Supplementary Online Content

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This supplementary material has been provided by the authors to give readers additional information about their work.

eMethods. Imaging and Neuropathological Analyses

MRI acquisition and processing

Structural MRI was acquired at the UCSF Neuroimaging Center. Fifty months prior to death, the patient underwent a high-resolution T1-weighted magnetization prepared rapid gradient echo (MPRAGE) sequence acquired on a 3T Siemens Prisma Fit (sagittal slice orientation; 160 slices; slice thickness = 1.0 mm; in-plane resolution = 1.0 × 1.0 mm; matrix = 240 × 256; repetition time = 2300 ms; echo time = 2.9 ms; inversion time = 900 ms; flip angle = 9°). For positron emission tomography (PET) reference region definition and spatial warping, the MPRAGE sequence was processed using Freesurfer 5.3 (http://surfer.nmr.mgh.harvard) and Statistical Parametric Mapping version 12 (SPM12, https://www.fil.ion.ucl.ac.uk/spm/) software.[1] Visual assessments were performed blinded to autopsy results but not to clinical history.

PET acquisition and processing

Fifty-two months prior to death, the patient underwent PET scans with ^{18}F-Flortaucipir (tau-PET), ^{11}C-PIB (Aβ-PET), and ^{18}F-FDG (glucose metabolism) at Lawrence Berkeley National Laboratory on a Siemens Biograph 6 Truepoint PET/CT scanner in 3D acquisition mode. Attenuation correction was performed using a low-dose CT/transmission scan acquired prior to each PET scan. The ^{18}F-FTP scan was acquired from 0–100 min after intravenous injection of 9.9 mCi of ^{18}F-FTP. A dynamic ^{11}C-PIB scan was acquired for 90 minutes immediately after intravenous injection of 14.8 mCi. ^{18}F-FDG PET was acquired for 30 minutes (6x5min frames) after 30 minutes of eyes-open quiet rest following intravenous injection of 9.8 mCi of ^{18}F-FDG. Scans were reconstructed using an ordered subset expectation maximum algorithm with weighted attenuation and were smoothed using a 4mm Gaussian kernel with scatter correction (calculated image resolution 6.5 x 6.5 x 7.25 mm using Hoffman). A voxelwise standardized uptake value ratio (SUVR) image was created in native MRI space for ^{18}F-FTP, using data acquired 80- to 100-minutes post-injection and inferior cerebellar gray matter as the reference region. The inferior cerebellar region was created by combining FreeSurfer-derived (FreeSurfer 5.3) cerebellar parcellation and inferior cerebellar parcels from the SUIT template.[2,3] ^{18}F-FDG-PET SUVR image was created using data acquired 30- to 60-minutes post-injection and pons (manually cleaned from FreeSurfer-derived brainstem) as the reference region. For ^{11}C-PIB, a Distribution Volume Ratio (DVR) image was estimated using Logan graphical analysis[4] with FreeSurfer-derived cerebellar gray matter as the reference region. Amyloid positivity at ^{11}C-PIB was assessed both visually by an expert reader (GDR) and via quantification using a neocortical composite score ^{11}C-PIB DVR score <1.08).[5]

W-score map estimation

A voxelwise ^{18}F-FTP W-map was estimated using previously-described methods.[1] This approach generates a single-subject statistical deviation map (W-map) considering normal controls and covariates of interest. W-score distribution is analogous to Z-score distribution and can thus be evaluated considering properties of the Gaussian curve. In the present study, W-maps were estimated considering the nuisance effects of age, which is known to affect ^{18}F-Flortaucipir binding in both striatal and cortical regions.[6] As a control group, we included N=88 cognitively-normal subjects from the Berkeley Aging Cohort Study (mean±sd age: 66.1±19.9, 50% female). Within the control group, amyloid-negativity was confirmed for all the >60 years old cognitively-normal subjects via semi-quantification (neocortical ^{11}C-PIB DVR score <1.08).[5] The patient age-corrected ^{18}F-FTP W-map was evaluated at three different significance levels, from more liberal to more stringent ($p<0.05$, $p<0.01$, $p<0.001$, corresponding to $W>1.65$, $W>2.33$, $W>3.1$), to enhance sensitivity and avoid over-correction.

Neuropathology

Diagnostic assessment

The brain was processed at the UCSF Neurodegenerative Brain Bank as previously described.[7] Histological blocks were sampled from 24 standard regions of interest taken the left cerebral hemisphere. Immunohistochemistry (IHC) for hyperphosphorylated tau (CP13, anti-tau pSer202, gift from Peter Davies), amyloid beta (MAB5206), transactive response DNA binding element 43 (TDP-43, ProteinTech), and alpha-synuclein (LB509) was performed, as previously described.[7] Neuropathological diagnoses for Chronic Traumatic Encephalopathy, limbic-predominant age-related TDP-43 encephalopathy, argyrophilic grain disease, and Alzheimer's disease were rendered according to published criteria.[8–11]

Quantitative tau immunohistochemistry

Histological slides stained with CP13 and counterstained with hematoxylin were digitally scanned on an Axio Scan Z1. Images were down-sampled by a factor of four in both dimensions prior to analysis, then transformed from RGB colorspace to YCrCb (luma, chroma-red, chroma-blue) colorspace. Tau-positive pixels were identified in YCrCb colorspace. Tissue area masks were generated using a low threshold of (1, 1, 1) and a high threshold of (190, 255, 255) also in YCrCb space before application of morphological closing and opening with a 3-pixel structuring element to remove small fragments of tissue. Specific anatomical regions of interest for correlation with PET were delineated by an expert in neuropathology (SS) on the extracted tissue area masks using the Fiji image analysis platform in order to match the imaging ROIs, selected to match our standard neuropathological block sampling scheme.[7,12] Area fractions were calculated as fraction of tau-positive pixels in these regions of interest. All analysis was performed using Python 3.6 bindings for the OpenCV package on an Ubuntu 16.04 workstation.

Imaging-pathology regional quantitative correlation

We extracted average FTP SUVR values from left hemisphere template space regions-of-interest (ROIs) derived from the Human Brainnetome Atlas or hand drawn as previously described.[7] These ROIs correspond to our standard neuropathological block sampling scheme.[7] We excluded the putamen, caudate, globus pallidus, and thalamus from our analysis due to known off-target FTP binding.[6] Subgenual cingulate gyrus, insula, and subthalamic nucleus ROIs were excluded because of proximity with areas with known off-target binding. Brainstem ROIs were also excluded. Brainnetome parcel 103 was used as ROI for the fusiform gyrus.[13] Spearman's rank correlation coefficient was calculated between regional FTP SUVR and tau area fraction as measured by quantitative IHC.

eReferences

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eTable. Neuropsychological testing results at age 68

	Raw score	Z-score/Range
Global Cognition		
MMSE (30)	30	WNL
Memory		
CVLT-II immediate recall (16)	6, 8, 8, 9, 10	0.00
CVLT-II short delay free recall (16)	10	0.50
CVLT-II short delay cued recall (16)	10	0.00
CVLT-II long delay free recall (16)	10	0.50
CVLT-II long delay cued recall (16)	10	0.00
CVLT-II long delay recognition (16)	16 (7 false positives)	0.00
Modified Benson figure recall (17)	13	0.47
Modified Benson figure recognition?	yes	WNL
Language		
Abbreviated BNT (15)	15	WNL
BDAE Sentence Comprehension (5)	4	WNL
Repetition (5)	5	WNL
Abbreviated PPVT (16)	16	WNL
Visuospatial		
Benson figure copy (17)	16	0.67
VOSP Number Location (10)	10	0.60
Calculations (5)	5	WNL
MMSE Pentagons (1)	1	WNL
Attention, Speed, Working Memory, and Executive Functions		
MMSE WORLD backwards (5)	5	WNL
Digit forwards	5	-2.09
Digits backwards	4	-1.36
DKEFS Trail Making Test (number-letter switching)	65 seconds (0 errors)	1.00
Stroop naming	81 (0 errors)	-0.41
Stroop inhibition	49 (1 error)	-0.19
Lexical (D-word) fluency	8	-1.48
Semantic (Animal) fluency	14	-1.51
DKEFS Design Fluency (filled dots)	9 (1 repetition)	-1.00
Psychiatric		
Geriatric Depression Scale (30)	18	Severe range
Neuropsychiatric Inventory – Questionnaire (12)	5	

Neuropsychological testing at age 68. The patient demonstrated impairments in frontally mediated tasks including attention and verbal fluency in the context of relatively preserved episodic memory, language, visuospatial skills, and other examined aspects of executive functioning. His endorsement of depressive symptoms was in the severe range and was characterized by loss of interest, low mood, worry about the future, and cognitive concerns. His informant reported moderate levels of apathy and mild levels of agitation, depression, anxiety, and nighttime behaviors. Abbreviations: BDAE, Boston Diagnostic Aphasia Examination; BNT, Boston Naming Test; CVLT-II, California Verbal Learning Test-II; DKEFS, Delis-Kaplan Executive Function System; MMSE, Mini Mental Status Examination; PPVT, Peabody Picture Vocabulary Test; VOSP, Visual Object and Space Perception Battery; WNL, within normal limits.

eFigure. Timeline