**Supplementary material section**

1. **Neuropathological assessment**

All neuropathological evaluations were performed by the neuropathologists of the ADNI neuropathology core, which is directed by Dr. John C. Morris, and co-directed by Dr. Nigel Cairns, and maintains a central laboratory at the Knight Alzheimer’s Disease Research Center, Washington University School of Medicine, St. Louis, to provide uniform neuropathological assessments of deceased ADNI participants (<http://adni.loni.usc.edu/about/#core-container>).

Neuropathological assessment in the ADNI neuropathology core follows the NIA-AA guidelines for the neuropathological assessment of AD1 and is captured in the format of the Neuropathology Data Form Version 10 of the National Alzheimer Coordinating Center (NACC) and its associated “Neuropathology Data Set Coding Guidebook” (http://adni.loni.usc.edu/methods/).

These guidelines specify the procedures to obtain AD neuropathological change scores, including regional distribution of β-amyloid deposits according to Thal phase (A score), staging of tau pathology according to Braak & Braak (B score), and scoring of neuritic plaque density according to CERAD (C score). In addition, standardized methods for assessing commonly co-morbid conditions such as Lewy body pathology, vascular brain injury, hippocampal sclerosis, and TDP-43 pathology are provided. Evidence of Lewy body pathology was assessed according to McKeith et al.2 as (number of cases):

0 – None (n = 28)

1 - brainstem predominant (n = 2)

2 - limbic (n = 3)

3 – neocortical (n = 19)

4 - amygdala predominant (n = 10)

Of note, extending McKeith et al.2 the Lewy body pathology scoring differentiated within the limbic category between the score 2 for Lewy body pathology in limbic areas (including the amygdala) and the score 4 for Lewy body pathology in the amygdala with paucity of Lewy body pathology in the other regions. Due to the low numbers within some of the subcategories we dichotomized Lewy body pathology into 0-absent (score 0) vs. 1-present (scores 1 through 4).

Scoring for TDP-43 pathology in the ADNI Neuropathology Core followed a previously established classification scheme3 (number of cases):

A – TDP-43 in spinal cord (n = 0)

B – TDP-43 in amygdala (n = 21)

C- TDP-43 in Hippocampus (n = 17)

D – TDP-43 in entorhinal cortex/inferior temporal gyrus (n = 22)

E –TDP-43 pathology in neocortex (n = 6)

21 cases had score B, i.e. they had TDP-43 in the amygdala; a subgroup of 16 of these cases had additional TDP-43 in hippocampus, corresponding to score C. Only one case had score C but not score B, i.e. this case had hippocampal, but not amygdala TDP-43. Twenty of the 21 score B cases had also score D with TDP-43 in entorhinal cortex/inferior temporal gyrus. Only two cases had score D but not score B. Six of the score B cases had also score E. There was no score E case who had not also score B. Thus, in most cases these findings conform with the proposed sequence of TDP-43 pathology scoring in the Limbic-predominant age-related TDP-43 encephalopathy consensus4. Due to the largely overlapping scores with only three deviating cases we decided to dichotomize the TDP-43 scoring into 0-absent (no TDP-43) vs. 1-present (TDP-43 scores B through E).

In addition to these global pathologic scales assessing general presence and regional distribution of pathologic hallmarks, semi-quantitative neuropathological ratings are provided for a total of 22 brain regions, including the Nucleus basalis Meynert (NbM) and medial temporal regions evaluated in the current study.

An overview of the staining methods used by the ADNI neuropathology core for the diverse neuropathological assessments is provided in the ADNI documentation (http://adni.loni.usc.edu/methods/) and published in5. Citing from these resources, the following histological stains were performed: “hematoxylin and eosin, a modified Bielschowsky silver impregnation, and immunohistochemistry (IHC) using antibodies that bind the following antigens: phospho-tau (PHF1, a gift of P. Davies, North Shore-Long Island Jewish Health System), b-amyloid (10D5; Eli Lilly); phospho-a-synuclein (Cell Applications Inc.), and phospho-TDP-43 (Cosmo Bio USA).” According to the described procedures, assessment of Lewy body pathology followed the standard protocol established by McKeith et al. (2005) 2, which includes assessment of both Lewy bodies and Lewy neurites.

Semiquantitative scoring within a given region for Aβ and pTau followed NIA-AA criteria (e.g. C score for neuritic plaques: C0 = none, C1 = 1-5 NP/1mm2, C2 = > 6 < 20, C3 = > 20), and for scoring of P-α-Syn (Lewy body pathology, LB) and pTDP (neuronal cytoplasmic inclusions, NCI) the following criteria were used: 0, none; 1, <1 LB/NCI x10 field; 2, 1-3 LB/NCI; 3, 4-10 LB/NCI; 4, >10 or numerous LB/NCI. Rating of neuronal loss was conducted analogue to the molecular neuropathological markers along the following 4-point scale: 0=0 (none) ; 1=1 (mild) ; 2=2 (moderate) ; 3=3 (severe).

In respect to regional sampling, NbM pathology was evaluated on a coronal slice at the level of the anterior commissure, so it does not include specific subsections of the NbM, neither a specific assessment of the cholinergic neuron population within this area. The hippocampus, including CA1 subfield and dentate gyrus, was sampled on a coronal section at the level of the lateral geniculate nucleus. The same section was also used to sample the parahippocampal gyrus. The entorhinal cortex was sampled on a separate, more anterior section.

1. **Effect of combined neuropathological scores on volumes**

In a complementary analysis, we assessed the degree to which the detected univariate correlations with the diverse neuropathological markers were independent from each other.

Therefore, we calculated additional multiple regression models assessing **the relative contributions** of the neuropathological markers that showed significant effects on the respective MRI-derived volumes in the univariate analyses.

Thus, when considering the **relative contributions of NbM Lewy body pathology** and **Thal phases of amyloid progression** to BF volume loss, a multiple regression model including both predictors, controlling for age, sex, and the distance between MRI and death, revealed a significant effect of NbM alpha-synuclein load with only a trend level effect of Thal phases (supplementary Table 1).

When considering the **relative contributions of TDP-43 and NFT** **load in the dentate gyrus** to hippocampus volume loss, a multiple regression model including both predictors, controlling for age, sex, and distance between MRI and death, revealed a significant effect of NFT, with only a trend level effect of TDP-43 (supplementary Table 2).

**Supplementary Table 1: Combined neuropathological scores and basal forebrain volumes**

|  |  |  |
| --- | --- | --- |
| **Predictors** | **β coefficients** | **p <** |
| Thal phases | -0.221 | 0.070 |
| NbM Lewy body pathology | -0.271 | 0.028 |
| sex | 0.117 | 0.331 |
| age at MRI | 0.205 | 0.091 |
| time from MRI to death | -0.158 | 0.186 |

**Supplementary Table 2: Combined neuropathological scores and hippocampus volumes**

|  |  |  |
| --- | --- | --- |
| **Predictors** | **β coefficients** | **p <** |
| DG TDP | -0.238 | 0.058 |
| DG NFT | -0.367 | 0.006 |
| sex | 0.040 | 0.748 |
| age at MRI | 0.047 | 0.702 |
| time from MRI to death | 0.011 | 0.928 |

**References:**

1. Montine TJ, Phelps CH, Beach TG, et al. National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease: a practical approach. Acta neuropathologica 2012;123:1-11.

2. McKeith IG, Dickson DW, Lowe J, et al. Diagnosis and management of dementia with Lewy bodies: third report of the DLB Consortium. Neurology 2005;65:1863-1872.

3. Nag S, Yu L, Wilson RS, Chen EY, Bennett DA, Schneider JA. TDP-43 pathology and memory impairment in elders without pathologic diagnoses of AD or FTLD. Neurology 2017;88:653-660.

4. Nelson PT, Dickson DW, Trojanowski JQ, et al. Limbic-predominant age-related TDP-43 encephalopathy (LATE): consensus working group report. Brain 2019;142:1503-1527.

5. Franklin EE, Perrin RJ, Vincent B, et al. Brain collection, standardized neuropathologic assessment, and comorbidity in Alzheimer's Disease Neuroimaging Initiative 2 participants. Alzheimers Dement 2015;11:815-822.