Filamentous Tau in Oligodendrocytes and Astrocytes of Transgenic Mice Expressing the Human Tau Isoform with the P301L Mutation

Wen-Lang Lin, Jada Lewis, Shu-Hui Yen,
Michael Hutton, and Dennis W. Dickson

From the Mayo Clinic, Jacksonville, Florida

We recently reported a transgenic mouse line (JNPL3) that expresses mutant (P301L) tau and develops neurofibrillary tangles composed of filamentous tau aggregates. Here we show that these mice have abnormal tau filaments not only in neurons, but also in oligodendrocytes and astrocytes. Similar results were detected in another transgenic line (JNPL2+3+) that expresses the longest human tau isoform with the P301L mutation. The ultrastructure of the tau filaments and immunoreactivity with tau and ubiquitin antibodies were similar in glia and neurons. Given similarities of the lesions in the mice to human neuronal and glial inclusions, these transgenic mice appear to be a valuable model to study pathogenesis of the neurodegenerative tauopathies. (*Am J Pathol* 2003, 162:213–218)

Tau is a microtubule-associated protein involved in microtubule assembly and stability.¹ Originally considered to be a neuronal protein,² tau has also been shown to be present in nonneuronal cells, including oligodendrocytes.^{3–6} Microtubules play a major role in the maintenance of cell structure and transport. It follows that abnormalities in tau might lead to changes in microtubules that would lead to dysfunction of neurons and glia and manifest as a neurodegenerative disorder.

Filamentous cellular inclusions composed of abnormal tau proteins are the histopathological hallmark of a group of neurodegenerative disorders, collectively called the “tauopathies.”⁷ The neurodegenerative tauopathies include Pick’s disease, progressive supranuclear palsy, corticobasal degeneration, argyrophilic grain disease, and frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17). Ultrastructurally, tau filaments appear as paired helical filaments or straight filaments. Paired helical filaments have a widest diameter of 20 nm that narrows to 8 nm at crossover points that occur every 80 nm. Longer periodicity and wider filaments, referred to as twisted ribbons, also occur. The straight filaments have no periodic narrowing although they may be intermixed with paired helical filaments in Alzheimer’s

disease.⁸ Although most studies have focused on neuronal inclusions, there is growing attention to glial inclusions in the tauopathies.^{9–12}

The recent discovery of pathogenic *tau* gene mutations in FTDP-17^{13–19} has provided a means to develop transgenic models for the tauopathies. We and others have previously reported neurofibrillary tangles composed of tau filaments in neurons of transgenic mice expressing mutant (P301L) tau protein.^{20–22} Götz and co-workers²³ first reported tau filamentous lesions in oligodendrocytes of transgenic mice with inducible expression of another tau mutation, G272V. More recently, Higuchi and colleagues²⁴ described progressive accumulation of tau in both astrocytes and oligodendrocytes, but not in neurons, in transgenic mice generated with a construct containing a *tau* minigene with the shortest normal human tau (3R0N) and the $T\alpha 1$ α -tubulin promoter.

Here we provide light and electron microscopic evidence for filamentous tau inclusions in oligodendrocytes and astrocytes in addition to previously reported neuronal inclusions in JNPL3 transgenic mice, as well as another mouse line (JNPL2+3+) expressing the longest human tau isoform (4R2N) with the P301L mutation. Differences between the oligodendroglial filamentous inclusions in P301L and the other two transgenic mice^{23,24} are discussed.

Materials and Methods

Transgenic Constructs and Animals

These were essentially the same as previously reported,²⁰ except for the addition of exons 2 and 3 to the cDNA construct for the JNPL2+3+ mouse line. Human tau cDNA containing exons 1, 4, 5, 7, 9 to 13, intron 13, exon 14, and exons 1 to 5, 7, 9 to 13, intron 13, exon 14 were provided by Dr. Athena Andreadis (E. K. Shriver Center for Mental Retardation, Waltham, MA). Mutagenesis to introduce the P301L mutation into exon 10 was performed with the Gene Editor kit (Promega, Madison,

Supported by the National Institutes of Health (grants AG16574, AG17216, AG14449, AG03949) and the Mayo Foundation.

Accepted for publication October 1, 2002.

Address reprint requests to Dennis W. Dickson, M.D., Mayo Clinic Jacksonville, Birdsall Bldg., 4500 San Pablo Rd., Jacksonville, FL 32224. E-mail: dickson.dennis@mayo.edu.

WI) using the manufacturer's recommendations. The sequence was confirmed with the BigDye Terminator Sequencing Kit (Perkin Elmer, Emeryville, CA) on an ABI377 automated sequencer with Sequence Navigator software (Perkin Elmer). A *Sall* fragment of each tau cDNA construct was ligated into a *XhoI* linearized MoPrP vector. Constructs were linearized with *NotI*, gel purified, and digested with β -agarase according to the manufacturer's recommendations. DNA was filtered, concentrated, and diluted to between 3 to 7 ng/ml in microinjection buffer (5 mmol/L Tris-HCl, pH 7.4, 10 mmol/L NaCl, 0.1 mmol/L ethylenediaminetetraacetic acid) for microinjection into C57Bl/DBA2/SW fertilized mouse eggs. Founders were screened by polymerase chain reaction between exons 1 to 5 of the human tau cDNA against an internal PS-2 control. The experimental animals in this study were obtained by breeding the founder line onto SW (Taconic Farms, Germantown, NY) for one to three generations.

Electron Microscopy and Postembedding Immunoelectron Microscopy (IEM)

Mice were perfused with 0.9% saline followed by 4% paraformaldehyde in 0.1 mol/L of phosphate buffer. Brains and spinal cords were removed and immersed in the same fixative. For IEM, small pieces (1 mm³) of tissues were dehydrated in 30%, 50%, 70%, and 90% EtOH, infiltrated, and embedded in LR White resin (Polysciences, Warrington, PA). Ultrathin sections collected on Formvar-coated nickel grids were incubated in primary antibodies overnight at 4°C, followed by secondary antibodies conjugated with colloidal gold particles. Antibodies used were CP13 (a mouse monoclonal antibody to phospho-tau, used undiluted, from Dr. Peter Davies, Albert Einstein College of Medicine, Bronx, NY), E1 (a polyclonal antibody to human tau, 1:200 dilution),²⁵ a polyclonal antibody to glial fibrillary acidic protein (1:20 dilution; BioGenex, San Ramon, CA) and rabbit antiserum to ubiquitin (UH19, 1:20 dilution, from Dr. Hanna Ksiezak-Reding, Mt. Sinai School of Medicine, New York, NY).²⁶ The sections were stained briefly with uranyl acetate and lead citrate before examination with a Philips 208S electron microscope. For routine ultrastructural studies, tissues were further fixed in 2.5% glutaraldehyde and 0.1 mol/L of cacodylate buffer, pH 7.4, postfixed in 1% O₃O₄-H₂O, *en bloc* stained in 1% uranyl acetate and 50% EtOH, dehydrated in ethanols and propylene oxide, infiltrated, and embedded in Epon 812 (Polysciences, Warrington, PA). Ultrathin sections were stained with uranyl and lead.

Immunohistochemistry (IHC) and Silver Staining

These were the same as previous reports.²⁰

Results

Inclusions in oligodendrocytes were detected at the light and electron microscopic level. Oligodendrocytes were identified according to ultrastructural features described

Figure 1. A: Three cells in the posterior horn are shown, with the largest cell being a neuron with a prominent nucleus (Nu), and lipofuscin granules (Lp). Note in-foldings of the nuclear membrane (**short arrows**). The middle cell is an oligodendrocyte (Olg) with clumped chromatin along the nuclear periphery and a prominent perinuclear cistern. Note the filamentous cytoplasmic inclusion (**long arrow**). The third cell may be a protoplasmic astrocyte of possibly a glial progenitor cell. **B:** Higher magnification of the filamentous inclusion in **A** shows straight and wavy filaments (**arrows**) with a diameter of 15 to 20 nm. Note the clear spaces between filaments. Scale bars, 0.2 μ m.

by Peters and colleagues,²⁷ including cells with a round nucleus, dense heterochromatin, a prominent perinuclear cistern, and scanty cytoplasm. Perineuronal satellite oligodendrocytes and interfascicular oligodendrocytes were examined. Filamentous cytoplasmic inclusions were detected in oligodendrocytes in both locations (Figure 1). The inclusions were composed of randomly oriented, 15- to 20-nm-diameter filaments. Most of the filaments appeared straight or slightly wavy. No twisted ribbons or paired helical filaments were detected. Some filaments were associated with focal electron-dense material. The filaments were otherwise free of additional structural elements. There was a gradual clearance of organelles from the cytoplasmic regions where filamentous aggregates formed. In areas with sparse filaments, organelles such as polysomes, endoplasmic reticulum, and mitochondria were intermixed with the filaments. Organelles were decreased in areas with the densest filamentous aggregates, and the interfilamentous space became electron lucent, making the inclusions stand out from the surrounding dense cytoplasm. In favorably oriented sections, several filaments appeared to perpendicularly abut the nuclear membrane. Oligodendroglial inclusions, like neuronal inclusions previously reported, were not mem-

Figure 2. A: An oligodendrocyte and several myelinated axons (Ax). **B:** Boxed area in A showing immunogold labeling of filaments by a human-specific tau antibody, E1 (short arrows). Note the close proximity of filaments to the nuclear membrane (long arrows). Scale bars: 1 μm (A); 0.2 μm (B).

brane-bound; however, large masses of tightly packed filaments, which were frequently present in neurons,^{20,21} were not detected in oligodendrocytes.

The filaments were composed of tau based on immunoreactivity with monoclonal and polyclonal tau antibodies (CP13 and E1) (Figure 2). Because E1 is specific to human tau protein,²⁴ it is certain that at least some, if not all, of the filamentous tau is derived from human mutant tau from the transgene. The filaments also contained abnormally phosphorylated tau because CP13 is specific to a phosphoepitope characteristic of abnormal tau in Alzheimer's disease and tauopathies. In addition to tau, the filaments were immunolabeled with a polyclonal antibody to ubiquitin (UH19) (Figure 3). The diameter of filaments in IEM appeared slightly smaller than those in transmission EM because of the reported shrinkage that occurs in LR White-embedded tissue.²⁸

The affected oligodendrocytes were found in the spinal cord, brainstem, and entorhinal cortex of both JPNL3 and JPNL2+3+ mice. They were more readily detected in the spinal cord than in the brain of both mouse lines, and they appeared to be more abundant in JPNL2+3+ mice; however, formal quantitative analysis remains to be done.

Tau inclusions in oligodendrocytes were not readily detected with routine light microscopy, including hematoxylin and eosin-stained paraffin sections and toluidine blue-stained plastic sections; however, they were detected with Gallyas silver stains and IHC with tau antibodies, most notably CP13 (Figure 4). Oligodendroglial inclusions were most numerous in the white matter of the spinal cord and brainstem, and they bore a striking re-

Figure 3. A: A satellite oligodendrocyte next to a neuron (Nu). **B:** Boxed area in A showing filaments labeled by ubiquitin antibody (arrows). A cluster of filaments about the nuclear membrane (open arrow). Scale bars: 1 μm (A); 0.2 μm (B).

semblance to the argyrophilic oligodendroglial coiled bodies that are found in a host of neurodegenerative tauopathies. They increased in frequency with disease severity and had a similar distribution to neurofibrillary tangles, being most abundant in spinal cord and brain-

Figure 4. a: Tau immunostain shows immunoreactivity of the cytoplasm of a subpial astrocyte. **b:** Oligodendroglia in spinal white matter have filamentous lesions that coil around the nucleus. **c** and **d:** Oligodendroglial inclusions in transgenic mice are argyrophilic with Gallyas silver stain similar to coiled bodies in human tauopathies.

Figure 5. **A:** A subpial astrocyte in white matter of the spinal cord. **B:** Boxed area of the cell process in **A** showing tau filaments that are labeled with immunogold and a human-specific antibody, E1 (arrows). The astrocytic nature of the cell is confirmed by the presence of bundles of characteristic glial fibrils (asterisks). Note the close association of these two types of filaments. Scale bars: 1 μm (**A**); 0.125 μm (**B**).

stem, infrequent in limbic cortices and amygdala, and absent from neocortex and basal ganglia.

IHC also revealed tau-immunopositive astrocytes in the subpial and perivascular spaces in the spinal cord of transgenic, but not control mice (Figure 4). Tau-positive astrocytes were not detected in the brain. They were also readily detected only in mice with marked spinal cord neuronal and glial pathology. In contrast to oligodendroglial inclusions, tau-positive astrocytes were not argyrophilic with the Gallyas silver stain. Nevertheless, IEM revealed that astrocytes in the spinal cord subpial space contained tau filaments that were immunolabeled by human-specific tau antibodies and were intermingled with normal glial fibrils (Figure 5). Tau filaments in astrocytes did not form aggregates, and this may account for the lack of argyrophilia.

Discussion

The present study demonstrates by IEM that tau filamentous inclusions can be found in oligodendrocytes and astrocytes in two transgenic mouse lines (JNPL3, JNPL2+3+) expressing human tau isoforms (4R0N and 4R2N, respectively) with the P301L mutation. The prion promoter drives expression of the transgene in both lines. Whereas the prion promoter used in the present study

has been considered to be primarily expressed in neurons,²⁹ prion protein expression is more ubiquitous and has also been reported in glial cells^{30–33} as well as epithelial and endothelial cells³⁴ of transgenic mice. Differences in the filamentous aggregates in neurons and oligodendrocytes may reflect differences in transgene expression in the different cell types.³⁵

The glial tau inclusions were composed of mostly straight filaments with a diameter ranging from 15 to 20 nm and were immunolabeled with a human-specific tau antibody. Although some filaments appeared wavy, there was neither periodic narrowing nor a twisted ribbon appearance. These ultrastructural findings are compatible with previous reports of fibrils in neurofibrillary tangles of JNPL3 transgenic mice.^{20,21} It is noteworthy that the oligodendroglial tau filaments formed aggregates that varied in their size, but never formed large tangle-like aggregates as in neurofibrillary tangles. This, plus the fact that oligodendrocytes are small in size with sparse perinuclear cytoplasm, may explain why they are difficult to detect by routine light microscopy; however, they were readily detected with tau IHC and Gallyas silver stains. Their appearance and staining properties are very similar to the coiled bodies in oligodendrocytes of human tauopathies.³⁶

The presence of ubiquitin immunoreactivity in the filaments in oligodendrocytes differs from tau inclusions in human tauopathies, in which ubiquitin immunoreactivity is minimal with IHC.³⁷ In addition, less ubiquitin immunolabeling has been detected on filaments isolated from corticobasal degeneration, a sporadic 4R tauopathy, than Alzheimer's disease.³⁸ The variability in ubiquitin immunoreactivity might be because of different tau isoform composition in different disorders. Disorders with less ubiquitin immunoreactivity tend to be 4R tauopathies, with glial as well as neuronal tau inclusions, whereas ubiquitin immunoreactivity is more abundant in Alzheimer's disease (a 3R and 4R tauopathy) and Pick's disease (a 3R tauopathy).³⁹

There are very few reports of tau filaments in rodent oligodendrocytes. Using the pre-embedding immunoperoxidase method, Papasozomenos and Binder³ observed tau immunostaining in the cytoplasm of neurons and perineuronal oligodendrocytes in normal rat and monkey brains. At the ultrastructural level, tau was localized to microtubules as well as ribosomes and polysomes. Using postembedding immunogold methods Migheli and co-workers⁴ also found tau protein in microtubules within interfascicular oligodendrocytes of rat brains.

A number of transgenic mice that express human tau isoforms have been developed.^{40–45} They have tau immunoreactivity in neurons and their processes, but only one study has demonstrated immunolabeling of astrocyte-like cells at the light microscopic level.⁴² Another study⁴³ showed aggregates of 10- to 20-nm straight filaments in myelinated spinal cord axons. The filaments were immunolabeled by antibodies to tau, tubulin, and neurofilaments. Tau filamentous inclusions were not reported in the cell bodies of neurons or glial cells. The lack of convincing tau filaments in these mice has limited their use as models of neurodegenerative disease. The present report of tau fila-

ments in two lines of transgenic mice (JNPL2+3+ and JNPL3)^{20,21} demonstrates the impact of the P301L mutation on tau filament formation in neurons and glia, and it provides further substantiation that these mice have a number of histopathological features similar to human neurodegenerative tauopathies.

Recently, Götz and co-workers²³ reported thioflavin-S-positive and tau-immunoreactive inclusions in oligodendrocytes and neurons of transgenic mice with inducible expression of mutant G272V tau. In the spinal cord, human tau levels were fourfold greater in oligodendrocytes than in neurons. Tubulofilamentous aggregates composed of 17- to 20-nm-wide filaments, either straight or twisted with a periodicity of 75 nm, were demonstrated with electron microscopy. Unfortunately, neither neuronal nor oligodendroglial inclusions were detected with Gallyas or Bielschowsky silver stain, limiting the usefulness of this model.

More recently, Higuchi and colleagues²⁴ showed tau immunoreactivity in neurons and glial cells, as well as Gallyas silver-positive inclusions in oligodendrocytes of old transgenic mice expressing the shortest normal human tau. In contrast to the present study in which both neuronal and glial lesions were detected, Higuchi and colleagues²⁴ did not detect tau filaments in neurons, but showed 15- to 20-nm-wide straight filaments in oligodendrocytes and astrocytes. They concluded that the glial rather than neuronal tau lesions produced the observed motor deficit. In our transgenic mice expressing P301L tau^{20,22} argyrophilic inclusions composed of 15- to 20-nm-diameter tau filaments were detected in both neurons and glia. It is unclear why previous transgenic mice other than those reported in the present study have failed to display both neuronal and glial inclusions, which are characteristic of the human diseases the mice attempt to model. Whether these discrepancies are because of differences in transgene constructs, mouse genetic background differences, or difference in expression levels remain to be determined.

Tau immunoreactivity in astrocytes was shown by IHC in the present study, but inclusions were not argyrophilic with Gallyas stain. IEM revealed that the tau filaments were mixed with glial fibrils. Although the mechanism of silver staining is unknown, it is possible that tightly packed glial fibrils may interfere with the silver interaction with the more dispersed tau filaments.

Although both JNPL3 and JNPL2+3+ mice showed similar results, inclusion-bearing oligodendrocytes were more readily detected in the JNPL2+3+ than the JNPL3 mice. Preliminary results also suggest that JNPL+2+3 mice also have more extensive neuronal pathology, including neurons with ballooning degeneration. The significance of the two N-terminal inserts encoded by exons 2 and 3 is currently unknown; however, *in vitro* studies have shown that the 4R2N isoform of tau assembles into filaments faster than 4R0N.⁴⁶

In summary, the present study demonstrates filamentous tau lesions in astrocytes and oligodendrocytes of transgenic mice expressing P301L mutant human tau, a model also characterized by neurofibrillary tangles, neuronal loss and progressive amyotrophy. The tau filaments

formed argyrophilic inclusions in oligodendrocytes, but not in astrocytes. These results provide support for the validity of this transgenic mouse model for the human neurodegenerative tauopathies.

Acknowledgments

We thank Dr. Peter Davies, Albert Einstein College of Medicine, Bronx, NY, and Dr. Hanna Ksiezak-Reding, Mt. Sinai School of Medicine, New York, NY, for their generous donation of tau and ubiquitin antibodies.

References

1. Delacourte A, Buée L: Normal and pathological tau proteins as factors for microtubule assembly. *Int Rev Cytol* 1997, 171:167–224
2. Binder LI, Frankfurter A, Rebhun L: The distribution of tau in the mammalian nervous system. *J Cell Biol* 1985, 101:1371–1378
3. Papasozomenos SC, Binder LI: Phosphorylation determines two distinct species of tau in the central nervous system. *Cell Motil Cytoskeleton* 1987, 8:210–226
4. Migheli A, Butler M, Brown K, Shelanski M: Light and electron microscope localization of the microtubule-associated tau protein in rat brain. *J Neurosci* 1988, 8:1846–1851
5. Shin R-W, Iwaki T, Kitamoto T, Tateishi J: Hydrated autoclave pretreatment enhances tau immunoreactivity in formalin-fixed normal and Alzheimer's disease brain tissues. *Lab Invest* 1991, 64:693–702
6. LoPresti P, Szuchet S, Papasozomenos SC, Zinkowski RP, Binder LI: Functional implications for the microtubule-associated protein tau: localization in oligodendrocytes. *Proc Natl Acad Sci USA* 1995, 92:10369–10373
7. Hong M, Trojanowski JQ, Lee VM-Y: Tau-based neurofibrillary lesions. *Neurodegenerative Dementias: Clinical Features and Pathological Mechanisms*. Edited by CM Clark, JQ Trojanowski. New York, McGraw-Hill, 2000, pp 161–175
8. Tolnay M, Probst A: Tau protein pathology in Alzheimer's disease and related disorders. *Neuropathol Appl Neurobiol* 1999, 25:171–187
9. Feany MB, Dickson DW: Neurodegenerative disorders with extensive tau pathology: a comparative study and review. *Ann Neurol* 1996, 40:139–148
10. Chin SS-M, Goldman JE: Glial inclusions in CNS degenerative diseases. *J Neuropathol Exp Neurol* 1996, 55:499–508
11. Ludwin SK: The pathobiology of the oligodendrocyte. *J Neuropathol Exp Neurol* 1997, 56:111–124
12. Ikeda K, Akiyama H, Arai T, Nishimura T: Glial tau pathology in neurodegenerative diseases: their nature and comparison with neuronal tangles. *Neurobiol Aging* 1998, 19:S85–S91
13. Hutton M, Lendon CL, Rizzu P, Baker M, Froelich S, Houlden H, Pickering-Brown S, Chakroverty S, Isaacs A, Grover A, Hackett J, Adamson J, Lincoln S, Dickson D, Davies P, Petersen RC, Stevens M, deGraaff E, Wauters E, van Baren J, Hillebrand M, Joosse M, Kwon JM, Nowotny P: Association of missense and 5'-splice-site mutations in tau with the inherited dementia FTDP-17. *Nature* 1998, 393:702–705
14. Spillantini MG, Murrell JR, Goedert M, Farlow MR, Klug A, Ghetti B: Mutation in the tau gene in familial multiple system tauopathy with presenile dementia. *Proc Natl Acad Sci USA* 1998, 95:7737–7741
15. Dumanchin C, Camuzat A, Campion D, Verpillat P, Hannequin D, Dubois B, Saugier-Verber P, Martin C, Penet C, Charbonnier F, Agid Y, Frebourg T, Brice A: Segregation of a missense mutation in the microtubule-associated protein tau gene with familial frontotemporal dementia and parkinsonism. *Hum Mol Genet* 1998, 7:1825–1829
16. Poorkaj P, Bird TD, Wijsman E, Nemens E, Garruto RM, Anderson L, Andreadis A, Wiederholt WC, Raskind M, Schellenberg GD: Tau is a candidate gene for chromosome 17 frontotemporal dementia. *Ann Neurol* 1998, 43:815–825
17. Clark LN, Poorkaj P, Wszolek Z, Geschwind DH, Nasreddine ZS, Miller B, Li D, Payami H, Awert F, Markopoulou K, Andreadis A, D'Souza I, Lee VM-Y, Reed L, Trojanowski JQ, Zhukareva V, Bird T,

- Schellenberg G, Wilhelmsen KC: Pathogenic implications of mutations in the tau gene in pallido-ponto-nigral degeneration and related neurodegenerative disorders linked to chromosome 17. *Proc Natl Acad Sci USA* 1998, 95:13103–13107
18. Rizzu P, Van Swieten JC, Joosse M, Hasegawa M, Stevens M, Tibben A, Niermeijer MF, Hillebrand M, Ravid R, Oostra BA, Goedert M, van Duijn CM, Heutink P: High prevalence of mutations in the microtubule-associated protein tau in a population study of frontotemporal dementia in The Netherlands. *Am J Hum Genet* 1999, 64:414–421
19. Iijima M, Tabira T, Poorkaj P, Schellenberg GD, Trojanowski JQ, Lee VM-Y, Schmidt ML, Takehashi K, Nabika T, Matsumoto T, Yamashita Y, Yohioka S, Ishino H: A distinct familial dementia with a novel missense mutation in the tau gene. *NeuroReport* 1999, 10:497–501
20. Lewis J, McGowan E, Rockwood J, Melrose H, Nacharaju P, Van Slegtenhorst M, Gwinn-Hardy K, Murphy MP, Baker M, Yu X, Duff K, Hardy J, Corral A, Lin W-L, Yen S-H, Dickson DW, Davis P, Hutton M: Neurofibrillary tangles, amyotrophy and progressive motor disturbance in mice expressing mutant (P301L) tau protein. *Nat Genet* 2000, 25:402–405
21. Lin W-L, Lewis J, Corral AR, Dickson DW, Hutton M: Ultrastructural pathology of neurofibrillary tangles in transgenic mice carrying mutant (P301) human tau gene. *Microsc Microanal* 2000, 6(Suppl 2): S584–S585
22. Götz J, Chen F, Barmettler R, Nitsch RM: Tau filament formation in transgenic mice expressing P301L tau. *J Biol Chem* 2001, 276:529–534
23. Götz J, Tolnay M, Barmettler R, Chen F, Probst A, Nitsch RM: Oligodendroglial tau filament formation in transgenic mice expressing G272V tau. *Eur J Neurosci* 2001, 13:2131–2140
24. Higuchi M, Ishihara T, Zhang B, Hong M, Andreadis A, Trojanowski JQ, Lee VM-Y: Transgenic mouse model of tauopathies with glial pathology and nervous system degeneration. *Neuron* 2002, 35:433–446
25. Crowe A, Ksiezak-Reding H, Liu W-K, Dickson DW, Yen S-H: The N terminal region of human tau is present in Alzheimer's disease protein A68 and is incorporated into paired helical filaments. *Am J Pathol* 1991, 139:1463–1470
26. Lee S, Park D, Yen S-HC, Ksiezak-Reding H, Goldman JE, Dickson DW: A study of infantile motor neuron disease with neurofilament and ubiquitin immunocytochemistry. *Neuropediatrics* 1989, 20:107–111
27. Peters A, Palay SL, Webster HD: *The Fine Structure of the Nervous System: Neurons and Their Supporting Cells*. New York, Oxford University Press, 1991
28. Lawton DM, Oswald WB, McClure J: The biological reality of the interlacunar network in the embryonic, cartilaginous, skeleton: a thiazine dye/absolute ethanol/LR White resin protocol for visualizing the network with minimal tissue shrinkage. *J Microsc* 1995, 178:66–85
29. Borchelt DR, Davis J, Fischer M, Lee MK, Slunt HH, Ratovitsky T, Regard J, Copeland NG, Jenkins NA, Sisodia SS, Price DL: A vector for expressing foreign genes in the brains and hearts of transgenic mice. *Genetic Anal* 1996, 13:159–163
30. Moser M, Colello RJ, Pott U, Oesch B: Developmental expression of the prion gene in glial cells. *Neuron* 1995, 14:509–517
31. Verghese-Nikolaki S, Michaloudi H, Polymenidou M, Groschup MH, Papadopoulos GC, Sklaviadis T: Expression of the prion protein in the rat forebrain-an immunohistochemical study. *Neurosci Lett* 1999, 272:9–12
32. Haeberle A-M, Ribaut-Barassin C, Bombarde G, Mariani J, Hunsmann G, Grassi J, Bailly Y: Synaptic prion protein immunoreactivity in the rodent cerebellum. *Microsc Res Tech* 2000, 50:66–75
33. Laine J, Marc M-E, Sy M-S, Axelrad H: Cellular and subcellular morphological localization of normal prion protein in rodent cerebellum. *Eur J Neurosci* 2001, 14:47–56
34. Lemaire-Vieille C, Schulze T, Pödevin-Dimster V, Follet J, Bailly Y, Blanquet-Grossard F, Decavel J-P, Heinen E, Cesbron J-Y: Epithelial and endothelial expression of the green fluorescent protein reporter gene under the control of bovine prion protein (PrP) gene regulatory sequences in transgenic mice. *Proc Natl Acad Sci* 2000, 97:5422–5427
35. Ford MJ, Burton LJ, Li H, Graham CH, Frobert Y, Grassi J, Hall SM, Morris RJ: A marked disparity between the expression of prion protein and its message by neurons of the CNS. *Neuroscience* 2002, 111: 533–551
36. Braak H, Braak E: Cortical and subcortical argyrophilic grains characterize a disease associated with adult onset dementia. *Neuropathol Appl Neurobiol* 1989, 15:13–26
37. Feany MB, Mattiace LA, Dickson DW: Neuropathologic overlap of progressive supranuclear palsy, Pick's disease and corticobasal degeneration. *J Neuropathol Exp Pathol* 1996, 55:53–67
38. Yang L-S, Ksiezak-Reding H: Ubiquitin immunoreactivity of paired helical filaments differs in Alzheimer's disease and corticobasal degeneration. *Acta Neuropathol* 1998, 96:520–526
39. Lee VM, Goedert M, Trojanowski JQ: Neurodegenerative tauopathies. *Annu Rev Neurosci* 2001, 24:1121–1159
40. Götz J, Probst A, Spillantini MG, Schäfer T, Jakes R, Bürki K, Goedert M: Somatodendritic localization and hyperphosphorylation of tau protein in transgenic mice overexpressing the longest human brain tau isoform. *EMBO J* 1995, 14:1304–1313
41. Brion J-P, Tremp G, Octave J-N: Transgenic expression of the shortest human tau affects its compartmentalization and its phosphorylation as in the pretangle stage of Alzheimer's disease. *Am J Pathol* 1999, 154:255–270
42. Ishihara T, Hong M, Zhang B, Nakagawa Y, Lee MK, Trojanowski JQ, Lee VM-Y: Age-dependent emergence and progression of a tauopathy in transgenic mice overexpressing the shortest human tau isoform. *Neuron* 1999, 24:751–762
43. Spittaels K, Van den Haute C, Van Dorpe J, Bruynseels K, Vandezande K, Laenen I, Geerts H, Mercken M, Sciort R, Van Lommel A, Loos R, Can Leuven F: Prominent axonopathy in the brain and spinal cord of transgenic mice overexpressing four-repeat human tau protein. *Am J Pathol* 1999, 155:2153–2165
44. Probst A, Götz J, Wiederhold KH, Tolnay M, Mistl C, Jaton AL, Hong M, Ishihara T, Lee VM-Y, Trojanowski JQ, Jakes R, Crowther RA, Spillantini MG, Bürki K, Goedert M: Axonopathy and amyotrophy in mice transgenic for human four-repeat tau protein. *Acta Neuropathol* 2000, 99:469–481
45. Duff K, Knight H, Refolo LM, Sanders S, Yu X, Picciano M, Malester B, Hutton M, Adamson J, Goedert M, Bürki K, Davis P: Characterization of pathology in transgenic mice over-expressing human genomic and cDNA tau transgenes. *Neurobiol Dis* 2000, 7:87–98
46. King EK, Gamblin TC, Kuret J, Binder LI: Differential assembly of human tau isoforms in the presence of arachidonic acid. *J Neurochem* 2000, 74:1749–1757