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# **Tau as a biomarker of neurodegenerative diseases**

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## **Abstract Summary**

**The microtubule associated protein Tau is mainly expressed in neurons of the central nervous system 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 of these diseases named Tauopathies, including Alzheimer's disease. The molecular diversity of Tau is very useful when analysing it in the brain or in the peripheral fluids. Immunohistochemical and biochemical characterisation of Tau aggregates in the brain allows the post-mortem classification and differential diagnosis of Tauopathies. As peripheral biomarker 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 brain and in peripheral fluids will lead to novel promising biomarkers for differential diagnosis of dementia and monitoring of therapeutics.**

**Author Keywords** Alzheimer's disease ; biomarker ; microtubule-associated Tau protein ; neurofibrillary degeneration ; phosphorylation ; tauopathies

## **Introduction**

Tau protein is mainly expressed in the neurons of the central nervous system 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 of 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 pattern 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 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) (for review see [1]). The diagnosis of these diseases remains difficult because of the heterogeneity and the partial overlap of their clinical presentations. With the aging of 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 from post-translational modifications, makes it well suited as a biomarker of these neurodegenerative disorders. The morphological and biochemical characterisation of Tau inclusions has lead to a huge improvement of the post-mortem 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 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 the 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.

## **THE MICROTUBULE ASSOCIATED PROTEIN TAU FROM THE GENE TO THE ISOFORMS**

Tau is encoded by a single gene named MAPT, located on chromosome 17q21 (Figure 2A). MAPT gene belongs 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 greater than 0,8 (<http://www.hapmap.org>) and define an extended haplotype that cover the entire MAPT gene (Figure 2B) [3]. Only twenty of them show a LD measure smaller than 0,4. This haplotype even spans to a region covering ~1,8Mb [4]. The H2 haplotype is much more rare than the H1 haplotype in healthy individuals, showing different prevalence among ethnic groups and results

from H1 by the inversion of a ~900 kb segment resulting from a rearrangement between LCRs [2]. In the central nervous system (CNS), two transcripts of 2 kb and 6 kb arise from utilization of two alternative polyadenylation sites, the 2 kb mRNA targets Tau to the nucleus and the 6 kb encodes the major form in axons [5, 6]. 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 molecular weight of ~37-46 kDa (Figure 2D). Brain Tau proteins can be subdivided in four regions: an amino-terminal region named projection domain which is acidic, a proline-rich region followed by imperfect microtubule binding repeat motifs (encoded by exons 9 – 12) and a short carboxy-terminal region. Each isoform is characterized by the length of the N-terminal domain and by the presence of three 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 posttranslational modifications: phosphorylation (developed further), O-glycosylation, ubiquitination, nitration, glycation (for review, see [7]).

## **TAU FUNCTION IN THE CNS**

One major biological function of Tau is to build an ordered microtubule network in axons, which is essential for the axonal transport. The large carboxy-terminal microtubule binding domain promotes microtubule assembly and maintains the stability of the previously formed microtubules through repetitive sequences. The amino-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 [8] and dynein/dynactin complex [9] suggest a role in the dynamic of axonal transport. The binding to SH3 containing proteins such as Fyn, a Src family kinase [10], phospholipase C-gamma 1 or p85- $\alpha$  subunit of PI-3K [10] supports a role of Tau in neuronal signal transduction. Association of Tau amino-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 amino-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 4 repeats (4R-Tau) bind to microtubules with a greater affinity (for review see [1]) and are more efficient at promoting microtubule assembly than isoforms containing 3 repeats (3R-Tau). This suggests that Tau isoforms have specific functions [11]. 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 [12].

## **TAU PHOSPHORYLATION**

Eighty-five 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 [13] and that of one previously published review [14], 71 among the 85 putative phosphorylation sites that 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 [15].

For some of these kinases, the effect on Tau function has been described. The proline directed protein kinases (PDPK) like the 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 (for review, [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 insertm-00375314, version 1 - 14 Apr 2009 14/04/2009 14:04:54 7 them, MARK kinases are strictly involved in the regulation of Tau binding to microtubules by phosphorylating specific motifs (KXGS) within the microtubule-binding domains [16]. Noteworthy, GSK3 $\beta$  phosphorylates MARK and inhibits MARK activity, making a functional link between the two groups of kinases and showing that GSK3 $\beta$  and MARK both contribute to regulated the binding of Tau to microtubule. Regarding the tyrosine protein kinases, studies have determined that human Tau Tyr18 and Tyr29 are phosphorylated by the Src family tyrosine kinase Fyn [17, 18]. More importantly, GSK3 $\beta$  is shown to phosphorylate Tau and reduce the binding of Fyn, PLC- $\gamma$  1, p85 $\alpha$  subunit of PI3K [10]. Finally, cotransfection of Tau and tyrosine protein kinases showed that even if Tyr-18 is the major site for Fyn phosphorylation, Tyr-394 is the main residue for Abl. [19].

## **TAU ALTERATIONS IN NEUROLOGICAL DISEASES**

### **MAPT DELETION AND 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 [20-23], the prevalence of this new syndrome has been estimated to 1/13000 and 1/20000 [20]. One microduplication reciprocal to the microdeletion was described in a case of severe psychomotor developmentally delay with dysmorphic features [24]. This suggests that dosage sensitive genes are present in this

chromosomal region, perhaps 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 very rare autosomal dominant inherited dementia named FTDP-17 (Frontotemporal dementia with parkinsonism linked to chromosome 17) (Tau mutations are listed on [www.molgen.ua.ac.be/FTDmutations](http://www.molgen.ua.ac.be/FTDmutations)) [25]. Approximately one hundred families with MAPT mutations have been reported in the world. The clinical features of these dementia are variable but are usually characterized initially 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, N279K and one splice site mutation (exon10 +16) (Figure 2B) [25]. MAPT missense mutations have also been identified in PSP, CBD and Pick's disease. The vast majority of these mutations are located in the coding region or close to the splice-donor site of the 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 these familial cases, accumulation of hyperphosphorylated Tau protein in neurons has been observed (for review, see [26]).

## **MAPT GENE POLYMORPHISMS**

The first evidence that the H1 haplotype is associated to an increased risk for several Tauopathies was shown for PSP (progressive supranuclear palsy). Further studies were then investigated in other Tauopathies like AD, FTD, CBD or Pick's disease (for review, see [3, 27]) : except for CBD where a significant overrepresentation of H1 haplotype in affected patients was observed, results among studies were controversial and do not allow to conclude insert-00375314, version 1 - 14 Apr 2009 14/04/200914:04:54 9 that Tau gene polymorphism is a risk factor for these diseases. In Parkinson's disease, two recent studies showed that the H1 haplotype was associated with a significantly increased risk for Parkinson's disease (PD) [28, 52]. Moreover, the sub-haplotype H1c, located in the promoter region of Tau gene, has been shown to be a more specific risk factor for PSP [29]. Association analysis of this H1c subhaplotype with AD [30, 31] is controversial. 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, showed significantly greater expression in vitro of Tau proteins than H2 [32] but this was not confirmed in vivo on unaffected post-mortem human brain [33]. However, the H1 haplotype, appears to express up to 43% more exon 10+ transcripts than H2 [34-36]. Interaction between MAPT gene and other potential pathogenic genes was also reported. For instance, the combination of risk genotypes of both MAPT and APOE has been recently shown to approximately double the risk for the development of PD [37].

## **ALTERED MAPT SPLICING**

Dysregulation of splicing can be caused either by alteration of cis-sequences on MAPT gene or alteration of MAPT splicing factors. The importance of MAPT splicing in the pathophysiology 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.

Myotonic dystrophy (DM) is the most prevalent form of adult-onset muscular dystrophy. Beside the cardinal muscle symptoms (myotony, progressive myopathy), other common troubles affect multiple organ systems (heart, genital tract, eyes, endocrine system), including the central nervous system : 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 [38, 39]. 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 [40]. 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") [41-45]. 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). Missplicing of exon 10 in DM1 is inconstantly observed [43, 44]. 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 [46] implicating a RNA-mediated trans mechanism (for review, see [40, 47]).

Several studies suggest that altering MAPT splicing could be responsible of central nervous dysfunction by altering the Tau isoform ratio: overexpression of Tau 4R in neuroblastoma cells showed enhanced susceptibility to cell death [48] and animal models with excess of exon 2/3 inclusion causes gliopathy and spinal cord degeneration [49]. Thus, abnormal Tau splicing is sufficient to cause neurodegeneration, although the exact mechanism that leads to clinical symptoms is not clear. A disruption of proper balance of Tau

isoforms is also observed in several sporadic Tauopathies, including FTD, PSP and CBD [50 ], PiD [51 ], PD [52 ] and is inconstantly observed in AD [53 -56 ]. 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 [6 ].

## **TAU AGGREGATION**

In more than 20 neurodegenerative disorders, referred to as Tauopathies, Tau proteins aggregate in the affected cortical and subcortical brain regions [57 ], forming intraneuronal inclusions. The most 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. During long time, it was admitted that Tau aggregation correlates with neuron loss and neuron toxicity; this was suggested by the fact that Tau aggregate spreading in AD brain increases with cognitive decline. More recently, some studies suggested that Tau aggregation can be dissociated from neuronal loss [58 , 59 ] and can be even be protective [60 ] as suggested for Huntingtin in Huntington disease [61 ]. Small Tau oligomers could be involved in neurodegeneration [62 ].

## **ALTERED TAU CONFORMATION**

Specific conformational changes of Tau are observed at 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 [63 ]. When the aggregation occurs, fibrils take a very well structured organization corresponding to paired helical filaments (PHF) in neurofibrillary tangles for AD.

## **TAU HYPERPHOSPHORYLATION**

The most prominent modification of Tau in all Tauopathies is abnormal phosphorylation characterized by i) high level of phosphorylation on epitopes localized at the half-N-terminus and C-terminus outside the microtubule binding domains (e.g. epitopes pTau181, pTau199, pTau231 pTau396 and pTau404) and ii) additional pathological phosphorylation sites (e.g epitope pTau422) (phosphorylation sites on PHF-Tau are reviewed in [14 ]).

One consequence is that the most striking difference in between post-mortem Tau and PHF-Tau proteins is their electrophoretic profiles. Normal "post-mortem" Tau proteins are resolved as 6 main bands (45-67 kDa) whereas more acidic hyperphosphorylated PHF-Tau are resolved as four bands comprised between 60 and 74 kDa (see further, Figure 4 ). In normal brains, the pTau epitopes are rapidly dephosphorylated during the post-mortem 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 1) the aggregation of Tau proteins into filaments that render them inaccessible to phosphatases; 2) phosphatases that are no more activated in degenerating neurons or 3) phosphatases activity is already decreased [64 ]. Nevertheless, hyperphosphorylation and abnormal phosphorylation may 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 hippocampus. Phosphorylation at threonine 181 and serine 199 occurs later, and these are only found to any appreciable extent in intracellular tangles [65 ]. Reactivity to TG3, an antibody that recognizes phosphothreonine 231, is found in pretangles, intracellular tangles and extracellular tangles and so 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 Serine 396 and Serine 404 or AT100 that recognizes the phosphorylated Threonines 212-217 and Serine 214. Nonetheless, to generate the pathological phospho-sites at AT100 epitope a sequential phosphorylation by two kinases [66 ] is necessary suggesting that several kinases may be deregulated in AD. Among the kinases described above, GSK3- $\beta$  and cdk5 play an important role in regulating Tau phosphorylation under pathological conditions.

Hyperphosphorylated Tau-epitopes are not the same in all Tauopathies: in PiD, Pick bodies, the neuropathological feature of this disease and composed of aggregated Tau proteins, 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 [67 , 68 ]. 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 [69 ]. Tau hyperphosphorylation is a relatively early event in the development of AD [70 ]. Phosphorylated Tau appears to be more resistant to proteolysis by different proteases [71 ] 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 [72 , 73 ] and calpains [74 ] and is degraded by the proteasome [75 ] . Tau fragments generated by different proteases may differ in their ability to assume pathological conformations, to aggregate and to induce neurotoxicity [76 , 77 ] . It has been shown that successive cleavage events occur on Tau protein during the course of NFT evolution in AD [76 ] . In AD and in other neurodegenerative disorders, Tau truncation by caspases is an early event [78 ] . Calpains also become over-activated because of elevation of cytosolic Ca<sup>2+</sup> levels. Inhibition of the proteasome leads surprisingly to an increased Tau degradation [79 ] . Once proteolysed, Tau fragments turn into effectors of apoptosis and initiate and accelerate DNF development [80 , 81 ] . 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 [78 ] . 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 overrepresented: it was detectable in PSP, CBD and 4R forms of FTDP-17 but was absent of controls, AD and PD brains [82 ] . 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: neurofibrillary tangles, neuropil threads, dystrophic neurites of neuritic plaques and Pick bodies. 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 “Bar Code” for neurodegenerative disorders [15 , 83 ] .

### **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 [84 ] . This pathological Tau quartet corresponds to the aggregation of the six Tau isoforms [85 , 86 ] . This classification is summarized on Figure 4 . Adjacent to the Western blot image are the isoform composing each bands (For review see: [1 ] ). Using histochemistry, aggregates of class I Tauopathies positively react with AD2, and antibodies directed against exon2 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 corticobasal degeneration (CBD), Argyrophilic grain dementia (AGD), progressive supranuclear palsy (PSP), and Frontotemporal dementia linked to chromosome 17 due to Tau gene mutations [87 , 88 ] . PSP, CBD and AGD are rare atypical Parkinsonism disorders which classification was recently updated by the Consortium for Frontotemporal lobar degeneration [89 ] .

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

Class III of Tauopathies includes PiD and autosomal dominant inherited FTDP-17 (Figure 4C ) . Pick's disease is a rare form of neurodegenerative disorder characterized by a progressive dementing process. Early in the clinical course, patients show signs of frontal disinhibition [90 ] . 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-exon2 antibodies and negative with anti-exon10 antibody.

### **Class IV: Tau isoform lacking exon 2, 3 and 10 principally aggregate**

Class IV is represented by myotonic dystrophy of type I and type II (Figure 4D ) . Neuropathological lesions, such as neurofibrillary tangles, 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 69kDa. This typical pathological Tau profile is reflected by a reduced number of Tau isoform expression in the brain of individuals with DM1, at both the protein and mRNA levels [44 ] . The analysis of multiple brain regions of one genetically confirmed DM2 patient aged of 71 years, showed some neurofibrillary degenerating processes. Using specific immunological probes against amino acid sequences corresponding to exon 2 and exon 3 corresponding, the neurofibrillary lesions were shown to be devoid of Tau isoforms with Nterminal inserts (Figure 4D ) [45 ] . 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 central nervous system 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 lead to a better understanding and detailed characterization of Tau in normal and diseased brain, but has also advanced considerably our understanding of Tau as biomarker in circulating fluids for a number of neurological conditions. While the first observations on the presence of Tau in CSF were based on several sandwich immuno-assays, improvement in highly sensitive western-blotting techniques and the use of immuno-capture mass-spectrometric techniques has lead to the unequivocal characterization of Tau in CSF.

### **CSF TOTAL TAU : A BIOMARKER OF NEURONAL DEGENERATION**

The presence of Tau in cerebrospinal fluid (CSF) was first described in 1993. Quantitative analysis of total Tau in CSF (CSF-tTau) was developed, using ELISA assays with different Tau antibodies that detect all Tau isoforms independently of their phosphorylation state (see Figure 5 for antibodies used in these assays). These ELISAs showed that CSF-tTau displays an age-associated increase in non-demented control individuals [91 ] 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 2-3 times higher compared to healthy controls (for review, see [92 ]). The most commonly used is a commercial sandwich ELISA kit (AT270-HT7), that shows a good specificity (90%) and sensitivity (81%) discriminating AD from age-matched controls [93 ]. Age-adjusted reference values have been established to improve discrimination between AD and controls: <300 pg/ml in the group 21- 50 years of age, <450 pg/ml in the group 51-70 years of age and <500 pg/ml in the group 71- 93 years of age [ 94 ]. There is evidence that CSF-tTau reflect the presence of the neuropathological hallmarks of AD from neuropathological studies [95 ].

The increase of CSF-tTau comes probably from leakage of Tau from damaged neurons into CSF and therefore reflects the intensity of neuronal damage and degeneration. This is assumed from the fact that in acute conditions as stroke or Creutzfeldt Jacob disease (CJD), there is a marked increase in CSF-tTau. After acute ischemic stroke, CSF-tTau level increases transiently (Table 1 ). 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 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 good as the established 14-3-3 marker for CJD [96 ]. Elevated values for CSF-tTau have also been found in bovine variant of CJD [97 ]. Increase of CSF-tTau levels are observed in several other acute neurological conditions, like severe malaria [98 ], Wernicke's encephalopathy [99 ], pediatric patients with brain tumor, hydrocephalus or serious CNS infections [100 ].

Studies assessing CSF-tTau in non-AD dementia such as FTD, PSP, CBD, Lewy Body Dementia (LBD), PDD and Amyotrophic Lateral Sclerosis (ALS) give contradictory results. In FTD cases, CSF-tTau has been found increased by some investigators and reported normal or reduced by others [101 -103 ]. In LBD, CSF-tTau has been found to be increased in some studies [104 ] and not in others [105 , 106 ] including one on an autopsy verified series [107 ]. Increased CSF-tTau has been also reported for PDD patient [108 , 109 ]. In PSP and CBD syndrome, CSF-tTau levels were found within normal range [109 ] or increased [92 ]. In ALS, inconstant increased CSF-tTau levels are described [110 , 111 ]. 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 [92 ]. These conflicting results might be caused by selection bias as high concomitant AD pathology was observed at autopsy in patients clinically diagnosed as VaD [112 ].

Normal CSF-tTau are found in several neurological diseases such as other type of dementia (alcoholic dementia), chronic neurological disorders (parkinson's disease [without dementia], multiple sclerosis) and psychiatric disorders (depression) [113 , 114 ].

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, and 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 phospho-dependent antibodies showed that phosphorylated Tau is recovered in the CSF and that several phospho-epitopes are present (for review see [92 ], such as Thr181, Thr235, Ser396 and Ser404. A moderate to marked increase in CSF-pTau compared to controls has been found for all the different ELISA methods based on these antibodies (for review, see [92 ], [93 ]). 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% [115]. A study compared the diagnostic performances of pTau181, pTau199 and pTau231 in the same samples [116]: all three pTau assays discriminated as well AD from normal aging.

There is no change in CSF-pTau after acute stroke [92], 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 [117]. 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 (parkinson's disease, multiple sclerosis or ALS) and psychiatric disorders (depression) [91, 118] and also in most cases of other dementia such as VaD, FTD and LBD [92, 116]. This implies that the specificity of pTau to differentiate from other dementias is higher than for total Tau, reaching more than 80% [119]. However, differences were observed between phospho-epitopes in their potential to discriminate AD from other dementias: the best discrimination between AD and FTD being obtained with pTau231, between AD and DLB with pTau181 [119, 120]. The use of phospho-Tau as a marker to distinguish dementia with Lewy bodies from fronto-temporal lobe dementia [120] also shows promising results, which needs to be further confirmed and extended [102, 121]. 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 TOTAL TAU AND pTAU LEVELS**

Interindividual variations of CSF Tau and pTau levels are important in controls and in AD patients. Therefore, some investigators studied the impact of polymorphisms known to be associated to an increased risk of developing AD on these levels. Neither apoEεε nor MAPT H1/H2 haplotype influenced CSF of total Tau or phospho-Tau [122-126]. Concerning association of MAPT H1c haplotype with CSF-tTau levels, results are contradictory [126, 127]. 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 disease. Finally, a very interesting recent observation was that a polymorphism of the kinesin light chain 1 gene (KNS2), that has been demonstrated to be associated to an increased risk of developing AD [128], was associated with high CSF pTau levels in MCI patients who converted to AD [129].

### **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 pre-tangle PHF and mature neurofibrillary tangles that are present within the brain even before the onset of AD [70]. 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 mild cognitive impairment (MCI). Patients with MCI can evolve to AD or remain with their MCI. The former are named converters or prodromal Alzheimer patients [130].

Several studies showed that high CSF-tTau and CSF-pTau levels were found already in early AD and the performance of these biomarkers in AD cases with MMSE (Mini-Mental State Examination) scores above 23-25 have been found to be similar to those in more advanced AD [131]. 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 [132]. 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 Ser 199 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% [133-139]. It is important to note that as only ~15% of MCI cases progress to AD each year [140], only very extensive follow up (more than 5 years) are needed to ascertain which patients will not develop dementia. Until today, most studies were done on 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 familiar AD showing that increased CSF-tTau and CSF-pTau levels are elevated in presymptomatic PSN-1 or APP mutation carriers [141]. 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 AND 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 the sustained unchanged levels over longer periods of time during the disease process (see Table 1 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 CVs ranging from 6.1% in a 6 month follow-up study to 21% in a 1 year follow-up. Studies in other neurodegenerative diseases such as brain trauma, neurotoxicity, alcohol dementia the increase in CSF-tTau levels is transient (Table 1). While some of these studies in neurodegenerative diseases associated with neuronal/axonal loss seem controversial, 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 [142], Guillain-Barré syndrome [143] or traumatic brain injury [144] suggest that degree of elevated Tau is related to poor outcome and thus may have prognostic value. Thus 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 Creutzfeld-Jacob disease where the elevation of Tau is high and relatively stable, the increase of Tau levels has diagnostic and prognostic value [96 , 130 , 145 ].

### **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. Especially, the association of CSF amyloid peptide A $\beta$  that reflect the presence of amyloid plaques in AD brains increases the diagnostic power of Tau proteins (for review, see [92 , 146 -148 ]).

Stable increased tTau/pTau levels do not only have value in differential dementia diagnosis [149 ], but might also be useful in early diagnosis. Also in this early stage of the disease the levels of CSF-tTau/pTau is stable over time (Table 1 [135 , 139 , 145 , 150 ]). 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 [151 ]. Moreover, the ratio of pTau to total CSF-tTau was found to discriminate CJD from other neurodegenerative disease without any overlap [117 ]. Finally, pTau and/or pTau/tTau ratio might be important markers to differentiate subtypes of CJD [97 , 152 ].

### **CSF-tTAU AND pTAU AS THERAPEUTIC MONITORING BIOMARKERS**

Stable levels over longer periods of time are not only useful in early diagnosis but offers also potential as a surrogate biomarker. The definition of a surrogate endpoint is that a biomarker is intended to substitute for a clinical endpoint. It is expected that such an endpoint predicts clinical benefit based on epidemiologic, therapeutic, pathophysiological, or other scientific evidence [153 ]. Several small drug studies have already studied CSF-tTau/pTau in relation to drug treatment in AD patients and a number of these studies suggest that Tau/pTau levels can be 'normalized/decreased' upon treatment (Table 2 ). In particular the reduction of CSF-tTau in immunotherapy might be particularly relevant. Furthermore, association studies with single nucleotide polymorphisms suggest that the use of Tau measurements can further be improved taking into account those associations (Table 2 ). Also discoveries in other drug treatments such as the association of Tau mRNA levels with sensitivity to Paclitaxel treatment of breast cancer [154 ], might also be of relevance for Tau-related disorders [155 ] (Table 2 ). However, clearly this field of biomarker research is 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 kDa to 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 55kDa and 33kDa [109 ]. The 55kDa band contains a large domain of Tau extending from the N-terminal region (amino acid 159) to the carboxy-terminal region (amino acid 432). In contrast, the 33kDa band shares a similar amino-terminal epitope with that of the 55kDa band, but is truncated at the C-terminus of Tau. Those Tau fragments were shown to be present in the brain tissue [82 ]. A 14kDa fragment of Tau was also evidenced by Zemlan and collaborators using C-terminal Tau antibodies [156 ]. 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 3 specific of unique Tau isoforms [157 ]. 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, Tau ratio 33 kDa/55 kDa was significantly decreased when compared to controls and AD, FTD and CBD. This is consistent with the recent study in PSP brain identifying a Tau fragment specific of 4R isoforms [82 ]. 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 [157 , 158 ] may lead to simple biochemical tests that may assist in sometimes-difficult neurological diagnosis.

### **FUTURE PERSPECTIVE**

For the use of Tau as biomarker in large clinical trials or in clinical practice, one important goal in the future years will be to develop sensitive methods to detect the very low serum-00375314, version 1 - 14 Apr 2009 14/04/200914:04:54 25 concentration of Tau in the blood (~30 pMol) and even in urine. One promising technique will be immunoPCR. High-confidence protein identifications in the HUPO plasma proteome have identified Tau in two paired plasma/serum samples, suggesting the presence of Tau in some of the samples [159 ]. Indeed a number of early reports using the tTau ELISA (AT270-HT7) assay or an assay for truncated Tau have reported increased Tau-like immunoreactivity in serum/plasma [160 -164 ] of patients with severe neurodegeneration. However, not all studies have reported increased Tau levels in serum [165 ], suggesting that sample pre-treatment 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 like xMAP (Luminex®) allowing the simultaneous analysis of as much as hundred

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

## **EXECUTIVE SUMMARY**

### **The microtubule associated protein Tau**

- alternative splicing and posttranslational modifications leads 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**

- MAPT mutations are responsible of rare forms of dementia
- MAPT polymorphisms are associated with some dementia like PSP, CBD, PD or AD
- Altered MAPT splicing is observed in genetic forms of dementia and in sporadic Tauopathies. The consequently 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
- Tau abnormal hyperphosphorylation is a main stigmat observed in Tauopathies.
- Loss of Tau protein expression is observed in some types of FTDs.
- 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 usefull for post-mortem differential diagnosis.

### **Tau proteins as peripheral biomarkers of neurodegenerative diseases**

- CSF total Tau levels has a clear diagnostic value for AD but is insufficient for differential dementia diagnosis
- CSF phospho-Tau levels have an improved diagnostic value for AD compared to total Tau levels and a 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 has a diagnostic value in AD and CJD and a prognostic value in transient acute neuronal damage syndroms.
- The combination of CSF Tau 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 new identified multiple fragments of Tau in brain and CSF are promising candidates for differential diagnosis of Tauopathies.

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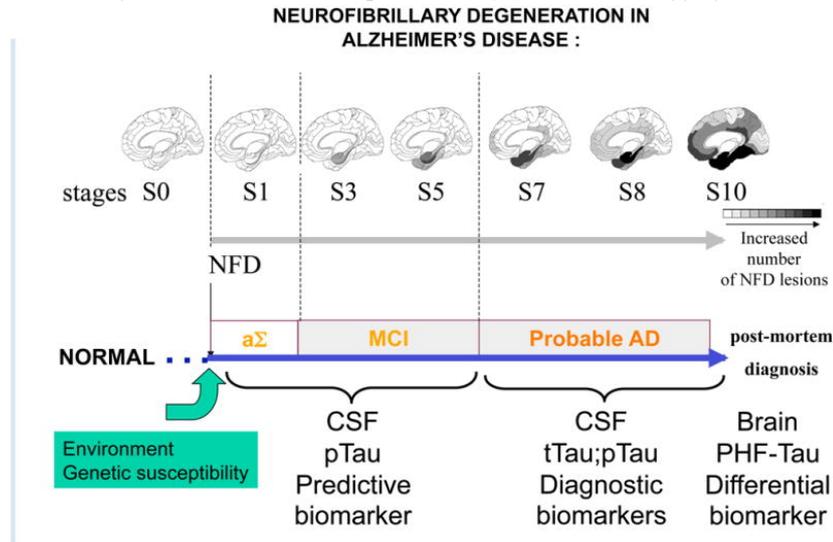
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**Figure 1**

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

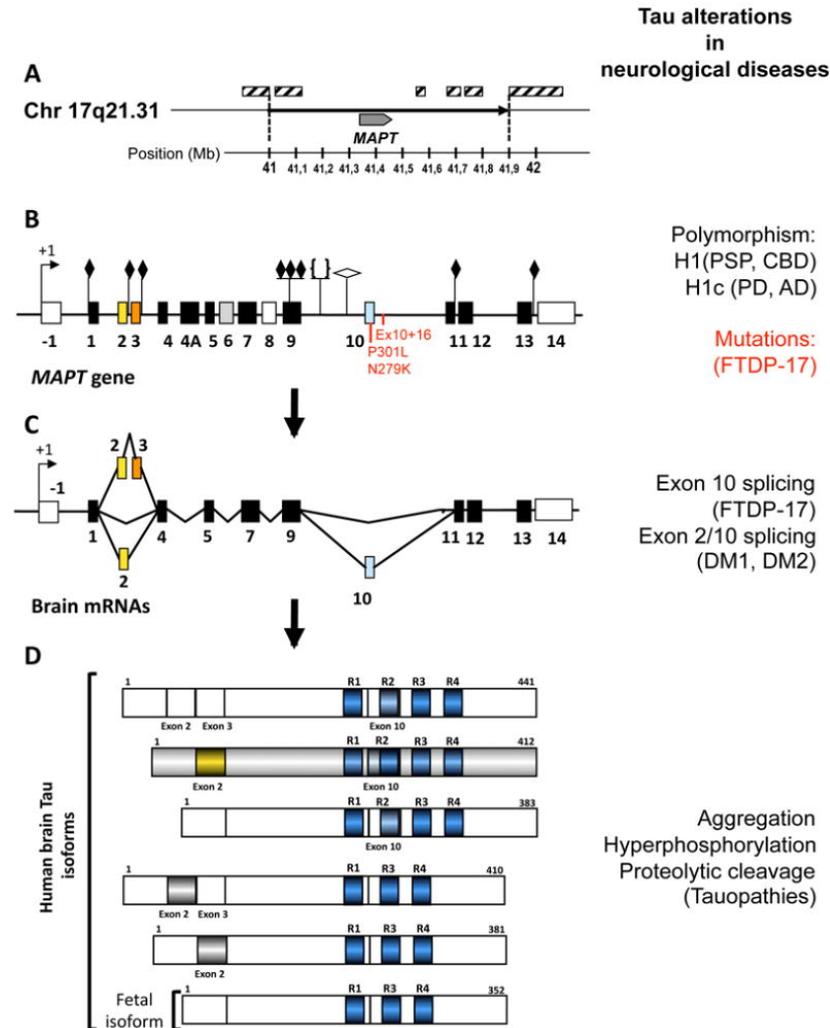
AD lesions, namely neurofibrillary degeneration, follow a stereotyped, sequential, hierarchical pathway. The progression is categorized into 10 stages according to the brain regions affected: transentorhinal cortex (S1), entorhinal (S2), hippocampus (S3), anterior temporal cortex (S4), inferior temporal cortex (S5), medium temporal cortex (S6), polymodal association areas (prefrontal, parietal inferior, 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 (MCI): 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 disease course. Only the post-mortem analysis of brain with the presence of the two characteristic lesions of AD (amyloid plaques and NFD) allows 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.



## Figure 2

### Microtubule-associated Tau gene, RNAs and human brain isoforms

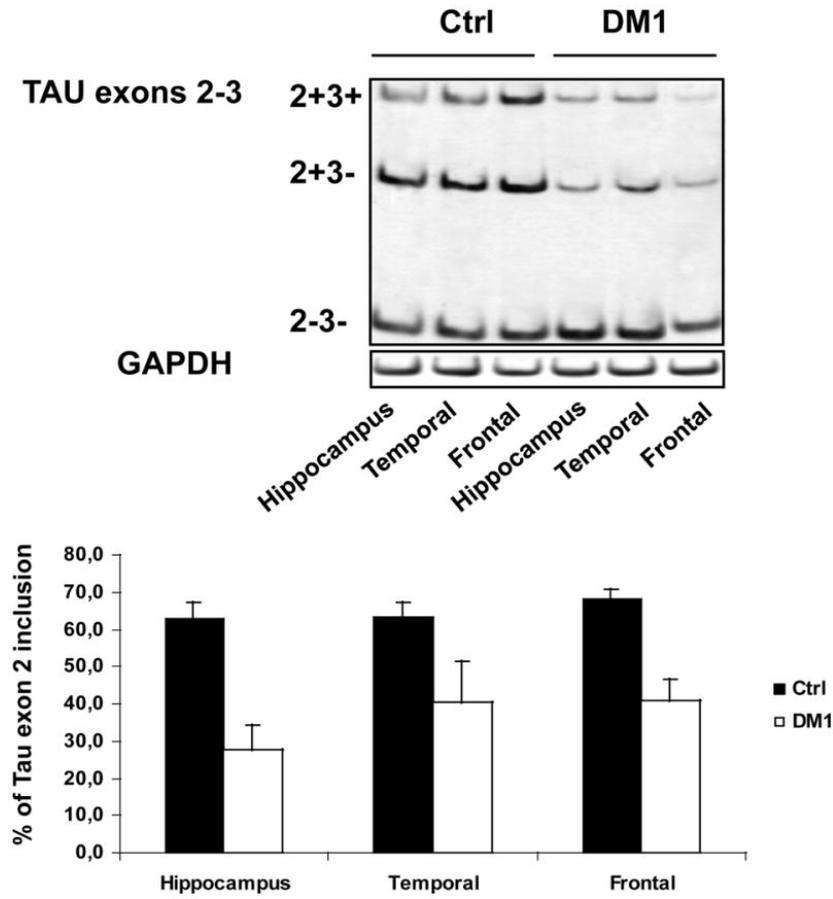
(A) Genomic architecture of the 17q21.31 region encompassing *MAPT* gene and flanked by low copy repeats (rectangles) that are susceptible to chromosomal rearrangements such as deletions, duplications or inversion. Dotted lines illustrate the breakpoints responsible for the inversion of a ~900kb segment resulting in the H1/H2 polymorphism. (B) Tau gene *MAPT* spans more than 130 kb and is composed of 16 exons. The most studied polymorphism associated to the H1/H2 haplotype are 8 SNP (♦), 1 (TG)<sub>n</sub> microsatellite ({}), and a 238pb insertion/deletion (◊). H1 haplotype is associated with PSP and CBD. The three most frequent mutations responsible of FTDP-17 are in red. (C) Several mRNAs are generated by alternative splicing of exons 2, 3, 4A 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. The exon 10 encodes an additional microtubule-binding motif numbered R1 to R4. Half of Tau proteins contain three microtubule-binding motifs and the other half has four microtubule-binding motifs.



**Figure 3**

Semi-quantitative analysis of Tau exon 2 in control and DM1 brains

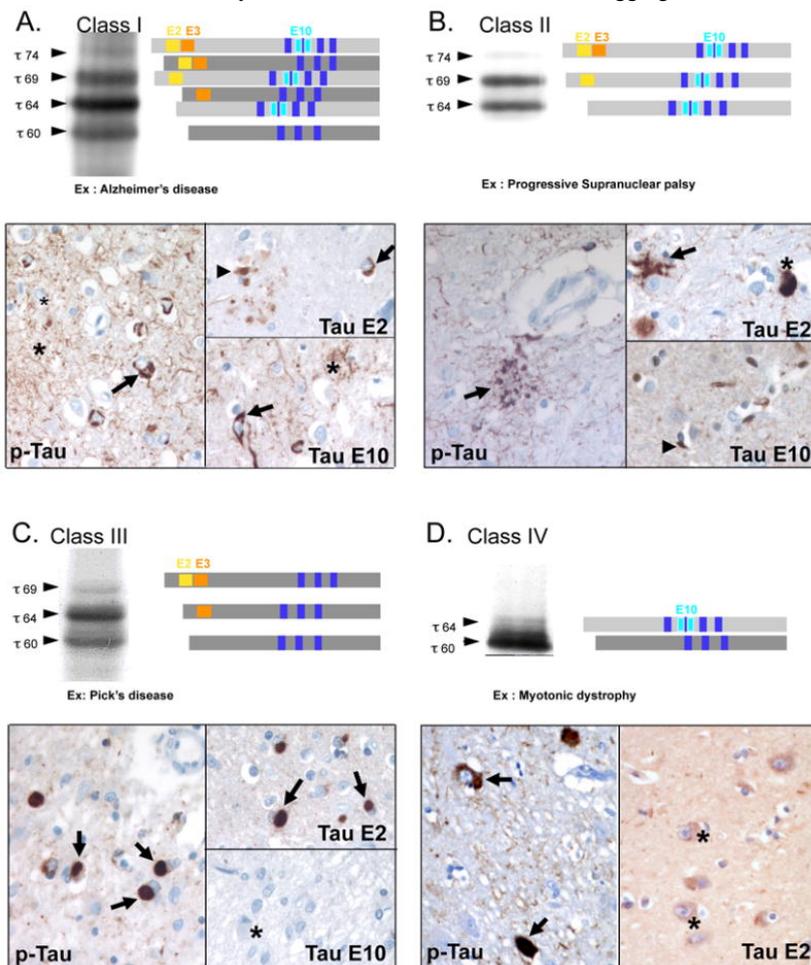
Reduced Tau exon 2 inclusion in DM1 brains. Tau exons 2 and 3 are alternatively spliced. Splicing of these two exons generated 3 Tau transcripts named 2+3+, 2+3- and 2-3-. The proportion of Tau transcripts including exon 2 and 3 was analysed by RT-PCR in three brain area (hippocampus, temporal and frontal cortex) in two control and in one DM1 patients. (B) Histogram of the percentage of transcripts including exon 2. The bands were quantified and the results expressed as the percentage of transcripts including exon 2. The mean and standard deviation were calculated from three individual experiments. Histograms are representative of the mean +/- standard deviation of three independent experiment.



## Figure 4

### The "Bar code" 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 two-dimensional 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 degenerative 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 i.e 1) intraneuronal somatic neurofibrillary tangles (NFT) 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 (NP). 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 "globoïd" neurofibrillary tangles 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 FTDP-17, corresponding to Class III diseases. Pick bodies are stained by anti-pTau and Tau E2 (arrows showing spherical 10µm bodies) but not by Tau E10 (in the lower right panel, a Pick body is readily seen but unstained, asterisk). D. At last, Class IV is characterized by the aggregation of Tau isoforms lacking sequences encoded by exons 2 and 3. Uptoday, the only known class IV diseases DM1 and DM2. On the left panel aggregates of hyperphosphorylated Tau are labelled with AD2. On the right panel, only unphosphorylated, non aggregated normal Tau are stained by anti-Tau E2 which in turn labels no aggregate.



### Figure 5

#### Characterization of Tau in cerebrospinal fluid

Sequence of the largest form of brain-derived Tau, containing all alternatively spliced exons, is shown. Boxes in colour are the alternatively spliced exons and the microtubule-binding repeats: yellow, exon2; green exon 3, light blue exon 10 and the boxes in blue are the four tubulin-binding repeat domains. Boxes indicate the epitopes of some antibodies commonly used i) in immuno-assays to quantify Tau in human CSF: for total Tau HT7, AT120 and BT2 are used, HT7-AT270, Tau-1-PHF-1 and Tau-1/CP27 are used in phospho-specific assays. [116 , 166 ] ii) in brain tissue analysis : AD2, AT100 or 12E8 for Tau phospho-epitopes. Letters in red indicate the 8 sequences identified using an optimized immunoprecipitation (BT2) mass spectrometry analysis of 16 peptides [157 ].

```
10      20      30      40      50      60
MAEPRQEFEV MEDHAGTYGL GDRKDQGGYT MHQDQEGDTD AGLKESPLQT PTEDGSEEPG
70      80      90     100     110     120
SETSDAKSTP TAEDVTAPLV DEGAPGQAA AGPHTKIPES TAEAEAGIGD TPSLEDEAAG
130     140     150     160     170     180
HVTQARMYSK SKDGTGSDDK KAKGADGKTK IATPRGAAPP GQKQANATR IPAKTTPAPK
190     200     210     220     230     240
TPPSSGPEPK SGRSGYSSP GSPGTPGSRG RTPSLPTPEPT REPKKVAVVR TPPKSPSSAK
AT270 BT2/Tau-1 AT100 AT120 CP27
250     260     270     280     290     300
SRLQTAPVPM PDLKNVYSKI GSTENLKHQP GGGKVQIINK KLDLSNVQSK CGSKDNIKHV
12E8 EXON 10
310     320     330     340     350     360
FGGGSVQIVY KVDLSKVTG KCGSLGNIHH KPGGGQVEVK SEKLDFKDRV QSRIGSLQNT
12E8
370     380     390     400     410     420
KIVFQGNKK IETHKLTFRE NAKAKTDHGA EIVYKSPVVS GDTSEPRHLSN VSSTGSIDMV
PHF-1 AD2
430     440
DSPQLATLAD EVSASLAKQG L
```

**Table 1**

Follow-up studies of Tau and pTau levels during chronic and acute neurodegenerative disorders.

Study	Diagnostic group	Follow-up	tTau baseline (pg/ml)	tTau follow-up	Ptau baseline (pg/ml)	Ptau follow-up
<b>Stable Tau/pTau levels in CSF of AD and CJD patients</b>						
[167]	9 AD	18.6 mo (2 – 43)	847 ± 253	878 ± 452	NA	
[168]	192 AD	1 yr	21% CV		NA	
[169]	29 AD	2 yrs	548 ± 355	577 ± 275	NA	
[135]	8 MCI	1 yr			530 ± 450*	560 ± 480*
[123]	20 AD	3-8 yrs	568 ± 365	570 ± 435	NA	
[170]	53 AD	6 mo	425 ± 30	431 ± 30 (6.1% CV)	93 ± 6	94 ± 6 (4.4% CV)
[150]	50 AD	21 ± 8 mo	731 ± 363	791 ± 419	84 ± 34	85 ± 35
[139]	22 MCI-AD	2.1 ± 0.5	750 ± 553	667 ± 327	49 ± 31	52 ± 29
[145]	12 MCI-AD	2 yrs	700 (240 -1700)	710 (270 – 1300)	86 (47-150)	86 (48-150)
[171]	66 CJD	6 wks	tTau Not significant		NA	
<b>Transient increase in CSF-tTau/pTau levels in conditions with neuronal/axonal loss</b>						
[172]	Brain trauma, n=9		770 ± 160 (wk 3)	250 ± 40 (3-5 mo)	37 ± 8 (wk 3)	29 ± 3 (3-5 mo)
[173]	Neurotoxicity, n=6		535-776 on day 8	<300 after 200 days	NA	
[99]	Alcoholic dementia		CSF-tau/P-tau significantly elevated in acute Wernicke's encephalopathy (WE, n=10) but not in chronic WE and after longterm follow-up			

**Table 2**

Tau as a potential surrogate biomarker: from mRNA to protein

As a protein Tau/pTau in CSF has been monitored in AD 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.

Tau	Drug	Effect	Reference
Protein	AChE-inhibitors (exelon, rivastigmine, galantamine)	No effect on CSF-tTau/Ptau levels	[174]
	Memantine	Decreased (=normalization) CSF-pTau levels (n=11)	[175]
	AN1792 (Aβ vaccine)	CSF-tau levels decreased in antibody responders (n=11) vs placebo subjects (n=10, p<0.001)	[176]
	Statins	Simvastatin-treatment, but not pravastatin, results in reduced CSF-pTau levels in all subjects (n=10)	[177]
SNP-gene expression		Tau haplotype affects CSF-tTau levels in FTD	[178]
		Tau haplotype is associated with increased 4-repeat tau gene expression in brain	[34]
mRNA		Minor SNP alleles are associated with increased CSF-tTau/pTau levels in the presence of amyloidbeta deposition	[126]
	Paclitaxel (taxanes) for treatment of breast cancer	Tau mRNA down-regulation is sensitive to drug	[154]
	Paclitaxel in PrP T44 Tg mice	Ameliorate motor impairment	[179]