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Pathologic correlations of [F-18]-AV-1451 imaging in non-Alzheimer tauopathies

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Abstract

Objective—Recent studies have shown that PET tracer AV-1451 exhibits high binding affinity for paired helical filament (PHF)-tau pathology in Alzheimer's brains. However, the ability of this ligand to bind to tau lesions in other tauopathies remains controversial. Our goal was to examine the correlation of *in vivo* and postmortem AV-1451 binding patterns in three autopsy-confirmed non-Alzheimer tauopathy cases.

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Author contributions:

1) conception and design of the study: MM and TG-I; 2) acquisition and analysis of data: MM, MDN, ACM, MSTC, NVA, AA-F, WEK, CAM, MDI, MD, EAB, CRV, IC, SM, SLD, DHO, SNG, JHG, KD-R, DEL, BCD, MPF, BTH, KAJ and TG-I; 3) drafting the text or preparing the figures: MM, MDN, ACM, BCD, MPF, BTH, KAJ, TG-I.

Potential Conflict of Interest:

Bradley T. Hyman reports personal fees from Lilly, the company that owns [F-18]-AV-1451, outside the submitted work.

Keith A. Johnson reports personal fees from Lilly/Avid, the company that owns [F-18]-AV-1451, outside the submitted work.

All other authors have nothing to disclose.

Methods—We quantified *in vivo* retention of [F-18]-AV-1451 and performed autoradiography, [H-3]-AV-1451 binding assays and quantitative tau measurements in postmortem brain samples from two Progressive Supranuclear Palsy (PSP) cases and a *MAPTP301L* mutation carrier. They all underwent [F-18]-AV-1451 PET imaging prior to death.

Results—The three subjects exhibited [F-18]-AV-1451 *in vivo* retention predominantly in basal ganglia and midbrain. Neuropathologic examination confirmed the PSP diagnosis in the first two subjects; the *MAPTP301L* mutation carrier had an atypical tauopathy characterized by grain-like tau-containing neurites in grey and white matter with heaviest burden in basal ganglia. In all three cases, autoradiography failed to show detectable [F-18]-AV-1451 binding in multiple brain regions examined with the exception of entorhinal cortex (reflecting incidental age-related neurofibrillary tangles) and neuromelanin-containing neurons in the substantia nigra (off-target binding). The lack of a consistent significant correlation between *in vivo* [F-18]-AV-1451 retention and postmortem *in vitro* binding and tau measures in these cases suggests that this ligand has low affinity for tau lesions primarily made of straight tau filaments.

Interpretation—AV-1451 may have limited utility for *in vivo* selective and reliable detection of tau aggregates in these non-Alzheimer tauopathies.

Introduction

PET ligands for tau have the potential to improve diagnostic accuracy and allow reliable *in vivo* quantification of brain tau burden in Alzheimer's disease (AD) and related tauopathies.^{1–4} [F-18]-AV-1451 (or T807) was developed to detect tau in the form of paired helical filaments (PHF).⁵ Recent studies show that patients with mild cognitive impairment (MCI) and AD dementia exhibit significantly higher [F-18]-AV-1451-PET retention than cognitively normal controls.^{6–12} Importantly, [F-18]-AV-1451-PET patterns correlate with disease severity and seem to match the predicted topographic Braak staging¹³ of neurofibrillary tangles (NFT) in AD.^{6, 12, 14, 15} Moreover, [F-18]-AV-1451-PET retention correlates with increased tau and decreased A β ₄₂ in cerebrospinal fluid in AD patients and normal controls.^{7, 10, 16} All these data suggest that [F-18]-AV-1451 is a promising biomarker for *in vivo* detection of tau lesions in AD.

Its usefulness as a biomarker in non-Alzheimer tauopathies, however, remains controversial. Some studies report increased [F-18]-AV-1451-PET retention in patients clinically diagnosed with Progressive Supranuclear Palsy (PSP)^{17–19} and Chronic Traumatic Encephalopathy (CTE),^{20, 21} while others notice PET patterns that cannot be reliably distinguished from controls.²² These discrepancies highlight the need for neuroimaging–pathologic studies conducted on postmortem material from individuals imaged while alive.

We recently conducted an analysis of [F-18]-AV-1451 autoradiographic and *in vitro* binding patterns in legacy postmortem brain samples representing a broad spectrum of neurodegenerative pathologies.²³ Our observations suggested that [F-18]-AV-1451 exhibits high binding affinity to tau lesions in AD but low affinity for tau inclusions in non-Alzheimer tauopathies. Importantly, we observed robust off-target binding to neuromelanin- and melanin-containing cells that should be carefully taken into account when interpreting [F-18]-AV-1451-PET patterns.²³ Other authors have made similar observations.^{24, 25}

Here we expand the characterization of this tracer by examining the correlation between *in vivo* antemortem and postmortem [F-18]-AV-1451 binding patterns in three autopsy-confirmed non-Alzheimer tauopathy cases (two PSP²⁶ cases and a *MAPTP301L* mutation^{27, 28} carrier). Our results suggest that [F-18]-AV-1451 holds great promise as surrogate marker for detection of tau pathology in AD but exhibits low affinity for tau aggregates in these non-Alzheimer tauopathy cases.

Subjects and Methods

The study was approved by local Institutional Review Board. Informed consent for neuroimaging and brain autopsy was obtained for each subject.

In vivo [F-18]-AV-1451 PET imaging

Subjects underwent [F-18]-AV-1451-PET scans and structural MRI. PET data were acquired using a Siemens/CTI (Knoxville, TN) ECAT HR+ scanner (3D mode; 63 image planes; 15.2 cm axial field of view; 5.6 mm transaxial resolution and 2.4mm slice interval) from 80–100 minutes after a 9 to 11 mCi bolus injection in 4 × 5-minute frames. PET data were reconstructed and attenuation corrected. Each frame was evaluated to verify adequate count statistics and absence of head motion. T1-weighted MRI images were acquired on a 3T Tim Trio (Siemens) and reconstructed using the MGH Freesurfer (FS) as previously described.^{29–31} [F-18]-AV-1451 specific binding was expressed in FS regions of interest (ROIs) as standardized uptake value ratios (SUVR) using cerebellar grey matter as reference.

Regional correlation between [F-18]-AV-1451 PET imaging and postmortem tissue ROIs

PET images were co-registered and fused with 3-Tesla MRI images. A manual method identified and matched 16–22 ROIs defined on postmortem 10 mm-thick coronal brain slabs and aligned them visually to the corresponding coronal T1-weighted MRI images. To optimize correspondence between ROIs defined on PET images and their pathological substrate, we used the gyral and ventricular morphology on tissue slabs and used a dynamic resampling of MRI in planes of view that matched the tissue blocks planes of sectioning. ROIs were drawn manually on MRI-T1 images using the medical software AMIDE v.1.0.5 (A Medical Image Data Examiner, <http://amide.sourceforge.net>),^{32, 33} expanded to sample a 10 mm-slice depth at the identified ROI location and saved to represent an object map on the MRI. The object map was then used to sample the previously co-registered PET data. Mean [F-18]-AV-1451-PET relative SUVRs in each ROI sampled were obtained.

Brain tissue samples

Brains were cut at time of removal on standard anatomic landmarks and areas of interest were identified and frozen at –80°C. Tissue blocks were fixed in formalin for one week before being embedded in paraffin and cut at 8-μm. Diagnostic histological evaluation was performed on 19 regions representative for a spectrum of neurodegenerative diseases and in accordance with published guidelines.^{34–36} Paraffin-embedded blocks were stained with Luxol fast blue and hematoxylin-eosin; selected blocks were stained for Bielschowsky silver stain and amyloid-β (Aβ), α-synuclein, ubiquitin, TDP-43, and phospho-tau

immunoreactivity. Frozen brain tissue blocks containing the ROIs for neuroimaging-pathologic correlation analyses were sectioned in a cryostat at 10- μ m and used for immunohistochemistry and autoradiography. Fresh-frozen homogenates prepared from the same tissue material were used to assess tau content by SDD-AGE and for [H-3]-AV-1451 *in vitro* binding assays.

[F-18]-AV-1451 phosphor screen and nuclear emulsion autoradiography

[F-18]-AV-1451 phosphor screen and nuclear emulsion autoradiography experiments were conducted following our previously published protocol.²³ To obtain autoradiographic information at cellular resolution level, frozen cryostat sections, adjacent to those used for phosphor screen autoradiography, were coated with liquid photographic emulsion followed by immunostaining using appropriate primary antibodies - anti-tau PHF1 (1:100, mouse, kind gift of Dr. Peter Davies), anti A β (1:50, rabbit, IgG Affinity Purify, IBL) and anti TDP-43 (1:100, rabbit, Protein Tech) – as described in detail elsewhere.²³ All assays were done in duplicate.

***In vitro* [H-3]-AV-1451 binding assays**

Fresh-frozen brain samples were homogenized at room temperature (RT) for 30 seconds in PBS at a concentration of 10 mg tissue/ml. Homogenates were diluted 10-fold in binding buffer (150 mM Tris-HCl, pH 7.0, containing 10% ethanol to enhance AV-1451 solubility) to a final concentration of 1 mg tissue/ml. 500 μ l of either zero or 6 μ M unlabeled AV-1451 were combined with 400 μ l of [H-3]-AV-1451 (44 Ci/mmol; gift of Avid Radiopharmaceuticals, Inc.) in a volume of 900 μ l of binding buffer. 100 μ l of the 1 mg tissue/ml was added to achieve a final concentration of 0.10 mg tissue/ml. The final concentration of [H-3]-AV-1451 was 1 nM and the final concentration of unlabeled AV-1451 was 3 μ M. After incubation at RT for 60 minutes, the binding mixture was filtered through a Whatman GF/B glass filter via a Brandel M-24R cell harvester and washed three times with 3 ml binding buffer. The filters were counted in Cytoscint-ES after sitting in the cocktail overnight. Complete (100%) inhibition of binding is defined as the number of counts displaced by 3 μ M non-radioactive AV-1451. All assays were done in triplicate.

Tau burden quantification by immunohistochemistry

10- μ m-thick frozen sections adjacent to those used in autoradiography were stained with PHF-1 antibody (1:100, mouse) and analyzed with an upright Olympus BX51 microscope (Olympus, Denmark) using the CAST software (Visiopharm, 2004, Denmark). Photomicrographs from each section were captured and a threshold of optical density was obtained using the ImageJ software (National Institute of Health). Manual editing eliminated artifacts. Tau pathology burden, defined as total percentage of area covered by PHF-1 immunostaining, was calculated in each ROI.

Semi-denaturing detergent agarose gel electrophoresis (SDD-AGE)

SDD-AGE was carried out as previously described with slight modifications.^{37, 38} 250 mg of frozen tissue containing each ROI was homogenized in cold PBS with Protease Inhibitor Cocktail (1x, Roche). Homogenates were spun at 3000g for 5 minutes at 4°C and

supernatant was collected. 1.5% agarose gel was prepared by dissolving agarose in Buffer G (20 mM Tris-Base, 200 mM glycine) and adding 10% SDS to a final concentration of 0.02%. 60 µg of lysate protein was incubated with 0.02% SDS sample buffer for 7 minutes at RT before loading. SDD-AGE was run in Laemmli buffer (Buffer G with 0.1% SDS) at 35V for 16 hours at 4°C. Protein was transferred via capillary action using 20 pieces of thick Whatman (GB 005) and 8 pieces medium Whatman (GB 003) to Immobilon polyvinylidene fluoride (PVDF) membrane (Millipore) at 4°C for 16–24 hours. Membranes were blocked in 5% non-fat dry milk (NFDM) in 1XTris-buffered saline-Tween (TBS-T) for 1 hour and probed for total tau (rabbit antibody 1:4000, Dako) and for PHF-tau (mouse PHF-1 antibody 1:4000) overnight at 4°C. Membranes were washed five times with TBS-T, probed with goat anti-rabbit HRP conjugated secondary antibody (1:2000, Biorad) or goat anti-mouse HRP (1:2000) respectively for 1 hour in 5% NFDM in TBS-T at RT, and washed five times with TBS-T. Membranes were developed using Enhanced Chemiluminescence (ECL) Substrate (Pierce) for 1 min and exposed to film (GE Healthcare). Images were quantified using Image J software (National Institute of Health).

Results

Clinical case description

Subject #1: 77 year-old male with a 7-year history of progressive speech and cognitive symptoms. He was clinically diagnosed with agrammatic/non-fluent variant of Primary Progressive Aphasia.³⁹ He subsequently developed dysphagia, gait instability and falls, hand tremor, right upper extremity rigidity and limited vertical eye movements, fulfilling clinical criteria for PSP.⁴⁰ [F-18]-AV-1451-PET scan was performed 19 months pre-death; at that time he was mildly demented. His motor phenotype worsened further over time. Subject #2: 68 year-old male with a 7-year history of progressive hand tremor and gait instability. He developed repeated falls, dysarthria, dysphagia, diplopia with limitation of vertical eye movements and dysautonomic symptoms, meeting clinical criteria for probable PSP.⁴⁰ [F-18]-AV-1451-PET scan was performed 5 months pre-death. At that time, he was wheelchair-bound and mildly demented. Subject #3: 56 year-old male with a 3-year history of progressive cognitive and behavioral symptoms. He was clinically diagnosed with behavioral variant Frontotemporal Dementia.⁴¹ His family history was consistent with an autosomal dominant pattern. Genetic testing confirmed the *MAPTP*301L mutation.^{27, 28} 8 months pre-death he underwent [F-18]-AV-1451-PET scan. At that time, he exhibited moderate dementia and mild parkinsonian signs.

In vivo [F-18]-AV-1451-PET imaging

PET images from the three subjects are shown in Figure 1A–C. Images from a mild AD patient and a cognitively normal are shown for comparison (Figure 1D–E). Subject #1's PET scan showed elevated retention predominantly in basal ganglia and midbrain, and lower signal in frontal and temporal cortices, white matter (left-lateralized), and cerebellar dentate nuclei (right-lateralized). Subject #2's PET scan showed elevated retention most prominent in basal ganglia and midbrain, and lower signal in medial and lateral prefrontal and anterior temporal cortices, white matter (right-lateralized), and cerebellar dentate nuclei (left-lateralized). Subject #3's PET scan showed elevated retention predominantly in basal

ganglia and midbrain, and lower signal in prefrontal, temporal and parietal cortices and white matter (Figure 1A–C). Overall tracer retention in these cases was much lower than that observed in AD patients.^{6, 11, 16}

Neuropathological examination

Subject #1 showed the expected neuropathologic lesions of PSP with tau-immunoreactive inclusions in neurons and glia in cerebral cortex, white matter and other grey matter regions including pallidum, midbrain, basis pontis and cerebellar dentate nucleus. NFT were observed in entorhinal cortex (EC) and subiculum, consistent with age-related NFT Braak stage II.¹³ In subject #2, histopathological exam revealed abundant tau-immunoreactive inclusions, mainly glial, in the form of tufted astrocytes, coiled bodies and interfascicular threads. Their distribution included brainstem, cerebellum, thalamus, pallidum, limbic regions and neocortex, consistent with the diagnosis of definite PSP.⁴² Incidental age-related NFT were found in the EC and the CA1 hippocampal subfield. Subject #3 demonstrated an unusual histopathological phenotype in the setting of the *MAPTP301L* mutation. Rather than the expected presence of NFT and neuritic processes, abundant small grain-like tau inclusions were present in cortex and white matter with the heaviest burden in basal ganglia, resembling a *MAPTS305I* mutation carrier phenotype previously reported.⁴³ Scattered age-related NFT in the EC, sparse diffuse A β -plaques in the EC and temporal cortices, and rare temporal lobe TDP-43 inclusions were also detected.

[F-18]-AV-1451 phosphor screen and nuclear emulsion autoradiography

Phosphor screen autoradiography, using [F-18]-AV-1451 aliquots from material prepared for routine *in vivo* imaging on the same day, showed nearly identical binding patterns in the three cases (Figure 2A, 2C and 2E). As expected, strong binding was detected in tangle-containing regions from AD tissue used as positive control (data not shown). No autoradiographic signal was detected across multiple cortical and subcortical regions known to contain abundant tau inclusions in these non-Alzheimer tauopathy cases, with the exception of the EC (reflecting incidental age-related tangle pathology) and the substantia nigra (off-target binding). Of note, no [F-18]-AV-1451 binding was detected in basal ganglia, despite elevated [F-18]-AV-1451-PET signal in this region (Figure 1A–C).

To confirm these observations and obtain cellular resolution, we dipped adjacent sections in a photographic nuclear emulsion, followed by immunohistochemistry with appropriate tau antibodies. In agreement with our previous observations,²³ tangle-containing sections from AD exhibited strong concentration of silver grains matching the distribution of PHF-tau aggregates (data not shown) while brain slices from the three non-Alzheimer tauopathy cases showed no detectable silver grain accumulation (Figure 2B, 2D and 2F).

In vitro [H-3]-AV-1451 binding assays

In vitro [H-3]-AV-1451 binding assays conducted in brain homogenates derived from the same tissue material used in autoradiography revealed no significant correlation between regional [F-18]-AV-1451-PET retention and *in vitro* tracer binding in any of the three cases (Figure 3). Of note, with the exception of basal ganglia, some of the regions that exhibited

high *in vitro* binding (e.g. superior temporal, anterior cingulate, superior frontal), showed very low [F-18]-AV-1451-PET signal (Figure 3).

Tau burden quantification

Quantification of tau burden in immunostained sections (Figure 4A) revealed robust loads of tau lesions in multiple ROIs analyzed. However, no significant correlation could be detected between [F-18]-AV-1451-PET retention and postmortem tau burden in matching ROIs in any of the three cases (Figure 4B).

Measurement of tau levels by SSD-AGE

SDD-AGE revealed the presence of tau in the form of low molecular weight (LMW) and high molecular weight (HMW) species. Representative SSD-AGE images are shown in Figure 5A (subject #1), Figure 5C (subject #2), and Figure 5E (subject #3). As expected, levels of total tau and phosphorylated tau were substantially lower in the non-Alzheimer tauopathy cases in comparison to AD. In subjects #1 and #3, no significant correlation was detected between tau levels and [F-18]-AV-1451-PET retention in matching ROIs (Figures 5B and 5F). In subject #2, a statistically significant correlation was noticed between total-tau (HMW) and phospho-tau (LMW and HMW) levels and regional [F-18]-AV-1451-PET retention ($R^2=0.369$, $p=0.04$; $R^2=0.588$, $p=0.004$, and $R^2=0.383$, $p=0.032$, respectively) (Figure 5D). Of note, this significance was driven by the basal ganglia, and the correlation was lost when excluding this region from the analysis.

Discussion

We have carefully examined the correlation of *in vivo* and postmortem regional- and substrate-specific binding patterns of PET tau tracer [F-18]-AV-1451 in three autopsy-confirmed non-Alzheimer tauopathy cases (two PSP and one *MAPTP301L* mutation carrier) who underwent imaging prior to death. Our observations are derived from [F-18]-AV-1451 high-resolution autoradiography, [H-3]-AV-1451 *in vitro* binding assays and tau measurements, as reported by immunohistochemistry and SDD-AGE, in brain samples obtained at autopsy. We analyzed the correlation between these postmortem measures and antemortem [F-18]-AV-1451-PET retention in matched ROIs. In agreement with our previous observations,²³ autoradiography experiments revealed strong binding of [F-18]-AV-1451 to NFT primarily made of PHF-tau in AD tissue, but no detectable binding to tau inclusions (mainly composed of straight filaments) in these three non-Alzheimer tauopathy cases. The lack of a consistent and robust correlation between [F-18]-AV-1451 *in vivo* regional retention and topographical distribution of tau inclusions, levels of tracer binding in *in vitro* assays or tau measurements at postmortem in these three cases, suggest that [F-18]-AV-1451 may not have sufficient sensitivity to reliably detect tau aggregates in these non-Alzheimer tauopathies.

[F-18]-AV-1451 came out of compound library screens that used NFT-containing sections from AD brains.⁵ Thus, it is not completely surprising that this tracer preferentially binds to tau filaments that compose NFT and dystrophic neurites in AD, and medial temporal lobe age-related NFT, but it exhibits low affinity for tau inclusions in non-Alzheimer tauopathies

such as Pick's disease, PSP or CBD, or to β -amyloid, α -synuclein or TDP-43 deposits, as recently reported by us and others.^{23–25} It is possible that tau conformation, composition and/or post-translational modifications are critical for [F-18]-AV-1451 binding. Of note, tau lesions in AD contain all six human tau isoforms including 3 and 4 repeat isoforms (3R and 4R), and adopt the twisted ribbon structure of PHF.⁴⁴ The same is the case of tau filaments that form as a function of age, and of a number of other tauopathies including some *MAPT* mutations (like V337M⁴⁵ and R406W⁴⁶). However, in tauopathies like Pick's disease, PSP, CBD or other *MAPT* mutations (like P301L), tau aggregates exist as straight filaments with either predominantly 3R or 4R isoforms.^{26–28, 35, 47}

Recent [F-18]-AV-1451 PET imaging studies consistently show significantly higher tracer retention in patients with MCI and AD dementia compared to controls.^{6–12} Emerging PET data in non-Alzheimer tauopathies, however, provide conflicting results. Some studies report elevated [F-18]-AV-1451-PET retention predominantly in hippocampus, basal ganglia and midbrain of individuals clinically diagnosed with PSP and CTE.^{17–21, 48, 49} However, we and others have noticed that this pattern, also observed in the three cases studied here, seems heavily influenced by the apparent nonspecific retention of this tracer in basal ganglia and off-target binding to neuromelanin-containing neurons in the substantia nigra²³ (Figure 1). Other studies also suggest that this tracer has high affinity for MAO-A and MAO-B, enzymes abundantly present in the human brain, that might also contribute to its off-target binding.^{5, 50} Interestingly, although our PSP cases exhibited very robust tau pathology for example in the pallidus, thalamus and rostral midbrain, where elevated *in vivo* [F-18]-AV-1451-PET retention was noticed, numerous tau inclusions were also present in cerebellum, limbic regions and neocortex where tracer signal was low. Similarly, in the *MAPT*P301L mutation carrier, the brain exam revealed predominant basal ganglia involvement, but abundant tau aggregates were also present in many other regions in cortex and white matter, where no elevated *in vivo* signal was noticed.

To date, only a few [F-18]-AV-1451 neuroimaging-pathologic single case reports have been published.^{51–53} In a R406W *MAPT* mutation carrier, antemortem [F-18]-AV-1451 retention correlated with postmortem distribution and semi-quantitative measures of tau pathology.⁵¹ Interestingly, this *MAPT* mutation is characterized by tau aggregates with PHF ultrastructure,⁵⁴ that contain all 6 isoforms of tau (3R and 4R)⁴⁶ resembling NFT found in AD. In two additional CBD cases *in vivo* uptake correlated with tau immunostaining at postmortem.^{52, 53} However, autoradiography was done only in one of them and showed minimal tracer binding.⁵²

To explore in depth the neuroimaging-pathologic correlation in our three non-Alzheimer tauopathy cases, we analyzed the relationship of *in vivo* [F-18]-AV-1451-PET retention and *in vitro* [H-3]-AV-1451 binding in matched ROIs. We could not find a significant correlation between these measures in any of the cases. Similarly, our sensitive-autoradiography results, showed no detectable binding of [F-18]-AV-1451 to brain regions with high tau burden, suggesting that most of the *in vivo* signal in medial temporal lobe and midbrain in these cases was likely driven by the presence of age-related tangles and off-target binding to neuromelanin-containing neurons in the substantia nigra, respectively, rather than by the 4R tau aggregates characteristic of these disorders. The underlying substrate for the [F-18]-

AV-1451-PET retention in basal ganglia in these and other cases continues to be intriguing. Several groups, including our own, have noticed elevated basal ganglia *in vivo* signal in older adults regardless of the clinical diagnosis, including cognitively intact controls whose brains are not expected to harbor tau pathology in this region.^{6–8, 12} This suggests that the *in vivo* PET signal in this region may represent, at least in part, nonspecific binding influenced by biological or technical factors that occur *in vivo* rather than underlying tau pathology. We cannot rule out with absolute certainty that the ethanol washing steps routinely used in autoradiography and *in vitro* binding assays to eliminate nonspecific binding, may have removed some weaker AV-1451 labeling from basal ganglia.

Importantly, quantification of tau burden in immunostained sections revealed no correlation between tau load at postmortem and [F-18]-AV-1541-PET retention in matching ROIs in any of the three cases. Further analysis of tau pools in ROIs by SDD-AGE allowed us to detect LMW- and HMW-tau aggregates, and compare their amount to the corresponding [F-18]-AV-1541-PET uptake. No significant correlation was detected between these measurements in subjects #1 and #3. Only in subject #2 we detected a significant correlation between the observed *in vivo* signal and amounts of HMW total tau, HMW PHF-tau and LMW PHF-tau. We interpret very cautiously these results because the significance in this case was driven by the basal ganglia, it was lost when this region was excluded from the analysis, and could not be confirmed in the other two cases. Extrapolation of *in vitro* results to *in vivo* imaging findings always requires a very prudent interpretation, especially in the setting of demonstrated off-target binding and potential nonspecific retention.

In conclusion, the results from this study further favor the idea that tau tracer [F-18]-AV-1451 binds with high affinity to tau aggregates in the form of classic PHF-tau tangles containing all six isoforms of tau (including 3R and 4R) present in AD brains and as a function of age. However, they also indicate that this ligand exhibits relatively low binding affinity to tau inclusions predominantly made of 4R isoforms that adopt a structure of straight filaments, like those seen in the autopsy-confirmed PSP and *MAPTP301L* mutation cases presented here. These data suggest that this PET tau tracer may have a limited utility for the reliable detection of tau lesions in these non-Alzheimer tauopathies, and highlight the need for further neuroimaging-pathologic correlation studies to accurately interpret what *in vivo* [F-18]-AV-1451 PET positivity means.

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Figure 1.

Coronal *in vivo* [F-18]-AV-1451 PET images from the three study subjects (A–C). PET images from a cognitively normal 85-yo male (D) and a 78-yo male clinically diagnosed with mild AD (E), using the same color scaling (SUVR values ranging from 1 to 2), are shown for comparison. The three study subjects exhibited increased [F-18]-AV-1451 *in vivo* retention predominantly in basal ganglia and midbrain (subject #1 PSP (A), subject #2 PSP (B) and subject #3 *MAPT*P301L mutation carrier (C)). Elevated uptake in basal ganglia and midbrain, and some lower retention in the medial and inferior temporal regions was also

observed in the 85-yo cognitively normal control (D). PET imaging from a 78-yo male clinically diagnosed with AD showed the typical *in vivo* AV-1451 distribution pattern of AD described in Johnson et al.,⁶ with high retention in the inferior/lateral temporal, parietal, occipital and precuneus/posterior cingulate regions (E). Abbreviations: AD = Alzheimer dementia; MAPT = microtubule-associated protein tau; PET = positron emission tomography; PSP = Progressive Supranuclear Palsy; yo = years old.

Representative images from phosphor screen (A, C, E) and nuclear emulsion autoradiography (B, D, F) of brain slices from the three study subjects. The three cases showed a similar autoradiographic pattern with non-detectable AV-1451 binding across multiple brain ROIs known to contain high burden of tau pathology, including the basal ganglia, a region that exhibited elevated *in vivo* retention. Only two regions in these brains exhibited a strong autoradiographic signal, the EC (asterisk) (reflecting age-related tangle pathology that served as internal positive control for our autoradiographic experiments), and midbrain sections containing the substantia nigra (asterisk) (reflecting tracer off-target binding) (A, C, E). In both regions [F-18]-AV-1451 binding was almost completely blocked after incubating the slides with 1 μ M unlabeled AV-1451. Microscopic images from slices

dipped in the nuclear emulsion and immunostained with mouse PHF-1 antibody (a kind gift of Dr. Peter Davies) showed negligible silver grain deposition colocalizing with PSP tau inclusions and tau grains in the MAPT P301L mutation carrier (B, D, F). As expected, neuromelanin-containing neurons of the substantia nigra were strongly labeled by silver grains (D, F). Scale bars = 20 μm (B right top and bottom images, D bottom images and F), 50 μm (B left top image and D top images), 1 cm (A, C, E). Abbreviations: EC = entorhinal cortex; HPC = hippocampus; MAPT = microtubule-associated protein tau; MRI = magnetic resonance imaging; NFT = neurofibrillary tangles; PET = positron emission tomography; PSP = progressive supranuclear palsy.

Abbreviations: MAPT = microtubule associated protein tau; MRI = magnetic resonance imaging, PET = positron emission tomography; PSP = progressive supranuclear palsy; SUVR = standardized uptake value ratio.

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Figure 4.

(A) Microphotographs from brain tissue slices stained with PHF-1 antibody (kind gift of Dr. Peter Davis, left) and corresponding images displaying the optical threshold used to calculate tau burden using ImageJ software (NIH, right) (representative images from subject #1, PSP: precentral cortex; subject #2, PSP: caudate; subject #3, *MAPT*P301L mutation carrier: putamen). (B) Correlation analysis between tau burden and *in vivo* [F-18]-AV-1451 PET uptake in matching ROIs. In subject #1, the highest tau burden was detected in basal ganglia (3.83%), frontal medial (2.92%) and precentral (2.46%) areas. In subject #2, the highest tau burden corresponded to basal ganglia (1.85%), dentate nucleus of the cerebellum (1.63%) and EC (1.77%). In subject #3, the highest load of tau grains was detected in superior frontal (4.22%), medial frontal (1.85%) and medial temporal areas (1.84%). No significant correlation was detected between postmortem burden of tau aggregates and *in vivo* binding of [F-18]-AV-1451 in matching brain regions (subject #1: $R^2=0.01$, $p=0.70$; subject #2: $R^2=0.09$, $p=0.48$; subject #3: $R^2=0.11$, $p=0.31$) (B). Numbers on the graphs correspond to the multiple ROIs analyzed: Subject #1: 1 = middle frontal, 2 = precentral, 3 = cingulate, 4 = superior temporal, 5 = insula, 6 = putamen, 7 = inferior temporal, 8 = HPC/EC, 9 = cerebellar cortex, 10 = superior colliculus, 11 = red nucleus, 12 = dentate nucleus; Subject #2: 1 = frontal, 2 = cingulate, 3 = basal ganglia, 4 = HPC/EC, 5 = midbrain, 6 = pons, 7 = dentate, 8 = cerebellum; Subject #3: 1 = HPC/EC, 2 = thalamus, 3 = insula, 4 = superior temporal, 5 = medial temporal, 6 = cingulate, 7 = prefrontal, 8 = superior frontal, 9 = medial frontal, 10 = inferior frontal, 11 = midbrain, 12 = cerebellum. Scale bar = 50 μ m. Abbreviations: EC = entorhinal cortex, HPC = hippocampus, MAPT = microtubule associated protein tau; PET = positron emission tomography; PSP = progressive supranuclear palsy, ROI = region of interest.

Figure 5.

Representative images of SDD-AGE membranes stained with total tau and PHF-1 antibodies (A, C, E) and correlation analysis between *in vivo* SUVR values and LMW and HMW tau measurements in matching ROIs (B, D, F). No significant correlation was detected between *in vivo* PET retention and postmortem measurements of total tau and PHF-tau assemblies, as reported by SDD-AGE, in subjects #1 and #3 (B and F). In subject #2, *in vivo* AV-1451 retention was significantly correlated with total tau HMW (R²=0.369, p=0.04), PHF-tau HMW (R²=0.383, p=0.032) and PHF-tau LMW (R²=0.588, p=0.004). Abbreviations: HMW

= high molecular weight; LMW = low molecular weight; PET = positron emission tomography; PHF = paired helical filaments; SDD-AGE = semi-denaturing detergent agarose gel electrophoresis; SUVR = standardized uptake value ratio.

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