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Review

# Tau and neurodegenerative disorders

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## Abstract

The mechanisms that render tau a toxic agent are still unclear, although increasing evidence supports the assertion that alterations of tau can directly cause neuronal degeneration. In addition, it is unclear whether neurodegeneration in various tauopathies occurs via a common mechanism or that specific differences exist. The aim of this review is to provide an overview of tauopathies from bench to bedside. The review begins with clinicopathological findings of familial and sporadic tauopathies. It includes a discussion of the similarities and differences between these two conditions. The second part concentrates on biochemical alterations of tau such as phosphorylation, truncation and acetylation. Although pathological phosphorylation of tau has been studied for many years, recently researchers have focused on the physiological role of tau during development. Finally, the review contains a summary of the significance of tauopathy model mice for research on neurofibrillary tangles, axonopathies, and synaptic alteration.

**Keywords:** frontotemporal lobar degeneration; microtubule; phosphorylation; tau; transgenic mice.

## Abbreviations

AD, Alzheimer's disease; 3R-tau, three-repeat tau; 4R-tau, four-repeat tau; E2, exon 2; E3, exon 3; MT, microtubule; *MAPT*, microtubule-associated protein tau; PSP, progressive supranuclear palcy; CBD, corticobasal degeneration; PiD, Pick disease; NFT, neurofibrillay tangle; GFT, glial fibrillary tangle; AGD, argyrophillic grain disease; bvFTD, behavioral variant frontotemporal dementia; AGs, argyrophillic grains; FTLD, frontotemporal lobar degeneration; FTDP-17, frontotemporal dementia and parkinsonism linked to chromosome 17.

## Introduction

Tau proteins are microtubule-associated proteins (MAPs) that are abundant in the central nervous system (CNS), where they are expressed predominantly in axons. Human tau proteins are encoded by a single gene consisting of 16 exons on chromosome 17q21 and CNS isoforms are generated by alternative mRNA splicing involving 11 of these exons. In adult human brain, alternative mRNA splicing of exons 2(E2), 3(E3), and 10 generates six tau isoforms ranging from 352 to 441 amino acids in length (Figure 1). Alternative splicing of exon 10 produces tau isoforms with either three (exon 10-) or four (exon 10+) repeat domains, known as 3R- and 4R-tau, respectively. The triplets of 3R-tau and 4R-tau isoforms differ as a result of alternative splicing of E2 and E3 to generate tau isoforms without (0N) or with either 29 (1N) or 58 (2N) amino acid inserts of unknown functions (1). In this review, we first summarize the current knowledge about clinicopathological findings of familial and sporadic tauopathies. Then we discuss the biochemical modifications of the tau and tau-related protein which includes phosphorylation, truncation, and acetylation. Finally, we discuss recent progress on tauopathy model mice. This comprehensive approach could be helpful in establishing a novel therapeutic strategy in tauopathies.

### Tauopathies

Tauopathies share abundant filamentous tau pathology and brain degeneration in the absence of extracellular amyloid deposits. They are divided into familial tauopathies, in which the causative gene was discovered to be the gene encoding microtubule-associated protein (*MAPT*), and sporadic tauopathies.

### Familial tauopathies

It is now more than 10 years since the first descriptions of pathogenic mutations within the *MAPT* gene as the cause of some cases of frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17) (2, 3). To date, 44 distinct pathogenic mutations in *MAPT* have been identified (Table 1, Figure 1) (4). The vast majority of tau mutations are missense, deletion, or silent mutations in the coding region, or intronic mutations located close to the splice-donor site of the intron following exon 10. Most coding region mutations are located in exons 9–12 or in exon 13 near the microtubule (MT)-binding region, as well as two mutations in exon 1 of tau (R5H and R5L, Figure 1, Table 1).

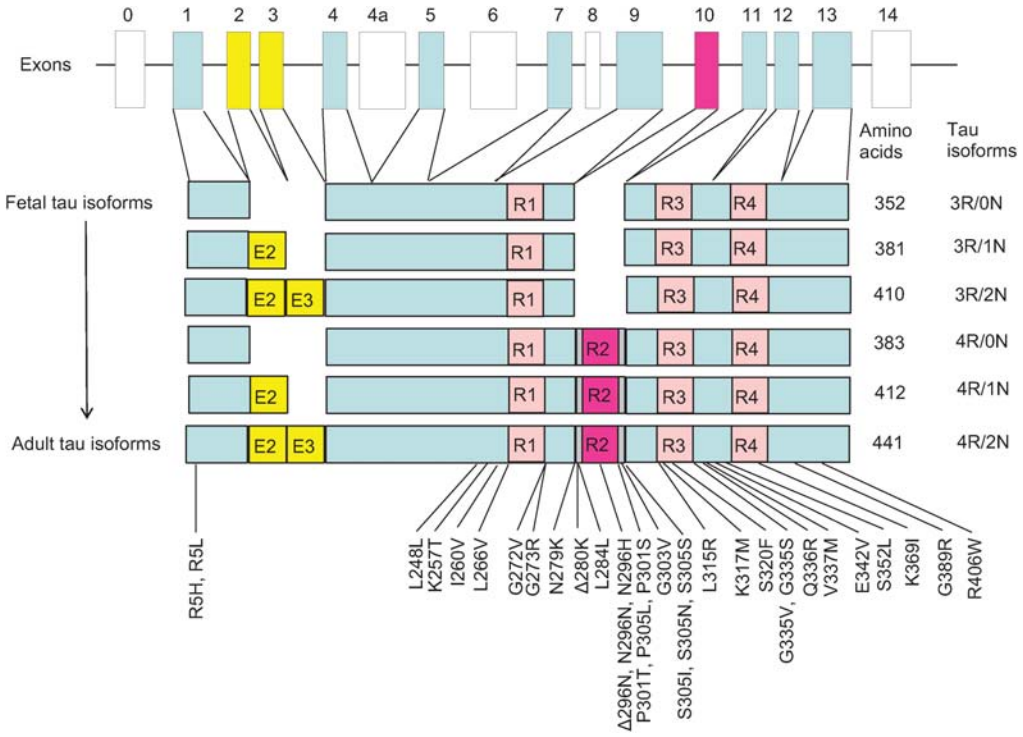


**Figure 1** Schematic representation of the human tau gene, the six central nervous system (CNS) tau isoforms, and FTDP-17 *tau* mutations. The human tau gene contains 16 exons. Exon 6 and exon 8 are not transcribed in the human CNS. E4a, which is also not transcribed in the human CNS, is expressed in the peripheral nervous system. Alternative splicing of exon 2, exon 3, and exon 10 produces the six tau isoforms. The shortest 352-amino acid isoform is found only in the fetal brain. The 18-amino acid microtubule (MT)-binding repeats (coded by exons 9, 10, 11, and 12) are referred to as R1–R4. The second MT-binding repeat is encoded by exon 10. Clearly, most FTDP-17 mutations are located in the MT-binding repeat.

**Molecular mechanism** Three distinct mechanisms have been proposed that support the hypothesis that FTDP-17 mutations lead to tau dysfunction and disease (5). First, intronic and some exonic mutations affect the alternative splicing of exon 10 and consequently alter the ratio of 4R- to 3R-tau. The other exonic mutations impair the ability of tau to bind to MTs and to promote MT assembly. The third mechanism is that missense mutations promote tau-tau interaction, consequently increasing tau aggregation.

**Exon 10 splicing** The majority of missense and deletion mutations that disrupt normal tau exon 10 splicing are located in exon 10 [N279K, ΔK280, L284L (CTC), N296N, ΔN296, N296H, P301T, P301L, P301S, G303V, S305I, S305N, S305S] and intron 10 (Figures 1 and 2). The majority of these mutations are located in exonic splicing enhancer, exonic splicing silencer, intronic splicing enhancer, or intronic splicing silencer and influence exon 10 splicing (6). The splicing mutations can cause FTDP-17 solely by disrupting the alternative splicing of exon 10 and consequently changing the ratio of 3R-tau/4R-tau, although other mutated tau proteins can cause disturbed MT binding or promoted aggregation. Biochemical analysis of insoluble tau extracted from autopsied FTDP-17 brain tissue of patients with these mutations reveals the presence of exclusively 4R-tau isoforms (Figure 3). Normally, adult human brain expresses approximately equal levels of 3R-tau and 4R-tau. The mechanism by which the imbalance of the 3R-tau/4R-tau ratio causes neurodegeneration is not currently understood. Because equal levels of 3R-tau and 4R-tau appear to be essential for the normal function of mature human brain, it is possible that a 1:1 ratio of 3R-tau/4R-tau bound to MTs is required to maintain the normal dynamics of MTs in mature neurons. Because the MT-binding and MT-assembling activities of 3R-tau are lower than those of 4R-tau, any changes in the 3R-tau/4R-tau ratio could alter the MT dynamics, leading to neuronal dysfunction (6). Clinically, neuroimaging studies demonstrated a difference between the patients with mutations that increase the splicing of exon 10 and those without these mutations. IVS 10+16, 10+3, N279K, and S305N mutations (which influence the alternative splicing) were associated with gray matter loss mainly in the medial temporal lobe, whereas atrophy of patients with P301L or V337M (mutations that affect the structures of tau protein) was found in the lateral temporal lobe (7).

**Genotype-phenotype correlation** The clinical phenotypes and topographical distributions of pathology in FTDP-17 vary. Some tau gene mutations cause a similar phenotype in different families or in different members of the same family. For instance, the majority of patients with the N279K mutation typically present a phenotype reminiscent of pro-

**Table 1** Phenotype and tau alteration in FTDP-17 patients.

| | Site | Clinical features | Insoluble tau | Exon 10 splicing | tau-MT interaction | tau-tau interaction |
|---|---|---|---|---|---|---|
| R5H | E1 | bvFTD | 4R>3R | No change | Reduced | ↑Filament formation |
| R5L | E1 | PSP-like | 4R>3R | No change | Reduced | N.D. |
| K257T | E9 | bvFTD | 3R | No change | ↓MT assembly | ↑3R filament formation |
| I260V | E9 | bvFTD | 4R | No change | ↓MT assembly | ↑Filament formation |
| L266V | E9 | bvFTD, PPA | 3R, 4R | No change | ↓MT assembly | N.D. |
| G272V | E9 | bvFTD | 3R | No change | ↓MT assembly | ↑Filament formation |
| G273R-10 | E9 | bvFTD, parkinsonism | N.D. | N.D. | N.D. | N.D. |
| | Intron | bvFTD | N.D. | ↑ | N.D. | N.D. |
| N279K | E10 | PPND | 4R | ↑ | No effect | ↑Filament formation |
| ΔK280 | E10 | bvFTD | N.D. | ↓ | ↓MT assembly | ↑Filament formation |
| L284L(CTC) | E10 | bvFTD | N.D. | ↑ | No effect | No effect |
| N296N | E10 | bvFTD, parkinsonism | N.D. | ↑ | No effect | No effect |
| ΔN296 | E10 | PSP-like | N.D. | ↑ | ↓MT assembly | No effect |
| N296H | E10 | bvFTD | 4R | ↑ | ↓MT assembly | No effect |
| P301T | E10 | bvFTD, PPA, PSP-like | N.D. | N.D. | N.D. | N.D. |
| P301L | E10 | bvFTD, PSP, parkinsonism | 4R | No change | ↓MT assembly | ↑Filament formation |
| P301S | E10 | bvFTD, parkinsonism | 4R | No change | ↓MT assembly | ↑Filament formation |
| G303V | E10 | PSP-like | 68, 64 K, PSP pattern | N.D. | N.D. | N.D. |
| S305I | E10 | AGD | 4R | ↑ | N.D. | N.D. |
| S305N | E10 | bvFTD | N.D | ↑ | No effect | No effect |
| S305S | E10 | PSP-like | N.D | ↑ | N.D. | N.D. |
| +3 | Intron | bvFTD, PSP-like | 4R | ↑ | N.D. | N.D. |
| +11 | Intron | MR, PSP-like | 4R | ↑ | N.D. | N.D. |
| +12 | Intron | bvFTD | 4R | ↑ | N.D. | N.D. |
| +13 | Intron | N.D. | 4R | ↑ | N.D. | N.D. |
| +14 | Intron | bvFTD | 4R | ↑ | N.D. | N.D. |
| +16 | Intron | bvFTD, PSP, PSG, AD | 4R | ↑ | N.D. | N.D. |
| +19 | Intron | bvFTD | No insoluble tau | ↓ | N.D. | N.D. |
| +29 | Intron | bvFTD | No insoluble tau | ↓ | ↓MT assembly | N.D. |
| L315R | E11 | bvFTD, PPA | 3R, 4R, 3R0N absence | N.D. | ↓MT assembly | No effect |
| K317M | E11 | Motor neuron disease | 64, 68 kDa | N.D. | N.D. | N.D. |
| S320F | E11 | bvFTD, PPA | 3R, 4R, 3R0N absence | N.D. | ↓MT assembly | N.D. |
| G335V | E12 | bvFTD | N.D. | N.D. | ↓MT assembly | ↑Filament formation |
| G335S | E12 | bvFTD | 3R, 4R | N.D. | ↓MT assembly | No effect |
| Q336R | E12 | bvFTD | 3R, 4R | N.D. | ↑MT assembly | ↑Filament formation |
| V337M | E12 | bvFTD | 3R, 4R | N.D. | ↓MT assembly | ↑Filament formation |
| E342V | E12 | bvFTD, PPA | Mainly 4R | N.D. | N.D. | N.D. |
| S352L | E12 | AR, respiratory failure | N.D. | N.D. | ↓MT assembly | N.D. |
| V363I | E12 | PPA | N.D. | N.D. | N.D. | N.D. |
| K369I | E12 | bvFTD | 3R, 4R | N.D. | ↓MT assembly | N.D. |
| G389R | E13 | bvFTD, CBS | 3R, 4R | N.D. | Reduced | N.D. |
| R406W | E13 | bvFTD, AD-like | 3R, 4R | N.D. | Reduced | ↑Filament formation |
| T427M | E13 | bvFTD | N.D. | N.D. | N.D. | N.D. |

FTDP-17, frontotemporal dementia and parkinsonism linked to chromosome 17; E, exon; bvFTD, behavioral variant frontotemporal dementia; PSP, progressive supranuclear palsy; PPA, primary progressive aphasia; AGD, argyrophilic grain dementia; AD, Alzheimer's disease; CBS, corticobasal syndrome; N.D., not described; 3R, 3 repeat tau; 4R, 4 repeat tau; MT, microtubule.

gressive supranuclear palsy (PSP) (8, 9). In contrast, both inter- and intrafamilial phenotypic heterogeneities with the same mutations have been reported on occasion. There are clinical and pathological variations in families with the intron 10+16 mutation that demonstrate a highly variable phenotype including PSP, progressive subcortical gliosis (PSG), behavioral variant FTD (bVFTD), and Alzheimer's disease (AD) (10–13). Even in a single family the proband showed only parkinsonism, whereas other family members suffered from PSP-like presentations or frontotemporal dementia (14). Intrafamilial clinical phenotypic heterogeneity has also been reported with other *tau* mutations: PSP or idiopathic Parkinson's disease (ΔN296) (15) and bvFTD, corticobasal degeneration (CBD), or levodopa-responsive parkinsonism (P301S) (16, 17), although these reports did not describe autopsied neuropathological examination results. Interestingly, Baba et al. examined nine patients with P301S mutation, and reported that the predominant phenotype was bvFTD in three patients and parkinsonism in six patients. All patients with the parkinsonism phenotype had the H1/H1 haplotype,

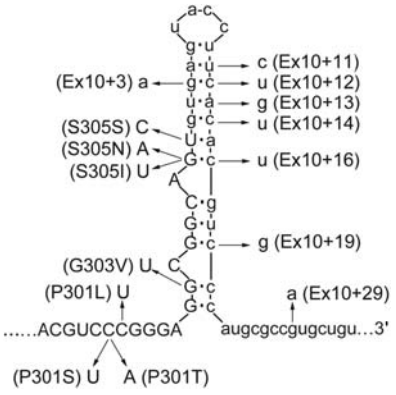


**Figure 2** Stem loop structure at the 3′-end of exon 10.
The exon-intron interface displays a high degree of self-complementarity, suggesting the presence of a stem loop. Eleven mutations are clustered in this region and all disrupt the complementarity and destabilize the stem loop structure, resulting in inclusion of exon 10.

whereas two patients with the bvFTD phenotype had H1/H2. These data suggest that the tau haplotype carrying the mutation and the tau genotype could be related to the clinical phenotype (18). Therefore, it appears that the manifestations of each mutation are subject to potential epigenetic and/or environmental influences and that the genotype-phenotype correlation is complex.

Recently, clinical phenotypes that differ from previously described tauopathies, such as Pick's disease (PiD), CBD, PSP and AD, were reported. Kovacs et al. reported that a patient with S305I novel *MAPT* mutation had neuropathological and biochemical features resembling argyrophilic grain disease (19). Another group reported that two pedigrees with K317M mutation in exon 11 presented with the motor neuron disease phenotype (20). The patients developed dysarthria, parkinsonism, and pyramidal signs and half of them had amyotrophy. Corticospinal tract degeneration and anterior horn neuron loss were identified in six out of seven autopsies.

## Sporadic tauopathies

Tauopathies have a biochemical signature; tau protein in these disorders is relatively insoluble and these insoluble species can be detected by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The insoluble fractions can be further characterized according to the pattern of tau isoforms (Figure 3). For example, in AD all six isoforms are abnormally hyperphosphorylated and migrate as three major bands (68, 64, and 62 kDa) and one minor band (72 kDa) when visualized by immunoblotting. Treatment with the enzyme alkaline phosphatase removes phosphate groups, and the tau isoforms appear as six bands (3R- and 4R-tau) (21). Thus, brain tissue from patients with FTLD where Pick bodies are present is characterized biochemically by a predominance of 3R-tau, whereas CBD, PSP, argyrophilic grain disease (AGD), and sporadic multiple system tauopathy with dementia are tauopathies with a predominance of 4R (22).

## 4R-tauopathies

**Progressive supranuclear palsy (PSP)** PSP is the most common 4R-tauopathy characterized by parkinsonism, vertical gaze palsy, and cognitive decline. Interestingly, the

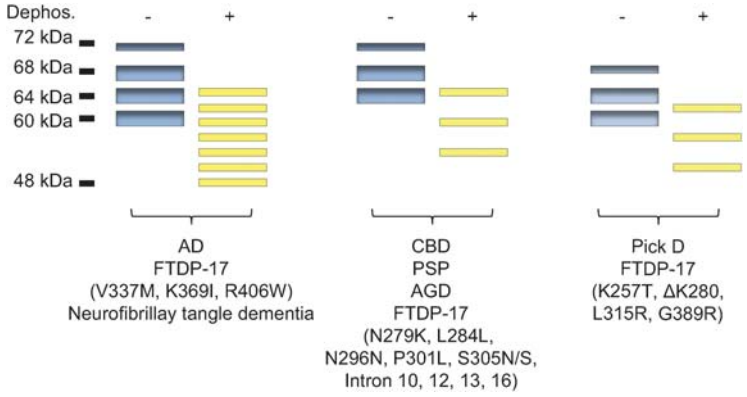


**Figure 3** Schematic representation of Western blot bands from insoluble tau of different tauopathies.
The image depicts the typical banding pattern of non-dephosphorylated and dephosphorylated insoluble (filamentous) tau from brains of patients with the diseases indicated following resolution by SDS-PAGE and immunoblotting with phosphorylation-independent anti-tau antibodies. Non-dephosphorylated insoluble tau from the brain of patients with AD and some FTDP-17 mutations that do not affect splicing (V337M, K369I, and R406W) runs as three major bands of 68, 64, and 60 kDa and as a minor, variable band of 72 kDa. When dephosphorylated, it resolves into six bands that correspond to the six isoforms. In corticobasal degeneration (CBD), progressive supranuclear palsy (PSP), and argyrophilic grain dementia (AGD), as well as FTDP-17 that alters exon 10 splicing, the two prominent 68- and 64-kDa insoluble tau bands are detected (the 72-kDa minor band is variably detected) and align with a four tandem repeat sequence (4R-tau) following dephosphorylation. In Pick's disease (PiD) and cases with some FTDP-17 mutations that do not affect splicing, the lower two 64- and 60-kDa insoluble tau bands predominate. Following dephosphorylation, a predominance of 3R-tau is observed. ''-'' and ''+'' indicate before and after alkaline phosphatase treatment.

majority of FTDP-17 mutations with PSP-like presentations were described as increasing splicing of exon 10 (Table 1). With regard to genetic predisposition to *MAPT*-associated disorders, Baker et al. described the association of PSP with the H1 haplotype, defined by a region of complete linkage disequilibrium spanning the entire coding sequence of *MAPT* (23). The H1 haplotype and its allelic counterpart, H2, were defined by a series of single nucleotide polymorphisms and a 238-bp deletion in intron 9 found only on the H2 background. In Caucasian populations, the frequency of H1 varies between 70% and 80%; in PSP cases, this frequency is usually over 90% (24). In postmortem analysis of normal brain tissue, H1 chromatin expresses up to 1.43-fold more 4R-tau mRNA than H2 in the globus pallidus, a brain region highly affected by PSP (25). However, in PSP autopsied brain, the H1 haplotype has no effect on the 4R to 3R ratio in detergent-insoluble tau fractions (26), soluble tau, or mRNA (27).

The most distinctive tau pathology is widespread neurofibrillary degeneration, with characteristic globose neurofibrillary tangles (NFTs), which particularly affects the striatum, pallidum, subthalamic nucleus, and brain stem as well as cortical regions (28). Under electron microscopy (EM), NFTs were recognized as compact accumulations of 14 nm straight tubules arranged in a roughly parallel manner (29).

It has not yet been confirmed whether exon 10 splicing is increased in sporadic PSP brains, although the majority of FTDP-17 cases with PSP-like presentation have mutations that enhance splicing of exon 10. In postmortem PSP brain tissue, the 4R/3R ratios of tau mRNA were increased in the brainstem (30), the globus pallidus (31), and the frontal cortex (27). However, another group reported that the 4R/3R ratios of soluble tau in PSP brains are one, whereas brains from FTDP-17 showed higher levels of soluble 4R-tau (32). This issue might be best addressed by analyzing soluble tau from various brain regions such as basal ganglia and brain stem.

**Corticobasal degeneration (CBD)** CBD is characterized clinically by parkinsonism, language dysfunction, and cognitive and behavioral abnormalities and, pathologically, by frequently asymmetric frontal and temporal atrophies with swollen achromatic neurons that are faintly tau-positive, and tau-positive NFTs. PSP and CBD share numerous clinical symptoms, neuropathological conditions, and biochemical abnormalities. Under EM, NFTs were recognized as compact aggregations of straight tubules (29). The most distinctive tau pathology of PSP and CBD is widespread distribution of glial fibrillary tangles (GFTs) in both astrocytes and oligodendrocytes. Tuft-shaped astrocytes are characteristic of PSP, which show a concentric arrangement of fine branching fibers (33). By contrast, astrocytic plaques typical of CBD demonstrate a corona-like arrangement and fuzzy short processes with collaterals. This morphological difference in GFTs between PSP and CBD can be explained by differences in the proteolytic fragments which arise from tau. An amino-terminal cleavage of a 33-kDa fragment is predominant in PSP, whereas CBD brains more often show that of a major 37-kDa fragment rather than a 33-kDa fragment (34). This 33-kDa fragment was found to be present in FTDP-17 cases with mutations that increase splicing of exon 10 but not in AD or PiD (35). The presence of a 33-kDa fragment in cerebrospinal fluid (CSF) might also be useful as a diagnostic tool in the near future.

**Argyrophilic grain disease (AGD)** AGD has been described as a degenerative dementia disease characterized by argyrophilic grains (AGs) in the entorhinal cortex, hippocampus, and amygdala. This disease appears to be sporadic. A single case bearing *tau* S305I mutation had AGD-like neuropathology (19). Golgi's method revealed that AGs are consistent with dendrite-derived appendages. Golgi's impregnation technique was developed more than 100 years ago to stain spines and axonal boutons using mercury chrolode, potassium dichromate, and potassium chromate. AGs and pre-tangle neurons contain hyperphosphorylated 4R-tau (36). This is associated with a typical 64 kDa and 68 kDa pattern (Figure 3) but is also accompanied by tau truncated forms of low molecular mass, probably resulting from thrombin-mediated proteolysis.

### 3R-tauopathies

**Pick disease (PiD)** PiD is characterized by behavioral abnormality and language dysfunction and the most distinctive neuropathologic feature consists of Pick bodies that are mainly present in the granule cells in the dentate gyrus, pyramidal cells of the hippocampus, subiculum, and entorhinal cortex (28). The brain tau isoform composition has been extensively analyzed by Western blotting for cases of sporadic FTLD where either Pick bodies or Pick-like bodies are present (5, 37, 38). Although the results were controversial, recent immunohistochemical analysis using 4R-tau- or 3R-tau-specific antibodies revealed that, in sporadic FTLD, Pick bodies contained only the 3R-tau isoforms (39).

### 3R- and 4R-tauopathies

**Neurofibrillary tangle dementia (NTD)** When NFTs alone are present, in the absence of Aβ plaques, and in the context of neuronal loss and gliosis, NTD, which is also called a tangle predominant form of senile dementia, and which, like AD tangles, contains all six isoforms of tau, is a diagnostic possibility (40).

## Biochemical modification

### Phosphorylation

The phosphorylation of tau plays a physiological role in regulating the affinity of tau for MTs. With its ability to modulate MT dynamics, tau contributes to key structural and regulatory cellular functions, such as maintaining neuronal processes and regulating axonal transport, respectively (41). The normal dynamic equilibrium of MT-bound tau is primarily determined by the phosphorylation state of tau. MT-

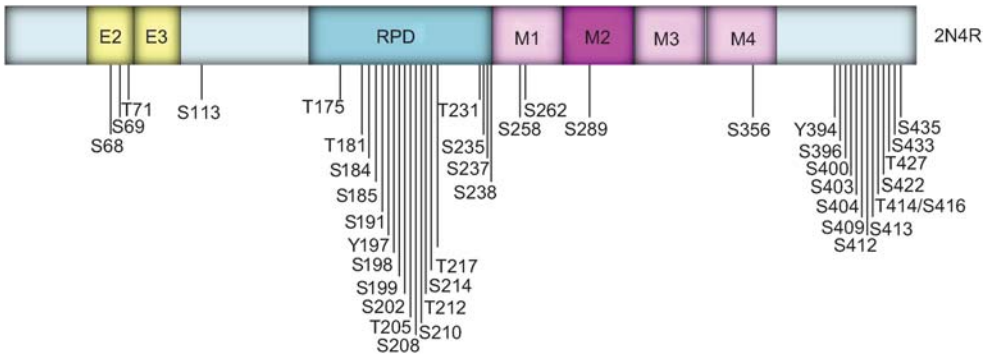


**Figure 4** Positioning of phosphorylation sites on tau from Alzheimer brain.
Approximately 45 sites have been identified and these seem to cluster in the proline-rich domain (RPD) and in the C-terminal region, with few sites evident within the microtubule-binding domain (M1–4) of tau.

bound tau is promoted by dephosphorylation of tau and detachment of tau from MT is promoted by phosphorylation of tau. There are six major isoforms in the adult human brain, all of which are derived from a single gene located on human chromosome 17 (Figures 1 and 4). The six tau isoforms differ from each other in the number of tubulin-binding repeats (either three or four, referred to as 3R- and 4R-tau isoforms, respectively) and in the presence or absence of either one or two 29-amino acid-long inserts at the N-terminal portion of the protein. The processes of phosphorylation and splicing of tau are developmentally regulated, with increased phosphorylation and only 3R-tau expression occurring at embryonic and early developmental stages (1, 42–44). Increased tau phosphorylation reduces the amount of MT-bound tau, and 3R-tau also binds less tightly than 4R-tau to MTs. These regulatory mechanisms can promote increased neuronal plasticity during embryogenesis and early development. Fetal tau is highly phosphorylated at ~18 sites (45) with stoichiometry (46) of phosphorylation similar to that in AD brain. However, fetal tau is not polymerized into NFTs. This raises the possibility that critical phosphorylation sites exist in addition to those fetal tau phosphorylation sites.

There are 85 putative phosphorylation sites (45 serines, 35 threonines, and 5 tyrosines) on the longest human tau isoform (441 amino acids). Using phosphorylation-dependent antibodies against tau and direct sequencing methods (mass spectrometric analysis or Edman degradation of phosphorpeptides), at least 45 phosphorylation sites on insoluble tau from AD brain have been identified (47, 48). It should be noted that not all of these 45 sites need to be phosphorylated on an individual tau molecule. With regard to alternative splicing, four sites in exon 2 (Ser46, Ser68, Ser69, and Thr71) and one site in exon 10 (Ser289) were identified in the AD brain (47). Phosphorylations in exons 2 and 10 might influence conformational change and MT-binding property. However, it is not clear whether phosphorylation level in each tau isoform leads to a certain tauopathy such as AD, PSP, CBD, or PiD.

In addition, it is likely that more phosphorylation sites in physiologically normal human brain exist than have been identified in postmortem materials because tau phosphorylation sites could be turned over rapidly in normal brain (49).

## Kinase and phosphatase

In addition to microtubule affinity-regulating kinase (MARK), many kinases have been shown to phosphorylate tau *in vitro* and in cells including Glycogen synthase kinase-3 (GSK-3β) (50, 51), cyclin-dependent kinase 5 (cdk5) (51), tau-tubulin kinase, mitogen-activated protein kinase, stress-activated protein kinase, $Ca^{2+}$/calmodulin-dependent protein kinase II (CaMKII), cyclic-AMP-dependent kinase (PKA), and casein kinases I and II [for review, see (47, 52, 53)]. Non-receptor tyrosine kinases are also associated with AD pathology (54, 55). Fyn, Syk, and c-Abl were found to phosphorylate tau at Tyr18, Tyr197, and Tyr394, respectively (56–58). Moreover, multiple kinases are probably involved in tandem phosphorylation of tau. For example, dual-specificity tyrosine-phosphorylation-regulated kinase 1A (DYRK1A) and cdk5 have been suggested to act as priming kinases for subsequent GSK-3β phosphorylation (59, 60).

Tau is dephosphorylated by protein phosphatase 2A (PP2A) and, to a lesser extent, by PP1, PP2B, and PP5 (61–64). These phosphatases are present in the brain and are developmentally regulated (65). In human brain, PPs PP2A, PP1, PP5, and PP2B account for approximately 71%, 11%, 10%, and 7% of the total, respectively (64). The mRNA expression and activity of some phosphatases are decreased in affected areas of AD brain (64, 66–70). Therefore, tau phosphorylation must be regulated by multiple tau kinases and phosphatases through both direct and indirect interactions.

## Effect of phosphorylation on MT interaction and organization

MT assembly is regulated by phosphorylation in the MT-binding domain. It is reported that phosphorylation at Ser262 and Ser356 dramatically reduced the affinity of tau for MTs *in vitro* (71–76). These phosphoepitopes do not contain proline residues but are characterized by the sequence Lys-(Ile/Cys)-Gly-Ser. This motif is located in each of the three or four repeated sequences that constitute the tau MT-binding domain (72). MARK was identified to phosphorylate this motif and this kinase activity induced detachment of tau from

MTs and destabilization of MTs (77). Phosphorylation outside the MT-binding domains, for example at Ser214 or Thr231, can also influence tubulin assembly by modifying the affinity between tau and MTs (78). The peptidyl-prolyl isomerase Pin1 (peptidyl-prolyl cis/trans isomerase, NIMA-interacting 1) binds to tau when phosphorylated at Thr231, which leads to a conformational change that restores the ability of tau to bind to MTs (79). Therefore, phosphorylations at these sites are critical for the maintenance of neuronal MT network.

### Effect of phosphorylation on cell fate

The overexpression of tau in cells causes changes in cell morphology, retards cell growth, and dramatically alters the distribution of various organelles transported by MT-dependent motor proteins (80, 81). Concomitant with the hyperphosphorylation of tau, the axonal transport of the cells was prominently damaged, and the outgrowth of axon-like cell processes was inhibited (82). Furthermore, transgenic mice overexpressing wild-type human tau develop axonal degeneration (83).

*In vitro* studies demonstrated that phosphorylation of tau could affect proteasome activity. In HEK293, extensive phosphorylation of tau by inhibition of PP2A or activation of PKA inhibits the proteasome activity, whereas moderate phosphorylation stimulates the activity (84). In HT22 neuronal cells, recombinant human tau was rapidly degraded by the 20 S proteasome but tau phosphorylation by GSK3β significantly inhibited proteolysis (85).

Several groups have recently demonstrated that tau phosphorylation can promote re-entry of the neuron into the cell cycle and thus can lead to neurodegeneration (86, 87).

## Truncation

In addition to phosphorylation and glycosylation, tau undergoes multiple truncations and conformational changes from an unfolded protein to the structured polymer characteristic of NFT. The presence of truncated tau in paired helical filaments (PHF) was first reported by Wischik et al. (88). Recently, it has been reported that the truncation of tau accelerates its assembly into fibrils *in vitro* (89–91), increases its association with MTs (92), and promotes abnormal MT assembly *in vitro* (93). Moreover, transgenic rats expressing human tau truncated both N- and C-terminally, tau151–391, showed a marked neurofibrillary pathology (93). Because tau truncated at either Glu391 or Asp421 was identified in human tauopathy brains (91, 94–97), it is possible that an apoptotic cascade is involved in neurofibrillary pathology and neuronal dysfunction. Indeed, tau truncated at Asp421 assembled more readily than the full-length molecule (91) and enhanced tau aggregation properties in the presence of Aβ (98). Although it is still unclear whether tau cleavage occurs before or after the aggregation of tau into NFT, Binder et al. suggested that truncation at the caspase site of Asp421 appears to occur after filaments form from full-length tau and the cleavage rapidly increases the amount of filaments in NFT by positively affecting nucleation or elongation (99). The phosphorylation of tau at Ser422, which is specifically phosphorylated in tau found in NFTs, inhibits the cleavage of tau by caspase (100). It was reported that c-jun N-terminal kinase (JNK) activation induced tau phosphorylation including Ser422 and caspase cleavage of tau at Asp421 (101). Only coexpression of GSK-3β and JNK, but not JNK alone, induced significant increases in SDS-insoluble tau and cytotoxicity (101). These results indicate that caspase activation and tau phosphorylation can have a synergistic influence on neurotoxicity and NFT formation.

## Tau and tubulin or histone acetylation

A member of the histone deacetylase family, HDAC6, functions as a tubulin deacetylase and it is localized exclusively in the cytoplasm, where it associates with the MT motor complex (102). Analysis of brain from patients with AD showed increased acetylation of the α-tubulin subunit mainly in neurons containing NFTs (103). Immunoprecipitation and immunoblotting of brain cell extracts from wild-type tau and from tau knockout mice indicates that tau binds to the HDAC6, decreasing its activity with a consequent increase in tubulin acetylation. Interestingly, nicotinamide, a histone deacetylase (HDAC) inhibitor, restores cognitive deficits and reduces a specific phosphor-species of tau (Thr231) that is associated with MT depolymerization in 3×Tg-AD mice (104). In addition, sodium 4-phenylbutyrate (4-PBA), a well-known HDAC inhibitor, reversed spatial learning and memory deficits in AD model mice (Tg2576) (105). The administration of 4-PBA restored brain histone levels and most probably as a consequence activated the transcription of synaptic plasticity markers such as the GluR subunit of the AMPA receptor, PSD95, and MAP-2. Both treatments have the potential to be novel and safe AD therapies because nicotinamide is the biologically active form of niacin (vitamin B3) which has been widely used clinically for >40 years and 4-PBA was approved for treatment of urea cycle disorders.

## Summary

It is probable that tau hyperphosphorylation is critical for the development of tau-related neurodegeneration during abnormal post-translational modifications because a constant dynamic equilibrium to modulate MT dynamics is primarily controlled by the phosphorylation state of tau. Other post-translational modifications, such as glycosylation (106, 107), glycation (108), and nitration (109), can also modulate MT dynamics in cooperation with phosphorylation. The events of tau truncation, ubiquitination (110), and sumoylation (111, 112) might occur after the equilibrium of tau binding to the MTs and an increased level of unbound tau in cytoplasm was produced. Although it is possible that most of these post-translational modifications play critical roles in the pathogenesis, it is still not completely understood how these modifications promote the formation of insoluble filamentous inclusions.

## Transgenic animal model

### Transgenic mice overexpressing wild-type tau

**Neurofibrillary tangles (NFTs)** The first transgenic (Tg) models used expression of wild-type human tau. These mice reproduced somatodendritic localization of tau and phosphorylation at disease-relevant epitopes but no evidence for cytoskeletal changes or formation of tau aggregates was obtained (113). Subsequently, stronger promoters were used to drive expression and this resulted in a phenotype such as motor abnormality (114, 115). Although the majority of these mice did not develop NFTs, Ishihara et al. reported that NFTs were present when the mice reached a very old age (116). NFTs were also observed in the mice with endogenous mouse tau null background (117).

**Axonopathies** Tg mice that overexpress the 4R-tau isoform develop axonal degeneration in brain and spinal cord (83, 117). Although Tg mice that overexpress the 3R-tau isoform also develop axonopathy, the level of 4R-tau required to induce this effect appears to be much lower than that for the 3R form (114). Axonal dilatations were observed at a young age (1–3 months) and were accompanied by accumulations of neurofilaments, mitochondria, and vesicles, which might indicate defects in axonal transport mechanisms. Interestingly, axonopathy is rescued when constitutively active GSK-3β kinase is coexpressed in the mice, which results in decreased binding of tau to MTs (118). The data suggest that excess protein tau binds in 4R-human-tau-expressing mice, which results in an ''overstabilization'' of the MTs.

**Behavioral abnormalities and synaptic alterations** Tg mice overexpressing 4R-tau (Wtau-tg) showed impaired spatial learning even though they did not develop NFTs (119). In Wtau-tg, the distribution of phosphorylated tau was correlated with synapse loss and neuronal activity in the parahippocampal region. In other Tg mice (htau mice), synaptic transmission and spatial learning were negatively affected in the hippocampal CA1 region of old (12 months) but not young (4 months) mice, which preceded neurodegeneration (120). Old htau mice showed moderate tau pathology, whereas young htau mice had only early stage tau pathology. The authors conclude that NFT formation (or alternatively a process upstream of NFT formation) can underlie the synaptic dysfunction and perhaps the cognitive decline. These data suggest that the temporal and regional distributions of synapse loss could lead to behavioral abnormality in Tg mice overexpressing wild-type tau.

### Mutant tau transgenic mice

**Neurofibrillary tangles (NFTs)** Several groups reported on the formation of NFTs in neurons in mice overexpressing pathogenic mutations in human tau (121–126). The first reported NFT-forming model expressed human P301L tau under the control of the murine PrP promoter and developed motor dysfunction (122). NFTs were present in brain and spinal cord and the number of motor neurons in spinal cord was reduced. Subsequently, other groups have succeeded in the generation of NFT-forming transgenic lines that showed impaired learning connected with parahippocampal formation (127–131).

To determine whether NFTs are central to the neurotoxic cascade in AD or represent a protective response, rTg($tau_{P301L}$)4510 mice were generated in which transgene overexpression could be reduced with a tetracycline-regulated transactivator (129). rTg($tau_{P301L}$)4510 mice progressively developed NFTs, neuronal cell loss, and age-related impaired learning. Following a reduction in P301L tau from 12- to 2.5-fold overexpression, memory function recovered and the number of neurons stabilized, but the number of NFTs continued to increase. These data indicate that NFTs are not sufficient to cause cognitive decline or neuronal death in this model. In this model, characterization of region-specific neuronal loss and neurofibrillary changes using stereological methods was described (132). Neuron loss and PHF-1-positive aggregate formation were assessed in the five regions including CA1, CA2/3, dentate gyrus, cortex, and striatum. Although, in CA1, there were both substantial accumulation of PHF-1-positive cells and neuronal loss, other regions showed a clear dissociation. In dentate gyrus, 53% of neurons were lost by 4 months of age, before PHF-1-positive neurons appeared. By contrast, in striatum, 11% of neurons at 7 months of age exhibited PHF-1 immunoreactivity, but there was no statistically significant loss of neurons. These results also indicate that neuron loss is independent of neurofibrillary pathology.

To investigate whether aggregation of tau can be transmitted, two tau-expressing lines were used (133). Diluted extracts of brain homogenates from 6-month-old transgenic mice, expressing mutant (P301S) human tau, in which NFTs had already developed were injected into the brains of 3-month-old ALZ 17 mice overexpressing wild-type human tau, which did not have NFTs. In the brains of injected 18-month-old ALZ 17 mice, silver-positive filamentous structures were induced. These findings demonstrate transmission of tauopathy between transgenic lines and similarities to prion disease. However, the authors did not describe the transmission of behavioral dysfunction and synaptic alteration.

**Glial fibrillary tangles (GFTs)** PSP, CBD, and some cases of FTDP-17 are characterized by a substantial glial pathology such as tufted astrocytes, astrocytic plaques, and coiled bodies. The first reported GFT-forming model expressed human G272V mutant tau under a PrP-driven expression system that resulted in a high level of expression of human G272V tau in neurons and oligodendrocytes (134). Tau filament formation was established using EM and thioflavin-S-positive fibrillary inclusions were identified in oligodendrocytes and motor neurons in spinal cord. In contrast, when human wild-type tau was overexpressed using minigene construct combined with the mouse Tα1-α-tubulin promoter, GFTs similar to astrocytic plaques and coiled bodies in humans were observed (135). Subsequently, Tg mice

expressing human P301L tau exclusively in oligodendrocytes were generated using the 2′,3′-cyclic nucleotide 3′-phosphodiesterase promoter (136). These mice developed thioflavin-S-positive tau inclusions in oligodendrocytes. It is interesting that structural disruption of myelin and axons preceded the emergence of thioflavin-S-positive tau inclusions in oligodendrocytes but that impaired axonal transport occurred even earlier, whereas motor deficits developed subsequently. Under the glial fibrillary acidic protein promoter, human-tau-expressing mice were also generated (137). These mice developed GFTs similar to tufted astrocytes in PSP, and blood-brain barrier disruption and focal neurodegeneration were present. The question of whether glial pathology contributes to the clinical features of FTLD might be best addressed by behavioral studies using marked GFT-forming mice.

**Axonal transport** Increasing evidence suggests that neuronal dysfunction resulting from failure of axonal transport is an important pathomechanism in neurodegeneration, including tauopathy (138). To determine whether reduced axonal transport is caused by mutated tau, the transported tau was quantified in mice carrying the R406W tau mutation (139). [$^{35}$S]-labeled methionine was microinjected into the L4–L6 anterior horns and radiolabeled tau proteins were monitored in ventral horn 7 days later. There was a significant reduction in radiolabeled tau protein in the distal segment of the ventral roots of adult R406W tau mice compared with that in human wild-type tau mice. These data were supported by the findings of reduced binding ability of R406W tau to MTs compared with that of wild-type tau. Subsequently, impaired antegrade axonal transport in brains, between substantia nigra (SN) and striatum (ST), was demonstrated by novel Tg mice carrying the K369I mutation (140). These mice developed parkinsonism, which was ameliorated by L-dopa, and the loss of dopaminergic neurons in the midbrain. When comparing protein levels between SN and ST, the levels of different cargos including amyloid β precursor protein (APP) and presenilin 1 (PS1), the motor protein kinesin, and the scaffold protein Jip1 were markedly reduced. These studies indicate that future therapeutic strategies that target axonal transport impairment in tauopathies could be beneficial.

**Comparison of the wild-type and mutated tau** It is challenging to compare the functions of wild-type tau and mutated tau using transgenic mice. However, to compare the effects of wild-type and P301L mutant human tau expressions, two strains were generated (125). Both expressed the longest human tau isoform at similar, moderate levels by the *thy1* gene promoter, with one bearing the P301L mutation and the other bearing the wild-type tau. They developed very different phenotypes. Wild-type mice had already become motor-impaired around the age of 6–8 weeks, accompanied by axonopathy but no tau aggregates, and survived normally. In contrast, P301L mice developed NFTs from the age of 6 months without axonal dilatations and despite only minor motor problems, and all the mice died before 13 months of age. It was concluded that excessive binding of wild-type tau as opposed to reduced binding of P301L tau to MTs might have been responsible for axonopathy and tauopathy, respectively. Furthermore, the conformational change of P301L tau could have been a major determinant in triggering the tauopathy. Recently, it was reported that, in aged P301L mice, progressive upper airway dysfunction is linked to NFTs in the midbrain and pontomedullary brainstem nuclei (141). Taken together, binding of tau to MTs is affected in all tauopathies; however, the underlying mechanism responsible for the effects of mutated and wild-type tau could differ.

**Triple transgenic mice** To generate an AD model with both NFTs and amyloid plaque, several triple transgenic mice were generated (142, 143). They coexpress mutant form of *PS1* or *PS2*, *APP* and *MAPT* in familial diseases. To determine functional consequence of the combined Aβ and tau, a proteomic analysis was performed using vesicular preparations from triple transgenic mice (144). A massive deregulation, of 24 proteins, was found and one-third were mitochondrial proteins mainly related to complexes I and IV. Interestingly, deregulation of complex I was tau-dependent, whereas deregulation of complex IV was Aβ-dependent. Recently, to investigate the relationship between tau, Aβ and α-synuclein, a mutant human α-synuclein transgene was introduced into 3×Tg-AD mice (145). The presence of Lewy body pathology in AD is associated with a more aggressive course and accelerated cognitive dysfunction. Thus, Aβ, tau and α-synuclein might interact synergistically to promote the accumulation of each other. As expected, transgenic mice that develop both dementia with Lewy bodies and AD pathologies exhibit accelerated cognitive decline associated with a Aβ, tau and α-synuclein pathology.

## Expert opinion

From a clinicopathological point of view, neurodegenerative diseases have been described as being caused by the dysfunction of various specific pathways linked to various anatomical brain parts. In addition, numerous biochemical analyses pointed out that tau phosphorylation altered MT binding and assembly, possibly leading to axonal transport defect. Finally, research on Tg mice has enabled the study of axonal transport defect or synaptic alteration and indicates that the process upstream of NFT formation or tau phosphorylation could affect these dysfunctions. Modifying specific phosphoepitopes of tau, which affect MT binding, could lead to recovery of axonal transport and establish a novel therapy for tauopathies.

## Outlook

We speculate that research on tau Tg mice will focus on axonal transport dysfunction or synapse loss rather than neuronal death. In addition, treatment of decreasing mRNA might be beneficial because the majority of FTDP-17 muta-

tions in exon 10 and intron 10 disrupt exon 10 splicing and cause 4R-tauopathies.

## Highlights

We conclude that phosphorylated tau or mutated tau could impair axonal transport, leading to neurodegeneration in various parts of the brain in tauopathies before or during developing NFTs.

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