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Aged chimpanzees exhibit pathologic hallmarks of Alzheimer's disease

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Abstract

Alzheimer's disease (AD) is a uniquely human brain disorder characterized by the accumulation of amyloid beta protein (A β) into extracellular plaques, neurofibrillary tangles (NFT) made from intracellular, abnormally phosphorylated tau, and selective neuronal loss. We analyzed a large group of aged chimpanzees (n = 20, ages 37–62 years) for evidence of A β and tau lesions in brain regions affected by AD in humans. A β was observed in plaques and blood vessels, and tau lesions were found in the form of pretangles, NFT, and tauimmunoreactive neuritic clusters. A β deposition was higher in vessels than in plaques and correlated with increases in tau lesions, suggesting that amyloid build-up in the brain's microvasculature precedes plaque formation in chimpanzees. Age was correlated to greater volumes of A β plaques and vessels. Tangle pathology was observed in individuals that exhibited plaques and moderate or severe cerebral amyloid

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angiopathy, a condition in which amyloid accumulates in the brain's vasculature. Amyloid and tau pathology in aged chimpanzees suggests these AD lesions are not specific to the human brain.

Keywords

Alzheimer's disease; amyloid beta protein; chimpanzee; neurofibrillary tangle; primate; tau

1. Introduction

Alzheimer's disease (AD) is a progressive, irreversible proteinopathy, resulting in cognitive impairment and characterized by pathological aggregations of the amyloid-beta (A β) and tau proteins in the form of plaques and neurofibrillary tangles (NFT) and selective neuronal loss (Montine et al., 2012). It has been suggested that humans are uniquely susceptible to AD, potentially due to genetic differences, changes in cerebral structure and function during evolution, and an increased lifespan (Hof et al., 2002; Rapoport and Nelson, 2011; Sherwood et al., 2011; Walker and Cork, 1999). While A β and tau pathology have been identified in nonhuman primates, these animals typically present with only amyloid plaque pathology (Head et al., 2001; Heuer et al., 2012; Hof et al., 2002; Oikawa et al., 2010; Walker and Cork, 1999). Additionally, aged primates demonstrate cognitive deficits in working memory, learning tasks, long-term retention, and cognitive flexibility similar to elderly humans (Joly et al., 2014; Lacreuse et al., 2014; Nagahara et al., 2010; Picq et al., 2015). Previous studies in aged monkeys and great apes confirmed the presence of diffuse and neuritic amyloid plaques as well as vascular amyloid (Gearing et al., 1997, 1996, 1994; Geula et al., 2002; Heuer et al., 2012; Kimura et al., 2003; Lemere et al., 2008, 2004; Martin et al., 1991; E J Mufson et al., 1994; Oikawa et al., 2010; Perez et al., 2016, 2013; Poduri et al., 1994; Rosen et al., 2008; Walker et al., 1987). The presence of abnormally phosphorylated tau has been reported in neurons and glia in rhesus monkeys (*Macaca mulatta*) and squirrel monkeys (*Saimiri sciureus*) as well as baboons (*Papio anubis*) (Elfenbein et al., 2007; Hartig et al., 2000; Oikawa et al., 2010; Schultz et al., 2000; Selkoe et al., 1987). In great apes, captive and wild gorillas (*Gorilla gorilla gorilla* and *Gorilla beringei beringei*) exhibited hyperphosphorylated tau-immunoreactive (ir) neurons in the neocortex in close association with A β plaques, but the perikarya lacked classic AD tau histopathologic abnormalities (Perez et al., 2016, 2013). Several pathological examinations of the chimpanzee (*Pan troglodytes*) and orangutan (*Pongo pygmaeus*) brain revealed diffuse A β plaques and vascular amyloid, but not tau-positive NFT and neuropil threads (Gearing et al., 1997, 1996, 1994). The only evidence of NFT and A β pathology coexisting in a primate species other than humans was reported in a 41-year-old female chimpanzee that suffered a stroke prior to death and had a history of high cholesterol (Rosen et al., 2008). This ape presented with scarce diffuse senile plaques and vascular amyloid, NFT, neuropil threads, and clusters of tau-positive neurites in the neocortex, which may have been the result of a rare ischemic event, as risk of dementia after stroke is increased two-fold in humans (Schneider and Bennett, 2010).

Chimpanzees share 100% sequence homology and all six tau isoforms with humans (Chimpanzee Sequencing and Analysis Consortium, 2005; Holzer et al., 2004; Nelson et al.,

2009). Moreover, amyloid precursor protein 695 (APP695) is more than 99% identical in chimpanzees and humans and can be cleaved into A β 40/42 peptides with 100% sequence homology to humans (Cervenáková et al., 1994; Götz and Ittner, 2008; Heuer et al., 2012). As one of our closest living primate relatives, chimpanzees demonstrate greater behavioral complexity and longer lifespans than non-primate AD animal models (Hakeem et al., 1996; Hill et al., 2001). In captivity, the average life span for chimpanzees ranges from 30 to 35 years with the oldest recorded age of 62 years old (Dyke et al., 1995). Therefore, chimpanzees are ideal candidates to investigate the pathobiology of AD from an evolutionary neurobiological perspective.

Brain samples from great apes, particularly aged individuals, are incredibly scarce. In the wild, chimpanzees have an average life expectancy of less than 15 years (maximum age noted was 53 years), and only 10% of captive male chimpanzees survive to age 45 and of captive females to age 55 (Dyke et al., 1995; Hill et al., 2001). Additionally, prior neuropathological studies in apes were limited to small samples sizes. The current study evaluated four brain regions affected by AD pathology in humans—prefrontal cortex (PFC), midtemporal gyrus (MTG), and CA1 and CA3 hippocampal subfields—in a large group of aged chimpanzees (Table 1) for A β -positive plaques, vascular amyloid, and tau lesions (i.e., pretangles, NFT, and neuritic clusters). We also developed an overall pathology scoring system based on clinical staging parameters for A β and NFT deposition in humans (see Pathology Identification and Scoring) (Attems et al., 2007; Braak et al., 2006; Braak and Braak, 1991; Mirra et al., 1991; Montine et al., 2012; Thal et al., 2002; Vonsattel et al., 1991). Here, we show that pretangles, NFT, and clusters of tau-ir dystrophic neurites (NC) co-occur with amyloid-positive plaques and vasculature in aged chimpanzees. These findings indicate that AD-like pathology is not limited to the human brain.

2. Materials and Methods

2.1 Specimens and Sample Processing

Postmortem brain specimens from 20 aged chimpanzees (Table 1) were acquired from Association of Zoos and Aquariums-accredited zoos or American Association for Accreditation of Laboratory Animal Care-accredited research institutions and maintained in accordance with each institution's animal care guidelines. Chimpanzee brain specimens were provided by the National Chimpanzee Brain Resource, and health information has been included when possible (Table S1). The chimpanzees in this study did not participate in formal behavioral or cognitive testing. Sample selection was balanced by sex for age as equally as possible. Samples from four brain regions, including prefrontal cortex (Brodmann areas 9 and 10), midtemporal gyrus (Brodmann area 21), and hippocampal subregions CA1 and CA3 (including subiculum and entorhinal cortex), were obtained from 8 male (ages 39–62) and 12 female (ages 37–58) chimpanzees.

Depending on availability samples were taken from the right or left hemispheres. Data examining interhemispheric distribution of AD and vascular pathology in humans found NFT and A β deposition was symmetrical even in early stages of neurodegeneration, indicating assessment of vascular and AD pathology in one hemisphere was sufficient (Giannakopoulos et al., 2009). Brains were collected postmortem (postmortem interval < 20

h) and immersion-fixed in 10% buffered formalin solution for at least 10 days. Specimens were transferred to a 0.1 M buffered saline solution containing 0.1% sodium azide at 4°C for storage prior to sectioning. Samples were cryoprotected in a graded series of 10, 20, and 30% sucrose solutions, and cut frozen into 40 µm-thick sections perpendicular to the main axis of the gyrus contained in each block using a Leica SM2000R freezing sliding microtome (Buffalo Grove, IL). Sections were placed into individual centrifuge tubes containing cryoprotection solution (30% dH₂O, 30% ethylene glycol, 30% glycerol, 10% 0.244 M phosphate buffered saline [PBS]), numbered sequentially, and stored at -20°C until histological or immunohistochemical processing. Every tenth section was stained for Nissl substance with a 0.5% cresyl violet solution to reveal cell somata and to define cytoarchitectural boundaries.

2.2 Identification and Regional Sampling

All regions were identified using Nissl-stained sections. Brain areas were selected based on Braak staging of NFT and Thal phases of Aβ deposition in humans with AD as well as prior studies in aged chimpanzees (Braak and Braak, 1991; Gearing et al., 1996, 1994; Montine et al., 2012; Rosen et al., 2008). Sampled regions included layer III in Brodmann's areas 9 and 10 of the dorsolateral PFC, layer III in Brodmann's area 21 of the MTG, and in stratum pyramidale in CA1 and CA3 subfields of the hippocampus. In AD, pyramidal neurons in layers III and V of the neocortex and stratum pyramidale in the hippocampus display extensive neuron and synapse loss, and the distribution of neuritic Aβ plaques and NFT is most prevalent in these cortical layers (Akram et al., 2008; Bussière et al., 2003; Hof et al., 1990; Scheff and Price, 2006).

2.3 Immunohistochemistry

Every twentieth section was immunohistochemically processed for tau, APP/Aβ, and Aβ42 (summary of antibodies and epitopes in Table S2) following previously established protocols utilizing the avidin-biotinperoxidase method and either 3,3'-diaminobenzidine (DAB) with nickel enhancement or Vector NovaRED (SK-4100/SK-4800, Vector Laboratories) (Raghanti et al., 2009, 2008). Additional sections were double immunostained for AT8/Aβ42 and AT8/GFAP to determine colocalization of pathologies and confirm tau NC contained astroglial inclusions. Neocortical tissue from the brain of a patient who died from AD was used as a positive control, and neocortical samples from young chimpanzees (ages 16–20) as well as omission of the primary antibody were used as negative controls. Immunoreactive profiles were not found in sections from young chimpanzees or in those without the primary antibody.

2.4 Thioflavin S

Sections were rinsed in a 0.1 M PBS and mounted on slides, air dried, placed in Citri-Solv for 10 min, and hydrated in a series of ethanol solutions (100/95/70/50%) followed by dH₂O (2 × 1 min) (Bussière et al., 2004; Guntern et al., 1992; Rajamohamedsait and Sigurdsson, 2012). Sections were incubated in a filtered 1% aqueous thioflavin S solution (Sigma, T1892) followed by dehydration in a graded series of ethanol solutions. Next, sections were placed in a filtered 1% Sudan Black B solution (Sigma, 19,966–4, dissolved in 70% ethanol) to eliminate autofluorescence and placed in Citri-Solv. Cover glass was applied with a

hydrophilic mounting media, and slides were stored at 4°C for 48 h before images were taken at 20x (N. A. 0.7) on an Olympus FV1000 Laser Scanning Confocal Microscope using Olympus FV10-ASW 3.0 Viewer. The excitation filter (DM 458/515) was selected based on available lasers and optimal excitation (~440–470 nm) and emission wavelengths for thioflavin S (~515–550 nm) (Schweers et al., 1995).

2.5 Data Acquisition

Quantitative analyses were performed using computer-assisted stereology with an Olympus BX-51 photomicroscope equipped with a digital camera and StereoInvestigator software version 11 (MBF Bioscience, Williston, VT) by a single observer. Initial subsampling techniques were performed for each probe to determine appropriate sampling parameters (Slomianka and West, 2005). Two-dimensional topographical mapping of APP/A β and A β 42 plaques and vessels (Fig. S1A) and AT8-ir profiles of pretangles, NFT, and NC (Fig. S1B) was completed in individuals with the most pathology (n = 7). Maps were used to determine regional progression of pathology for staging purposes (see Pathology Identification and Scoring). These spatial data indicated the highest number of pretangles, frank NFT, and NC were detected by AT8, and thus, quantification for each tau pathology type was performed using this antibody with this stain (Fig. S2).

AT8-positive pretangles, frank NFT, and NC densities were obtained using the optical disector probe at 40x (N.A. 0.75) under Köhler illumination. Grid size was set at 250 $\times$ 250 μ m with a disector height of 8 μ m and a guard zone of 2%. Beginning at a random starting point, three equidistant sections (every 10h or 20th section) per region of interest and individual were selected for analysis. Mounted section thickness was measured every fifth sampling site. A different marker for each pathology type was placed when encountered within the optical disector frame. Densities (per mm³) for each region were calculated as the population estimate of pretangle, NFT, and NC markers divided by planimetric volume of the disector (Sherwood et al., 2005). For each individual, densities for PFC and MTG were averaged to calculate neocortical (NC) density. The same process was executed for CA1 and CA3 to compute average hippocampal (HC) density. Densities for all four regions were averaged for total pretangle, NFT, and NC densities. To correct for tissue shrinkage in the z-axis, the height of the disector was multiplied by the ratio of section thickness to the actual weighted mean thickness after mounting and dehydration. No correction was necessary for the x and y dimensions because shrinkage in section surface area is minimal (Dorph-Petersen et al., 2001). The mean number of sampling sites for each area per individual was 93 $\pm$ 31. Penetration of antibodies was determined by examining each section through the z-axis.

Percentages (%) of volume occupied by APP/A β and A β 42-ir plaques and vessels were measured in all regions of interest using the area fraction fractionator (AFF) probe, founded on a Cavalieri point-counting system. Using a 10x objective (N.A. 0.25), markers were placed on a grid of points (300 $\times$ 300 μ m) overlaying the sampling area. Every point on the grid received one of three potential markers: non-A β , A β plaque, or A β vessel. Estimated area fractions were calculated by the AFF probe and reported as % for each region per individual. Percentage of volume occupied by APP/A β and A β 42-ir plaques for PFC and

MTG were summed to calculate neocortical percentage and the equivalent was performed for CA1 and CA3 percentages to determine hippocampal percentage. Percentages from all four regions were summed for total volume occupied by APP/A β and A β 42-ir plaques respectively. The same procedure was conducted for APP/A β and A β 42-ir vessel volumes. The mean number of sampling sites for each area per individual was 70 ± 15.2 . In addition, APP/A β and A β 42-ir vessel diameters were measured using the quick circle tool in StereoInvestigator during AFF data collection for every positive vessel within the grid. Diameters (μm) were averaged by region for every subject.

2.6 Pathology Identification and Scoring

The current study defined tau and A β pathology as previously outlined in Serrano-Pozo et al. (Serrano-Pozo et al., 2011). Amyloid plaques were defined as extracellular deposits of insoluble A β . Plaques were not distinguished between diffuse and dense-core, though both types were present and confirmed by thioflavin S staining. CAA was categorized by deposits of A β in the tunica media of leptomeningeal and cortical arteries and in small arterioles. Pretangles were defined as healthy-looking neurons with the presence of diffuse punctate tau staining in the cytoplasm, well-preserved dendrites, and a centered nucleus. NFT contained intraneuronal aggregates of hyperphosphorylated tau, and the nucleus was either displaced toward the periphery of the soma or absent. Dendrites and axons were distorted, shortened, or absent in tangles. Tau NC contained clusters of dystrophic neurites, consisting of AT8-ir swollen axons and dendrites, or diffuse, punctate staining.

To evaluate neuropathologic changes for each individual, a pathology score was computed utilizing a system adapted from staging guidelines for A β and NFT deposition in AD and CAA (Table 2) (Attems et al., 2007; Braak et al., 2006; Braak and Braak, 1991; Mirra et al., 1991; Montine et al., 2012; Thal et al., 2002; Vonsattel et al., 1991). The Consortium to Establish a Registry for AD (CERAD) protocol was used for scoring APP/A β -ir plaque and AT8-ir NC frequency (Mirra et al., 1991). To measure regional progression of APP/A β -ir plaque accumulation in the brain, a modified, four-point scale of Thal phases was incorporated (Montine et al., 2012; Thal et al., 2002). Staging of CAA was based on prior studies examining topographical distribution and frequency of A β in vasculature as well as intensity of staining (Attems et al., 2007; Vonsattel et al., 1991). NFT were evaluated using a revised Braak four-stage classification described in the National Institute on Aging-Alzheimer's Association guidelines for AD assessment (Braak and Braak, 1991; Montine et al., 2012).

For every animal, each of the four brain regions was given a score from 0 to 3. Regional scores then were summed for a total score per staging method ranging from 0 to 12. Scores for each of the five staging assessments were summed for a total pathology score with the maximum potential value of 60. Regional APP/A β -ir plaque and vessel volumes, AT8-ir pretangle, NFT and NC densities, topographical distribution maps of all five pathologies, and staining images were used for scoring purposes. Based on the CERAD protocol, APP/A β plaque volume was considered mild when less than 1% and moderate when more than 1%; no frequent cases were present in this sample. Besides the number of regions affected, CAA staging was evaluated using total APP/A β vessel volume. Less than 1% was scored as

minimal, 1–4% as mild, 5–15% as moderate, and more than 15% as severe. NFT stages were assigned based on location and severity of pathology. AT8-ir NC densities less than 100/mm³ were scored as sparse, 100–250/mm³ as moderate, and more than 250/mm³ as frequent.

2.7 Statistical Analyses

All densities and volumes were checked for linearity, and because of skewness from means close to zero, densities and volumes were transformed using the formula: $\arcsin(\sqrt{\text{volume or density}/1,000})$. The correlation matrix of the transformed measures was examined, and any variable that did not correlate with others ($r < 0.1$) was removed. To address potential errors due to redundancy and multicollinearity, variables that had extremely high correlations ($r > 0.9$) were excluded from analyses. PCA was performed to reduce the number of extraneous dimensions of variation in the system, and PCA-generated linear pathology components were employed for further analyses. For example, mean % of APP/A β and A β 42 plaque and vessel volumes measured the same underlying dimension; therefore, only APP/A β plaque and vessel volumes were retained. Other pairwise data reductions resulted from a lack of applicability to human pathologies, such as pretangle density which is not used as a diagnostic marker of AD pathology in humans and was excluded from the PCA-generated pathology factor.

PCA clarifies and reduces the covariance structure. The determinant of the correlation matrix was 0.09. The Kaiser-Meyer-Olkin measure of sampling adequacy was 0.62, and Bartlett's test of sphericity was significant ($\chi^2 = 39.98$, $p < 0.01$). Communalities were all above 0.45. The PCA-generated pathology component (weighted first component) served as the regressor in all subsequent models. To assess for sex differences in PCA-generated pathology factor, a two-sample t-test was utilized. Regression analyses were performed to determine relationships between tau densities and A β variables as well as with PCA-generated pathology factor and chronological age. Two-way ANOVAs with Bonferroni post hoc tests were conducted to examine sex and brain region differences in tau and A β pathologies. Statistical analyses were conducted using IBM SPSS Statistics, Version 22 (Armonk, NY), and level of significance (α) was set at 0.05.

3. RESULTS

3.1 APP/A β and A β 42 Plaque Volume

To evaluate A β protein aggregates in the aging chimpanzee brain, we measured volume occupied by plaques using immunostaining against two peptides, APP/A β and A β 42. Of the 20 chimpanzees, 13 had APP/A β -ir plaques (Fig. 1A-B, E) while five of those individuals also presented with A β 42-ir plaques (Fig. 1C-D, F). Of those with A β 42 plaques, four were the oldest individuals (57–62 years). A β 42 plaques were not found in the youngest subjects (39–44 years). Diffuse and dense-core plaques were identified in both sexes, although diffuse plaques displayed a weaker thioflavin S-positive histochemical intensity than densecore plaques (Fig. 2A-C). Plaques with an A β core surrounded by tau-ir dystrophic neurites were not observed. Plaques were distributed in all neocortical layers of the PFC and MTG and primarily in the pyramidal layer of hippocampal fields CA1 and CA3, and often

were adjacent to A β -positive vessels (Fig. 1M-O). In association with plaques, we did observe pretangles, NFT, and tau-ir neuritic clusters (NC) (Fig. 3). Two of the eldest individuals with the greatest A β deposition were recorded as Thal Phase 4 (i.e., a staging system used to determine the regional progression of A β plaques in AD patients).

Across the sample, total % of volume occupied by plaques was higher in neocortex (APP/A β = 0.28%, A β 42 = 0.24%) compared to the hippocampus (APP/A β = 0.12%, A β 42 = 0.08%), although factorial ANOVA did not detect a difference between regions ($F_{1,73} = 2.22$, $p = 0.14$; Fig. S3A). Volume occupied by APP/A β plaques did not differ significantly from A β 42 plaque volume ($F_{1,73} = 0.96$, $p = 0.33$). The interaction between region and APP/A β vs A β 42 was not significant ($F_{1,73} = 0.33$, $p = 0.57$). APP/A β plaque burden was similar in both sexes ($F_{1,72} = 0.35$, $p = 0.56$) regardless of region ($F_{3,72} = 1.21$, $p = 0.31$) with no interaction between sex and region ($F_{3,72} = 0.99$, $p = 0.40$; Fig. S4A). However, A β 42 plaque volume was significantly higher in males than females ($F_{1,66} = 5.13$, $p = 0.03$) across all brain areas ($F_{3,66} = 0.38$, $p = 0.77$) with a non-significant interaction ($F_{3,66} = 0.013$, $p = 0.99$; Fig. S4C).

3.2 APP/A β and A β 42 Vasculature

Given the substantial presence of A β -positive vasculature, we measured APP/A β - and A β 42-ir vessel volumes. All 20 chimpanzees displayed APP/A β and A β 42-ir microvessels including leptomeningeal, neocortical, and hippocampal arteries and smaller arterioles (Fig. 1G-J). The three oldest individuals (ages 57–62) displayed pathology similar to humans with severe CAA in both the neocortex and hippocampus. Total % of vessel volume occupied by amyloid ranged from 21–23% for APP/A β and from 16–26% for A β 42. A single case of moderate CAA was observed in a 58-year-old female chimpanzee with 5.2% total APP/A β and 3.8% A β 42 vessel volume. All other chimpanzees (ages 37–51 years) exhibited low CAA-like pathology with a total vessel volume of between 0.1–2.7% for APP/A β and 0.1–3.1% for A β 42. These individuals displayed decreased amyloid staining intensity as well as fewer reactive cortical vessels than older chimpanzees, whereas the hippocampal subfields lacked A β -positive microvessels. Cerebrovascular amyloid was also thioflavin S-positive (Fig. 2I-L) and frequently was bordered by NFT and tau NC (Fig. 3).

The total amyloid-positive vessel volume was greater in the neocortical regions (APP/A β = 3.0%, A β 42 = 2.6%) compared to the hippocampus (APP/A β = 1.2%, A β 42 = 1.3%) based on a two-way ANOVA, which revealed a significant main effect of region ($F_{1,73} = 4.96$, $p = 0.03$) but not peptide marker ($F_{1,73} = 0.00$, $p = 0.97$), with a nonsignificant interaction ($F_{1,73} = 0.18$, $p = 0.67$). A Bonferroni post hoc analyses revealed that the distribution of APP/A β and A β 42-ir vessels was comparable across brain regions (all p values > 0.10 ; Fig. S3B). Percentage of volume occupied by APP/A β and A β 42-ir vessels also did not differ between sexes (APP/A β : $F_{1,72} = 1.45$, $p = 0.23$; A β 42: $F_{1,66} = 1.08$, $p = 0.30$; Fig. S4B, D).

3.3 Pretangle, NFT, and NC Densities

In the early stages of tau deposition, neurons may contain pretangles that appear morphologically normal with minimal non-fibrillar tau and diffuse cytoplasmic staining (Uchihara, 2014). Intracellular accumulations of hyperphosphorylated tau protein aggregated

into insoluble PHF, which extend into dendrites, forming NFT (Braak et al., 2006; Grundke-Iqbal et al., 1986). To evaluate the occurrence of tau pathology, we quantified AT8-ir pretangles, NFT, and NC in neocortical regions (PFC and MTG) and hippocampus (CA1 and CA3) using stereology. Tau pathological markers and neuropil threads were identified in the pyramidal layer of CA1 and CA3. AT8-ir neurites extended from cell somata into the stratum radiatum and stratum oriens. Pretangles, NFT, and tau NC also were observed in the hilus of the dentate gyrus, subiculum, and EC (layers II, III, and V; Fig. S5). All three types of tau-related pathology were identified in neocortical layers II-VI, although pretangles and NFT were more concentrated in layers III and V. Additionally, tau lesions were associated with A β -positive vessels (Fig. 3).

All 20 apes demonstrated AT8-ir pretangles (Fig. 4A-F). Total average pretangle density was highest in neocortex (511/mm³) compared to hippocampus (103/mm³) ($F_{3,72} = 10.96$, $p = 0.01$; Fig. S7A; Table S3). No significant differences were found for sex ($F_{1,72} = 1.13$, $p = 0.29$) nor for an interaction between region and sex ($F_{3,72} = 0.71$, $p = 0.55$; Fig. S7A). Pretangle densities were higher in MTG (Brodmann area 21) (319/mm³) than PFC (Brodmann areas 9 or 10) (193/mm³) and in CA1 (85/mm³) compared to CA3 (17.73/mm³). CA3 was the least affected area with only half of the subjects displaying pretangles.

Five individuals presented with AT8-ir neurofibrillary tangles (Fig. 4G-L). Two chimpanzees displayed NFT in the PFC, though NFT were absent in the MTG. Four apes exhibited NFT in CA1 but only one displayed NFT in CA3. Braak stage V, in which NFT spread extensively from limbic regions into neocortex, was recorded in a 57-year-old male, while three cases (45–58 years old) were categorized as stage I or II, in which tangle pathology was located mainly in the EC or hippocampus. One animal with the highest NFT density did not follow Braak staging. Instead, this 39-year old male exhibited intense, clustered tau pathology exclusively in the PFC, and lacked amyloid plaque and vessel pathology. Unlike pretangle density, total NFT density was higher in the hippocampus (7.4/mm³) than neocortex (6.3/mm³), although ANOVA did not find a main effect of region ($F_{3,72} = 0.00$, $p = 1.00$; Fig. S7B; Table S4), sex ($F_{1,72} = 0.00$, $p = 1.00$; Fig. S7B), or their interaction ($F_{3,72} = 0.00$, $p = 1.00$). NFT were also thioflavin S-positive (Fig. 2E-H).

Tau pathology also was exhibited as clusters of dystrophic neurites evident in 12 apes (37–58 years old), which we categorized as AT8-ir NC (Fig. 4M-R). AT8-ir NC were found in both neocortical and hippocampal regions in six cases (37–58 years old), in the neocortex of three subjects (40–51 years old), and in the CA1 and CA3 subregions of the hippocampus of only three individuals (43–58 years old). Though AT8-ir NC densities were higher in the neocortex (81/mm³) than hippocampus (8.4/mm³), there was no significant effect of region ($F_{3,72} = 2.35$, $p = 0.08$; Fig. S7C), sex ($F_{1,72} = 3.39$, $p = 0.07$; Fig. S7C; Table S5), or interaction ($F_{3,72} = 2.04$, $p = 0.12$).

3.4 Age and Pathology Score Correlations

Principle components analysis (PCA) was used to compute a composite pathological score (PCA-generated pathology score) for A β and tau pathologies. To simplify correlational analyses of these pathologies, PFC and MTG were averaged (tau densities) or summed (A β volumes) for a neocortical value, CA1 and CA3 were averaged or summed for a

hippocampal value, and all four regions were averaged or summed for a total volume or density value. Five factors (i.e., neocortical and hippocampal AT8-ir NC densities, hippocampal NFT density, and total APP/A β plaque and vessel volumes) explained 57% of the variance. All variables had primary loadings between 0.67 and 0.87.

Age and PCA-generated pathology score were positively correlated ($r = 0.68$, $p = 0.01$). Neocortical, hippocampal, and total APP/A β and A β 42 plaque and vessel volumes significantly increased with age (all p values < 0.01) (Table S6). While total pretangle density also increased with age ($p = 0.02$), NFT and AT8-ir NC densities did not exhibit a significant relationship (all p values > 0.10). PCA-generated pathology score was positively associated with % of volume occupied by APP/A β and A β 42 plaques and vessels (all p values < 0.01). Unlike age, pathology score correlated with all three tau pathologies. Total and hippocampal pretangle and NFT densities, in addition to total, neocortical and hippocampal AT8-ir NC densities, were higher in individuals with PCA-greater pathology scores (all p values < 0.03), though the same relationship was not observed for neocortical pretangle and NFT densities (all p values > 0.68). Sex differences were not detected for PCA-generated pathology scores ($t = 1.18$, $p = 0.23$).

3.5 Co-occurrence of A β and Tau Pathologies

Total volume occupied by A β vessels (APP/A β = 4.2%, A β 42 = 3.9%) was significantly higher than total volume occupied by plaques (APP/A β = 0.4%, A β 42 = 0.3%) in aged chimpanzees ($r_s = 0.70$, $p = 0.01$; Fig. 5A). APP/A β and A β 42 plaque volumes were positively correlated ($p = 0.01$; Fig. 5B), indicating that A β 42 was prevalent in the chimpanzee brain. Percentage of volume occupied by APP/A β - and A β 42-ir plaques also was positively associated with APP/A β and A β 42 vessel volumes (all p values < 0.01 ; Figure 5C).

Neocortical and total pretangle densities were not correlated with changes in NFT or AT8-ir NC densities (all p values > 0.10). However, greater hippocampal pretangle density was accompanied by increases in hippocampal and total NFT density (all p values < 0.01) as well as larger neocortical, hippocampal, and overall AT8-ir NC loads (all p values < 0.03). Furthermore, hippocampal and total NFT densities were positively correlated with neocortical and total AT8-ir NC densities (all p values < 0.02).

Neocortical pretangle and total NFT densities were not associated with A β pathology (all p values > 0.25), but hippocampal and total average pretangle densities were positively correlated with neocortical, hippocampal, and total APP/A β vessel volumes (all p values < 0.05 ; Fig. 6A). Moreover, higher pretangle density was linked to increased APP/A β plaque volume in the hippocampus ($p = 0.03$; Fig. 6B). Greater neocortical and total APP/A β vessel volume was associated with increased hippocampal NFT density (all p values < 0.02 ; Fig. 6C) as well as hippocampal and total AT8-ir NC loads (all p values < 0.03 ; Fig. 6D). Hippocampal APP/A β vessel volume correlated with increased tau plaque loads (all p values < 0.03 ; Fig. 6E). A β 42 plaque and vessel volumes were not significantly correlated with changes in tau pathology (all p values > 0.05).

The severity of CAA was significantly associated with mean APP/A β plaque volume ($t = -4.63$, $p = 0.01$; Fig. 7A), pretangle density ($t = -3.30$, $p = 0.01$; Fig. 7B), and AT8-ir NC density ($t = -2.65$, $p = 0.02$; Fig. 7D), but not NFT density ($t = -0.74$, $p = 0.47$; Fig. 7C).

4. DISCUSSION

To date, less than 50 brains of aged apes have been examined for AD pathology across all prior studies (Finch and Austad, 2015). Here, we present an investigation of AD neuropathology in the largest cohort of any great ape species consisting of 20 chimpanzee brains aged 37 to 62 years old. Our findings establish that aged chimpanzees can exhibit the two main histological markers found in the brain of humans with AD, A β plaques and NFT.

APP/A β and A β 42-ir diffuse and dense-core plaques were most abundant in the temporal and frontal neocortex compared to the hippocampus; this progression aligns with Thal phases of A β deposition in humans (Thal et al., 2002). Similar to humans, diffuse plaques had an amorphous shape with ill-defined contours and were thioflavin S-negative. Dense-core plaques contained fibrillar A β deposits with a compact, spherical core and were associated with thioflavin S-positive staining (Itagaki et al., 1989). While diffuse plaques are observed in cognitively intact elderly people, neuritic plaques with dense cores are associated with cognitive impairment and AD-related dementia (Arriagada et al., 1992; Malek-Ahmadi et al., 2016). Volume occupied by A β 42-ir plaques was slightly less than APP/A β -ir plaques in all regions, and A β 42-ir plaques were absent or scarce in younger chimpanzees (ages 39–46) compared to older chimpanzees (ages 49–62) similar to previous findings in chimpanzees and baboons (Ndung'u et al., 2012; Rosen et al., 2008). This outcome differs from prior histological results in rhesus macaques and orangutans where A β 40 was the major form of A β in those species (Gearing et al., 1997, 1994). Similar to gorillas, amyloid plaques frequently were found adjacent to A β -ir vessels (Perez et al., 2016, 2013). Though not quantified, A β -ir vessels and rare plaques were observed in EC layers II, III, and V as well as the presubiculum of the three eldest chimpanzees with severe cerebral amyloid angiopathy (Fig. S5). Conversely, younger apes presented with A β -ir vessels in the medial temporal area but had few or lacked all immunoreactivity in the entorhinal cortex. Whether A β -ir vessels in the EC correlates with neuronal loss in chimpanzees remains to be determined, though studies in aged rhesus monkeys, a species shown to develop A β plaques, demonstrated preservation of layer II, III, and V neurons in the EC of juvenile, young adult, and aged monkeys (Gazzaley et al., 1997; Merrill et al., 2000; Elliott J. Mufson et al., 1994; West et al., 1993). Additionally, changes in cognition based on plaque burden were not observed in aged macaques (Sloane et al., 1997). Although A β 42 plaque volume was significantly greater in males than females, this variance may not be an accurate reflection of sex differences in chimpanzees due to the small sample size between sexes (two were males [57 and 62 years old] and one female [58 years old]). Furthermore, sex was not found to be a significant factor for APP/A β and A β 42 vessel volume or any tau pathological marker.

Approximately 80% of AD patients show A β accumulation in the brain's microvasculature (i.e., CAA) in addition to plaques (Serrano-Pozo et al., 2011). Aged chimpanzees exhibited A β and thioflavin S-positive vessels in the neocortex and hippocampus. Combining neocortical and hippocampal volumes, total mean volume occupied by APP/A β - and A β 42-

ir vessels was 4.2% and 3.9%, respectively. These data suggest that vessels accumulate fibrillar A β and that A β 42 may be the prevalent form of A β associated with CAA-like pathology in chimpanzees. A recent report has shown mostly A β 42-ir brain vasculature and plaques in aging squirrel and rhesus macaque monkeys (Ndung'u et al., 2012). Previous research in chimpanzees also demonstrated higher soluble and insoluble levels of A β 42 than A β 40 (Rosen et al., 2008). Therefore, aged chimpanzees may differ from humans who exhibit A β -positive vessels with stronger A β 40 than A β 42 immunoreactivity (Gravina et al., 1995). Amyloid deposition in CAA progresses in a regional pattern with the greatest accumulation in the occipital cortex followed by prefrontal cortex and hippocampus (Attems et al., 2007). While occipital cortex was not examined in this study, a higher percentage (e.g., 2- to 3-fold) of A β vessels were found in the neocortex than the hippocampus. In addition, all chimpanzees in this sample exhibited APP/A β and A β 42-positive vessels but only two-thirds had plaques, suggesting fibrillar A β vascular aggregation may be a precursor for plaque development in the aged chimpanzee brain.

CAA is more severe in demented patients and is an independent risk factor for cognitive decline (Attems et al., 2007; Fernando and Ince, 2004; Greenberg et al., 2004; Nicoll et al., 2004; Serrano-Pozo et al., 2011; Tian et al., 2004; Xu et al., 2003; Zekry et al., 2003). Tangle density also is dramatically higher in individuals with moderate or severe CAA compared to those with no or mild CAA, despite similar plaque densities in both groups (Yamada, 2002). The strong connection between CAA and neuritic pathology, which also is seen in AD where neuritic plaques surround amyloid-laden cortical microvessels, suggests that aged chimpanzees display an analogous CAA pattern to humans (Fig. 7) (Yamada et al., 1997). Total amyloidpositive vessel volume was significantly correlated with increased hippocampal pretangle, NFT, and NC pathology in aged chimpanzees. Conversely, hippocampal APP/A β plaque volume demonstrated a significant relationship with only hippocampal pretangle density and neocortical NC loads, but not NFT density. Interestingly, A β 42 plaque and vessel volumes were not correlated with tau pathology in chimpanzees. These data indicate that CAA, not plaque development, may play a key role in the precipitation of NFT in chimpanzees and that the combination of A β 40 and A β 42 peptides (i.e., APP/A β) is more neurotoxic than A β 42 alone in this ape.

Pretangle densities were highest in the neocortex, particularly MTG, compared to the hippocampus, as reported in a prior tauopathy study in a single chimpanzee (Rosen et al., 2008). Distinct from that case, though, NFT density was greater in the hippocampus in our group of aged chimpanzees. Both pretangle and NFT density patterns followed Braak staging of NFTs in AD patients (Braak et al., 2006; Braak and Braak, 1991). The composition and density of AT8-ir tau pathology changes in humans with age (Braak et al., 2011). AT8-ir pathology increases with age and is primarily in the form of pretangles in younger individuals, but after age 50, the predominant form of tau lesions is NFT. Chimpanzees also demonstrated increasing total pretangle densities with age; however, NFT densities as well as NC loads were not associated with age. This result may be due to the small number of individuals with this pathology in the current study. The entorhinal cortex and hippocampus are two of the first regions to display NFT during the onset of AD (Braak and Braak, 1991; Montine et al., 2012). In chimpanzees with extensive tau lesions, an intense focus of pretangles and NFT was observed in CA1, particularly near the border of

CA2. The CA3 was affected to a much lesser extent and exhibited primarily pretangles except for one ape with NFT. Though tau lesions were not quantified in the entorhinal cortex, pretangles, NFT, and tau NC were present in layers II, III, and V in a 45-year old female that suffered a stroke, a 57-year old male with severe cerebral amyloid angiopathy, and a 58-year old female with mild amyloid pathology (Fig. S5). Tau lesions were absent in the EC of younger individuals and two older chimpanzees, a 58-year old female and 62-year old male with severe cerebral amyloid angiopathy; however, AT8-ir glia were present in the EC of the two older apes. In AD, neuron densities in layer II of the EC are decreased substantially compared to non-demented controls between the ages of 60 and 90 (Gómez-Isla et al., 1996). Future quantification of neuron densities in the EC, CA1, and CA3 of these chimpanzees will address whether neuronal loss occurs with AD pathology, as is observed in humans, or with age (Padurariu et al., 2012). Although most of the apes followed human Braak staging, a 39-year old male chimpanzee presented with a high concentration of thioflavin S-positive NFT and NC in the prefrontal cortex and virtually none in the hippocampus. The location and intensity of tau lesions in the PFC, in addition to an absence of A β plaques and only an occasional, weakly positive vessel, suggests that A β accumulation did not initiate tangle formation in this chimpanzee. Rather, this ape may offer the first pathological evidence of a non-A β tauopathy in a species outside of humans. However, further analysis of additional brain regions and histopathological lesions (i.e., astroglial inclusions) is needed in this individual. In contrast to humans with AD, AT8-ir NC in chimpanzees were not associated with an A β core and were found in individuals with and without A β pathology (Hof et al., 1990; Kosik et al., 1986; Mirra et al., 1991). A previous study of tauopathy in a chimpanzee demonstrated similar neuritic clusters (Rosen et al., 2008) lacking astrocytes, which we also confirmed. These results also differ from those observed in a recent study of aged gorillas that exhibited Alz50-ir neurons in the neocortex in close association with A β plaques but lacked dystrophic neurites (Perez et al., 2016, 2013). Aged chimpanzees displayed tau NC (i.e., clusters of AT8-ir dystrophic neurites) near A β -ir vessels. Alz50 targets early stages of tau hyperphosphorylation in humans, such as pretangle neurons, and therefore, tau pathology found in the aged gorillas may have been at an earlier stage compared to the aged chimpanzees in our study.

In the present investigation, we noted subtle differences between chimpanzees that exhibited normal versus pathologic aging (Table S7). Whether amyloid pathology is part of the normal aging process in humans is a continuing debate (Arriagada et al., 1992; Fjell et al., 2014). However, the presence of A β -positive vessels in all apes within the current study suggests A β deposition may be part of the normal aging process in chimpanzees. APP/A β and A β 42 plaque and vessel volumes were significantly correlated with increasing chronological age and greater PCA-generated pathology scores, indicating amyloid lesions also contribute to pathologic aging in apes. In normal aging, plaques are fewer and diffuse in nature (Arriagada et al., 1992). During pathologic aging, plaque distribution was greater and consisted of diffuse and dense-core plaques (Selkoe et al., 1987). Indeed, younger individuals had fewer A β -ir plaques and vessels than older apes, while the four oldest chimpanzees had moderate or severe CAA-like pathology as well as higher plaque loads. Younger subjects also exhibited fewer A β -positive vessels with reduced staining intensity. Individuals with the largest distribution of A β pathology also displayed immunoreactive vessels in more of the

brain regions examined, in deeper neocortical layers and underlying white matter, and perivascular leakage. Normal aging in chimpanzees may be associated with an increase in hyperphosphorylation of tau, as indicated by the significant, positive association between total pretangle density and chronological age. However, formation of NFT and NC in chimpanzees was not correlated with chronological age, implying these pathologies are not part of the normal aging process. Rather NFT and NC were related to the overall pathology score, suggesting that they are more likely associated with pathologic aging in chimpanzees. Apes with the most A β plaque and vessel pathology also had the most severe tau pathology except for one chimpanzee with only tau lesions present. While statistically significant, some correlations were based on a low number of individuals with the most severe pathology. These individuals were included as they represent part of the pathology continuum, which can range from mild to severe levels. However, it should be noted that small sample sizes may lack statistical power, and correlational analyses based on larger sample sizes may change. The mechanisms that cause a transition from normal aging to pathologic aging remain unknown. Although similar to humans, age is probably the greatest risk factor for both pathological aging and neurodegenerative disease.

AD dementia is a clinical disorder confirmed at autopsy by the presence of amyloid and tau brain pathology and selective neuronal loss. Thus, antemortem behavioral and cognitive testing accompanied by postmortem neuropathological evaluation is required to determine whether amyloid and tau lesions are associated with cognitive decline in aged chimpanzees. Most cognitive aging research in primates has been performed in rhesus monkeys, which have shown functional deficits in short-term memory, spatial memory, executive function, attention, and recognition memory (Bachevalier et al., 1991; Bartus et al., 1978; Gallagher and Rapp, 1997; Herndon et al., 1997; Lacreuse and Herndon, 2009; Moore et al., 2006, 2003; Moss et al., 1997; Presty et al., 1987; Rapp and Amaral, 1991). Only a handful of studies on aging and cognition have been performed in great apes due to the rarity of the animals, expense and difficulty of maintaining them, and required special adaptations of cognitive tests and equipment (Lacreuse and Herndon, 2009). The few investigations of cognitive aging in apes has focused on chimpanzees and gorillas. Using object discrimination trials, young (11–19 years old, $n = 8$) and old (28–40 years old, $n = 8$) chimpanzees were tested on their rate of learning, memory, and response variability. Age differences were not found in any of the tasks (Bernstein, 1961). A second study in 19 chimpanzees (ages 7–14 years) also confirmed no effect of age on two different object discrimination tasks designed to stimulate novel responses. However, an age-related decline in performance was observed on delayed response and fourchoice oddity tasks (Riopelle and Rogers, 1965). A group of 38 female chimpanzees (ages 10–54 years) were tested for three years in 12 tests of physical and social cognition (Lacreuse et al., 2014). While an age-related decline was not observed in physical cognition tests, a significant decline in performance was found in four individuals more than 50 years old in spatial memory, attention-getting, and gaze-following tasks. Performance on the delayed response task worsened with longer delays and higher numbers of choices in aged gorillas ($n = 16$), though not statistically significant (Kuhar, 2004). The ability to detect “numerousness” in (ages 6–43 years, $n = 11$) did not differ between young and old groups, though the aged apes did respond significantly

slower which was attributed to cognitive slowness rather than motor speed variances since the two age groups responded at the same speed prior to training (Anderson et al., 2005).

While age-related decline in cognition and memory in apes and monkeys appears similar to the mild memory loss observed in humans during the normal aging process, the profound memory impairment found in AD patients has not been demonstrated yet in nonhuman primates. This disparity may be a result of humans being exceptionally vulnerable to neurodegenerative diseases due to genetic differences in apolipoprotein E, structural and functional differences in A β and tau between species, and an increased lifespan (Walker and Jucker, 2017). Another explanation, however, is the complete lack of studies with both antemortem cognitive data in conjunction with postmortem neuropathologic analyses in the same apes to distinguish between normal and pathologic aging processes. Unfortunately, cognitive data that could directly inform our analyses were not collected for these chimpanzees. The current scarcity of research in these rare animals precludes the opportunity to resolve the significant question of whether humans are uniquely susceptible to AD. Therefore, further cognitive longitudinal studies of great apes are of the utmost importance in establishing whether AD neuropathologic change, including amyloid and tau deposits as well as neuronal loss, leads to the development of cognitive impairment and AD in great apes.

Conclusions

Tau and A β lesions were identified in the neocortex and hippocampus in a large cohort of aged chimpanzees. Surprisingly, AT8-ir pretangles and A β -positive vessels were exhibited in all 20 apes, while A β plaques were found in two-thirds of individuals. We also demonstrated thioflavin S-positive NFTs in aged chimpanzees, though electron microscopic analysis is planned to confirm filament structure. Neuritic plaques with an A β core were absent, but clusters of AT8-ir tau neurites were observed. Most importantly, the presence of both A β and tau markers in aged chimpanzees indicates that a co-occurrence of the two classic hallmark lesions of AD is not specific to the human brain. Future investigations will study possible neuronal loss in association with AD pathology, the structural and cellular composition of tau-ir neurite clusters, association of DNA sequence polymorphisms in the APOE, APP, presenilin, and tau genes with observed neuropathology, and the potential coexistence of neurodegenerative pathologies, such as Lewy bodies and synaptopathy, in aged chimpanzees.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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HIGHLIGHTS

- Comparative studies assessing neuropathology between humans and apes are rare.
- This study demonstrates AD-like pathology in a large group of aged chimpanzees.
- Tau lesions include neurofibrillary tangles and tau neuritic clusters.
- Vascular amyloid was associated with tau lesions in the chimpanzee brain.
- Coexistence of both lesions indicates that AD pathology is not limited to humans.

Figure 1.

APP/A β and A β 42-ir plaques and vessels in aged chimpanzees (subject 12: A-B,D-F,H,M-O; subject 9: C; subject 20: G,J,L; subject 19: I,K): (A-B,E) APP/A β -ir plaques, (C-D,F) A β 42-ir plaques, (G) APP/A β -ir cortical artery and arterioles, (H) APP/A β -ir leptomeningeal arteries, (I) A β 42-ir cortical artery (J) A β 42-ir cortical arterioles, (K) APP/A β -ir cortical arterioles, (L) A β 42-ir cortical arterioles, and (M-O) APP/A β -ir plaques (black arrows) near immunopositive vessels (white arrows). Scale bars = 250 μ m (A, D, G, J-L, M-O) or 25 μ m (B-C, E-F, H-I).

Figure 2.

Thioflavin S staining in the temporal cortex (A-B, I-J, L), CA1 subfield of the hippocampus (C-F, H, K), and prefrontal cortex (G) of aged chimpanzees (subject 19: A,D-F,H-J; subject 20: B,L; subject 12: C,K; subject 13: G): (A) diffuse plaque, (B) dense-core plaque, (C) plaque near positive vessel (white arrow), (D) positive arteriole, (E-G) NFT, (H) NFT (yellow arrow) near vessel, (I-J) cortical arteries, and (K-L) cortical arterioles. Scale bars = 20 μ m (A-B, E-H) or 200 μ m (C-D, I-L).

Figure 3.

Co-occurrence of Aβ and tau reactivity in hippocampus (CA1, pyramidal layer) of aged chimpanzees (subject 19: A-E; subject 12: F): (A-F) AT8-ir pretangles (red arrows, NFT (yellow arrows), and NC (blue arrows) (DAB with nickel enhancement, black) associated with Aβ42-ir (NovaRED) vessels (white arrows) and plaques (black arrows). Scale bars = 25 μm (A-B) or 250 μm (C-F).

Figure 4.

AT8-ir lesions in the prefrontal cortex (C, H, J-K, L-N, P-R) and hippocampus (A-B, D-G, I, O) of aged chimpanzees (subject 11: A,F; subject 19: B-E,G,I,M-R; subject 13: H,J-L): (A-F) pretangles, (G-J) NFT, and (M-R) AT8-ir NC. Scale bars = 250 μ m (A, D, G, J, M, P) or 25 μ m (B-C, E-F, H-I, K-L, N-O, QR).

Figure 5.

Plaque and vessel volumes in 20 chimpanzees aged 37–62 years old (mean age = 46 years): (A) Average total plaque and vessel volume (%) ($r_s = 0.70$, $p = 0.01$, small circles represent outliers), (B) correlation of total APP/A β plaque volume vs total A β 42 plaque volume across brain regions ($R^2 = 0.54$, $p < 0.01$), and (C) total plaque volume vs total vessel volume by type (APP/A β : $R^2 = 0.66$, A β 42: $R^2 = 0.87$, p 's < 0.01).

Figure 6.

Significant correlations of APP/Aβ (%) and tau (mm³) lesions (p 's < 0.05): (A) HC pretangle density vs NC and HC APP/Aβ vessel volume (NC: $R^2 = 0.22$, HC: $R^2 = 0.18$), (B) HC pretangle density vs HC APP/Aβ plaque volume ($R^2 = 0.19$), (C) HC NFT density vs NC APP/Aβ vessel volume ($R^2 = 0.23$), (D) HC AT8-ir tau NC density vs NC and HC APP/Aβ vessel volume (NC: $R^2 = 0.20$, HC: $R^2 = 0.20$), and (E) NC AT8-ir tau NC density and HC APP/Aβ plaque volume ($R^2 = 0.27$).

Figure 7.

Severity of CAA was associated with higher A β plaque volume (%) and increasing levels of tau lesions (per mm³) in aged chimpanzees: (A) total APP/A β plaque volume vs CAA stage (p = 0.01), (B) total pretangle density vs CAA stage (p = 0.01), (C) total NFT density vs CAA stage (p = 0.47), and (D) total AT8-ir tau NC density vs CAA stage (p = 0.02).

Table 1.

Subject demographics

Subject	Age	Sex	Pathology Score	A β Plaque	A β Vessel (CAA)	Pretangle	NFT (Braak)	Tau NC (CERAD)
1	37	F	4	Phase 1	Minimal	+	-	Sparse
2	39	F	0	-	Minimal	+	-	-
3	40	F	2	Phase 1	Minimal	+	-	-
4	40	F	0	-	Minimal	+	-	-
5	41	F	0	-	Minimal	+	-	-
6	43	F	7	Phase 2	Mild	+	-	Sparse
7	44	F	0	-	Minimal	+	-	-
8	45	F	5	Phase 1	Mild	+	Stage II	Sparse
9	49	F	3	Phase 1	Minimal	+	Stage I	-
10	51	F	4	Phase 1	Minimal	+	-	Sparse
11	58	F	11	Phase 3	Moderate	+	-	Moderate
12	58	F	9	Phase 1	Severe	+	Stage I	Sparse
13	39	M	9	-	Minimal	+	+	Moderate
14	40	M	1	-	Minimal	+	-	Sparse
15	41	M	4	Phase 1	Minimal	+	-	Sparse
16	41	M	4	-	Mild	+	-	Sparse
17	41	M	0	-	Minimal	+	-	-
18	46	M	3	Phase 2	Minimal	+	-	Sparse
19	57	M	19	Phase 4	Severe	+	Stage V	Moderate
20	62	M	10	Phase 4	Severe	+	-	-

A β (amyloid-beta) includes APP/A β and A β 42, CAA (cerebral amyloid angiopathy), CERAD (Consortium to Establish a Registry for AD), F (female), M (male), NFT (neurofibrillary tangle), NC (neuritic cluster), Thal (clinical phases for accumulation of A β plaques), Braak (clinical staging system used to classify tangle distribution)

+

positive for pathology

*

does not follow staging pattern.

Table 2.

Scoring system for AD pathology in chimpanzees

Pathology	A β plaques		A β vasculature	NFT	Tau NC
Staging	CERAD	Thal phases	CAA stages	Braak stages	CERAD
Scoring	0 Absent	0 Absent	0 Absent/minimal	0 Absent	0 Absent
	1 Sparse	1 Stages 1/2	1 Mild CAA	1 Stages I/II	1 Sparse
	2 Moderate	2 Stage 3	2 Moderate CAA	2 Stages III/IV	2 Moderate
	3 Frequent	3 Stages 4/5	3 Severe CAA	3 Stages V/VI	3 Frequent