Enhanced Neurofibrillary Degeneration in Transgenic Mice Expressing Mutant Tau and APP

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JNPL3 transgenic mice expressing a mutant tau protein, which develop neurofibrillary tangles and progressive motor disturbance, were crossed with Tg2576 transgenic mice expressing mutant β-amyloid precursor protein (APP), thus modulating the APP-AB (β-amyloid peptide) environment. The resulting double mutant (tau/APP) progeny and the Tg2576 parental strain developed AB deposits at the same age; however, relative to JNPL3 mice, the double mutants exhibited neurofibrillary tangle pathology that was substantially enhanced in the limbic system and olfactory cortex. These results indicate that either APP or AB influences the formation of neurofibrillary tangles. The interaction between AB and tau pathologies in these mice supports the hypothesis that a similar interaction occurs in Alzheimer’s disease.

Alzheimer’s disease (AD) is pathologically characterized by senile plaques, largely composed of extracellular deposits of AB peptide, and neurofibrillary tangles (NFTs), composed of intracellular filamentous aggregates of hyperphosphorylated tau protein. Since the initial molecular characterizations of these lesions (1–3), there has been controversy over how these lesions and their constituent molecules are pathogenically related to each other and to the neuronal and synaptic losses that characterize the disease (4–6). A key part of this debate has been the observation that the pathogenic mutations that underlie the autosomal dominant forms of the disease—mutations in APP or in the presenilins PS-1 and PS-2 (7–9)—lead to increased production of the Aβ42 peptide in tissues from affected individuals (10), in transfected cells (11–13), and in transgenic animals (12, 14–18). Some transgenic mouse models for AD, overexpressing mutant human APP alone or with mutant PS-1, develop senile plaques; however, these mice lack NFTs and exhibit little neuronal loss (14–18). This has limited their use as models of disease and fueled the notion that senile plaques and NFTs are generated by independent processes.

Neurofibrillary pathology is also a feature of other neurodegenerative diseases, including FTD-PD-17 (frontotemporal dementia and Parkinsonism linked to chromosome 17). Mutations in the tau gene underlie FTD-PD-17, hence tau dysfunction is sufficient to cause neurodegeneration (19). Furthermore, JNPL3 transgenic mice with the Pro301L four-repeat tau (P301L)–mutant APP (APPSw) (hereafter termed TAPP mice), mutant tau (JNPL3), mutant APP (Tg2576), and nontransgenic animals. TAPP mice had amyloid plaques similar in number and distribution to those of comparably aged Tg2576 mice. Plaques were detected as early as 6 months of age but became numerous only in older TAPP and Tg2576 mice (8.5 to 15 months) in the olfactory cortex, cingulate gyrus, amygdala, entorhinal cortex, and hippocampus (Fig. 1) (30). NFTs were morphologically similar in TAPP and JNPL3 mice and appeared in the spinal cord and pons as early as 3 months of age, but were consistently present and numerous only in older animals (Figs. 2 and 3). Some of the NFTs were fluorescent when stained with thioflavin-S, and all were intensely positive for Gallyas silver stain and immunoreactive with a panel of antibodies to tau protein, including antibodies to phosphorylation-dependent and conformational epitopes (24–28) (Fig. 1). Ultrastructurally, NFTs in the TAPP mice were also similar to those in JNPL3 mice and were composed of straight filaments, 17 to 22 nm

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in diameter, that sometimes formed complex arrangements with a herringbone appearance similar to those described in Pick’s disease (Fig. 1) (31). Tau filaments occupied a large proportion of the cell volume in neurons with NFTs, displacing the nucleus and cytoplasmic organelles and compressing the Golgi apparatus.

Although NFTs were morphologically similar in the TAPP and JNPL3 (mutant tau) mice, older female TAPP mice (9 to 11 months) had a marked increase in NFTs in limbic areas, most notably the olfactory cortex, entorhinal cortex, and amygdala. This enhanced neurofibrillary degeneration occurred as early as 6 months of age. The density of NFTs in these regions in female TAPP mice (9 to 11 months) was greater than in female JNPL3 littermates by a factor of more than 7 ($P < 0.012$ to $P < 0.0009$) (Figs. 2 and 3). NFTs were also detected in the subiculum, hippocampus, and occasionally isocortex in the TAPP animals, areas that rarely or never had NFTs in JNPL3 mice. The number and distribution of pretangles was also increased in female TAPP mice (9 to 11 months) in limbic areas and cerebral cortex. In contrast, subcortical neurofibrillary pathology was similar in female TAPP and JNPL3 mice, with no increase in density of either NFTs or pretangles in the diencephalon, hindbrain, or spinal cord (Fig. 3). NFTs and pretangles were not observed in the basal ganglia in TAPP mice. In limbic areas with the most NFTs, there was a concomitant increase in astrocytosis (Fig. 2) in TAPP mice. In contrast, in the ventral diencephalon, hindbrain, and spinal cord of JNPL3 and TAPP mice, gliosis was equally severe. Enhanced neurofibrillary pathology in female TAPP mice (9 to 11 months) in limbic regions is of interest because these are the areas in which Aβ pathology first develops in Tg2576 mice (15).

However, even in areas vulnerable to both types of lesions (such as the entorhinal cortex), NFTs were not typically increased in the immediate vicinity of amyloid deposits. The fact that amyloid plaques were not generally surrounded by tangle-bearing neurons suggests that either a high-Aβ environment or APP dysfunction, but not necessarily the formation of mature amyloid deposits, is responsible for the modulation and enhancement of the tau phenotype in the TAPP mice. Interestingly, male TAPP mice did not develop similar enhanced NFT pathology in limbic regions (Fig. 3). This likely reflects sex differences in the development of NFT pathology previously observed in the JNPL3 line; female mice develop NFT pathology significantly earlier than do males (32). However, the difference between female and male TAPP mice could also be caused by significant sex differences in amyloid burden previously noted in older Tg2576 mice (33) or could reflect hormonal changes in aging female TAPP mice. The latter possibility is interesting given the higher incidence of AD in women (34–36).

The morphology, distribution, and density of the amyloid plaques were similar in TAPP mice and age-matched Tg2576 mice (Fig. 1) (30). The amyloid deposits were immunoreactive for both Aβ40 and Aβ42 (30). In addition, dystrophic neurites immunoreactive for APP were associated with the senile plaques in both TAPP and Tg2576 mice (30). Some of the plaques had neurites that were immunoreactive with phospho-tau antibodies, but plaque-associated neurites were not detected with antibodies specific to conformational tau epitopes, such as Alz50 and MC1, or antibodies specific to NFTs, such as Ab39 (Fig. 1) (30). To date, we have not identified plaque-associated dystrophic neurites containing tau-immunoreactive filamentous structures at the ultrastructural level.

Brain Aβ1-40 and Aβ1-42 were measured in hemibrains from 9- to 11-month-old TAPP mice and from Tg2576 littermates (15, 37–39). Aβ40 and Aβ42 levels were similar in the brains of TAPP mice (553 pmol/g and 352 pmol/g, respectively) and Tg2576 littermates (560 pmol/g and 421 pmol/g, respectively), consistent with the absence of a detectable difference in Aβ plaque burden in

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**Fig. 1.** NFTs and amyloid plaques in TAPP mice. (A and C) Adjacent sections of entorhinal cortex viewed with thioflavin-S fluorescent microscopy (A) or Gallyas silver stain (C) show both amyloid plaques and NFTs in female TAPP mice. (B and D) Amyloid plaques are immunostained with a rabbit antibody to Aβ42 and double-immunostained with mouse antibodies to tau. In (B), a tangle-specific mAb (Ab39) identifies a NFT (left) but no neurites in the plaque. In (D), double staining of a diffuse amyloid deposit in the frontal cortex shows local phospho-tau (PG5) immunoreactivity in neurons and neuronal cell processes associated with the plaque. (E) Ultrastructural studies of entorhinal NFTs reveal aggregates of criss-crossing straight filaments that are about 20 nm in diameter. (F) Granulovacular bodies (arrowheads) are detected in neurons in the amygdala, entorhinal cortex, and subiculum with phospho-tau antibodies (TG3). Mice were aged 9.5 to 10.5 months. Magnifications: (A) and (C), 200×; (B), (D), and (F), 400×.
the TAPP mice compared with Tg2576 mice at the same age. A larger series of animals, however, will be needed to determine whether there are significant differences in the initial levels of Aβ in young TAPP mice and in the rate of Aβ deposition as the mice age.

Levels of total soluble endogenous and transgenic tau protein (40) and tau mRNA (41) were similar in TAPP and JNPL3 brains (30). In addition, in situ hybridization analysis showed no difference in tau transgene expression pattern between TAPP and JNPL3 mice. These data indicate that there was no global or region-specific increase in tau expression that could explain the enhanced neurofibrillary pathology in the TAPP mice.

Sarkosyl-insoluble tau extracted from both TAPP and JNPL3 mice contained a major hyperphosphorylated species migrating at 64 kD (Fig. 3) that increased as a proportion of the total insoluble tau with age. Deaphosphorylation studies in JNPL3 mice and in humans with AD and FTDP-17 have shown that the 64-kD band contains hyperphosphorylated tau of the same isoform expressed by the tau transgene (20). The 64-kD insoluble tau species (42, 43) extracted from the cortex/limbic fraction (fraction 1) of the mouse brain was increased in female TAPP mice (9.5 to 11 months) relative to female JNPL3 mice, but not in the fraction (fraction 2) containing the subcortical regions, brainstem, and cerebellum (Fig. 3). This elevation of 64-kD insoluble tau in the female TAPP animals (9.5 to 11 months) correlates with histopathologic evidence of enhanced neurofibrillary pathology in the limbic system of the oldest female TAPP mice. At earlier time points (3 and 6 months), insoluble tau could be detected in both fractions in TAPP and JNPL3 mice; however, enhanced insoluble tau in the cortex/limbic fraction was not observed in TAPP mice. This correlates with the observation that significant increases in limbic NFT pathology are detected only after 9 months in female TAPP mice (Fig. 3).

In addition to increased NFTs, granulovacular degeneration was observed in neurons in the amygdala, entorhinal cortex, and subiculum in female TAPP mice (Fig. 1). Granulovacular degeneration was characterized by optically clear vacuoles with dense cores that contained phosphorylated epitopes recognized by TG3 and MPM-2 monoclonal antibodies (mAbs) (44, 45). In contrast, only neurons in the amygdala of JNPL3 mice showed rare granulovacular degeneration.

TAPP mice developed motor disturbances similar to their JNPL3 littermates, with identical range in age of onset, including progressive hindlimb weakness, hunched posture, eye irritations, reduced vocalization, and decreased grooming (20). The motor phenotype most likely is associated with the spinal cord and neuromuscular pathology that was similar in both TAPP and JNPL3 mice.

Our results reveal an interaction between APP or Aβ and tau (46) that leads to increased NFT formation and distribution in regions of brain vulnerable to these lesions. Most important, the findings for TAPP mice show that improved rodent models of AD are possible using an APP-tau cross-breeding strategy. These models should allow therapies to be developed and tested that address not only amyloid deposition but also NFT formation and neuronal loss, features of AD that previous transgenic mice have failed to recapitulate.

Note added in proof: Götz et al. (47) injected Aβ42 fibrils into the brains of P301L mutant tau transgenic mice and noted a factor of 3 increase in the numbers of NFTs in the amygdala from where neurons project to the injection sites. These data are consistent with our own observations in TAPP mice and further support the hypothesis that there is an interaction between the Aβ and tau pathologies in AD.

References and Notes

Fig. 2. Enhanced limbic neurofibrillary pathology in TAPP mice. Gallyas silver-stained preparations show enhanced neurofibrillary degeneration in the amygdala and adjacent entorhinal cortex of female TAPP mice (A and C) relative to age-matched JNPL3 mice (B and D); boxes in (A) and (B) correspond to regions in (C) and (D), respectively. Note the pyknosis of non-tangle-bearing neurons in (C). Immunostaining for GFAP also reveals gliosis in female TAPP mice (E) that is minimal in female JNPL3 mice (F). Sections are from 9-month-old mice. Magnifications: (A) and (B), 40×; (C) and (D), 400×; (E) and (F), 200×.
Double mutant mice were generated by crossing Neurosci. News mice NFTs were largely restricted to cerebral areas in female TAPP but not JNPL3 mice (A). By 6 to 7 months of age, TAPP mice had consistent NFTs in limbic areas, but in JNPL3 mice NFTs were largely restricted to spinal cord and pons (B); however, there were no statistically significant differences at this age range. There were statistically significant increases ($P < 0.012$ to $P < 0.0009$) in NFTs in the amygdala, anterior and posterior entorhinal cortex, olfactory cortex, subiculum, and dorsal hippocampus CA1 in the oldest female TAPP mice relative to age-matched female JNPL3 mice (C); the oldest female TAPP mice had significantly more NFTs than the oldest male TAPP mice (ranging from $P < 0.012$ to $P < 0.0005$) in all regions except the spinal cord and pons. (D to F) Sarkosyl-insoluble tau was immunoblotted with a human-specific tau antibody, E1. JNPL3 and TAPP mice had sarkosyl-insoluble tau, including a 64-kD hyperphosphorylated species, as early as 3 months of age (D). At all ages, female JNPL3 and TAPP mice had more insoluble tau than did male mice. In the younger age groups [(D) and (E)], a greater amount of insoluble tau was extracted from brain fraction 2 (basal ganglia, diencephalon, brainstem, and cerebellum) than from fraction 1 (cortex, amygdala, and hippocampus). In contrast, 9.5- to 11-month-old female TAPP mice had more sarkosyl-insoluble tau in fraction 1 than did female JNPL3 mice, consistent with the enhanced neurofibrillary pathology in these areas (F). Abbreviations: SC, spinal cord; Amyg, amygdala; ERCA, anterior entorhinal cortex; ERCp, posterior entorhinal cortex; Olf, olfactory cortex; Subic, subiculum; CA1, dorsal hippocampus CA1; F, female; M, male; Tau, JNPL3.
Formation of Neurofibrillary Tangles in P301L Tau Transgenic Mice Induced by Aβ42 Fibrils

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β-Amyloid plaques and neurofibrillary tangles (NFTs) are the defining neuropathological hallmarks of Alzheimer’s disease, but their pathophysiological relation is unclear. Injection of β-amyloid Aβ42 fibrils into the brains of P301L mutant tau transgenic mice caused fivefold increases in the numbers of NFTs in cell bodies within the amygdala from where neurons project to the injection sites. Gallyas silver impregnation identified NFTs that contained tau phosphorylated at serine 212/threonine 214 and serine 422. NFTs were composed of twisted filaments and occurred in 6-month-old mice as early as 18 days after Aβ42 injections. Our data support the hypothesis that Aβ42 fibrils can accelerate NFT formation in vivo.

Transgenic mice that express P301L mutant human tau form abnormally tau-containing filaments in brain (1, 2). These filaments have striking similarities with the NFTs of several non-Alzheimer’s disease tauopathies, including Alzheimer’s disease (AD) and frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17), but their numbers are considerably lower than these commonly found in human disease (3). To determine whether β-amyloid can accelerate NFT formation, we injected synthetic Aβ42 fibrils into the somatosensory cortex and the hippocampus of 5- to 6-month-old P301L tau transgenic mice (4) and nontransgenic littermates (5–7). For the control peptide, we used the reversed sequence, Aβ42-1–41, derived from the identical source (6). Aβ42 fibrils were generated by incubation at 37°C with shaking and were confirmed by electron microscopy (Fig. 1, A and B) (5, 6). Aβ42 fibrils were stable in vivo in both P301L transgenic and wild-type control mice and were readily detectable at least until 45 days after the injections (Fig. 1C). As expected, brain amyloid deposits were accompanied by reactive astrogliosis at both the injection sites (Fig. 1D) and the amygdala (Fig. 1E) (8); these were seen in both Aβ42- and control-injected transgenic mice and persisted for at least 45 days after injection. This reaction may be related to the fact that neurons in the amygdala project to the injection sites, as shown by retrograde transport of Texas red–conjugated dextran from the injection site in the somatosensory cortex to cell bodies in the amygdala (Fig. 1F) (8).

Eighteen days after the injections of Aβ42, Gallyas silver impregnation (9) re-

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42. For analysis of sarkosyl-insoluble tau, brain tissue (sectioned into cortex- limbic and subcortical-basal ganglia-cerebellar fractions) was homogenized in tris-buffered saline (TBS); a small sample was removed for the analysis of total tau, and the remainder was centrifuged at 100,000g for 1 hour at 4°C. The pellet was homogenized in 0.8 M NaCl and 10% sucrose in TBS. After centrifugation at 150,000g for 15 min, the supernatant was brought to 1% sarkosyl and incubated at 37°C for 1 hour. The mixture was then centrifuged at 150,000g for 30 min and the precipitate was collected as the sarkosyl-insoluble fraction. Equal values (μg) of insoluble tau pre-

30. For supplemental data, see Science Online (www. sciencemag.org/cgi/content/full/293/5534/1487/ DC1).


28. Studies of the JNPL3 mice have indicated that there are sex differences in the development of NFT patho-

27. For itation in the central nervous system relative to males (in situ hybridization, Northern, and Western analysis) and develop tau pathology and motor disturbance at an early age (24). Similar levels of tau expression in female mice have also been observed in a second transgenic mouse line expressing the longest 4R tau isoform with the P301L mutation.


22. For Aβ1-42 measurements, one hemisphere was dounce-


19. For total soluble tau Western blots, mouse brains were harvested and snap-frozen; immunoblotting was performed by dilution in buffer EC (0.02 M NaH 2PO 4, 0.002 M EDTA, 0.4 M NaCl, 0.2% BSA, 0.05% CHAPS, 0.04% BlockAce, 0.05% NaPO 4, pH 7.0). Aβ1 values were determined by sandwich enzyme-linked immuno-

18. Studies of the JNPL3 mice have indicated that there are sex differences in the development of NFT patho-

17. For Aβ1-42 measurements, one hemisphere was dounce-


14. For total soluble tau Western blots, mouse brains were harvested and snap-frozen; immunoblotting was performed by homogenizing half of a mouse brain in buffer containing protease inhibitor (1 μM phenylmethyisulfonyl fluoride, 20 μM aprotinin, 10 μM leupeptin, and 1 mM EGTA) and phosphatase inhibitor (5 mM sodium pyrophosphate, 30 mM β-glycerophosphate, and 30 μM sodium fluoride). Homogenates were dissolved in sample buffer and run on a 10% SDS–polyacrylamide gel electrophore-

13. For Northern analysis, total RNA was extracted from crushed whole mouse brains using Trizol (Life Technolo-

12. RNA (15 μg) was electrophoresed on a denatur-

11. RNA (15 μg) was electrophoresed on a denatur-

10. RNA (15 μg) was electrophoresed on a denatur-

9. RNA (15 μg) was electrophoresed on a denatur-


4. For analysis of sarkosyl-insoluble tau, brain tissue (sectioned into cortex- limbic and subcortical-basal ganglia-cerebellar fractions) was homogenized in tris-buffered saline (TBS); a small sample was removed for the analysis of total tau, and the remain-


2. Studies of the JNPL3 mice have indicated that there are sex differences in the development of NFT patho-


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