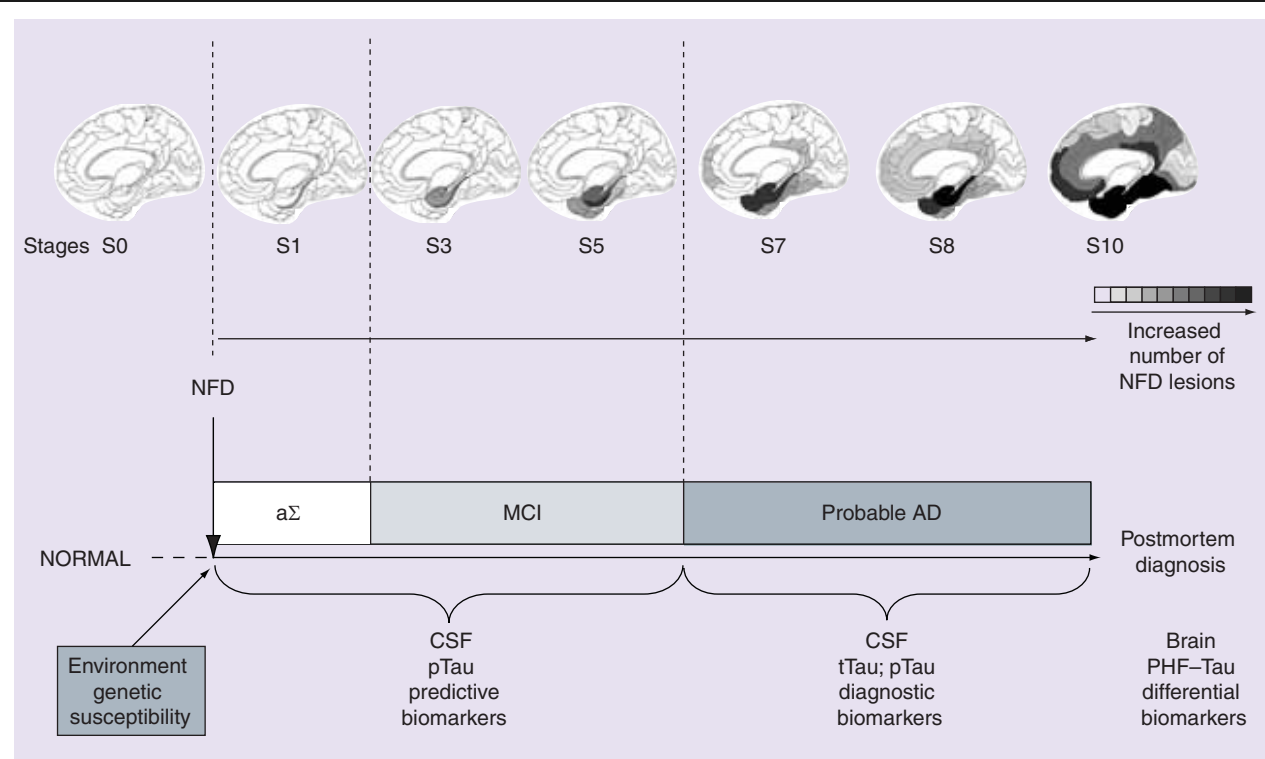
The microtubule-associated protein Tau is mainly expressed in neurons of the CNS and is crucial in axonal maintenance and axonal transport. The rationale for Tau as a biomarker of neurodegenerative diseases is that it is a major component of abnormal intraneuronal aggregates observed in numerous tauopathies, including Alzheimer's disease. The molecular diversity of Tau is very useful when analyzing it in the brain or in the peripheral fluids. Immunohistochemical and biochemical characterization of Tau aggregates in the brain allows the postmortem classification and differential diagnosis of tauopathies. As peripheral biomarkers of Alzheimer's disease in the cerebrospinal fluid, Tau proteins are now validated for diagnosis and predictive purposes. For the future, the detailed characterization of Tau in the brain and in peripheral fluids will lead to novel promising biomarkers for differential diagnosis of dementia and monitoring of therapeutics.

Tau protein is mainly expressed in the neurons of the CNS, where it exerts a role in stabilizing microtubules, key components of axonal transport and in signal transduction. Tau alterations are observed in numerous neurodegenerative diseases at different levels: at the gene level with mutations responsible for rare familial dementia or polymorphisms associated with the increased risk of developing syndromes with parkinsonism; at the mRNA level, with altered alternative splicing leading to abnormal patterns of Tau isoforms in the brain; and at the protein level with, in particular, abnormal phosphorylation and cleavage leading to intraneuronal aggregation of Tau. These alterations are found among more than 20 different neurodegenerative disorders named tauopathies, including Alzheimer's disease (AD) and a non-Alzheimer's type of dementia (progressive supranuclear palsy [PSP], frontotemporal dementia with parkinsonism linked to chromosome 17 [FTDP-17], corticobasal degeneration [CBD], frontotemporal dementia [FTD] and Pick's disease [PiD] [1]). The diagnosis of these diseases remains difficult because of the heterogeneity and partial overlap of their clinical presentations. With the aging of the population, the incidence of some of these diseases, mainly AD, is rapidly increasing and the need of biomarkers for a reliable diagnosis is tremendous. The molecular diversity of Tau, evolving from alternative splicing and post-translational modifications, makes it well suited as a biomarker of these neurodegenerative disorders. The morphological and biochemical characterization of Tau inclusions has led to a huge improvement

of the postmortem differential diagnosis of these diseases. However, more recently, the development of sensitive assays for quantifying Tau in cerebrospinal fluid (CSF) also demonstrated its great interest as a peripheral biomarker of neurodegenerative diseases, mainly AD. Tau inclusions in the brain associated with neuronal damages lead to the leakage of abnormal forms of Tau in the CSF, resulting in quantitative and qualitative changes in CSF-Tau composition. As the brain lesions develop very early during the disease course even before the first clinical symptoms appear, CSF-Tau is not only a useful diagnostic marker in advanced stages of the disease but also a prognostic marker in the earliest stages when clinical expression is weak. An illustration of the different applications of Tau protein analysis at different stages of AD disease is illustrated in Figure 1 and shows the broad possibilities presented by these biomarkers.

Microtubule-associated protein Tau: from the gene to the isoforms

Tau is encoded by a single gene, *MAPT*, located on chromosome 17q21 (Figure 2A). The *MAPT* gene belongs, along with several other genes, to a chromosomal region flanked by low copy repeats (LCRs) that are susceptible to chromosomal rearrangements such as deletions, duplications or inversion [2]. From the 5'UTR to the end of the 3'UTR, it spans 133.9 kb and contains 16 exons. There are more than 200 single nucleotide polymorphisms (SNPs) covering *MAPT*. More than 150 are in complete linkage disequilibrium (LD) with each other, with an LD measure

Figure 1. Stages in the neuropathology of Alzheimer's disease. Implication for the use of Tau as a biomarker during the course of the disease.

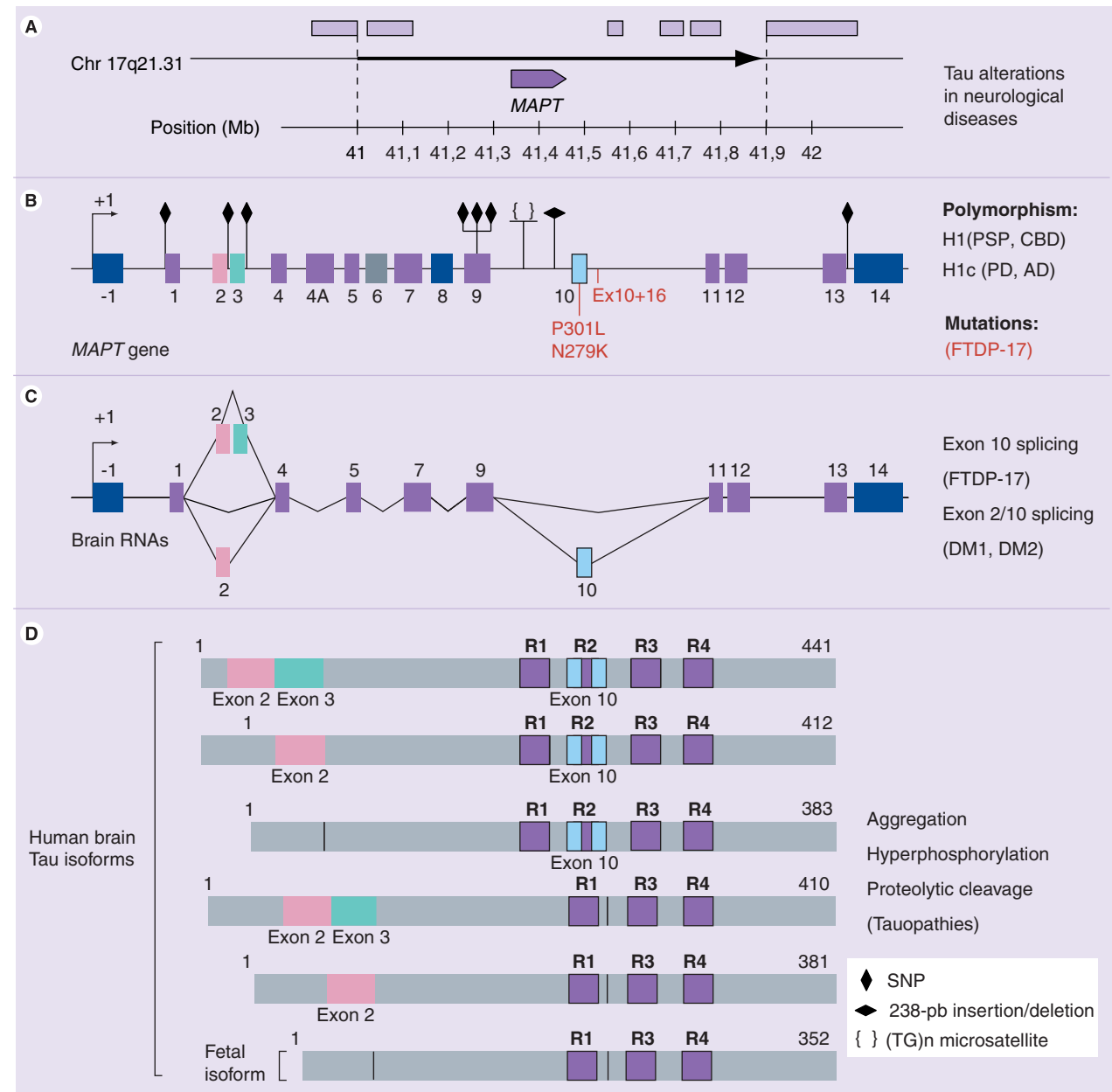
AD lesions, namely neurofibrillary degeneration, follow a stereotyped, sequential and hierarchical pathway. The progression is categorized into ten stages according to the brain regions affected: transentorhinal cortex (S1), entorhinal (S2), hippocampus (S3), anterior temporal cortex (S4), inferior temporal cortex (S5), medium temporal cortex (S6), polymodal-association areas (prefrontal, parietal inferior and temporal superior) (S7), unimodal areas (S8), primary motor (S9a) or sensory (S9b, S9c) areas and all neocortical areas (S10). Up to stage 6, the disease can be asymptomatic or paucisymptomatic (mild cognitive impairment); the CSF levels of pTau are, however, already altered and are useful for predictive diagnosis. In the more advanced stages when clinical criteria of AD are fulfilled, CSF-tTau and CSF-pTau are altered and stable during the disease course. Only the postmortem analysis of the brain with the presence of the two characteristic lesions of AD (amyloid plaques and NFD) allows one to obtain the definite diagnosis of AD. The electrophoretic analysis of PHF-Tau aggregates is a useful tool for differential diagnosis of AD in brain tissue.

AD: Alzheimer's disease; CSF: Cerebrospinal fluid; MCI: Mild cognitive impairment; NFD: Neurofibrillary degeneration; PHF: Paired helical filament.

greater than 0.8 [201], and define an extended haplotype that cover the entire *MAPT* gene (Figure 2B) [3]. Only 20 of them show a LD measure smaller than 0.4. This haplotype even spans to a region covering approximately 1.8 Mb [4]. The H2 haplotype is much rarer than the H1 haplotype in healthy individuals, showing different prevalence among ethnic groups, and results from H1 by the inversion of an approximately 900-kb segment resulting from a rearrangement between LCRs [2]. Tau is mainly expressed in the CNS within neurons and various glial cell types, including astrocytes and oligodendrocytes, although at much lower levels than neurons [5]. In the CNS, two transcripts of 2 and 6 kb, respectively, arise from the utilization of two alternative polyadenylation sites; the 2-kb

mRNA targets Tau to the nucleus and the 6-kb transcript encodes the major form in axons [6,7]. Alternative splicing is tissue specific and developmentally regulated: in the CNS, exons 2, 3 and 10 are alternatively spliced (Figure 2C), leading to one fetal isoform, which is still expressed in adult stage, and five additional adult isoforms. The six Tau isoforms are made of 352–441 amino acids with a molecular weight of approximately 37–46 kDa (Figure 2D). Brain Tau proteins can be subdivided into four regions: a N-terminal region named the projection domain that is acidic, a proline-rich region followed by imperfect microtubule binding repeat motifs (encoded by exons 9–12) and a short C-terminal region. Each isoform is characterized by the length of the N-terminal domain and by the presence of three

Figure 2. Microtubule-associated Tau gene, RNAs and human brain isoforms.



(A) Genomic architecture of the 17q21.31 region encompassing *MAPT* gene and flanked by low copy repeats (rectangles) that are susceptible to chromosomal rearrangements such as deletions, duplications or inversions. Dotted lines illustrate the breakpoints responsible for the inversion of an approximately 900-kb segment, resulting in the H1/H2 polymorphism. **(B)** The Tau gene *MAPT* spans more than 130 kb and is composed of 16 exons. The most studied polymorphism associated with the H1/H2 haplotype are eight single nucleotide polymorphisms, one (TG)_n microsatellite and a 238-pb insertion/deletion. The H1 haplotype is associated with PSP and CBD. The three most frequent mutations responsible of FTDP-17 are shown in red. **(C)** In the human CNS, several mRNAs are generated by alternative splicing of exons 2, 3 and 10. **(D)** In the human brain, six major Tau isoforms are generated from the alternative splicing of exons 2, 3 and 10. Exon 3 is always included with exon 2. Microtubule-binding motifs are numbered from R1 to R4. Half of Tau proteins contain three microtubule-binding motifs and the other half has four microtubule-binding motifs. AD: Alzheimer's disease; CBD: Corticobasal degeneration; FTDP-17: Frontotemporal dementia with parkinsonism linked to chromosome 17; PD: Parkinson's disease; PSP: Progressive supranuclear palsy.

or four repeat motifs, depending, respectively, on exon 2/3 or exon 10 alternative splicing. The diversity of Tau isoforms is further increased by

various post-translational modifications: phosphorylation (developed further), O-glycosylation, ubiquitination, nitration and glycation [8].

Tau function in the CNS

One major biological function of Tau is to build an ordered microtubule network in axons, which is essential for axonal transport. The large C-terminal microtubule binding domain promotes microtubule assembly and maintains the stability of the previously formed microtubules through repetitive sequences. The N-terminal region, together with the proline-rich domain, projects from the microtubules' surface to adjacent microtubules and is proposed to determine spacing between microtubules. Tau proteins would therefore contribute to the parallel ordered organization of microtubules in axons.

More recently, other important roles of Tau are suggested by the interactions of the N-terminal domain with protein partners. Interactions with motor proteins such as kinesin-1 [9] and dynein/dynactin complex [10] suggest a role in the dynamics of axonal transport. The binding to SH3-containing proteins such as Fyn, a Src family kinase [11], phospholipase C- γ 1 or p85- α subunit of PI-3K [11] supports a role of Tau in neuronal signal transduction. In oligodendrocytes, Tau and its regulation by Fyn are proposed to be involved in process outgrowth [12]. Association of Tau N-terminal region with several of its interacting partners is regulated by phosphorylation and is further discussed in the phosphorylation section. Finally, Tau proteins interact with the plasma membrane or with cytoskeleton proteins such as actin, spectrin and neurofilament proteins, suggesting a role in neuronal cell architecture.

The polypeptide sequences encoded by alternative spliced exons modulate specific Tau functions: the N-terminal inserts encoded by exons 2 and 3 affect the microtubule spacing, and the fourth microtubule domain encoded by exon 10 modulates interactions to microtubules. Tau isoforms containing four repeats (4R-Tau) bind to microtubules with a greater affinity [1], and are more efficient at promoting microtubule assembly than isoforms containing three repeats (3R-Tau). This suggests that Tau isoforms have specific functions [13]. The ratio of isoforms is probably important for correct cell function and is not the same in all neurons. For example, Tau mRNAs containing exon 10 are not found in granular cells of the dentate gyrus [14].

Tau phosphorylation

A total of 85 potential phosphorylation sites are distributed along the longest Tau isoform and are essentially located in regions surrounding the repeat-binding motifs. According to the latest

extensive analysis of Tau phosphorylation [15] and that of one previously published review [16], 71 among the 85 putative phosphorylation sites can be phosphorylated in physiological or pathological conditions. A total of more than 20 protein kinases can phosphorylate Tau proteins; an extensive review of these kinases is found in [17].

For some of these kinases, the effect on Tau function has been described. The proline-directed protein kinases (PDPKs), such as cyclin-dependant kinases 1 and 5, MAPK and GSK families are involved in modulation of Tau binding to microtubules (Thr231 and Ser396) and also in signal transduction [1]. The non-PDPK includes a number of kinases also involved in signal transduction, where Tau may act as a linker or modulator. However, among them, MARK kinases are strictly involved in the regulation of Tau binding to microtubules by phosphorylating specific motifs (KXGS) within the microtubule-binding domains [18]. Of note, GSK3 β phosphorylates MARK and inhibits MARK activity, making a functional link between the two groups of kinases and showing that GSK3 β and MARK both contribute to regulate the binding of Tau to microtubules. Regarding the tyrosine protein kinases, studies have determined that human Tau Tyr18 and Tyr29 are phosphorylated by the Src family tyrosine kinase Fyn [19,20]. More importantly, GSK3 β is shown to phosphorylate Tau and reduce the binding of Fyn, PLC- γ 1 and p85 α subunit of PI3K [11]. Finally, cotransfection of Tau and tyrosine protein kinases showed that even if Tyr18 is the major site for Fyn phosphorylation, Tyr394 is the main residue for Abl [21].

Tau alterations in neurological diseases *MAPT deletion & duplication*

Both duplication and deletion of the 17q21.31 region containing the *MAPT* gene have been reported. *De novo* microdeletions have been identified in several patients with developmental delay, hypotonia and learning disability [22–25]; the prevalence of this new syndrome has been estimated to be between 1/13,000 and 1/20,000 [22]. One microduplication reciprocal to the microdeletion was described in a case of severe psychomotor development delay with dysmorphic features [26]. This suggests that dosage-sensitive genes are present in this chromosomal region, perhaps within the *MAPT* gene. Further studies will be needed to evaluate the potential detrimental effects of Tau haplo-insufficiency or triplication.

MAPT gene mutations

In AD, no *MAPT* mutation has been described. However, study of familial cases of non-Alzheimer's tauopathies has led to the discovery since 1994 of at least 43 *MAPT* mutations that cause a very rare autosomal dominant inherited dementia, FTDP-17 (Tau mutations are listed in [202]) [27]. Approximately 100 families with *MAPT* mutations have been reported in the world. The clinical features of these dementia are variable but are usually initially characterized by behavioural and motor disturbances that are later associated with cognitive impairment. The most common mutations, accounting for approximately 60% of known cases, are two missense mutations, P301L and N279K, and one splice site mutation (exon 10 + 16) (Figure 2B) [27]. *MAPT* missense mutations have also been identified in PSP, CBD and PiD. The vast majority of these mutations are located in the coding region or close to the splice donor site of exon 10. The primary effect of most mutations appears to be a reduced ability of Tau to interact with microtubules, but alternatively some of them can affect exon 10 splicing, leading to an imbalance in the expression of the different Tau protein isoforms. Furthermore, increased Tau self-assembly has also been detected in most of these mutated Tau proteins. In almost all of these familial cases, accumulation of hyperphosphorylated Tau protein in neurons has been observed [28].

MAPT gene polymorphisms

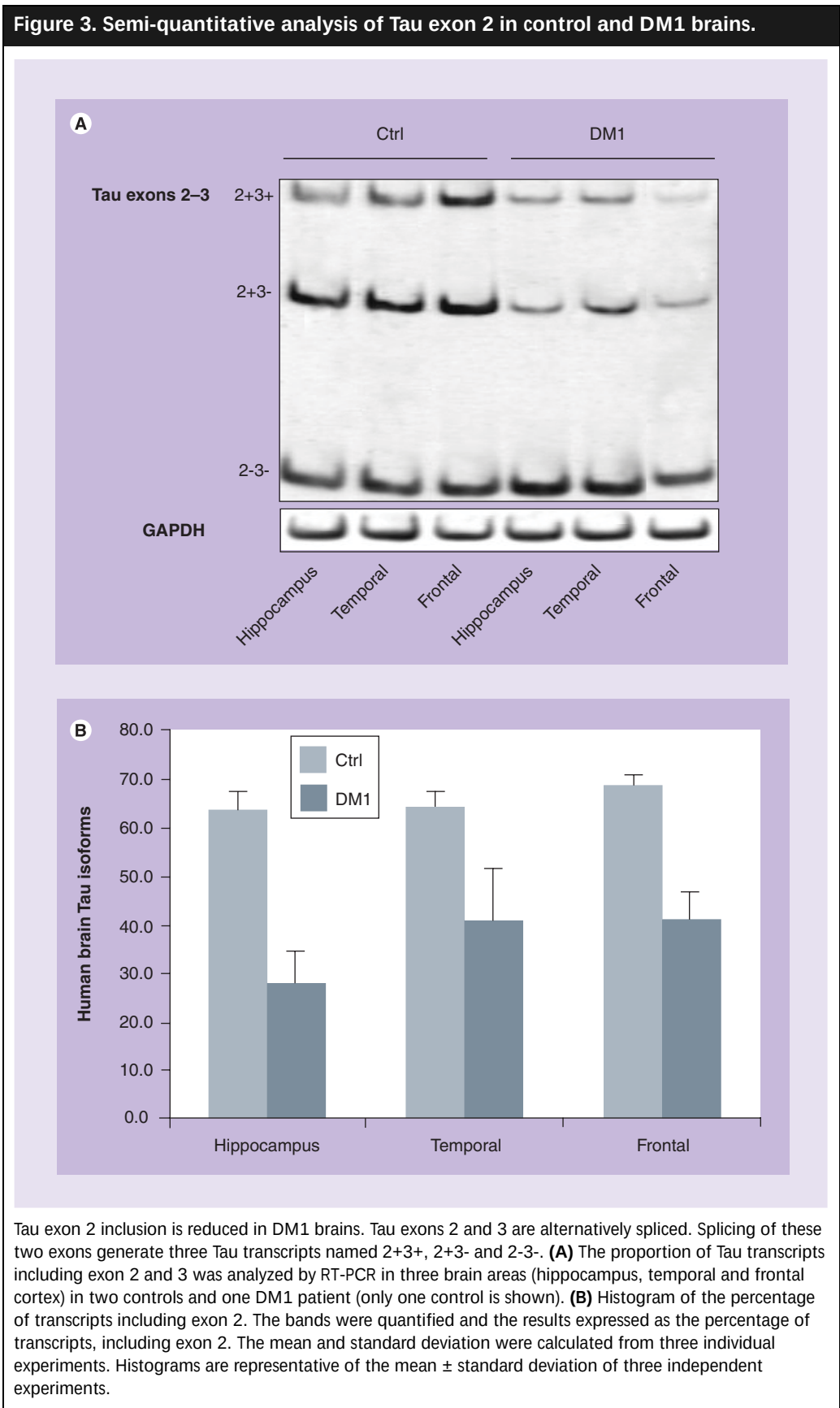
The first evidence that the H1 haplotype was associated with an increased risk for several tauopathies was shown for PSP. Further studies were then investigated in other tauopathies such as AD, FTD, CBD or PiD [3,29], except for CBD, where a significant over-representation of H1 haplotype in affected patients was observed. Results among studies were controversial and do not allow us to conclude that the Tau gene polymorphism is a risk factor for these diseases. In Parkinson's disease (PD), two recent studies showed that the H1 haplotype was associated with a significantly increased risk for PD [30,31]. Moreover, the subhaplotype H1c, located in the promoter region of the Tau gene, has been shown to be a more specific risk factor for PSP [32]. Association analysis of this H1c subhaplotype with AD is controversial [33,34]. The exact pathogenic mechanism by which *MAPT* polymorphisms can cause an increased risk is unknown, but one hypothesis is that they can

influence Tau expression and splicing. The H1 haplotype, and particularly the H1c subhaplotype, showed significantly greater expression *in vitro* of Tau proteins than H2 [35], but this was not confirmed *in vivo* on unaffected postmortem human brains [36]. However, the H1 haplotype, appears to express up to 43% more exon 10+ transcripts than H2 [37–39]. Interaction between the *MAPT* gene and other potential pathogenic genes was also reported. For instance, the combination of risk genotypes of both *MAPT* and α -synuclein has been recently shown to approximately double the risk for the development of PD [40].

Altered MAPT splicing

Dysregulation of splicing can be caused either by alteration of *cis*-sequences on the *MAPT* gene or alteration of *MAPT* splicing factors. The importance of *MAPT* splicing in the physiopathology of tauopathies was first highlighted by FTDP-17 mutations, located within or in the vicinity of exon 10, affecting exon 10 Tau splicing and placing it upstream of the dementia process. Regarding alteration of *MAPT* splicing factors, the disease model is myotonic dystrophy (DM).

Myotonic dystrophy is the most prevalent form of adult-onset muscular dystrophy. Beside the cardinal muscle symptoms (myotony and progressive myopathy), other common problems affect multiple organ systems (e.g., heart, genital tract, eyes and endocrine system), including the CNS: cognitive impairment, including memory, visuo-spatial recall, verbal scale, with cortical atrophy of the frontal and the temporal lobe and white matter lesions are often described in both DM1 and DM2 [41,42]. DMs are inherited autosomal dominant disorders caused by dynamically unstable CTG or CCTG expansions in the 3'UTR of DMPK or the first intron of ZNF9 in DM1 and DM2, respectively [43]. A disruption of *MAPT* splicing in DM1 and DM2 is observed together with a pathological aggregation of Tau proteins (further developed in the paragraph 'Tau as biomarkers of brain pathology') [44–48]. In several cortical brain areas of a DM1 patient, exon 2 splicing is altered (Figure 3A), with a reduced inclusion of exon 2 and a relative increase of 2–3 transcripts (Figure 3B). Mis-splicing of exon 10 in DM1 is inconsistently observed [46,47]. Splicing alteration in DM1 and DM2 are due to a loss of function of a *MAPT* splice factor, MBNL1, caused by the DM1 or DM2 mutation [49], implicating a RNA-mediated *trans* mechanism [43,50].



Several studies suggest that altering *MAPT* splicing could be responsible for central nervous dysfunction by altering the Tau isoform ratio: overexpression of Tau 4R in neuroblastoma cells showed enhanced susceptibility to cell death [51] and animal models with excessive exon 2/3 inclusions cause gliopathy and spinal cord degeneration [52]. Thus, abnormal Tau splicing is sufficient to cause neurodegeneration, although the exact mechanism that leads to the clinical symptoms is not clear. A disruption of proper balance of Tau isoforms is also observed in several sporadic tauopathies, including FTD, PSP, CBD [53], PiD [54], PD [30] and is inconstantly observed in AD [55–58]. This suggests that a common toxic mechanism is shared in all these tauopathies, and that restoring the isoform balance may be a novel therapeutic strategy [7].

Tau aggregation

In more than 20 neurodegenerative disorders, referred to as tauopathies, Tau proteins aggregate in the affected cortical and subcortical brain regions [59], forming inclusions within both glial and neuronal cell types. The most well-known is AD and other tauopathies include PSP, FTDP-17, CBD, FTD and PiD. Biochemically, the inclusions contain aggregated Tau proteins characterized by abnormal phosphorylation and/or abnormal splicing. All the isoforms are capable of polymerization into fibrillar structures such as those present in AD. Over a long time, it was found that Tau aggregation correlates with neuron loss and neuron toxicity; this was suggested by the fact that Tau aggregate spreading in the AD brain increases with cognitive decline. More recently, some studies suggested that Tau aggregation can be dissociated from neuronal loss [60,61] and can even be protective [62], as suggested for Huntington in Huntington disease [63]. Small Tau oligomers could be involved in neurodegeneration [64].

Altered Tau conformation

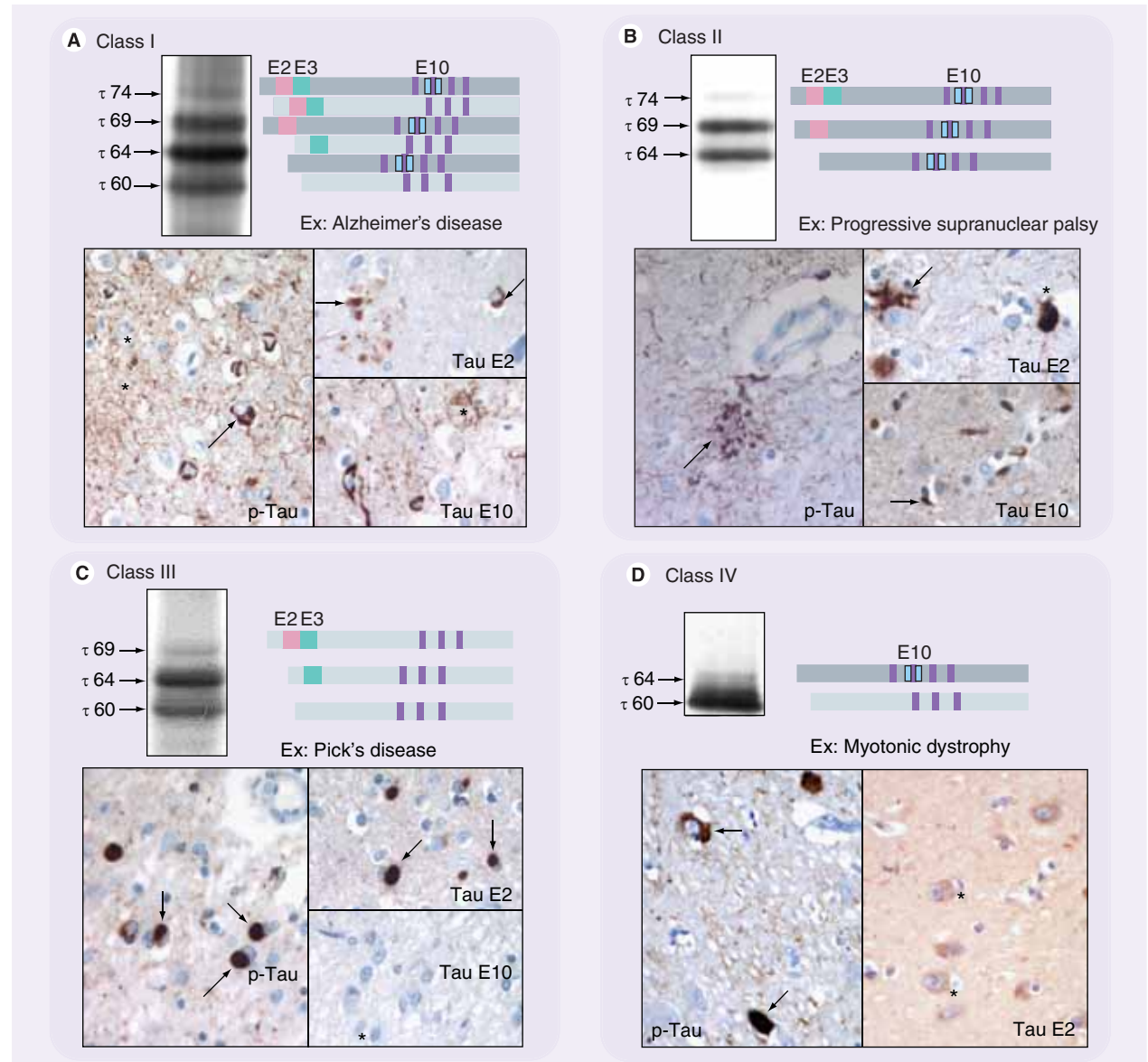
Specific conformational changes of Tau are observed in the early stages of AD. In FTDP-17, Tau proteins are altered. The conformational changes may render Tau more susceptible to phosphorylation. Abnormal conformation of Tau has been reported to be toxic in cell models and in transgenic mouse models [65]. When the aggregation occurs, fibrils take a very well structured organization corresponding to paired helical filaments (PHF) in neurofibrillary tangles (NFTs) for AD.

Tau hyperphosphorylation

The most prominent modification of Tau in all tauopathies is abnormal phosphorylation characterized by a high level of phosphorylation on epitopes localized at the half-N terminus and C terminus outside the microtubule binding domains (e.g., epitopes phosphorylated Tau [pTau]181, pTau199, pTau231 pTau396 and pTau404) and additional pathological phosphorylation sites (e.g., epitope pTau422) (phosphorylation sites on PHF–Tau are reviewed in [16]). One consequence is that the most striking difference between postmortem Tau and PHF–Tau proteins is their electrophoretic profiles. Normal ‘postmortem’ Tau proteins are resolved as six main bands (45–67 kDa) whereas more acidic hyperphosphorylated PHF–Tau are resolved as four bands of between 60 and 74 kDa (Figure 4). In normal brains, the pTau epitopes are rapidly dephosphorylated during the postmortem delay: this effect is likely due to the rapid drop of ATP and activation of phosphatases. Conversely, in AD brains, this dephosphorylation does not occur. It may be due to [66]:

- The aggregation of Tau proteins into filaments that render them inaccessible to phosphatases
- Phosphatases that are no longer activated in degenerating neurons
- Phosphatase activity is already decreased

Nevertheless, hyperphosphorylation and abnormal phosphorylation may be useful as diagnostic markers. The most studied phospho-epitopes in CSF are pTau181 and pTau231. It is well established that pTau231 appears early in AD, occurring before the formation of PHF in the neurons of the hippocampus. Phosphorylation at threonine (Thr)181 and serine (Ser)199 occurs later, and these are only found to any appreciable extent in intracellular tangles [67]. Reactivity to TG3, an antibody that recognizes phosphothreonine 231, is found in pretangles, intracellular and extracellular tangles, and thus is present at all stages of the disease. The additional pathological phosphorylation sites can be detected using antibodies that recognize PHF–Tau and not normal or ‘native’ Tau, such as AD2 that recognizes the phosphorylated Ser396 and Ser404 or AT100 that recognizes the phosphorylated Thr212–217 and Ser214. Nonetheless, to generate the pathological phospho-sites at AT100 epitope a sequential phosphorylation by two kinases [68] is necessary,

Figure 4. The ‘barcode’ of neurodegenerative diseases.

Aggregated Tau proteins from the brain tissue of patients suffering from different neurodegenerative disorders were resolved by 1D gels. Four main patterns of Tau bands were observed and the isoform content was determined using Tau exon-specific antibodies or 2D gel electrophoresis. A classification is proposed according to Tau isoforms composing each of four biochemical patterns. **(A)** Class I, which encompasses the largest number of neurodegenerative diseases with Tau aggregation, is characterized by the aggregation of six Tau isoforms. This class is characterized by the occurrence on the brain samples of the hallmarks of Alzheimer's disease (AD) i.e., 1) intraneuronal somatic neurofibrillary tangles (NFTs), as shown on the left panel by an antibody against pSer396–404 of Tau (AD2, arrows); 2) neuropile threads (NT), corresponding to neuritic processes filled of aggregated Tau (asterisks); and 3) neuritic plaques (NPs). Antibodies raised against the sequences coded either by exon 2 (Tau E2, upper right panel) or by exon 10 (Tau E10, lower right) stain similarly NFTs (arrows), NTs (asterisks) and NPs (arrowhead) in AD and in other class I tauopathies. **(B)** Only Tau isoforms containing four microtubule-binding domains aggregate in class II disorders. They are represented here by a PSP case. AD2 anti-pTau labels the so-called astrocytic tufts, corresponding to intra-cytoplasmic Tau aggregation in astrocytes (arrow, left panel). Tau E2 and Tau E10 show glial Tau pathology as well, astrocytic (upper right, arrow) or oligodendrial (arrowhead, lower right). The 'globoid' NFTs are seen here in the substantia nigra (upper right, asterisk) and are stained by both Tau E2 and Tau E10. **(C)** Tau isoforms with three microtubule-binding domains are found in Pick's disease and few frontotemporal dementias with parkinsonism linked to chromosome 17, corresponding to class III diseases. Pick bodies are stained by anti-pTau and Tau E2 (arrows showing spherical 10 μm bodies) but not by Tau E10 (in the lower right panel, a Pick body is readily seen but unstained, asterisk). **(D)** Finally, class IV is characterized by the aggregation of Tau isoforms lacking sequences encoded by exons 2 and 3. To date, the only known class IV diseases are DM1 and DM2. On the left panel, aggregates of hyperphosphorylated Tau are labeled with AD2. On the right panel, only unphosphorylated, nonaggregated normal Tau are stained by anti-Tau E2, which in turn labels no aggregate.

suggesting that several kinases may be deregulated in AD. Among the kinases described above, GSK3- β and cdk5 play an important role in regulating Tau phosphorylation under pathological conditions.

Hyperphosphorylated Tau epitopes are not the same in all tauopathies: Pick bodies, the neuropathological features of PiD, are composed of aggregated Tau proteins. Pick bodies are not detected by the monoclonal antibody 12E8 raised against the phosphorylated residue Ser262/Ser356, whereas in other neurodegenerative disorders, this phosphorylation site is detected [69,70]. The lack of phosphorylation at Ser262 and 356 sites is likely to be related to either a kinase inhibition in neurons that degenerate in PiD or an absence of these kinases within degenerating neurons.

Hyperphosphorylation is associated with a loss of microtubule binding capacity and a consequent accumulation in neuronal bodies. Tau hyperphosphorylation correlates with neurodegeneration in the brain of transgenic mice overexpressing an FTDP-17 mutant [71]. Tau hyperphosphorylation is a relatively early event in the development of AD [72]. Phosphorylated Tau appears to be more resistant to proteolysis by different proteases [73], and therefore could accumulate in neurons and contribute to Tau toxicity.

Tau catabolism

Tau is cleaved *in vivo* by at least two groups of proteases, caspases [74,75] and calpains [76], and is degraded by the proteasome [77]. Tau fragments generated by different proteases may differ in their ability to assume pathological conformations, to aggregate and to induce neurotoxicity [78,79]. It has been shown that successive cleavage events occur on Tau protein during the course of NFT evolution in AD [78]. In AD and in other neurodegenerative disorders, Tau truncation by caspases is an early event [80]. Calpains also become overactivated because of elevation of cytosolic Ca²⁺ levels. Inhibition of the proteasome leads surprisingly to an increase in Tau degradation [81]. Once proteolysed, Tau fragments turn into effectors of apoptosis and initiate and accelerate NFD development [82,83]. The number of aggregates positive for caspase 6-truncated Tau fragments has been inversely correlated to cognitive score during aging and early stages of AD [80]. More recently, a 35-kDa C-terminal Tau fragment, lacking the N terminus of Tau but containing four microtubule-binding

repeats was shown to be present only in neurodegenerative disorders in which 4R was over-represented: it was detectable in PSP, CBD and 4R forms of FTDP-17, but was absent in controls, AD and PD brains [84]. All these observations suggest that Tau fragments are potential biomarkers of AD.

Tau proteins as biomarkers of brain pathology

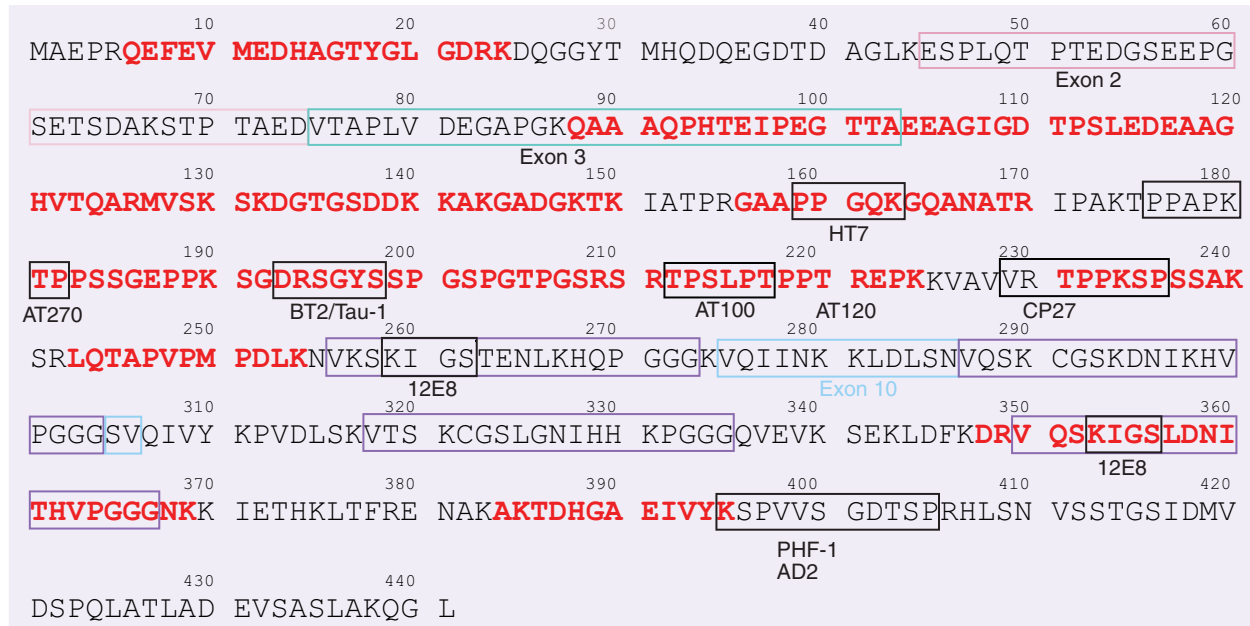
Tau aggregates form intraneuronal inclusions of typical morphologic features specific of the tauopathies: NFTs, neuropil threads, dystrophic neurites of neuritic plaques and Pick bodies. In addition to neuronal Tau inclusions, some tauopathies, including PSP and CBD, develop glial Tau inclusions such as 'tufted' astrocytes [85], coiled bodies in oligodendrocytes [86] or astrocytic plaques [87,88]. The generation of specific antibodies using antibodies that recognize PHF-Tau and not normal or 'native' Tau, such as AT100, and against amino acid sequences corresponding to alternative spliced exons 2, 3 and 10 allowed the characterization of Tau aggregates in tauopathies using immunoblotting and immunohistochemistry. Tau aggregates differ in both Tau phosphorylation and isoform content, which enables a molecular classification of tauopathies. We propose a classification composed of four classes of tauopathies, defined depending on the type of typical Tau electrophoretic profiles of aggregates, constituting disease-specific biochemical signatures or 'barcodes' for neurodegenerative disorders [16,17].

Class I: all brain Tau isoforms are aggregated

Class I is characterized by a pathological Tau quartet at 60, 64 and 69 kDa, and a minor pathological Tau at 72/74 kDa [89]. This pathological Tau quartet corresponds to the aggregation of the six Tau isoforms [90,91]. This classification is summarized in Figure 4. Adjacent to the western blot image is the isoform composing each band [1]. Using histochemistry, aggregates of class I tauopathies positively react with AD2, and antibodies directed against exon 2 and exon 10 (Figure 4A).

Class II: Tau isoforms containing the exon 10-encoding sequence aggregate

The class II profile is characterized essentially by the aggregation of Tau with four microtubule binding domains (Figure 4B). This pathological Tau profile is observed in CBD, argyrophilic grain dementia (AGD), PSP and FTD linked to

Figure 5. Characterization of Tau in cerebrospinal fluid.

chromosome 17 owing to Tau gene mutations [92,93]. PSP, CBD and AGD are rare atypical parkinsonism disorders, for which classification was recently updated by the Consortium for Frontotemporal Lobar Degeneration [94].

Class III: Tau isoforms lacking the exon 10-encoding sequence aggregate

Class III of tauopathies includes PiD and autosomal dominant inherited FDT-17 (Figure 4C). PiD is a rare form of neurodegenerative disorder characterized by a progressive dementing process. Early in the clinical course, patients show signs of frontal disinhibition [95]. Neuropathologically, Pick's disease is characterized by the presence of typical spheroid inclusions in the soma of neurons called Pick bodies. Pick bodies are labeled by Tau antibodies, with a higher density in the hippocampus than in the frontal and temporal cortices. The pathological Tau profile of PiD contrasts with that of class II tauopathies, with the pathological Tau isoforms consisting essentially of the 3R Tau isoforms. Immunohistologic staining of those aggregates is positive with AD2 and anti-exon 2 antibodies and negative with anti-exon 10 antibodies.

Class IV: Tau isoforms lacking exon 2, 3 and 10 principally aggregate

Class IV is represented by DM type I and type II (Figure 4D). Neuropathological lesions, such as NFTs, have been observed in adult DM1 individuals over 50 years of age. The pathological Tau profile of DM1 is characterized by a strong pathological Tau band at 60 kDa and, to a lesser extent, a pathological Tau component at 64 and 69 kDa. This typical pathological Tau profile is reflected by a reduced number of Tau isoform expressions in the brain of individuals with DM1, at both the protein and mRNA levels [47]. The analysis of multiple brain regions of one genetically confirmed DM2 patient aged 71 years, showed some neurofibrillary degenerating processes. Using specific immunological probes against amino acid sequences corresponding to exon 2 and exon 3, the neurofibrillary lesions were shown to be devoid of Tau isoforms with N-terminal inserts (Figure 4D) [48]. An altered splicing of Tau with a reduced expression of Tau isoforms containing the N-terminal inserts characterizes both DM1 and DM2. Overall, it demonstrates that the CNS is affected and that DMs are real tauopathies. The direct relationship between the altered splicing of

Tau and neurofibrillary degeneration in DM remains to be established. Indeed, such an altered splicing of Tau is commonly observed in FTDP-17 and considered as reminiscent to neurofibrillary degeneration and tauopathies.

Tau proteins as peripheral biomarkers of neurodegenerative diseases

The generation and characterization of highly sensitive and specific antibodies to the microtubule-associated protein Tau has not only led to a better understanding and detailed characterization of Tau in normal and diseased brain, but has also considerably advanced our understanding of Tau as a biomarker in circulating fluids for a number of neurological conditions. While the first observations on the presence of Tau in the CSF were based on several sandwich immunoassays, improvement in highly sensitive western blotting techniques and the use of immunocapture mass spectrometric techniques have led to the unequivocal characterization of Tau in the CSF.

CSF total Tau: a biomarker of neuronal degeneration

The presence of Tau in the CSF was first described in 1993 [96]. Quantitative analysis of total Tau (tTau) in the CSF (CSF-tTau) was developed, using ELISA assays with different Tau antibodies that detect all Tau isoforms independently of their phosphorylation state (refer to Figure 5 and Table 1 for antibodies used in these assays). These ELISAs showed that CSF-tTau displays an age-associated increase in nondemented control individuals [97], and therefore age-adjusted reference values should be used when

CSF-tTau is used diagnostically. All the data of more than 50 studies, including over 2500 AD patients and 1300 controls have consistently demonstrated increased CSF-tTau levels in AD, with mean levels two- to three-times higher compared with healthy controls [98]. The most commonly used is a commercial sandwich ELISA kit (AT120-HT7/BT2) that shows a good specificity (90%) and sensitivity (81%) discriminating AD from age-matched controls [99]. Age-adjusted reference values have been established to improve discrimination between AD and controls: less than 300 pg/ml in the group 21–50 years of age, less than 450 pg/ml in the group 51–70 years of age and less than 500 pg/ml in the group 71–93 years of age [100]. There is evidence that CSF-tTau reflect the presence of the neuropathological hallmarks of AD from neuropathological studies [101,102].

The increase of CSF-tTau probably comes from the leakage of Tau from damaged neurons into the CSF and therefore reflects the intensity of neuronal damage and degeneration. This is assumed from the fact that in acute conditions such as stroke or Creutzfeld–Jacob disease (CJD), there is a marked increase in CSF-tTau. After acute ischemic stroke, CSF-tTau levels increase transiently (Table 2). The highest CSF-tTau levels with values of 10–50-times higher than in controls were revealed in CJD, a disorder with very intense neuronal loss. Using a cut-off value of 1300 pg/ml, several studies on the differentiation of CJD from AD and other dementia showed that CSF-tTau level is a highly discriminative marker, which has recently been confirmed in a neuropathological study to be as

Table 1. Tau antibodies.

Antibody	Epitope	Phospho-independent site	Dephosphorylated site	Phosphorylated site
HT7	159–163	Yes	–	–
CP27	130–150	Yes	–	–
AT120	218–224	Yes	–	–
Tau 1	195/198/199/202	–	Yes	–
BT-2	Ser202	–	Yes	–
AD2/PHF1	Ser396–Ser404	–	–	Yes
AT8	Ser202–Thr205	–	–	Yes
AT270	Thr181	–	–	Yes
AT180/CP9/TG-3	Thr231	–	–	Yes
AT100	Thr212–Ser214	–	–	Yes
12E8	Ser262–Ser356	–	–	Yes

PHF: Paired helical filaments; Ser: Serine; Thr: Threonine.

Table 2. Follow-up studies of tTau and pTau levels during chronic and acute neurodegenerative disorders.

Diagnostic group	Follow-up	tTau baseline (pg/ml)	tTau follow-up (pg/ml)	pTau baseline (pg/ml)	pTau follow-up (pg/ml)	Ref.
Stable Tau/pTau levels in CSF of AD and CJD patients						
9 AD	18.6 months (2–43)	847 ± 253	878 ± 452	NA	NA	[184]
192 AD	1 year	21% CV	21% CV	NA	NA	[185]
29 AD	2 years	548 ± 355	577 ± 275	NA	NA	[186]
8 MCI	1 year			530 ± 450	560 ± 480	[146]
20 AD	3–8 years	568 ± 365	570 ± 435	NA	NA	[130]
53 AD	6 months	425 ± 30	431 ± 30 (6.1% CV)	93 ± 6	94 ± 6 (4.4% CV)	[187]
50 AD	21 ± 8 months	731 ± 363	791 ± 419	84 ± 34	85 ± 35	[161]
22 MCI–AD	2.1 ± 0.5 years	750 ± 553	667 ± 327	49 ± 31	52 ± 29	[150]
12 MCI–AD	2 years	700 (240–1700)	710 (270–1300)	86 (47–150)	86 (48–150)	[156]
66 CJD	6 weeks	tTau not significant	tTau not significant	NA	NA	[188]
Transient increase in CSF–tTau and pTau levels in conditions with neuronal/axonal loss						
Brain trauma (n=9)		770 ± 160 (week 3)	250 ± 40 (3–5 months)	37 ± 8 (week 3)	29 ± 3 (3–5 months)	[189]
Neurotoxicity (n=6)		535–776 on day 8	<300 after 200 days	NA	NA	[190]
Alcoholic dementia		CSF–Tau/pTau significantly elevated in acute WE (n = 10) but not in chronic WE and after long-term follow-up				[106]

AD: Alzheimer's disease; CJD: Creutzfeld–Jakob disease; CSF: Cerebrospinal fluid; CV: Coefficient of variation; MCI: Mild cognitive impairment; n: Number; NA: Not applicable; pTau: Phosphorylated Tau; tTau: Total Tau; WE: Wernicke's encephalopathy.

good as the established 14–3–3 marker for CJD [103]. Elevated values for CSF–tTau have also been found in a bovine variant of CJD [104]. Increases of CSF–tTau levels are observed in several other acute neurological conditions, such as severe malaria [105], Wernicke's encephalopathy [106], pediatric patients with brain tumor, hydrocephalus or serious CNS infections [107].

Studies assessing CSF–tTau in non-AD dementia such as FTD, PSP, CBD, Lewy body dementia (LBD), Parkinson's disease dementia (PDD) and amyotrophic lateral sclerosis (ALS) give contradictory results. In FTD cases, CSF–tTau has been found to be increased by some investigators and reported normal or reduced by others [108–110]. In LBD, CSF–tTau has been found to be increased in some studies [111] and not in others [112,113], including one on an autopsy-verified series [114]. Increased CSF–tTau has also been reported for PDD patients [115,116]. In PSP and CBD syndrome, CSF–tTau levels were found within the normal range [116] or increased [98]. In ALS, inconsistent increased CSF–tTau levels are described [117,118]. This suggests that presence of Tau pathology does not necessarily result in higher CSF–tTau levels. In vascular dementia (VaD),

some studies have reported normal CSF–tTau levels, whereas others have found an elevation [98]. These conflicting results might be caused by selection bias as high concomitant AD pathology was observed at autopsy in patients clinically diagnosed as VaD [119].

Normal CSF–tTau levels are found in several neurological diseases such as other types of dementia (alcoholic dementia), chronic neurological disorders (PD [without dementia] and multiple sclerosis) and psychiatric disorders (depression) [120,121].

In conclusion, CSF–tTau has a clear diagnostic value over that of clinical criteria of AD for the discrimination of AD from normal aging, depression, chronic neurological disorders, alcoholic dementia and possibly VaD. However, for differential diagnosis from other non-AD dementias (FTD, CBD and LBD), the diagnostic value of CSF–tTau is insufficient.

CSF–pTau: a biomarker of hyperphosphorylation

The use of phosphodependent antibodies showed that pTau is recovered in the CSF and that several phosphoepitopes are present [98],

such as Thr181, Thr235, Ser396 and Ser404. A moderate to marked increase in CSF-pTau compared with controls has been found for all the different ELISA methods based on these antibodies [98,99]. Pooling 13 different papers including more than 1000 AD patients and 500 controls, the mean sensitivity to discriminate AD from controls was 81% and the specificity 91% [122]. A study compared the diagnostic performances of pTau181, pTau199 and pTau231 in the same samples [123]: all three pTau assays discriminated AD from normal aging equally well.

There is no change in CSF-pTau after an acute stroke [98], although there is a marked increase in total Tau. Furthermore, CSF-pTau levels are normal or only mildly increased in CJD, despite a huge increase in total Tau [124]. This suggests that pTau is probably not simply a marker of neuronal loss but reflects the phosphorylation state of Tau. Normal CSF levels of pTau are found in chronic neurological disorders (PD, multiple sclerosis or ALS), psychiatric disorders (depression) [97,125] and also in most cases of other dementias such as VaD, FTD and LBD [98,123]. This implies that the specificity of pTau to differentiate from other dementias is higher than for total Tau, reaching more than 80% [126]. However, differences were observed between phosphoepitopes in their potential to discriminate AD from other dementias: the best discrimination between AD and FTD being obtained with pTau231, and between AD and dementia with Lewy bodies with pTau181 [126,127]. The use of pTau as a marker to distinguish dementia with Lewy bodies from FTD [127] also shows promising results, which needs to be further confirmed and extended [109,128]. Finally, it is interesting to note that Tau pathology and hyperphosphorylation does not necessarily result in increased CSF-pTau levels.

Influence of gene polymorphisms on tTau & pTau levels

Interindividual variations of CSF Tau and pTau levels are important in controls and in AD patients. Therefore, some investigators have studied the impact of polymorphisms known to be associated with an increased risk of developing AD on these levels. In some studies, neither the apoE ϵ 4 allele nor MAPT H1/H2 haplotype influenced the CSF of tTau or pTau [129–133]. However, there are studies that have found an association between the apoE polymorphism and CSF Tau levels [134–137]. Concerning the associa-

tion of MAPT H1c haplotype with CSF-tTau levels, results are contradictory [133,138]. One study showed that two very rare H1 haplotypes were correlated with higher CSF-tTau levels. However, a combined genotype and CSF-tTau or CSF-pTau may be useful for the diagnosis or prognosis of other neurodegenerative diseases. Finally, a very interesting recent observation was that a polymorphism of the kinesin light chain 1 gene, which has been demonstrated to be associated with an increased risk of developing AD [139], was associated with high CSF-pTau levels in mild cognitive impairment (MCI) patients who converted to AD [140].

pTau: a predictive biomarker of AD

The rationale for CSF-tTau and CSF-pTau as predictive biomarkers of AD is that pTau is a major component of pretangle PHF and mature NFTs that are present within the brain even before the onset of AD [72]. During this preclinical period, there is a gradual loss of axons and neurons, and at a certain threshold the first symptoms, most often impaired episodic memory, appear. At this stage, patients do not fulfil the criteria for dementia and may be diagnosed with MCI. Patients with MCI can evolve to AD or remain with their MCI. The former are named converters or prodromal Alzheimer patients [141].

Several studies have demonstrated that high CSF-tTau and CSF-pTau levels were already found in early AD, and the performance of these biomarkers in AD cases with Mini-Mental State Examination scores above 23–25 have been found to be similar to those in more advanced AD [142]. Moreover, in MCI cases that developed AD during clinical follow up, these biomarkers are already altered with sensitivities similar to those found in AD cases with clinical dementia [143]. Longitudinal studies over periods ranging between 2 and 6 years in subjects with MCI showed that pTau levels were significantly increased only in the MCI converters, and that pTau at different epitopes, including Thr181, Thr231 and Ser199, is the most reliable predictor of the decline from MCI to AD, with a sensitivity for prediction of 66–100% and a specificity of 66–78% [144–150]. It is important to note that as only approximately 15% of MCI cases progress to AD each year [151], very extensive follow up (more than 5 years) is needed to ascertain which patients will not develop dementia. To date, most studies have been carried out with shorter follow-up periods; it is probable that the specificity data

are too low. Finally, these data on sporadic AD are confirmed by studies of familial AD, showing that increased CSF-tTau and CSF-pTau levels are elevated in presymptomatic presenilin-1 or amyloid protein precursor mutation carriers [152]. The very early diagnosis of AD at a presymptomatic stage of the disease will be the basis for initiating preventive treatments in the future.

Clinical significance of CSF Tau & pTau levels in neurodegenerative diseases

Dementia disorders are characterized by progressive cognitive and functional decline. While the increase in CSF levels of Tau in patients with AD was rapidly confirmed, its use as a diagnostic marker is based on sustained unchanged levels over longer periods of time during the disease process (see Table 2 for an overview of published data). In most studies, no significant changes in CSF-tTau and CSF-pTau have been reported in follow-up studies in mid- and late-stage AD, with coefficients of variation ranging from 6.1% in a 6-month follow-up study to 21% in a 1-year follow up. In studies on other neurodegenerative diseases such as brain trauma, neurotoxicity and alcohol dementia, the increase in CSF-tTau levels is transient (Table 2). While some of these studies in neurodegenerative diseases associated with neuronal/axonal loss seem controversial, the timing of CSF sampling with respect to the transient change is obviously crucial to the interpretation of the results. Indeed, longitudinal follow-up studies in patients with multiple sclerosis [153], Guillain-Barré syndrome [154] or traumatic brain injury [155] suggest that degree of elevated Tau is related to poor outcome, and thus may have prognostic value. Therefore, in neurodegenerative diseases in which axonal damage is related to clinical outcome, quantifying CSF-tTau might have prognostic value, while in AD, MCI-AD and CJD, where the elevation of Tau is high and relatively stable, the increase of Tau levels has diagnostic and prognostic value [103,141,156].

Increased power of CSF Tau biomarkers when combined together or with other biomarkers

The combination of several CSF markers will increase the specificity of tests for discriminating dementia. In particular, the association of CSF amyloid peptide A β 1–42, that reflect the presence of amyloid plaques in AD brains increases the diagnostic power of Tau proteins [98,157–159].

Stable increased tTau and pTau levels do not only have value in differential dementia diagnosis

[160], but might also be useful in early diagnosis. Also in this early stage of the disease, the levels of CSF-tTau and pTau levels are stable over time (Table 2) [146,150,156,161]. To improve the reliability of early diagnosis of AD, a combination of biomarkers will be required.

The association of 14–3–3 protein with CSF-tTau increases test specificity for the diagnosis of CJD [162]. Moreover, the ratio of pTau to CSF-tTau was found to discriminate CJD from other neurodegenerative diseases without any overlap [124]. Finally, pTau and/or pTau/tTau ratio might be important markers to differentiate subtypes of CJD [104,163].

CSF-tTau & pTau as therapeutic monitoring biomarkers

Stable levels over longer periods of time are not only useful in early diagnosis but also offer potential as a surrogate biomarker. The definition of a surrogate end point is that a biomarker is intended to substitute for a clinical end point. It is expected that such an end point predicts clinical benefit based on epidemiologic, therapeutic, pathophysiological or other scientific evidence [164]. For this purpose, CSF biomarker levels should be indices of the intensity of a specific pathogenic process in the AD brain and reflect the rate of clinical worsening. Several studies highlight a relationship between CSF-Tau levels and cognitive change. Some data have shown a significant correlation between high CSF-tTau levels and a more severe cognitive dysfunction [165,166] and others not [111]. One follow-up study shows that high CSF-tTau levels are associated with shorter survival [166]. More recently, a longitudinal study observed increasing CSF-pTau levels during cognitive decline and conversion to dementia [167]. Similarly, significant positive correlations between tTau and pTau levels and change in ventricular [168] and hippocampal volume [169] were observed. Finally, in a recent study, CSF-tTau and pTau correlated with slowing of cognitive speed as measured by relative electroencephalogram θ power [170].

Several small drug studies have already studied CSF-tTau and pTau levels in relation to drug treatment in AD patients, and a number of these studies suggest that tTau or pTau levels can be ‘normalized/decreased’ upon treatment (Table 3). In particular, the reduction of CSF-tTau in immunotherapy might be particularly relevant. Furthermore, association studies with SNPs suggest that the use of Tau measurements can fur-

ther be improved, taking into account those associations (Table 3). Additionally, discoveries in other drug treatments, such as the association of Tau mRNA levels with sensitivity to paclitaxel treatment of breast cancer [171], might also be of relevance for Tau-related disorders (Table 3) [172]. However, this field of biomarker research is clearly in its early phases, and longitudinal studies are required to definitely demonstrate the relationship between change in Tau/pTau levels and clinical outcome.

CSF Tau fragments: promising differential biomarkers of dementia

Tau in CSF consists of partially proteolytic fragments ranging from 14–55 kDa and full-size Tau. Based on identification of specific peptides it is clear that all isoforms found in the brain are present in the CSF. Tau fragments are mainly composed of two major polypeptides of 55 and 33 kDa, respectively [116]. The 55-kDa band contains a large domain of Tau extending from the N-terminal region (amino acid 159) to the C-terminal region (amino acid 432). By contrast, the 33-kDa band shares a similar N-terminal epitope with that of the 55-kDa band, but is truncated at the C terminus of Tau. Those Tau fragments were shown to be present in the brain tissue [84]. A 14-kDa fragment of Tau was also evidenced by Zemlan and collaborators using C-terminal Tau antibodies [173]. More recently, a characterization

by mass spectrometry of Tau in human CSF identified 19 tryptic fragments, of which 16 are common to all isoforms and three specific to unique Tau isoforms [174]. The sequence of the largest form of human brain Tau is shown in Figure 5 and summarizes the characterization of Tau in CSF.

In CSF of PSP patients, the Tau ratio 33/55 kDa was significantly decreased when compared with controls and AD, FTD and CBD. This is consistent with the recent study in PSP brain identifying a Tau fragment specific to 4R isoforms [84]. The multiplicity of Tau fragments in CSF represents a great potential for differential diagnosis of dementia and therefore their analysis should be developed further in independent studies and on larger patient series. Thus, a detailed characterization of Tau in CSF of neurodegenerative diseases [174,175] may lead to simple biochemical tests that may assist in sometimes difficult neurological diagnosis.

Future perspective

For the use of Tau as a biomarker in large clinical trials or in clinical practice, one important goal in the future will be to develop sensitive methods to detect very low concentrations of Tau in the blood (~30 pMol) and even in urine. One promising techniques will be immuno-PCR. High-confidence protein identifications in the HUPO plasma proteome have identified

Table 3. Tau as a potential surrogate biomarker: from mRNA to protein.

Tau	Drug	Effect	Ref.
Protein	AChE-inhibitors (exelon, rivastigmine and galantamine)	No effect on CSF-tTau and pTau levels	[191]
	Memantine	Decreased (normalization) CSF-pTau levels (n = 11)	[192]
	AN1792 (Aβ vaccine)	CSF-Tau levels decreased in antibody responders (n = 11) vs placebo subjects (n = 10, p <0.001)	[193]
	Statins	Simvastatin treatment, but not pravastatin, results in reduced CSF-pTau levels in all subjects (n = 10)	[194]
SNP-gene expression		Tau haplotype affects CSF-tTau levels in FTD	[195]
		Tau haplotype is associated with increased 4-repeat Tau gene expression in brain	[37]
		Minor SNP alleles are associated with increased CSF-tTau/pTau levels in the presence of amyloid-β deposition	[133]
mRNA	Paclitaxel (taxanes) for treatment of breast cancer	Tau mRNA downregulation is sensitive to drug	[171]
	Paclitaxel in PrP T44 Tg mice	Ameliorate motor impairment	[196]

As a protein, Tau/pTau in CSF has been monitored in AD (Alzheimer's disease) patients treated with AD drugs, while the effect of SNP-gene expression and mRNA have only been observed in clinical conditions and drug treatments in other conditions or in a transgenic animal.

AChE: Acetylcholinesterase; CSF: Cerebrospinal fluid; FTD: Frontotemporal dementia; pTau: Phosphorylated Tau; tTau: Total Tau.

Tau in two paired plasma/serum samples, suggesting the presence of Tau in some of the samples [176]. Indeed, a number of early reports using the tTau ELISA (AT120-HT7/BT2) assay or an assay for truncated Tau have reported increased Tau-like immunoreactivity in serum/plasma [177–181] of patients with severe neurodegeneration. However, not all studies have reported increased Tau levels in serum [182], suggesting that sample pretreatment and handling will be crucial in developing a reliable Tau assay in blood/plasma. The stable increased levels of CSF–Tau/pTau might be a unique feature of AD that will be reflected in the plasma of patients. A multiparametric approach will probably be needed to solve the problem of differential diagnosis of neurodegenerative diseases. Therefore, the development of new technologies such as xMAP (Luminex®) allowing the simultaneous analysis of as much as a

hundred biomarkers is promising. The diagnosis of neurodegenerative diseases will very probably rely on the definition of disease-specific protein profiles.

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Executive summary
The microtubule-associated protein Tau
• Alternative splicing and post-translational modifications lead to multiple Tau isoforms. • Tau proteins have major functions in the CNS in axonal transport and possibly in signal transduction. • Tau phosphorylation modulates its activity in the CNS.
Tau alterations in neurodegenerative diseases
• <i>MAPT</i> mutations are responsible for rare forms of dementia. • <i>MAPT</i> polymorphisms are associated with some dementias such as progressive supranuclear palsy, corticobasal degeneration, Parkinson's disease or Alzheimer's disease (AD). • Altered <i>MAPT</i> splicing is observed in genetic forms of dementia and in sporadic tauopathies. The consequent imbalance of Tau isoforms could be responsible of neuron dysfunction. • Tau aggregation is a major feature of a class of dementia named tauopathies. • Altered Tau conformation is observed in tauopathies. • Abnormal hyperphosphorylation of Tau is a main stigma observed in tauopathies. • Tau protein cleavage is observed in the brain of patients with tauopathies.
Tau proteins as biomarkers of brain pathology
• Immunohistology of Tau inclusions and electrophoretic analysis of Tau aggregates allows a classification of tauopathies useful for postmortem differential diagnosis.
Tau proteins as peripheral biomarkers of neurodegenerative diseases
• CSF total Tau levels have a clear diagnostic value for AD, but are insufficient for differential dementia diagnosis. • CSF phospho (p)Tau levels have an improved diagnostic value for AD compared with total (t)Tau levels and the potential for differential diagnosis depending on phosphorylation sites. • ApoE has no effect on CSF–tTau and pTau. Kinesin polymorphism is associated with high CSF–pTau levels. • CSF–pTau levels are altered very early during the disease course of AD and are therefore useful predictive biomarkers. • CSF–tTau levels have a diagnostic value in AD and Creutzfeld–Jacob disease, and a prognostic value in transient acute neuronal damage syndromes. • The combination of CSF–tTau and pTau levels together or with other biomarkers increases sensitivity and specificity of diagnosis. • Stable levels of CSF–tTau and pTau are potentially useful for monitoring novel therapeutic assays. • The newly identified multiple fragments of Tau in the brain and CSF are promising candidates for differential diagnosis of tauopathies.

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