Tauopathy With Paired Helical Filaments in an Aged Chimpanzee

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ABSTRACT

An enigmatic feature of age-related neurodegenerative diseases is that they seldom, if ever, are fully manifested in nonhuman species under natural conditions. The neurodegenerative tauopathies are typified by the intracellular aggregation of hyperphosphorylated microtubule-associated protein tau (MAPT) and the dysfunction and death of affected neurons. We document the first case of tauopathy with paired helical filaments in an aged chimpanzee (Pan troglodytes). Pathologic forms of tau in neuronal somata, neuropil threads, and plaque-like clusters of neurites were histologically identified throughout the neocortex and, to a lesser degree, in allocortical and subcortical structures. Ultrastructurally, the neurofibrillary tangles consisted of tau-immunoreactive paired helical filaments with a diameter and helical periodicity indistinguishable from those seen in Alzheimer's disease. A moderate degree of Aβ deposition was present in the cerebral vasculature and, less frequently, in senile plaques. Sequencing of the exons and flanking intronic regions in the genomic MAPT locus disclosed no mutations that are associated with the known human hereditary tauopathies, nor any polymorphisms of obvious functional significance. Although the lesion profile in this chimpanzee differed somewhat from that in Alzheimer's disease, the copresence of paired helical filaments and Aβ-amyloidosis indicates that the molecular mechanisms for the pathogenesis of the two canonical Alzheimer lesions—neurofibrillary tangles and senile plaques—are present in aged chimpanzees. J. Comp. Neurol. 509:259–270, 2008. © 2008 Wiley-Liss, Inc.

Indexing terms: Aβ; aging; amyloid; Alzheimer’s disease; cerebral amyloid angiopathy; neurodegeneration; neurofibrillary tangles; Pan troglodytes; proteopathy; senile plaques; tau

The tauopathies are a class of human neurodegenerative disorders characterized by the intracellular aggregation of abnormally phosphorylated tau protein and selective neuronal loss (Lee et al., 2001). Tau dysfunction likely plays a primary role in disease pathogenesis, in that mutations in the microtubule-associated protein tau (MAPT) locus are associated with several of these tauopathies (Goedert and Jakes, 2005). In the mammalian nervous system, tau normally interacts with tubulin to stabilize microtubules and promote their assembly (Mandelkow et al., 2007). In the neurodegenerative tauopathies, tau is hyperphosphorylated by intracellular kinases, which impede the normal binding of the protein to cytoskeletal elements and thereby augments its tendency to polymerize into higher molecular weight assemblies such as neurofibrillary tangles (Ballatore et al., 2007; Mandelkow et al., 2007). More than 20 human tauopathies are known, each with a disease-specific clinical presentation and pat-
tern of atypical tau aggregation in neurons and/or glial cells (Goedert, 2004; Lee et al., 2001; Williams, 2006).

The most frequently occurring tauopathy is Alzheimer’s disease (AD), a dementing disorder in which specific regions of the brain are beset by neurofibrillary tangles as well as deposits of the Aβ peptide in senile plaques and in the walls of cerebral blood vessels (Duyckaerts et al., 1998; Hardy and Orr, 2006). The neurofibrillary tangles in AD consist mostly of paired helical filaments, twisting ribbons of tau that have a helical half-periodicity of ~80 nm and an apparent diameter that alternates between ~8 and 20 nm (Crowther, 1991). Although genetic and biochemical evidence indicates that the pathogenic cascade of AD is initiated by aberrant Aβ, particularly the 42-amino-acid form (Aβ42; Hardy and Selkoe, 2002), the degree of tau pathology correlates strongly with cognitive decline (Arriagada et al., 1992; Crystal et al., 1988; Giannakopoulos et al., 2007; Wilcock and Esiri, 1982).

Humans are particularly, and perhaps uniquely, susceptible to AD and other tauopathies (Nelson et al., 1996; Walker and Cork, 1999). However, amino acid sequence similarities in extant species indicate that both Aβ-precursor protein (APP) and tau protein are highly conserved evolutionarily. The amino acid sequence of APP695 is >100% identical in humans and chimpanzees (NCBI Q5IS80), and the tau sequence is 100% identical in the two species (Holzer et al., 2004). Additionally, all six tau isoforms that are found in the human brain also have been identified in nonhuman primates (Holzer et al., 2004; Nelson et al., 1996).

In the normal course of senescence, many mammalian species exhibit cerebral Aβ-amyloidosis (Cork et al., 1988; Elfenbein et al., 2007; Erwin et al., 2000; Gearing et al., 1996, 1997; Geula et al., 2002; Hartig et al., 2000; Kimura et al., 2003; Mestre-Frances et al., 2000; Nakamura et al., 1996, 1997; Poduri et al., 1994; Roertgen et al., 1996; Walker and Cork, 1999; Walker et al., 1990), and Aβ accumulation in the brains of aged nonhuman primates can sometimes reach levels comparable to those in AD (Elfenbein et al., 2007; R.F. Rosen, unpublished data). Aggregated tau has been histologically identified in glia and neurons of a number of aged mammals (Braak and Braak, 1994; Cork et al., 1988; Hartig et al., 1997; Kiattipattanasakul et al., 2000; Nelson et al., 1993, 1994; Nelson and Saper, 1995; Roertgen et al., 1996; Schultz et al., 2001), but no documented case of nonhuman tau pathology fully recapitulates the degree and localization of intraneuronal tau accumulation seen in the human tauopathies. Furthermore, neuronal tau pathology with AD-like paired helical filaments has never been identified in a nonhuman primate (Erwin et al., 2000; Walker and Cork, 1999). Here we present the first evidence of tauopathy with human-like paired helical filaments in an aged chimpanzee.

**MATERIALS AND METHODS**

**Subjects**

A 41-year-old, socially housed female chimpanzee (*Pan troglodytes; CO494*) at the Yerkes National Primate Research Center spontaneously developed acute lethargy and rapidly progressive motor dysfunction suggestive of stroke. A T2-weighted magnetic resonance (MR) scan revealed a massive, left-hemispheric lesion involving mainly the temporal, parietal, and occipital lobes (Fig. 1). At necropsy, gross examination of the brain confirmed a substantial region of ischemic (nonhemorrhagic) necrosis in the left hemisphere. The right hemisphere was grossly normal. The total brain weight was 287.5 g, less than the average weight (354.8 g) of an adult female chimpanzee brain (Herndon et al., 1999), possibly because of necrosis in the left hemisphere. The previous medical history was unremarkable except for a chronic systolic heart murmur.
first diagnosed at 15 years of age, moderate obesity (weight at death 61.5 kg), and high serum cholesterol (total cholesterol levels in 1995, 2000, and 2005 were 262, 244, and 359 mg/dl, respectively). A T1-weighted MR scan performed in 1995 showed no obvious abnormalities of the brain (Fig. 1).

For comparison, age-related Aβ and tau lesions were examined in available archival neocortical and/or hippocampal tissue samples from eight additional chimpanzees aged 30–56 years (Table 1). Furthermore, as a reference group for the MAPT genotyping, frozen cerebellum from seven chimpanzees were selected from the Yerkes archives for tau gene sequence analysis. All studies were conducted in accordance with federal and local guidelines for the humane care and use of animals.

Collection and preparation of cerebral tissue samples

Fresh, unfixed tissue blocks (~500 mg) were excised from the right (noninfarcted) superior temporal cortex for analysis by enzyme-linked immunosorbance assay (ELISA). (Unfixed tissue samples for ELISA also were taken from the superior temporal cortex of five end-stage AD cases for comparison; Table 2). The brain then was coronally slabbed at AD cases for comparison; Table 2). The brain then was taken from the superior temporal cortex of five end-stage (ELISA). (Unfixed tissue samples for ELISA also were analysis by enzyme-linked immunosorbance assay from the right (noninfarcted) superior temporal cortex for for the humane care and use of animals. conducted in accordance with federal and local guidelines for tau gene sequence analysis. All studies were conducted in accordance with federal and local guidelines for the humane care and use of animals.)

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Antibodies and reagents

The following antibodies were used for immunohistochemistry. AT8 (1:5,000) mouse IgG1 monoclonal antibody from Pierce Biotechnology (Rockford, IL; catalog No. MN1020), in PBS, raised against partially purified human PHF-tau, has an epitope in the region around phospho-serine 202/phosphothreonine 205 and does not cross-react with normal tau (Goedert et al., 1995). CP13 (1:10,000) mouse IgG1 monoclonal antibody, a generous gift from Dr. Peter Davies (Albert Einstein College of Medicine, Bronx, NY), was produced from mice immunized with PHF-tau purified from AD brain tissue by the method described in detail by Jicha et al. (1999). The antibody was selected for reactivity with the phosphopeptide GYSSPG(phosphoS)-PGPGSR, where the phosphoS is phosphoserine 202 of tau. No reactivity with any other phosphoserine of tau was detected (Jicha et al., 1999). PHF1 (1:10,000) mouse IgG1 monoclonal antibody, also from Dr. Davies, was raised against detergent-extracted PHF preparations and has epitope mapped to the region around phosphoserine 396/404 (Greenberg et al., 1992; Otvos et al., 1994). Specificity was confirmed with Western blots of transfected cell lines (Otvos et al., 1994). MC1 (1:10,000) mouse IgG1 monoclonal, also from Dr. Davies, was raised against Alz50-immunopurified PHFs and then epitope mapped to conformation-specific regions similar to those for Alz50, but not FAC1 (Jicha et al., 1997; in the same study, Western blot and dot blot showed specificity of MC1 to PHFs of tau protein). 6E10 (1:5,000) mouse IgG1 monoclonal antibody from Covance (Princeton, NJ; catalog No. SIG-39320), protein G-purified, in PBS, was raised against residues 1–16 of Aβ peptide, with an epitope at residues 3–8 (Kim et al., 1988). 4G8 (1:5,000) mouse IgG2b monoclonal antibody from Covance (catalog No. SIG-39220), protein G-purified, in PBS, was raised against residues 17–24 of Aβ peptide, with an epitope at residues 18–22 (Kim et al., 1988). The specificity of both 6E10 and 4G8 antibodies was characterized in the source reference. Rabbit polyclonal antibodies R361 and R398 (both at 1:15,000), a generous gift from Dr. Pankaj Mehta (Institute for Basic Research on Developmental Disabilities, Staten Island, NY), were raised against synthetic Aβ32–40 and Aβ33–42 (AnaSpec, San Jose, CA), respectively, conjugated to keyhole limpet hemocyanin in PBS. Specificity of these antibodies was examined by sandwich ELISA and Western blot analysis (Potempa et al., 1999). All antibodies to phosphorylated tau and Aβ were tested immunohistochemically with human AD tissue sections and specifically stained only abnormal tau accumulations and parenchymal and vascular amyloid deposits, respectively. Antiubiquitin (1:10,000) rabbit polyclonal antibody from Dako (Carpinteria, CA; catalog No. Z0458) was raised against ubiquitin isolated from cow erythrocytes and conjugated to chicken gamma globulins with glutaraldehyde, purified by solid-phase absorption with human plasma proteins (in Western blotting, the antibody labels bands corresponding to free ubiquitin and ubiquitin conjugates). Anti-GFAP (1:5,000) purified immunoglobulin fraction of rabbit antiserum from Dako (catalog No. Z0334) was raised against glial fibrillary acidic protein isolated from cow spinal cord and purified by solid-phase
absorption with human and cow serum proteins (shows no cross-reactivity with human plasma or cow serum by crossed immunoelectrophoresis and indirect ELISA). Immunohistochemistry with the antibody revealed the expected pattern of astrocytic staining in human AD and chimpanzee tissue sections. Anti-Iba1 (1:10,000) rabbit polyclonal antibody from Wako (Osaka, Japan; catalog No. 019-19741), in TBS, purified by affinity antigen chromatography from rabbit antiserum, was raised against a synthetic peptide (PTGPPAKKAISELP) corresponding to the C-terminus of Iba1, a 17-kDa EF hand protein that is specifically expressed in macrophages/microglia and is up-regulated during the activation of these cells. Specificity was confirmed by Western blot (Imai et al., 1996) and immunohistochemistry with the antibody revealed the expected pattern of microglial staining in human AD and chimpanzee tissue sections. Anti-α-synuclein (1:300) rabbit polyclonal antibody, a gift of Dr. Bernardino Ghetti (Indiana University, Indianapolis), was raised against a peptide containing residues 119–137 (DPDNEYEMPSEGQODYE) of the C-terminus of α-synuclein (Piccardo et al., 1998). Specificity was confirmed by specific immunolabeling of Lewy bodies in Parkinson’s disease. Vectastain Elite kits (Vector Laboratories, Burlingame, CA) were used for ABC-based immunodetection of antigen-antibody complexes.

**Histochemistry**

Endogenous peroxidase in tissue sections was inactivated with 3% H2O2 in methanol for 10 minutes at room temperature. Nonspecific reagent binding was blocked with 2% normal serum in 0.2% Tween-20 for 1 hour at room temperature. For Aβ immunodetection, sections were pretreated for 10 minutes in 90% formic acid to expose antigenic sites. Sections were incubated in primary antibody (diluted in buffer with blocking serum) overnight at 4°C. After rinsing, sections were incubated for 1 hour at room temperature in biotinylated secondary antibody, rinsed, immersed for 30 minutes in avidin-biotin complex, and then developed with diaminobenzidine (DAB) or DAB-nickel (black), and the subsequent antibody was marked with DAB only (brown). Finally, we stained selected sections with the Congo red, Bielschowsky, and Campbell-Gallyas stains for AD-type lesions, with the Prussian blue (Perls) stain for iron, with hematoxylin and eosin, and with a gram stain for bacteria (there was no evidence of bacterial infection in any brain region). Light-microscopic photomicrographs were taken with a Leica DMLB microscope (Leica, Wetzlar, Germany) and Spot XPlorer and Flex digital cameras (Diagnosti c Instruments, Sterling Heights, MI). Confocal images were captured with a Zeiss LSM 510 laser scanning confocal microscope. All images were edited in Photoshop (Adobe) without any further manipulations.

**Quantitative mapping of tau and Aβ lesions**

Tau lesions (intranasometric neurofibrillary tangles and plaque-like clusters of immunoreactive neurites) and Aβ lesions (cerebral Aβ-amyloid angiopathy (CAA) and parenchymal (senile) Aβ plaques) were mapped and quantitated bilaterally in matched CP13-, R361-, and R398-immunostained sections from the prefrontal, temporal, and occipital cortices with the Neurolucida image analysis system (MBF Biosciences, Williston, VT). The prefrontal cortical sections were taken at the level of the rostral end of the middle frontal gyrus (Bailey and Bonin FE/FD) (Brodmann area 10/9/46) (Bailey et al., 1950; Brodmann, 1909); temporal cortical sections taken at the level anterior to the primary auditory cortex (11) (TA/TE, Brodmann area 20/21/22) and the occipital cortical sections were from a level between the lunate sulcus and the occipital pole, (reversed parenthetic phrase): containing both area V1 and extrastriate cortex (Brodmann area 18/17; QB/OC).

A single researcher used the Neurolucida system to count every tau or Aβ lesion found in each section. Every discrete cell soma or cluster of tau-immunoreactive elements was counted as a single lesion. Two different types of tau plaques were identified and counted: neuritic and punctate. Similarly, each distinct Aβ plaque or Aβ-reactive vascular profile was indicated on the tissue map. R361- or R398-positive vessels that were spatially continuous within the section were counted as a single lesion; otherwise, discrete vascular profiles were counted separately. The numeric densities of immunoreactive lesions were calculated from the total planar area of each section (Ellenbein et al., 2007). Lesion densities in the two hemispheres were compared statistically using paired t-tests, with a set limit of P < 0.05 for significance. Neuropil threads (tau-positive processes that were not organized into plaques) were profuse and widely distributed in brain (see Fig. 2) and were not quantitated histologically.

**Electron microscopy**

For ultrastructural analysis, tissue samples from the left prefrontal cortex were postfixed in 4% paraformaldehyde and 0.5% glutaraldehyde, washed in phosphate buffer (0.1 M, pH 7.4), and immersed in osmium tetroxide (1% in phosphate buffer) for 20 minutes. They were then rinsed in phosphate buffer and dehydrated in a graded
series of ethanol and propylene oxide. Uranyl acetate (1%) was added to the 70% ethanol (35-minute immersion) to improve contrast in the electron microscope. Numerous tau-immunoreactive processes (threads) occupy the intervening neuropil. Antibody AT8. B: Tau-immunoreactive neuron (arrowhead) in area CA1 of the right hippocampus; note the integrity of the nearby pyramidal cells. Antibody AT8, hematoxylin counterstain. C: Double-immunostained section showing tau (CP13/nickel-DAB, black, developed first) and Aβ (R398/DAB, brown) immunoreactivity in the left prefrontal cortex. A tau plaque (black arrow) and a tau-positive pyramidal neuron (arrowhead) are present. A focus of Aβ-immunoreactive CAA is indicated by the gray arrow. Note the absence of an Aβ-core in the tau plaques, which was typical of focal accumulations of tau neurites in this animal. D: Cerebral amyloid angiopathy (arrowheads denote two blood vessels) and a senile plaque (arrow) in the right temporal neocortex. Antibody 6E10. Scale bars = 100 μm in A,C; 50 μm in B; 200 μm in D.

Fig. 2. Tau and Aβ pathology in the aged chimpanzee. A: Tau-immunoreactive somata (two indicated by arrowheads) and neuritic tau plaques (one indicated by arrow) in the left prefrontal cortex. For immunogold EM, nonpostfixed sections were preincubated in PBS containing 5% nonfat dry milk and then washed in Tris-buffered saline (TBS)-gelatin buffer (0.02 M Tris, 0.15 M NaCl, 1 μl/ml fish gelatin, pH 7.6) to block nonspecific sites. Sections were then incubated for 48 hours at 4°C in CP13 antibody (1:10,000) diluted in PBS-BSA, rinsed in TBS-gelatin, and incubated for 2 hours at room temperature in gold-conjugated goat anti-mouse Fab’ fragments (dilution 1:100; Nanogold; Nanoprobes Inc., Yaphank, NY). Gold particles (1.4 nm) were silver enhanced with the HQ Silver kit (Nanoprobes). As a control for the specificity of immunolabeling, omission of the primary antibody from incubation solutions completely abolished immunostaining for the corresponding antigens. The tissue was then embedded and cut as described above. All thin sections were examined with a Zeiss EM10-C
electron microscope (Zeiss, Oberkochen, Germany), and digital images were captured in the Dual View camera (Gatan Inc., Pleasanton, CA).

**Aβ ELISA**

Unfixed, right temporal cortical tissue was Dounce homogenized in 5 volumes of homogenization buffer (50 mM Tris-HCl, 150 mM NaCl, and protease inhibitor tablets; Santa Cruz Biochemicals, Santa Cruz, CA) and then centrifuged at 100,000 g for 60 minutes at 4°C to generate the “soluble” supernatant. The pellet was probe sonicated in 70% formic acid and centrifuged at 14,000 rpm for 60 minutes at 4°C to generate the “insoluble” supernatant. Aβ40 and Aβ42 were measured in each extract by ELISA (The Genetics Company, Schlieren, Switzerland) according to the manufacturer’s instructions. Soluble extracts were diluted in sample buffer at a 1:50 dilution and insoluble extracts were neutralized in 1.0 M Tris base, pH 11 (1:20 dilution), and diluted in sample buffer to a 1:1,000 total dilution. All samples were run in duplicate. Plates were read at 450 nm, and the average optical density values for each extract were interpolated on a four-parameter standard curve to determine Aβ concentrations.

**DNA extraction and MAPT sequence analysis**

Genomic DNA was extracted from unfixed liver of the 41-year-old subject (and, as a reference group, from unfixed cerebellar tissue from seven additional chimpanzees). MAPT exons and flanking intronic regions were amplified (primers available on request), and amplicons were purified using large-scale, automated template purification systems employing solid-phase reversible immobilization (Agencourt Bioscience Corporation, Beverly, MA). The purified DNA samples were then sequenced by using ABI dye-terminator chemistry. All subsequent steps were based on sequencing by automated DNA sequencing methods. The ABI dye terminator sequence reads were run on ABI 3700/3730 (Applied Biosystems, Foster City, CA) machines, and the data were transferred to Linux machines. Base calls and quality scores were determined with the program PHRED (Ewing and Green, 1998; Ewing et al., 1998).

**RESULTS**

**Tau histopathology**

Post-mortem immunohistochemical analysis using antibodies to pathologic forms of tau revealed abnormal intracellular tau immunoreactivity in multiple cortical

### Table 3. Quantitative Mapping Data of Intrasmotic Neurofibrillary Tangles and Tau Plaque-Like Clusters of Immunoreactive Neurites in Location-Matched Tissue Blocks from the Bilateral Prefrontal, Temporal, and Occipital Cortices of Chimpanzee CO494

<table>
<thead>
<tr>
<th>Region</th>
<th>No. N-plaque</th>
<th>No. P-plaque</th>
<th>No. cell bodies</th>
<th>N-plaque density (No./mm²)</th>
<th>P-plaque density (No./mm²)</th>
<th>Cell body density (No./mm²)</th>
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<td>0.6132</td>
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<td>LTC</td>
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<td>25</td>
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<td>0.1436</td>
<td>0.1795</td>
</tr>
</tbody>
</table>

1Lesion densities are expressed as number of lesions per unit area of tissue analyzed. Antibody CP13. L, left; R, right; FC, prefrontal cortex; OC, occipital cortex; TC, temporal cortex; N-plaque, tau neuritic plaque; P-plaque: tau punctate plaque.

**Fig. 3.** Silver-stained lesions in the left prefrontal neocortex of the aged chimpanzee. **A:** Campbell-Gallyas-stained neuritic plaque (tortuous black neurites, arrow). This plaque was positive with the anti-tau antibody CP-13 in an adjacent section (not shown). **B:** Two cortical cells stained with the Bielschowsky silver method. Scale bar = 50 μm.
regions of both hemispheres and, to a lesser extent, in subcortical structures. Throughout the neocortex (the most severely affected brain structure), the lesions included tau-laden neurons, neuropil threads, and diverse plaque-like clusters of neurites (“tau plaques”) (Fig. 2A–C). The regional density of tau lesions varied among and within cortical areas, and there were foci of particularly intense pathology (Fig. 2A). The lesions were most abundant in the prefrontal cortical samples, followed by the temporal cortex and the occipital cortex, which had the least tauopathy of the neocortical regions examined (Table 3). In the hippocampus, occasional tau-positive neurons (Fig. 2B), neuropil threads, and tau plaques were present, but these were much less numerous than in most regions of neocortex. The tau plaques generally were not associated with a core of aggregated Aβ (Fig. 2C). Subcortically, tau immunoreactivity, mostly in thread-like processes, was present to varying degrees in the globus pallidus, neostriatum, and diencephalon and occasionally in white matter pathways. Scattered immunoreactive profiles also were present in the lower brainstem and, very infrequently, in the cerebellum. Overall, subcortical tauopathy was sparse relative to that in neocortex.

Ultrastructurally, the neurofibrillary tangles consisted of dense, intraneuronal bundles of paired helical filaments that were identical in size and helical periodicity to those in humans with AD (Crowther, 1991; Metuzals, 1986; Figs. 5, 6B). Immunogold labeling with antibody CP13 confirmed the presence of phospho-tau epitopes on the paired helical filaments (Fig. 5B,C).

**Aβ histopathology**

Immunohistochemistry with antibodies to Aβ disclosed a moderate degree of CAA (Fig. 2C,D) in all of the neocortical regions examined, as well as mild, focal Aβ-plaque pathology (Fig. 2D, Table 4). As in humans, Aβ deposits were sparse in the hippocampus compared with temporal neocortex and were essentially absent in the basal ganglia, diencephalon, and lower brainstem. The cerebellum manifested very mild CAA. In affected regions, most Aβ-immunopositive senile plaques were diffuse in nature, as confirmed by a paucity of Congo red staining/birefringence in parenchymal deposits. The number of Aβ42-positive plaques did not differ significantly from the number of Aβ40-positive plaques, but CAA was marginally more likely to be immu-

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**Fig. 4.** Double-fluorescence immunostaining of tau neurites and reactive glia in the left prefrontal cortex of the aged chimpanzee. By confocal microscopy, CP13-positive neurites (green) in tau plaques do not colocalize with GFAP-stained astrocytes (magenta; A), nor do they colocalize with Iba1-immunoreactive microglia (magenta; B). Neuropil threads also are negative for these glial markers. Scale bars = 20 μm.
noreactive for Aβ42 than for Aβ40 (t = 2.560; P = 0.0506). Surprisingly, the degree of total CAA was significantly greater in the right, nonstroke hemisphere than in the left hemisphere (t = 4.668; P = 0.043; Table 4).

**MAPT sequence analysis**

Sequence analysis of genomic DNA revealed no mutations of functional significance at the MAPT locus (including saitohin). Two synonymous base pair changes in exons 7 and 9 were identified, whereas all other polymorphisms were located in noncoding regions at distances from the nearest splicing sites ranging from 40 to 145 base pairs. One unusual, 4-base-pair deletion was identified in intron 3 only in this chimpanzee, but this deletion was deemed unlikely to be of pathogenic significance because it lies 67 base pairs from the nearest predicted splicing site.

**DISCUSSION**

This is the first report of cerebral tauopathy with paired helical filaments in an aged chimpanzee. The tau lesions included neurofibrillary tangles, neuropil threads, and neuritic tau plaques; ultrastructurally, the neurofibrillary tangles consisted of paired helical filaments that were indistinguishable from those occurring in humans with AD (Figs. 2, 5, 6; Crowther, 1991; Metuzals, 1986). This aged chimpanzee also exhibited a species-typical profile of cerebral β-amyloid angiopathy and infrequent Aβ-immunoreactive senile plaques (Gearing et al., 1994, 1996).

The impetus for human-like tau pathology in this chimpanzee is uncertain. Any influence on the expression or splicing of tau, whether environmental or genetic, could alter the probability of developing tauopathy (Hardy et al., 2006). Several mutations in the tau gene are associated with human primary tauopathies (Goedert and Jakes, 2005; Lee et al., 2001). However, sequencing of the exons and flanking intronic regions in the genomic MAPT locus of this subject disclosed no known tauopathy-associated mutations nor any other genetic changes of obvious functional significance. The two extended MAPT haplotypes that occur in human populations (H1 and H2) are thought to influence differentially the probability of developing neurofibrillary tangles (Hardy et al., 2006). The tau haplotype in chimpanzees, however, is a mixture of the human H1 and H2 haplotypes (Conrad et al., 2004; Holzer et al., 2004; Pittman et al., 2005), and at present it is unclear...
whether this gene structure affects the likelihood that chimpanzees will manifest tau lesions. While genetic or epigenetic changes might yet be discovered that regulate the pathogenicity of tau, our analysis shows that this animal did not harbor a sequence modification in the tau gene that would be expected to precipitate a human-like tauopathy. Old age is the most important risk factor for AD and other neurodegenerative diseases in humans (Kawas and Katzman, 1999), and age probably contributed to the emergence of tau pathology in this chimpanzee. The documented maximum life span of *Pan troglodytes* is 59 years (Herndon et al., 1999). Chimpanzees in their 40s and 50s have been shown previously to exhibit cerebral Aβ amyloidosis, primarily in the form of CAA (Cork and Walker, 1993; Erwin et al., 2000; Gearing et al., 1994, 1996; Walker and Cork, 1999), but significant intraneuronal tau pathology has not been documented previously in chimpanzees (Gearing et al., 1994, 1996; Walker and Cork, 1999). Although it is not unusual to encounter occasional tau-immunoreactive neurons and processes in older animals, immunohistochemical examination of archival, post-mortem tissue samples from eight additional chimpanzees ranging from 30 to 56 years of age revealed little abnormal tau immunoreactivity (R.F. Rosen, unpublished). Thus, although age probably played a role in the ontogeny of tauopathy in this 41-year-old animal, the paucity of tau lesions in other chimpanzees of similar or greater age suggests that additional factors are involved.

Tau pathology also could be related to conditions that engendered the ischemic stroke, which appears to be a rare occurrence in nonhuman primates (Borkowski et al., 2000; Fish et al., 2004). The chimpanzee in this study had high levels of cholesterol throughout her adult life, and hypercholesterolemia has been implicated as a risk factor both for stroke and tauopathy (Ohm and Meske, 2006). Furthermore, the ischemic lesion itself might have directly initiated AD-like tau pathology in this chimpanzee. In humans, epidemiological evidence suggests a relationship between cerebral ischemia and dementia, and nearly

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**TABLE 4. Quantitative Mapping Data of Aβ Parenchymal Plaques and CAA in Location-Matched Tissue Blocks From the Bilateral Prefrontal, Temporal, and Occipital Cortices of Chimpanzee CO494**

<table>
<thead>
<tr>
<th>Region</th>
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<th>Total Aβ42-CAA</th>
<th>Total Aβ40-plaques</th>
<th>Total Aβ42-plaques</th>
<th>Aβ40-CAA density (No./mm²)</th>
<th>Aβ42-CAA density (No./mm²)</th>
<th>Aβ40-plaque density (No./mm²)</th>
<th>Aβ42-plaque density (No./mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LFC</td>
<td>162</td>
<td>298</td>
<td>2</td>
<td>0</td>
<td>0.5512</td>
<td>1.0447</td>
<td>0.0068</td>
<td>0.0000</td>
</tr>
<tr>
<td>LOC</td>
<td>45</td>
<td>78</td>
<td>0</td>
<td>0</td>
<td>0.1723</td>
<td>0.2957</td>
<td>0.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td>LTC</td>
<td>67</td>
<td>112</td>
<td>12</td>
<td>10</td>
<td>0.2505</td>
<td>0.3329</td>
<td>0.0449</td>
<td>0.0297</td>
</tr>
<tr>
<td>RFC</td>
<td>358</td>
<td>666</td>
<td>57</td>
<td>26</td>
<td>1.2257</td>
<td>2.1810</td>
<td>0.1947</td>
<td>0.0851</td>
</tr>
<tr>
<td>ROC</td>
<td>289</td>
<td>966</td>
<td>3</td>
<td>0</td>
<td>0.9947</td>
<td>3.1774</td>
<td>0.0103</td>
<td>0.0000</td>
</tr>
<tr>
<td>RTC</td>
<td>118</td>
<td>284</td>
<td>43</td>
<td>50</td>
<td>0.8592</td>
<td>2.0315</td>
<td>0.3131</td>
<td>0.3577</td>
</tr>
</tbody>
</table>

Lesion densities are expressed as number of lesions per unit area of tissue analyzed. Antibodies R361 and R398 to Aβ40 and Aβ42, respectively. L, left; R, right; FC, prefrontal cortex; OC, occipital cortex; TC, temporal cortex.
neuronal threads, and neuritic tau plaques in an aged chimpanzee. The subject also exhibited a moderate degree of cerebral Aβ deposition, mainly in the brain vasculature. Despite tantalizing similarities, there are also important pathologic differences between this chimpanzee and humans with AD, notably in the rarity of neurofibrillary tangles in the hippocampus, the presence of unusual tau-only neuritic plaques lacking β-amyloid cores, and the paucity of parenchymal Aβ (senile) plaques. However, the occurrence of both tau and Aβ pathology indicates that the cellular and molecular machinery for generating two key hallmarks of AD—neurofibrillary tangles and Aβ-amyloidosis—is fully present in aged chimpanzees. In addition to providing evidence for biological similarities between humans and chimpanzees even late in the life span, these findings compel us to reconsider the assumption that humans are the only primates to manifest Alzheimer-like tauopathy with age.

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LITERATURE CITED


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