
54 Neurofibrillary Degeneration: Patterns of tau Isoform Expression

ANDRÉ DELACOURTE

INTRODUCTION

Tau proteins are the basic components of the pathological filaments that accumulate in neurons and glial cells affected by neurofibrillary degeneration (Iqbal et al., 1998; Buée et al., 2000). Tau pathology is observed in more than 20 different diseases, including Alzheimer's disease (AD), progressive supranuclear palsy (PSP), corticobasal degeneration (CBD), Pick's disease (PiD), myotonic dystrophy (MyoD), familial frontotemporal dementia with parkinsonism, associated with chromosome 17 (FTDP-17), etc. Tau is an outstanding marker, well correlated with clinical manifestations. Indeed, pathological tau proteins observed in the neocortical association areas are systematically associated with dementia. Also, tau pathology in the brain association areas is a degenerative process specific to humans. Taken together, tau pathology reveals precisely the intensity and the extent of the degenerative process (Delacourte and Buée, 2000). This pathological entity can be defined according to six different molecular parameters, which are presented here.

NEUROFIBRILLARY DEGENERATION (NFD)

A MODERN DEFINITION

NFD is a degenerative process, visualized at the histological level using silver stains, as demonstrated by Aloïs Alzheimer and colleagues at the beginning of the twentieth century (Alzheimer, 1907). This technique is still used for neuropathological examination, and reveals in detail the abnormal intracellular fibrils that accumulate in cell bodies and in neurites: neurofibrillary tangles, neuropile threads and dystrophic neurites of senile

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plaques. These lesions are composed of bundles of filaments that result from the aggregation of tau proteins. Using antibodies against tau, it has been shown that NFD is a degenerative process found in numerous neurodegenerative disorders. All approaches combined, the modern definition of neurofibrillary degeneration is as follows: a degenerating process characterized by the abnormal filamentous accumulation of tau proteins in neurons and glial cell.

PATHOLOGIES WITH NEUROFIBRILLARY DEGENERATION

NFD is observed in more than 20 other neurodegenerative disorders (Table 54.1). All these diseases are very different in that they are familial or sporadic, with different origins, from mutations on tau gene to traumatism. NFD is a many-sided pathological process that can preferentially affect either subcortical nuclei or neocortical areas, neurons or, in addition, astrocytes or oligodendrocytes. The pattern of NFD lesions is also different and characteristic, according to the type of cell affected and the subcellular

Table 54.1. Presentation of the different neurodegenerative disorders with a tau pathology, and their different biochemical tau signatures, from Class I to Class IV

Diseases	Classes of tau pathology
Ageing (hippocampal region, patients over 75 years)	I
Alzheimer's disease, familial and sporadic	I
Amyotrophic lateral sclerosis/parkinsonism–dementia complex of Guam	I
Argyrophilic grain dementia	
British type amyloid angiopathy	I
Corticobasal degeneration	II
Dementia pugilistica/autism with self-injury behavior	I
Down's syndrome	I
FTDP-17	II, I and III
Gerstmann–Straussler–Scheinker disease (rarely)	I
Hallervorden–Spatz disease	
Inclusion body myositis	
Multiple system atrophy	
Myotonic dystrophy	IV
Niemann–Pick disease type C	I
Pick's disease	III
Presenile dementia with tangles and calcifications	
Prion protein cerebral amyloid angiopathy	
Progressive supranuclear palsy	II
Post-encephalitic parkinsonism	I
Subacute sclerosing panencephalitis	
Tangle only dementia	

location, such as the Pick bodies of Pick's disease (PiD) or the neuritic plaques of AD. At the electron microscopic level, the filamentous material of NFD is either helical (5AD), twisted (PSP, CBD) or mainly straight (PiD) (Delacourte and Buée, 2000).

THE ROLE OF TAU PROTEINS

Tau proteins belong to the microtubule-associated proteins (MAP) family. The human tau gene is unique and located over 100 kb on the long arm of chromosome 17 at band position 17q21, and contains 16 exons. Exons 2, 3 and 10 are alternatively spliced and are adult brain-specific. In the human brain, the tau primary transcript gives rise to six mRNAs, three of them with exon 10. Translation of exon 10 adds a fourth repeated sequence, which is a binding site to tubulin dimers, the basic components of microtubules. The normal role of tau is to stabilize microtubules, which are the tracks of the intraneuronal transport. Stabilization of microtubules is dramatically increased by tau isoforms with four repeated binding sites (4R tau or tau E10+ isoforms). Conversely, phosphorylation of tau destabilizes microtubules, and it is suggested that abnormal phosphorylation, as observed in AD, provokes a collapse of the microtubule network and neurodegeneration (Buée et al., 2000).

THE PARAMETERS OF TAU PATHOLOGY IN THE HUMAN BRAIN

QUANTIFICATION OF TAU PATHOLOGY

Native tau proteins are normally phosphorylated on numerous serine or threonine sites. In a living cell, there are different pools of tau proteins with different states of phosphorylation. The more tau proteins phosphorylated, the less they bind to the microtubules. Also, the state of phosphorylation of tau proteins is probably different according to the cell compartments. Tau are less phosphorylated in axons, as demonstrated by monoclonal antibody tau 1. Also, phosphorylation of tau proteins is developmentally regulated. Antibodies such as AT8, AD2 and PHF-1, which are well known in the field of AD to label tau pathology, also strongly label native tau proteins (reviewed in Buée et al., 2000).

AD biochemistry is performed on post mortem human brains. From the work of Matsuo et al. (1994), we know that native tau proteins are almost totally dephosphorylated during post-mortem delays. Dephosphorylation results from the strong phosphatase activity which is released after cell death. But in parallel, aggregated tau proteins that constitute brain lesions are not dephosphorylated, because the phosphatase enzymes are unable to access

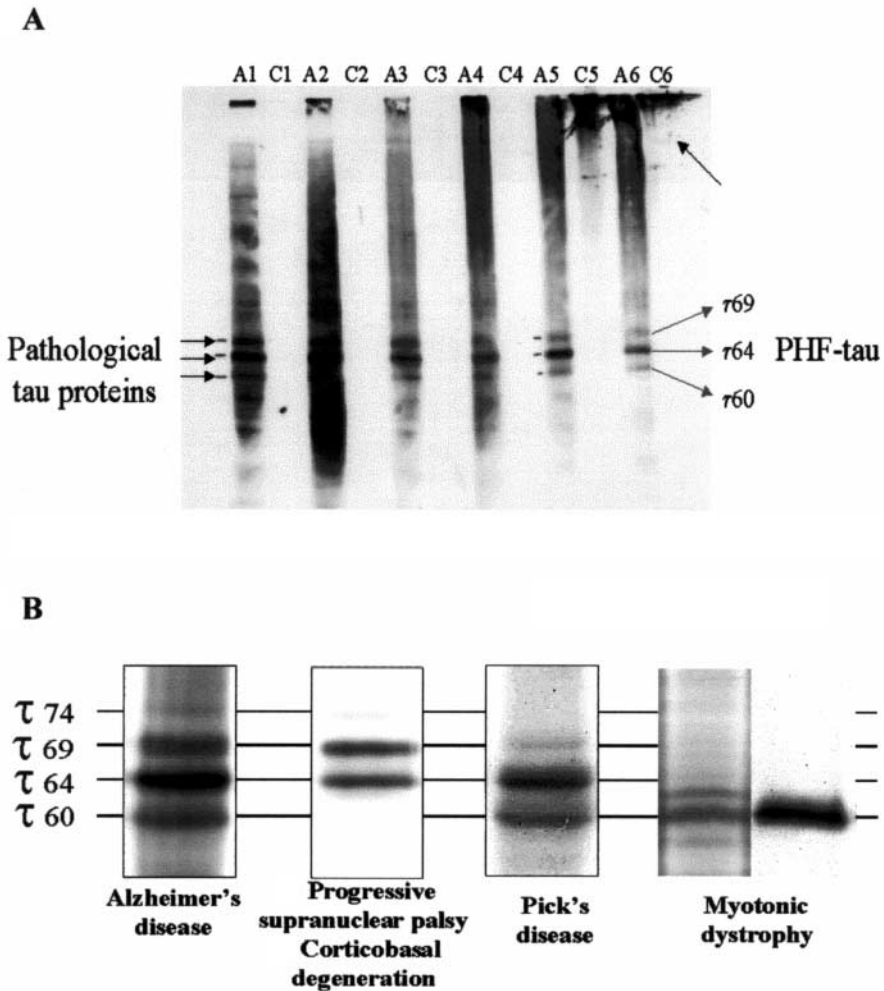


Figure 54.1. (A) Immunoblot detection of pathological tau protein in AD brain extracts, with AD2, a phosphodependent tau antibody, as described in Sergeant et al. (1999). Same amounts of total protein extracts were loaded in each well. The brain area analyzed is the parietal cortex. Controls were non-demented, aged-matched cases. Pathological tau proteins are detected, with a molecular mass of 60, 64 and 69 kDa, exclusively in AD brain extracts. Note the intensity of tau pathology, which is different among the AD patients. A1–A6, Alzheimer’s disease parietal cortex samples; C1–C6, control cortex samples. (B) The different tau signatures in several disorders. Note the main triplet of pathological tau proteins in AD, the upper doublet in progressive supranuclear palsy, the lower doublet in Pick’s disease and the main Tau 60 in myotonic dystrophy

phosphorylated sites that are buried deep in brain lesions. Therefore, antibodies such as AT8 or AD2 (Buée-Scherrer et al., 1996b) specifically detect tau pathology on post-mortem brain tissues (Figure 54.1).

As shown in Figure 54.1A, a characteristic triplet of pathological tau proteins is exclusively detected in AD brain extracts from polymodal association brain areas, while no trace of tau pathology is observed in the same brain areas from non-demented aged-matched controls. Pathological tau proteins from AD, named PHF-tau, are composed of three main electrophoretic variants designated tau 60, 64 and 69, as a function of their molecular mass. A minor fourth band, named tau 74, is also detected at 74 kDa (Mulot et al., 1994; Sergeant et al., 1999). Furthermore the intensity of the detection is proportional to the intensity of NFD. Therefore, a semi-quantification by Western blot is able to easily detect and quantify NFD, in good agreement with immunohistochemical observations.

DIFFERENT BIOCHEMICAL SIGNATURES IN AD, PSP, PiD, MYoD

As shown in the previous section, a characteristic triplet of electrophoretic bands is detected in AD brain homogenates. A different but also characteristic pattern is observed in other diseases with pathological tau and with tau-positive brain lesions (Figure 54.1B). Indeed, we demonstrated that pathological tau proteins from progressive supranuclear palsy (PSP) are composed of a main doublet (tau 64, tau 69; Flament et al., 1991) and a minor tau, 74 (Sergeant et al., 1999), while those from Pick's disease are composed of another doublet (tau 60, 64) and a minor tau, 69 (Delacourte et al., 1998).

Myotonic dystrophy (MyoD), a familial disease with abnormal CTG repeats on chromosome 19, is characterized by a tau pathology with a major band of 60 kDa. Electrophoretic bands at 64 and 69 kDa are also found, but in lower amounts (Vermersch et al., 1996).

TAU ISOFORMS IN BRAIN LESIONS: CLASSES I-IV

The analysis of the tau isoform content, using specific immunoprobe against isoforms with the peptidic regions expressed by exons 2, 3 or 10, combined with 2D gel electrophoreses and Western blots, enabled us to characterize the different patterns of pathological tau. Specific sets of tau isoforms aggregate to constitute four main classes of brain lesions: AD (all six isoforms); PSP/CBD (three E10⁺ isoforms) (Sergeant et al., 1999); PiD (three E10⁻ isoforms) (Delacourte et al., 1998) and MyoD (mainly the shortest tau) (Figure 54.2). These different patterns can be reconstructed in cellular models (Mailliot et al. 1998).

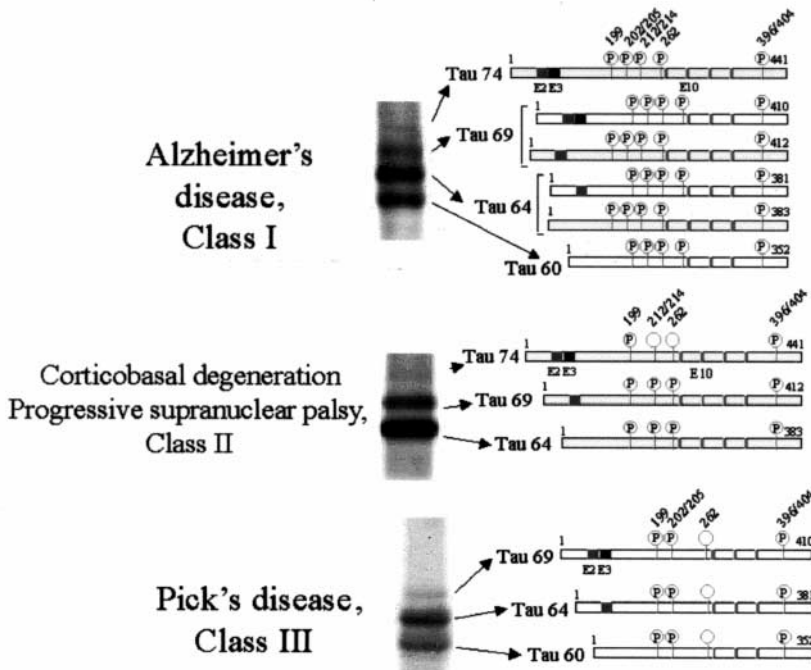


Figure 54.2. The correspondence between the biochemical signature of tau pathology and the tau isoform content. Note that PHF-tau from AD made up the six isoforms, while only 4R-tau isoforms are found in tau filaments of PSP and 3R-tau isoforms in the Pick's bodies

These different classes of tau isoform patterns can be explained at two levels:

1. Neuronal subsets could express specific sets of tau isoforms, e.g. it has been shown that the granule cells of the dentate gyrus do not normally express E10⁺ tau isoforms. If a given neuronal subset degenerate, the corresponding set of tau isoforms of this neuronal subset will aggregate to constitute a characteristic tau lesion, e.g. neurons that express only E10⁻ tau isoforms will produce Pick bodies if they are acted by NFD (Delacourte et al., 1998).
2. On the other hand, E10⁺ tau isoforms are able to dramatically increase the stability of microtubules and therefore to modify the physiological properties of the cell. It is likely that the expression of E10⁺ tau isoforms can be dysregulated in several diseases, due to an abnormal alternative splicing. This dysregulation is demonstrated in FTDP-17, following a pathogenic mutation in the intron 10 regulating the splicing of exon 10 (reviewed in Delacourte and Buée, 2000). This abnormal processing of

intron 10 could also be boosted by polymorphisms on the tau gene, such as those found in PSP (Conrad et al., 1997).

Together, we observe a number of important neurodegenerative diseases, with different etiologies and classes of tau pathology (Table 54.1). They have all in common a cognitive impairment when tau pathology is present in brain association areas. For some of them, tau is the etiological agent (FTDP-17). For many other diseases, tau pathology is not only a marker but also a motor of the degenerative process (AD, PSP, CBD) (Delacourte and Buée, 2000). For dementia pugilistica, tau pathology is probably a consequence, since the etiology is related to trauma.

HYPER- AND ABNORMAL TAU PHOSPHORYLATION IN DISEASES WITH NEUROFIBRILLARY DEGENERATION

tau Proteins in AD Brains are Hyperphosphorylated

tau Pathology in the brain at autopsy is visualized with antibodies against phosphorylated sites on tau. However, these phospho-dependent antibodies reveal an aggregation of tau but certainly not an abnormal phosphorylation, as frequently mentioned in the literature. Indeed, a Western blot analysis of a biopsy sample from a human brain reveals a tau triplet that is similar to the triplet of AD. The major difference is that no smears of aggregated tau are observed in the biopsy brain tissue. Following these observations, the question was to determine whether tau proteins in the AD brain are really hyperphosphorylated, as frequently suggested (Buée et al., 2000).

Sergeant et al. (1995) have been able to demonstrate directly the hyperphosphorylation of tau in AD brains, by comparing on 2D gel the isoelectric pattern of tau proteins from a biopsy of a normal human brain tissue with an autopsy sample of an AD brain. The 2D electrophoresis revealed that AD tau proteins are more acidic, and therefore more phosphorylated, than normal native biopsy tau proteins. These results unambiguously demonstrate that pathological tau proteins in AD are hyperphosphorylated (Sergeant et al., 1995).

tau Proteins are Abnormally Phosphorylated in Alzheimer's Disease

A few sites on tau proteins have been shown to be phosphorylated on PHF-tau, but not present on native tau (from biopsies). These sites are: Ser 212/Ser214, detected by AT100; Ser 231/Ser235, detected by PHF-27 and TG3; and Ser 422, detected by AP422 or Ab988. These monoclonal antibodies demonstrate the aberrant phosphorylation that occur in numerous neurodegenerative disorders (reviewed in (Buée et al., 2000).

Some Phosphorylation Sites are Disease-specific

Many normal phosphorylation sites, such as AT8 or AD2, are observed in all tau lesions of neurodegenerative diseases presented in Table 54.1. In the same way, phosphorylated pathological epitopes such as Ser422 are found in all tau lesions, whatever the pathology (Bussiere et al., 1999). However, there are some interesting specificities. Indeed, it has been shown at the immunohistological and biochemical levels that phosphorylated Ser262 is present in tau lesions of numerous neurodegenerative disorders, but not in the Pick bodies of PiD (Probst et al., 1996; Delacourte et al., 1998). Also, oligodendrocyte tau inclusions in multiple system atrophy do not contain pathological sites of phosphorylation (Cairns et al., 1997).

SPATIO-TEMPORAL DISTRIBUTION

The mapping of the spatio-temporal distribution of tau pathology in the different brain areas is important to understand how the disease spreads in the brain. Indeed, there is a precise biochemical pathway of tau pathology in aging and in AD. The progression of tau pathology is sequential, invariable, hierarchical and predictable. Ten stages (S1–S10) were defined, corresponding to the 10 brain areas sequentially affected. The degenerative process always starts in the hippocampal region (S1, transentorhinal cortex; S2, entorhinal cortex; S3, CA1 region of the hippocampus), followed by the temporal cortex (S4, temporal pole; S4, inferior temporal cortex; S6, mid-temporal cortex), then the polymodal brain association areas (S7–S8), and finally the primary regions (motor cortex and/or the occipital cortex) as well as many subcortical nuclei (S9–S10) (Delacourte et al., 1999).

These data show that tau pathology is systematically observed in the normal population aged over 75 years. They also demonstrate that tau pathology sometimes occurs independently of amyloid deposits and that the hippocampal area is the most vulnerable area of the human brain. The hippocampal vulnerability is probably a springboard for AD pathology, namely amyloid precursor protein dysfunction, which will exacerbate and extend tau pathology in other brain areas (Figure 54.3). Taken together, the neuropathological and biochemical data indicate that tau pathology is instrumental in AD.

From these data, it is now possible to precisely quantify tau pathology and amyloid deposition at the biochemical level and to determine the criteria to establish a biochemical diagnosis of AD (CEBDAD), that separate aging from preclinical AD, and preclinical AD from clinical AD (Delacourte et al., 1999). In that respect, it should be pointed out that recent criteria for the neuropathological diagnosis have rehabilitated tau pathology, in good agreement with Aloïs Alzheimer's observations and the natural history of AD (Working Group, 1997).

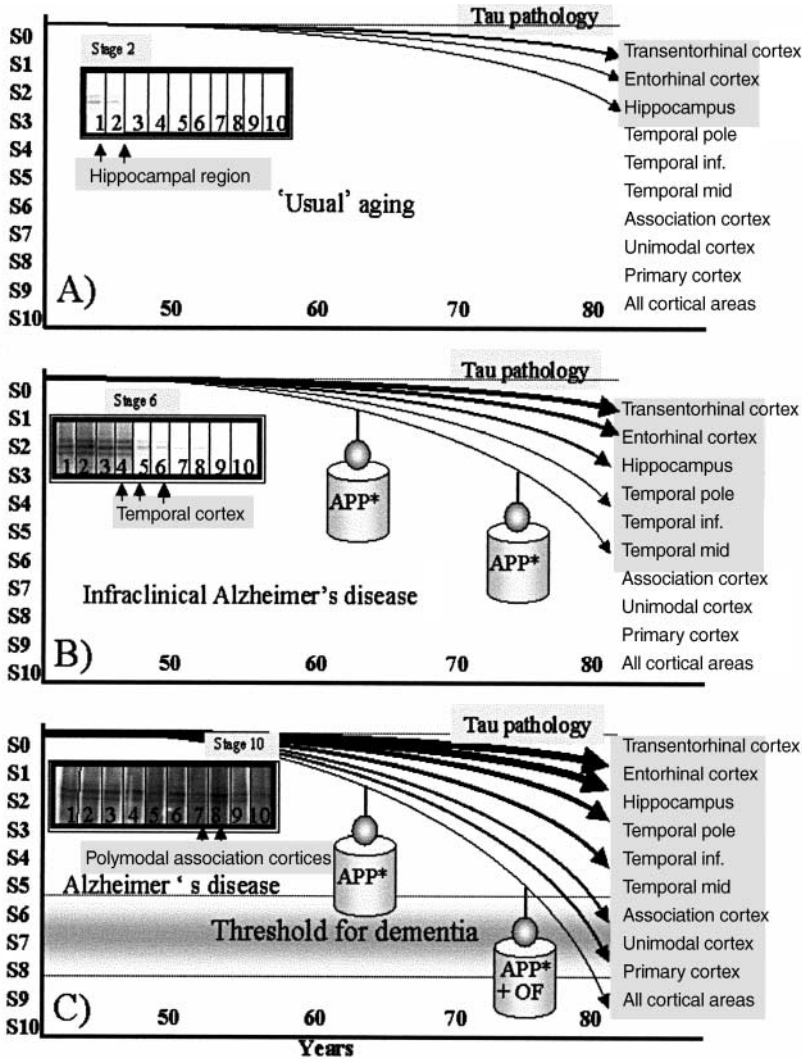


Figure 54.3. The biochemical pathway of tau pathology in aging and Alzheimer's disease (AD). Insert: Western blot analysis in the 10 brain areas that are successively affected in AD (Delacourte et al., 1999). (A) Tau pathology is systematically observed in the transentorhinal cortex (stage 1), or in addition in the entorhinal cortex (stage 2), and CA1 of hippocampus (stage 3) in non-demented patients aged over 75 years. (B) Tau pathology can be found in other brain areas, along a stereotypical pathway, at the infraclinical stage of AD (stages 4–6). (C) All brain areas are affected by tau pathology at the last stage of AD. The clinical symptoms occur when polymodal association areas are affected (stage 7). Hypothesis: the spreading of tau pathology is fuelled by APP dysfunctions (APP*), and also, progressively, by other factors (OF), such as microglial reaction, inflammation and oxidative stress

The pathway of tau pathology in PSP is different from that in AD, and is roughly opposite, emerging from the subcortical nuclei toward the neocortex, and especially the frontal motor cortex. The specific pattern of tau pathology in PSP, with the upper tau doublet (tau 64, 69), is observed in all brain areas affected, from the subcortical nuclei to the frontal neocortical regions (Vermersch et al., 1994; Buée Scherrer et al., 1996a).

In all types of neurodegenerative disorders, the spreading of tau pathology follows specific neuronal connections, like a precise neuronal chain reaction. There is also probably a 'dynamic' of spreading, fuelled by different factors, each of them being a lead for neuroprotection (Delacourte, 2000).

THE GENETIC PROFILE OF TAU PATHOLOGY

Tau Pathology as an Etiological Agent

The presence of brain lesions in neurodegenerative disorders usually prompts the same question: is this a cause or a consequence? Some answers are already available for tau pathology. The discovery that tau mutations are directly involved in numerous FTDP-17 cases has been dramatically documented. More than 20 different mutations have been spotted. Most of the pathogenic mutations are responsible for an increase of 4R tau isoforms, giving a class II tau pathology. 4R tau isoforms, with the additional peptide sequence of exon 10, have a much stronger affinity towards microtubules than 3R isoforms, and an excess could modify microtubule properties, which will be stiffer and less dynamic. The other mutations are missense mutations, which also affect microtubule polymerization and stability by decreasing tau-microtubule binding and lead to one of the class I, II or III tau pathologies. The striking feature of these familial tauopathies is the heterogeneity of the phenotype, which results from the different effects on tau (overexpression, loss of function). Surprisingly, for the same mutation in the same family, different onsets and different phenotypes can be observed, showing that numerous additional factors are modulating the clinical and neuropathological phenotypes (reviewed in (Delacourte and Buée, 2000)).

We note that the neuropathological profile in FTDP-17 is quite different from AD, with a special involvement of astrocytes and cortical white matter, but also with an important heterogeneity for each mutation.

However, not all diseases with a tau pathology have mutations on the tau gene. This has been verified for CBD, PSP, amyotrophic lateral sclerosis/parkinsonism dementia complex of Guam, and AD.

Tau as a Genetic Risk Factor

Conrad et al. (1998) identified a polymorphic dinucleotide repeat sequence in a Caucasian population with PSP. This polymorphism, named A0,

corresponding to an 11 TG dinucleotide repeat in intron 9 of the tau gene, is found in 95% of the PSP cohort (95.5%) and only in 57% of normal controls and 50% of patients with AD. Recently, these data were confirmed by several studies and extended to a haplotype, including a number of polymorphisms in linkage disequilibrium with A0 and named H1. This haplotype corresponds to A0 polymorphism, numerous single nucleotide polymorphisms along the entire tau gene and one intronic 238 bp deletion flanking exon 10. These polymorphisms may influence exon 10 splicing and thus the proportion of 4R:3R tau isoforms, leading to a class II tau pathology. It should be noted that these A0 polymorphisms or H1 haplotypes were recently described in other pathologies including CBD and Parkinson's disease. Some other polymorphisms in the tau gene were also described as being associated with a risk of AD, but these data are still controversial (reviewed in Delacourte and Buée, 2000).

SUMMARY

Tau is an outstanding biochemical marker of neurofibrillary degeneration (NFD), well correlated with clinical manifestations. Tau pathology concerns many familial or sporadic neurodegenerative disorders, such as Alzheimer's disease (AD), progressive supranuclear palsy (PSP), corticobasal degeneration (CBD), Pick's disease (PiD), FTDP-17, myotonic dystrophy (MyoD) and many other diseases. Molecular parameters of Tau pathology help to elucidate the involvement of tau in the physiopathology of these diseases and to set up diagnoses and therapeutic strategies. Six main features define tau pathology: (1) the quantitative aspects, using Western blots; (2) the different biochemical signatures observed in AD, PSP, CBD, PiD, MyoD (one to four immunodetected electrophoretic bands); (3) the specific tau isoform content of brain lesions; indeed, 'disease-specific' sets of tau isoforms aggregate to constitute four main categories of tau lesions—class 1, AD (all six isoforms); class 2, PSP/CBD (three exon10⁺ isoforms); class 3, PiD (three exon10⁻ isoforms); and class 4, MyoD, mainly, and sometimes exclusively, the shortest tau isoform; (4) the different states of phosphorylation of tau, with hyper- and abnormal phosphorylation, as well as disease-specific sites of phosphorylation (e.g. ser262); (5) the spatiotemporal progression of tau pathology, which is extremely well correlated with cognitive impairment; (6) the genetic aspects, with pathogenic tau mutations in FTDP-17 or characteristic polymorphisms in PSP. These results emphasize two important physiopathological points. First, neuronal populations are probably distinguished by the expression of different sets of tau isoforms. A dysregulation in the expression could generate vulnerability and neurodegeneration. Second, there is a 'dynamic process' that fuels the precise, sequential and hierarchical spreading of tau pathology in brain areas, along corticocortical projections. This process should be a

target for neuroprotection. Taken together, tau pathology is a many-sided degenerative process that will open diagnostic and therapeutic avenues

CONCLUSION

Taken together, the correlation between the distribution of tau pathology and clinical manifestation is excellent if we take into account the extent and the function of the brain areas that are affected. In AD, dementia is observed at stage 7 or above, when polymodal brain association areas are affected. In PSP and CBD, dementia is always observed when tau pathology is found in the frontal neocortical areas.

Other parameters of tau pathology might be important, such as the extent of tau phosphorylation and the location of the phosphorylated sites. Other post-translational events, such as glycosylation, that modulate tau functions or sorting, have still to be investigated (Buée et al., 2000). Tau pathologies are also influenced at the genetic level, either by polymorphisms or directly driven by mutations on the tau gene. In conclusion, tau pathology is a many-sided degenerative process that will open diagnostic and therapeutic avenues.

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Address for correspondence:

André Delacourte,
INSERM U422,
1 place de Verdun
59045 Lille cedex, France
Tel: +33 3 2062 2078; fax: +33 3 2062 2079;
e-mail: delacourte@lille.inserm.fr