BRAIN
ORIGINAL ARTICLE

Age-related and amyloid-beta-independent tau deposition and its downstream effects

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Amyloid-β (Aβ) is hypothesized to facilitate the spread of tau pathology beyond the medial temporal lobe. However, there is evidence that, independently of Aβ, age-related tau pathology might be present outside of the medial temporal lobe. We therefore aimed to study age-related Aβ-independent tau deposition outside the medial temporal lobe in two large cohorts and to investigate potential downstream effects of this on cognition and structural measures.

We included 545 cognitively unimpaired adults (40–92 years) from the BioFINDER-2 study (*in vivo*) and 639 (64–108 years) from the Rush Alzheimer's Disease Center cohorts (*ex vivo*). ^{18}F-RO948- and ^{18}F-flutemetamol-PET standardized uptake value ratios were calculated for regional tau and global/regional Aβ *in vivo*. Immunohistochemistry was used to estimate Aβ load and tangle density *ex vivo*. *In vivo* medial temporal lobe volumes (subiculum, cornu ammonis 1) and cortical thickness (entorhinal cortex, Brodmann area 35) were obtained using Automated Segmentation for Hippocampal Subfields packages. Thickness of early and late neocortical Alzheimer's disease regions was determined using FreeSurfer. Global cognition and episodic memory were estimated to quantify cognitive functioning.

In vivo age-related tau deposition was observed in the medial temporal lobe and in frontal and parietal cortical regions, which was statistically significant when adjusting for Aβ. This was also observed in individuals with low Aβ load. Tau deposition was negatively associated with cortical volumes and thickness in temporal and parietal regions independently of Aβ. The associations between age and cortical volume or thickness were partially mediated via tau in regions with early Alzheimer's disease pathology, i.e. early tau and/or Aβ pathology (subiculum/Brodmann area 35/precuneus/posterior cingulate). Finally, the associations between age and cognition were partially mediated via tau in Brodmann area 35, even when including Aβ-PET as covariate. Results were validated in the *ex vivo* cohort showing age-related and Aβ-independent increases in tau aggregates in and outside the medial temporal lobe. *Ex vivo* age-cognition associations were mediated by medial and inferior temporal tau tangle density, while correcting for Aβ density.

Taken together, our study provides support for primary age-related tauopathy even outside the medial temporal lobe *in vivo* and *ex vivo*, with downstream effects on structure and cognition. These results have implications for our understanding of the spreading of tau outside the medial temporal lobe, also in the context of Alzheimer's disease. Moreover, this study suggests the potential utility of tau-targeting treatments in primary age-related tauopathy, likely already in preclinical stages in individuals with low Aβ pathology.

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Introduction

Amyloid-beta (Aβ) plaques and tau neurofibrillary tangles, the primary hallmarks of Alzheimer's disease, can be present decades before the onset of symptoms.[1,2] According to the Aβ cascade hypothesis, Aβ accumulation is one of the earliest pathological changes to occur in Alzheimer's disease,[3,4] first appearing in the neocortex and spreading to the rest of the brain with disease progression.[5] Tau pathology (neurofibrillary tangles), on the other hand, occurs early in the medial temporal lobe (MTL) regions' transentorhinal and entorhinal cortex[6] before spreading to the neocortex with disease progression. This occurrence outside the MTL is hypothesized to be facilitated by Aβ.[7-9]

In primary age-related tauopathy (PART), however, tau pathology is thought to be present and confined to the MTL in individuals without or with very low levels of Aβ pathology.[10] PART has also been shown to be linked to neurodegeneration and memory decline,[10-13] but less severe than in Alzheimer's disease.[10] Interestingly, accumulating evidence from post-mortem and animal studies suggest that tau pathology in PART, thought to be independent of Aβ, might be present even outside the MTL.[14-17] In addition, tau seeding activity, which is the transcellular propagation of tau protein seeds, may be widespread in the brain even with limited observed tau pathology.[15,18-20] Based on this evidence, it is possible that PART, even in the absence of Aβ pathology, also occurs in regions outside the MTL. Prior *in vivo* PET studies have reported similar findings of age-related tau-PET uptake outside the MTL,[21-23] but not all.[24] However, most studies used first-generation tracers and, except for Lowe *et al.*,[22] had small sample sizes. Thus, investigating this in a large cohort with a second-generation tracer with less off-target binding[25] is important. Additionally, less is known about downstream effects of age-related Aβ-independent tau in this population, especially outside the MTL.

Based on this, we aimed first to assess age associations with Aβ-independent tau deposition within and outside the MTL in a large sample of cognitively unimpaired older adults ($n = 545$) and an Aβ-negative subgroup ($n = 418$). To this end, we investigated regions typically affected during either early, intermediate or late stages of Alzheimer's disease[1] and used a second-generation tau-PET tracer (^{18}F-RO948). Second, to investigate potential downstream effects of age-related tau deposition in ageing, we assessed associations of tau-PET uptake with (i) measures of neurodegeneration within the same regions; (ii) global cognition and episodic memory; and (iii) whether these downstream effects of tau deposition are independent of global and regional Aβ deposition. Third, to extend previous work, we aimed to replicate and validate the results *ex vivo* using an autopsy cohort with neuropathology data ($n = 587$). Fourth, we aimed to examine the validity of the tau-PET tracer with *in vitro* autoradiography.

Materials and methods

Participants

BioFINDER cohort: *in vivo*

We included 545 cognitively unimpaired adults older than 40 years from the Swedish BioFINDER-2 study (NCT03174938), recruited 2017–22, who underwent MRI, Aβ- and tau-PET. The study was approved

by the ethical review board in Lund, Sweden, and all study participants provided written informed consent. None of the included study participants fulfilled the diagnostic criteria for mild cognitive impairment or any type of dementia, determined after thorough clinical and cognitive assessment (described in Palmqvist *et al.*[26]). By design of the BioFINDER-2 study, cognitively unimpaired participants are enriched for *APOE*-ε4 allele carriership.[26]

Harmonized RADC cohorts: *ex vivo*

We used the data of 639 cognitively unimpaired individuals from four Rush Alzheimer's Disease Center (RADC) cohort studies: Religious Orders Study; Rush Memory and Aging Project; Minority Aging Research Study; and African American Clinical Core.[27] None of the included participants fulfilled the diagnostic criteria for mild cognitive impairment or any type of dementia. Aβ status was based on semi-quantitative estimates of neuritic plaque density as recommended by the Consortium to Establish a Registry for Alzheimer's Disease (CERAD).[28] The study was approved by an Institutional Review Board of Rush University Medical Center, all participants signed an informed consent, and a repository consent to share data and biospecimens.[29]

In vitro autoradiography in primary age-related tauopathy

To validate the tau-PET tracer for use in PART, we used tissue sections from fresh-frozen human brain with PART from Lund University, Sweden. This specimen was rated as Braak IV but showed no Aβ plaques deposition (CERAD neuritic plaque score[30] 0, Thal phase[5] 0). ^{3}H-RO948 was tritiated at Roche with a molar activity of 54.3 Ci/mmol and a radiochemical purity higher than 99%. The brain tissue sections were incubated with the radioligand (10 nM) in 50 mM Tris-HCl buffer pH 7.4 at room temperature for 30 min. After washing three times for 10 min at 4°C in 50 mM Tris-HCl buffer pH 7.4 and three quick dips in distilled H_2O at 4°C, the sections were dried at 4°C for 3 h. The sections were placed in a FujiFilm Cassette (BAS 2025), exposed to a FujiFilm Imaging Plate (BAS-IP TR 2025) for 5 days and scanned with a FujiFilm IP reader (BAS-5000) with a resolution of 25 µm per pixel. The autoradiograms were visualized with the software MCID Analysis (v7.0, Imaging Research Inc.). Immunostaining for co-localization of tau aggregates with ^{3}H-RO948 binding was assessed on the same sections using tau-specific antibody AT8 (AT8-Fluor594) conjugated with Alexa594 (7.5 µg/ml). In addition, the Aβ-specific antibody MOAB-2 (MOAB2-Fluor488) conjugated with Alexa488 (5 µg/ml) was used to assess the absence of Aβ within the samples. DAPI (4′,6-diamidino-2-phenylindole) staining was used to visualize nuclei of cells.

BioFINDER imaging protocols

MRI

T_1- and T_2-weighted images were acquired on a Siemens MAGENTOM Prisma 3 T MRI scanner (Siemens Medial Solutions) with a 64-channel head coil. For T_1-weighted images, a magnetization prepared-rapid gradient echo (MPRAGE) sequence [in-plane resolution = 1 × 1 mm^2, slice thickness = 1 mm, repetition time (TR) = 1900ms, echo time (TE) = 2.54 ms, flip-angle = 9°] was used. The T_2-weighted images were acquired with a turbo spin echo sequence (in-plane resolution = 0.4 × 0.4 mm^2, slice thickness = 2 mm, TR = 8240 ms, TE = 52 ms, flip-angle = 150°).

Tau- and Aβ-PET

Aβ- and tau-PET scans were acquired with a digital GE Discovery MI Scanner (General Electric Medical Systems). Tau-PET was performed 70–90 min post-injection of ∼370 MBq of ^{18}F-RO948. Aβ-PET was performed 90–110 min post injection of ∼185 MBq of ^{18}F-flutemetamol. The Swedish Medical Products Agency and the local Radiation Safety Committee at Skåne University Hospital, Sweden, approved the PET imaging.

Structural MRI processing and analysis

Using the Automated Segmentation of Hippocampal Subfields (ASHS) packages for T_1- and T_2-weighted MR images,[31] MTL subregions were automatically segmented to obtain hippocampal subfield volumes (from T_2-weighted MRI) and MTL cortical thickness (from T_1-weighted MRI) measures[32,33] using a new atlas[34] (Supplementary material).

Four MTL regions were selected since they are assumed to be affected early by tau pathology: subiculum, cornu ammonis (CA) 1, entorhinal cortex (ERC) and Brodmann area (BA) 35 (≈transentorhinal cortex). Volumes for the hippocampal subregions (subiculum, CA1) were directly obtained from the ASHS segmentation, averaged across hemispheres and corrected for intracranial volume (using volume-to-intracranial volume fractions). ERC and BA35 thickness measures were obtained using the graph-based multi-template thickness analysis pipeline applied to the T_1-ASHS segmentations[32,35] and averaged across hemispheres.

FreeSurfer 6.0 was used to parcellate the T_1-weighted MRI images. We analysed cortical thickness, averaged across hemispheres, from eight regions of interest (ROIs) (https://surfer.nmr.mgh.harvard.edu/): superior-frontal, orbitofrontal, anterior cingulate, inferior temporal, supramarginal, lateral occipital and two composite regions composed of precuneus/posterior cingulate and a motor composite (pre- and post-central cortex; control region).

All ROIs correspond to early (ERC, BA35, CA1, subiculum, precuneus/posterior cingulate), intermediate (inferior temporal, orbitofrontal, anterior cingulate, supramarginal cortex) or late (superior frontal, lateral occipital) involvement of Alzheimer's disease pathologies during disease progression. Thus, this is based on either the early occurrence of tau (e.g. ERC) and/or Aβ pathology (e.g. precuneus/posterior cingulate).

Tau-PET and Aβ-PET processing and analysis

Standardized uptake value ratio (SUVR) was calculated using an inferior cerebellar reference region for ^{18}F-RO948-PET (tau-PET)[36] and whole cerebellum for ^{18}F-flutemetamol-PET (Aβ-PET).[37] Using the geometric transfer matrix method,[38] partial volume correction was performed for both ^{18}F-RO948-PET and ^{18}F-flutemetamol-PET. See Leuzy *et al.*[39] for details of our processing pipeline.

For ^{18}F-flutemetamol, a neocortical composite SUVR was calculated comprising frontal, parietal and temporal lobe regions.[40] To determine Aβ status (low versus high), a cut-off of 1.03 SUVRs (corresponding to 11 centiloids) was used for global ^{18}F-flutemetamol-PET, previously determined by Gaussian mixture modelling.[26] For 11 individuals, no ^{18}F-flutemetamol-PET data were available, and in these cases Aβ status was determined based on CSF Aβ42/Aβ40 ratio (see 'CSF biomarkers' section). For ^{18}F-RO948-PET, a composite MTL SUVR was calculated using the neocortical MTL regions (ERC and BA35; here denoted ERC/BA35

tau-PET) and excluding the hippocampus to reduce the influence of off-target binding in the choroid plexus. We used putamen tau-PET SUVR in order to take potential off-target or non-specific binding into account.[41] For that purpose, we regressed out age effects on putamen tau-PET signal and used the resulting residuals as a covariate in all primary analyses when estimating regional tau-PET effects. Finally, tau-PET outliers were checked, and none were excluded.

CSF biomarkers

Handling procedure and analysis of CSF followed standardized protocol.[42,43] The concentration of Aβ42 and Aβ40 was measured with the Roche Elecsys platform (Roche Diagnostics International Ltd.) as described by Hansson *et al.*[44] To determine Aβ status, a cut-off of 0.080 (determined by Gaussian mixture modelling[26]) was used for CSF Aβ42/Aβ40 ratio in the 11 cases where ^{18}F-flutemetamol-PET was not available. Seventy-eight individuals did not have CSF Aβ42/Aβ40 ratio data and were excluded from analysis. CSF p-tau181[45] was measured using CSF electrochemiluminescence immunoassay on a fully automated cobase 601 instrument (Roche Diagnostics International Ltd.).

Cognitive assessments

BioFINDER cohort: *in vivo*

For the BioFINDER cohort, a modified version of the Preclinical Alzheimer's Cognitive Composite 5 (mPACC5)[46] was calculated using the Mini-Mental State Examination,[47] Alzheimer's Disease Assessment Scale-Cognitive subscale (ADAS)[48] delayed word list recall (double-weighted), Symbol Digit Modalities test[49], and animal fluency. For details, see Binette *et al.*[50] To examine episodic memory, we calculated the number of failures of the delayed recall and mean failures of the immediate recall of the ADAS word list (out of 10). All test scores were normalized based on the values of the Aβ− subgroup and, if applicable, reversed (for episodic memory), so a higher score equaled better performance for the mPACC5 and episodic memory score.

Harmonized RADC cohorts: *ex vivo*

For the RADC cohort, global cognition closest to death was estimated using a z-score, based on the mean and standard deviation of the combined cohorts baseline visit, average of 19 tests from five cognitive domains (Supplementary material).[51,52] A value lower than zero indicates a score worse than the baseline average of the combined cohorts. Episodic memory closest to death was estimated in the same manner with seven tests (Supplementary material).

Neuropathological assessment of RADC cohorts

All details concerning the neuropathological data have been described elsewhere.[29,51,53] Aβ load and paired helical filament (PHF) tau tangle density information from immunohistochemistry were used for main analyses, following the National Institute on Aging guidelines for neuropathologic assessment.[28] The neuropathological data contains burden of Aβ proteinopathy identified by immunohistochemistry using monoclonal antibodies against 1–40 and 1–42 Aβ, 4G8 (1:9000; Covance Labs), 6F/3D (1:50; Dako North America Inc.) and 10D5 (1:600; Elan Pharmaceuticals). The overall score is based on percentage area of cortex in eight brain regions: hippocampus, ERC, midfrontal, inferior temporal, angular gyrus, calcarine, anterior cingulate and superior frontal cortex.[53] Overall Aβ pathology load was calculated as the mean square root transformation of the score in these eight regions. PHF tau tangle density was assessed by stereology. Overall tau pathology burden scores were calculated using immunohistochemistry with AT8, an antibody specific to phosphorylated tau, and the square root taken. CERAD estimates were generated from modified Bielschowsky-stained sections. As a semiquantitative measure of neurofibrillary tangles, Braak stages were determined with Bielschowksy silver staining in frontal, temporal, parietal, ERC and hippocampus.[6,54] Aβ pathology was additionally quantified using Thal phases[5] for a subset of individuals. A high portion (31.4%) of individuals with low CERAD semiquantitative estimates of neuritic plaque density (no to possible Alzheimer's disease) was not quantified on Thal phases. Therefore, we used CERAD neuritic plaque scores to determine Aβ status.

Statistical analyses

Analyses were performed in R 4.0.2.[55] First, Pearson partial correlations were calculated ('ppcor' package[56]) to assess associations between (i) age and regional tau-PET including sex and age-independent putamen signal as covariates; and (ii) including sex, age-independent putamen signal, and regional Aβ-PET as covariates. Note that the residual of the association of age and putamen SUVR was included to adjust for age-independent effects of potential off-target binding. Only tau- and Aβ-PET measures within the same ROI were associated with each other. Analyses were repeated in the Aβ− subgroup. Second, associations between ROI thickness/volume (i) with age (covariates: sex, Aβ-PET); and (ii) with the corresponding regional tau-PET (covariates: sex, age-independent putamen signal, Aβ-PET) were estimated individually. Again, analyses were repeated in the Aβ− subgroup. Lastly, associations between regional tau-PET, age, regional thickness/volume, and cognitive measures were estimated (covariates: regional Aβ-PET, sex, education). These analyses were repeated, including age as an additional covariate. To investigate the MTL tau only, an ERC/BA35 tau-PET composite was used. All *P*-values were reported after false discovery rate (FDR, $P < 0.05$, Benjamini–Hochberg procedure).

Second, mediation models were fitted ('lavaan' package,[57] bootstrapping = 500). First, we fitted mediation models with regional Aβ-PET as the mediator of significant age-regional tau-PET SUVR associations (covariate: sex). Second, regional tau-PET SUVR as the mediator of significant age-volume/thickness associates was investigated (covariates: sex, regional Aβ-PET). Third, regional tau-PET SUVR as the mediator of significant age-cognition associations was in investigated (covariates: sex, education, regional Aβ-PET).

The same analyses were performed in the neuropathological cohort, using partial Spearman rank correlations due to the nature of the data. The associations between (i) age and regional tau tangles (covariate: sex); (ii) age and regional tau tangles (covariates: sex, Aβ); (iii) age with global cognition or episodic memory (covariates: sex, education, Aβ); (iv) tangles with global cognition (covariate: sex, education); and (v) tangles with global cognition (covariates: sex, education, Aβ) in the whole sample and the Aβ− subgroup were investigated. Additionally, we fitted mediation models investigating the mediating effects of regional tau tangles on the association of age and cognition.

Seven *in vivo* sensitivity analyses were performed. First, to potentially capture earlier Aβ accumulation, we repeated the analyses using the CSF Aβ42/Aβ40 ratio as covariate. Second, we repeated

analyses using global Aβ-PET as a covariate. Third, since the tau-PET tracer may show unspecific binding, we repeated the analyses excluding cases with high skull/meningeal binding (described in Pichet Binette *et al.*[58]). Fourth, we examine the associations between regional tau-PET and CSF p-tau181. Fifth, analyses were repeated including *APOE*-ε4 carriership as a covariate. Sixth, while model assumptions for Pearson partial correlations were met, we used robust regression models[59-61] to examine robustness for significant associations. Two *ex vivo* sensitivity analyses were performed. First, we repeated analyses defining Aβ status based on Thal phases. Second, we repeated analyses using global Aβ burden.

Data availability

Anonymized data from BioFINDER will be shared on request from a qualified academic investigator for the sole purpose of replicating procedures and results presented in the article and as long as the data transfer is in agreement with EU legislation on the general data protection regulation and decisions by the Swedish Ethical Review Authority and Region Skåne, which should be regulated in a material transfer agreement.

A request to receive the data of the RADC cohorts can be submitted under: https://www.radc.rush.edu/.

Results

Demographics

Demographics of the *in vivo* cohort with cognitively unimpaired older adults ($n = 545$, 53% female, mean age 65, mean education 13 years, 49% *APOE*-ε4 carriers), the *in vivo* Aβ− subgroup ($n = 418$, 52% female, mean age 61, mean education 13 years, 42% *APOE*-ε4 carriers; Table 1) and of the neuropathological dataset ($n = 639$, mean age 87.4 ± 7.00, 33% female, mean education 16 years) can be found in Table 1 and Supplementary Tables 1 and 2.

Autoradiography

The ^{3}H-RO948-PET tracer was validated *in vitro* with autoradiography using cortical tissue sections from a PART case (Fig. 1). The results show that the tracer binds to tau pathology (based on AT8 staining) in the parahippocampal gyrus even in the absence of Aβ pathology.

Aβ-independent associations of age and regional tau-PET signal within and outside the MTL

In vivo: BioFINDER-2 cohort

We first investigated the association between age and regional tau-PET signal in the whole sample. Age was positively associated with regional tau-PET signal in the MTL ($r_P = 0.36$, $P_{FDR} < 0.001$), as well as widespread neocortical ROIs (inferior temporal, superior frontal, orbitofrontal, anterior cingulate, posterior cingulate/precuneus, supramarginal and lateral occipital; $r_P = 0.15$–0.36, $P_{FDR} < 0.001$), adjusting for sex and tau-PET off-target putamen binding (Fig. 2A).

To examine if these associations are independent of Aβ, we adjusted the age and regional tau-PET associations for regional Aβ-PET signal (Fig. 2B and Supplementary Table 3). Age was still positively associated with medial temporal tau-PET ($r_P = 0.24$, $P_{FDR} < 0.001$) and interestingly, although with relatively small effect sizes, also with superior frontal, orbitofrontal, anterior cingulate, precuneus/posterior cingulate, and motor cortex tau-PET ($r_P = 0.11$–0.27, $P_{FDR} = 0.023$–<0.001), regions typically not implicated in early tau deposition. Mediation models show similar results (Supplementary Fig. 1).

In the Aβ− subgroup similar widespread associations between age and regional tau-PET tracer uptake were observed (Fig. 2E and F; $r_P = 0.14$–0.46, $P_{FDR} = 0.007$–<0.001), with the largest effect sizes for precuneus/posterior cingulate and ERC/BA35 and smallest effect sizes for inferior temporal, supramarginal and lateral occipital

Table 1 Sample characteristics of the BioFINDER (*in vivo*) and the RADC (*ex vivo*) cohorts for the whole cognitively unimpaired and Aβ− subgroup

BioFINDER cohort	Whole sample	Aβ−[a]
n	545	418
Sex, % female	52.6	52.3
Age, years	65.0 ± 11.7 [40–92]	60.9 ± 11.5 [40–88]
APOE-ε4 carrier, %	48.6	42.3
Education, years	12.8 ± 3.4	12.9 ± 3.3
mPACC5, z-scored	0.06 ± 0.81	0.17 ± 0.76
Episodic memory, z-scored	−0.23 ± 1.89	0.01 ± 1.81
CSF Aβ42/Aβ40 ratio	0.10 ± 0.028	0.11 ± 0.02
Global Aβ-PET SUVR	1.01 ± 0.35	0.85 ± 0.06
ERC/BA35 tau-PET SUVR	1.19 ± 0.49	1.05 ± 0.32
RADC cohorts	**Whole sample**	**Aβ−[b]**
n	639	318
Sex, *n* (% female)	205 (32.1)	112 (35.2)
Age at death, years	87.2 ± 7.2 [64–108]	86.0 ± 7.5 [64–108]
Education, years	16.3 ± 3.7	16.1 ± 3.7
APOE-ε4 carrier, *n* (%)	93 (16)	35 (11.0)
Braak stages, *n* (%)		
0	15 (2.4)	11 (3.5)
I	63 (9.9)	51 (16.0)
II	105 (16.5)	75 (23.6)
III	210 (32.9)	116 (36.5)
IV	198 (31.0)	–
V	47 (7.4)	–
VI	–	–
CERAD, *n* (%)		
Definite	103 (16.1)	–
Probable	218 (34.0)	–
Possible	71 (11.1)	71 (22.3)
No Alzheimer's disease	247 (38.7)	247 (77.7)
Thal phase, *n* (%)		
0	84 (12.9)	84 (26.1)
1	110 (16.9)	92 (28.6)
2	27 (4.2)	16 (5.0)
3	188 (29.0)	22 (6.8)
4	82 (12.6)	3 (0.9)
5	53 (8.2)	4 (1.2)
Aβ load, mean	1.12 ± 1.04	0.36 ± 0.57
Total tangle density, mean	3.33 ± 3.78	2.24 ± 2.38
Global cognition, z-scored	0.14 ± 0.45	0.20 ± 0.44
Episodic memory, z-scored	0.38 ± 0.57	0.44 ± 0.53

Continuous variables are displayed as mean ± standard deviation; age range is presented within square brackets. Categorical variables are displayed as *n* (%). mPACC5 = modified Preclinical Alzheimer's Cognitive Composite; RADC = Rush Alzheimer's Disease Center; SUVR = standardized uptake value ratio.
[a]Aβ− is defined, in BioFINDER, as a global SUVR <1.03 or <0.08 on CSF Aβ42/Aβ40 ratio if PET was not available.
[b]In the RADC cohorts, the Aβ− subgroup is based on semiquantitative estimates of neuritic plaque density as recommended by the Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Sensitivity analyses are done defining the Aβ− subgroup according to Thal phases (0–1).

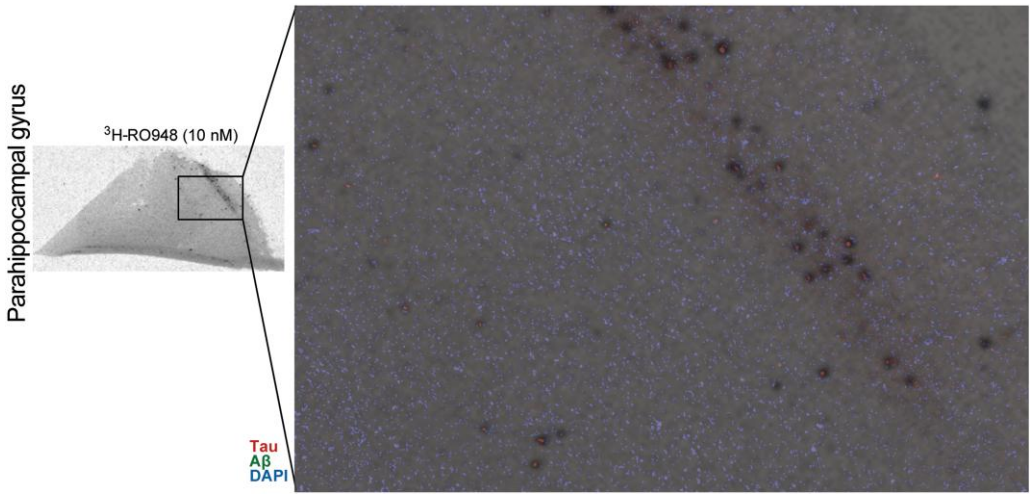

Figure 1 ^{3}H-RO948 binds to tau pathology in the parahippocampal gyrus in PART. DAPI = 4′,6-diamidino-2-phenylindole.

cortex. All associations remained statistically significant when adjusting for regional Aβ-PET (r_P = 0.12–0.42, P_{FDR} = 0.022–<0.001), except for motor cortex tau-PET.

Ex vivo: RADC cohort

In the neuropathological dataset, age and tau tangle score were positively associated for all regions except lateral occipital tau tangles (Supplementary Table 6; rho_P = 0.10–0.39, P_{FDR} = 0.020–<0.001). After adjusting for regional Aβ load, age was significantly associated with CA1 and ERC tau tangles and with smaller effect sizes to inferior temporal and anterior cingulate cortex tau tangle load (Fig. 2C and D; rho_P = 0.11–0.37, P_{FDR} = 0.024–<0.001). This supports the *in vivo* results of potential Aβ-independent age-related tau deposition within and outside the MTL.

In the Aβ– subgroup, Aβ-independent associations between age and tau tangles were found for CA1 and entorhinal, inferior temporal, and anterior cingulate cortex, suggesting a potential age-tau tangle association in and outside the MTL (rho_P = 0.15–0.39, P_{FDR} = 0.032–<0.001), where weaker, but statistically significant correlations were observed for the latter two. It should be noted that tau tangle burden was not available for parietal regions (Fig. 2).

Association between age and regional structural measures is partially mediated by tau-PET signal

In the next step we investigated the associations of tau-PET SUVR with regional volume and ROI thickness, while adjusting for sex, regional Aβ-PET, and the off-target variable (Fig. 3A and for details see Supplementary Table 7). Higher ERC/BA35 tau-PET SUVR was associated with lower subiculum (r_P = –0.30, P_{FDR} < 0.001) and CA1 (r_P = –0.19, P_{FDR} = 0.002) volume and BA35 thickness (r_P = –0.24, P_{FDR} < 0.001; Fig. 3A). For neocortical regions, higher regional tau-PET SUVR was associated with lower cortical thickness for inferior temporal cortex (r_P = –0.15, P_{FDR} = 0.002) and, regions typically associated with early Aβ accumulation, precuneus/posterior cingulate cortex (r_p = –0.20, P_{FDR} < 0.001; Fig. 3A). This suggests potential Aβ-PET-independent associations between tau-PET signal and neurodegeneration in these regions. While not focus of this paper, we adjusted the analyses for age and found that the results remained significant. This indicates that there are also age-independent tau-PET and volume/thickness relationships (Fig. 3B).

To further focus on the role of age in the tau and volume/thickness relationships, we examined whether tau-PET signal mediates the association between age and structure volume/thickness (Fig. 3D). The mediation models indicate that regional tau-PET partially mediates this association in an Aβ-independent manner for subiculum (c-c′ = –0.06, 95% CI: –0.08:–0.03, P < 0.001, 15%) and BA35 (c-c′ = –0.04, 95% CI: –0.06:–0.02, P = 0.001, 15%), and for precuneus and posterior cingulate in the whole cognitively unimpaired sample (c-c′ = –0.02, 95% CI: –0.03:–0.01, P = 0.034, 5%; Fig. 3D). Note that the tau-PET mediating effects explained only a small portion of the variance of the age-structure relationship.

In the Aβ– subgroup, higher ERC/BA35 tau-PET was significantly associated with lower volume in subiculum (r_P = –0.23, P_{FDR} < 0.001) and CA1 (r_P = –0.18, P_{FDR} = 0.003), and lower BA35 (r_P = –0.30, P_{FDR} < 0.001) and ERC (r_P = –0.12, P_{FDR} < 0.001) thickness; higher precuneus/posterior cingulate tau-PET was associated with reduced thickness in that region (r_P = –0.19, P_{FDR} < 0.001; Fig. 3 and Supplementary Table 7), again adjusting for Aβ-PET and sex. This indicates direct regional tau-PET effects of the age-structure association appear to be Aβ-independent for these regions. The association between ERC/BA35 tau-PET and BA35 and ERC thickness survived adjusting for age. Mediation models examining the role of age in the tau and volume/thickness relationships in this subgroup, indicate that ERC/BA35 tau-PET partially mediates the association between age and regional volume/thickness for BA35 (c-c′ = –0.06, 95%CI: –0.09:–0.03, P < 0.001, 18%; Fig. 3F).

MTL tau-PET signal partially mediates the association between age and global cognition

In vivo: BioFINDER-2 cohort

Next, we explored the association of imaging measures and cognitive performance (global cognition and episodic memory). Analyses were only conducted for regions with significant Aβ-independent effects of tau-PET signal on volume/thickness (i.e. subiculum, CA1, BA35, inferior temporal, and precuneus/posterior cingulate cortex; Fig. 4).

In the whole sample of cognitively unimpaired older adults, significant associations with global cognition (mPACC5) and episodic memory were found for higher age, ERC/BA35 tau-PET, subiculum and CA1 volume, and BA35, inferior temporal, and precuneus/

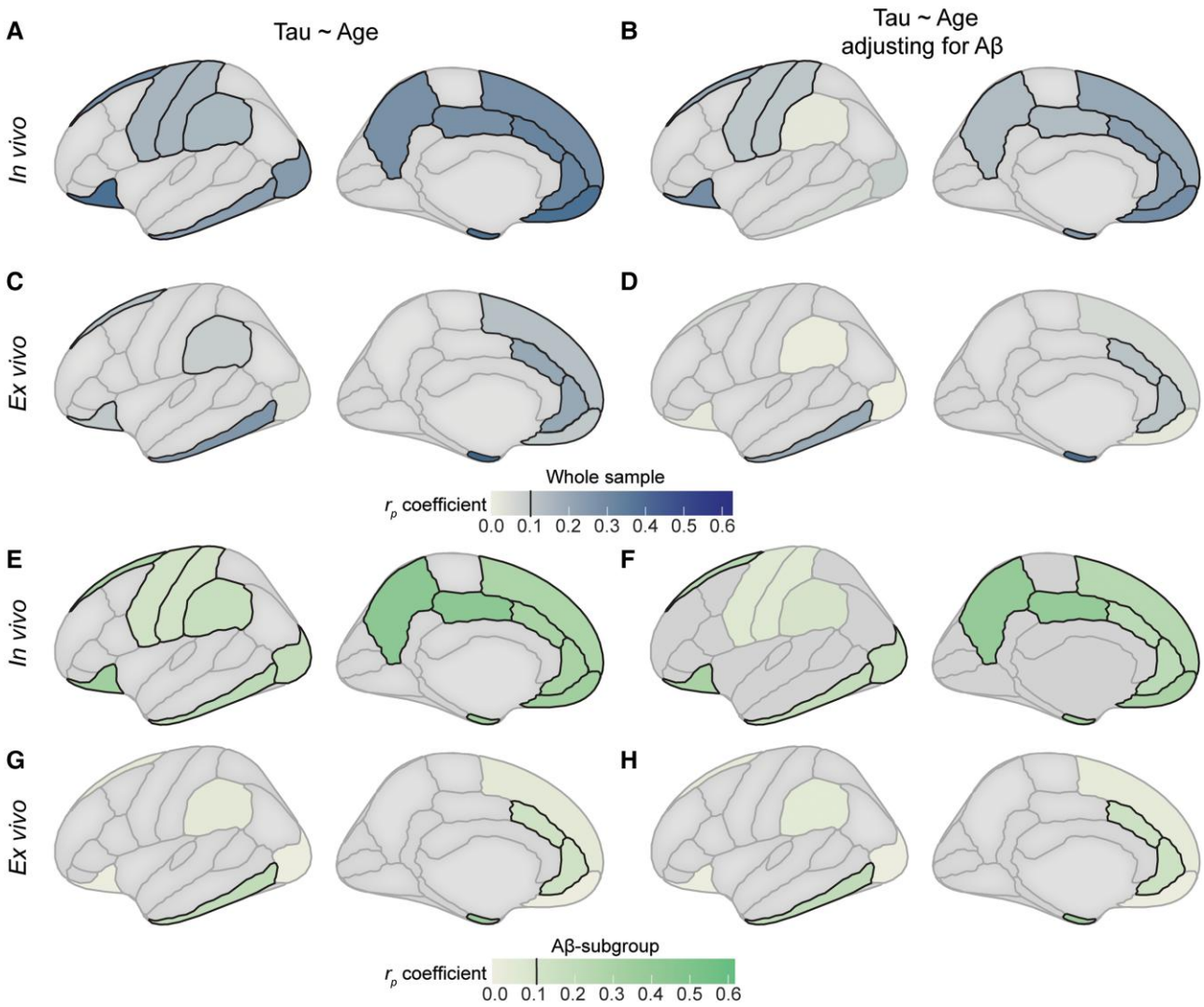

Figure 2 Association between age and regional tau-PET ^{18}F-RO948 uptake (*in vivo*) or regional tau tangles (*ex vivo*) in the whole samples and Aβ− subgroups. (**A**) Increasing age is associated with increased regional tau-PET ^{18}F-RO948 tracer uptake in medial temporal, parietal, and frontal regions. (**B**) Aβ-PET independent associations between age and regional tau-PET signal in regions from **A**. (**C**) Increasing age is associated with increased regional tau tangles in medial temporal, parietal and frontal regions. (**D**) Aβ-PET independent associations between age and regional tau tangles in temporal and frontal regions. **E**–**H** show the results of the same analysis as **A**–**D** but in the Aβ− subgroup. Pearson partial correlation coefficients/Spearman rank correlations > 0.10 are $P_{FDR} < 0.05$ (dark outline). Light grey regions were not investigated. Similar results are found using global Aβ-PET.

posterior cingulate thickness (see Supplementary Table 8 for details). Higher inferior temporal and precuneus/posterior cingulate tau-PET SUVRs were only associated with reduced global cognition but not episodic memory. Most of these correlations were relatively weak. While, again, not primary focus of this paper, when additionally adjusting for age, only ERC/BA35 tau-PET was significantly associated with reduced global cognition and episodic memory scores ($r_P = -0.16$, $P_{FDR} = 0.001$; $r_P = -0.14$, $P_{FDR} = 0.002$). This suggests that there is also an age-independent association between ERC/BA35 tau-PET and cognition.

Further focusing on the role of age in the tau and cognition relationship, we performed mediation analyses of the age-cognition association with ERC/BA35 tau-PET. Similar to the mediation models of age-thickness/volume associations, mediation models showed that there is a partial mediation of ERC/BA35 tau-PET on the age-global cognition association (Fig. 4B; $c\text{-}c' = -0.04$, 95% CI: −0.06:−0.01, $P = 0.002$, 10%) partially independent of Aβ. Mediations of the association between age and episodic memory showed a partial mediation of ERC/BA35 tau-PET, independent of regional Aβ, sex, and education (Fig. 4D; $c\text{-}c' = -0.03$, 95% CI: −0.06:−0.01, $P = 0.013$, 9%). These partial mediations hold also when adding subiculum volume or BA35 thickness as a covariate, indicating that this is independent of atrophy in these regions. Imaging measures in other regions were not associated with the cognition measures (Supplementary Table 8). Again, it should be noted that only a small portion of the variance of the age-cognition relationship was explained by the mediating effects of tau-PET.

In the Aβ− subgroup, higher age was significantly associated with global cognition ($r_P = -0.36$, $P_{FDR} < 0.001$) and episodic memory ($r_P = -0.26$, $P_{FDR} < 0.001$). Higher ERC/BA35 and precuneus/posterior cingulate tau-PET were significantly associated with reduced global cognition (Supplementary Table 8). ERC/BA35 tau-PET was also significantly associated with reduced episodic memory. Subiculum and CA1 volume, as well as BA35, inferior

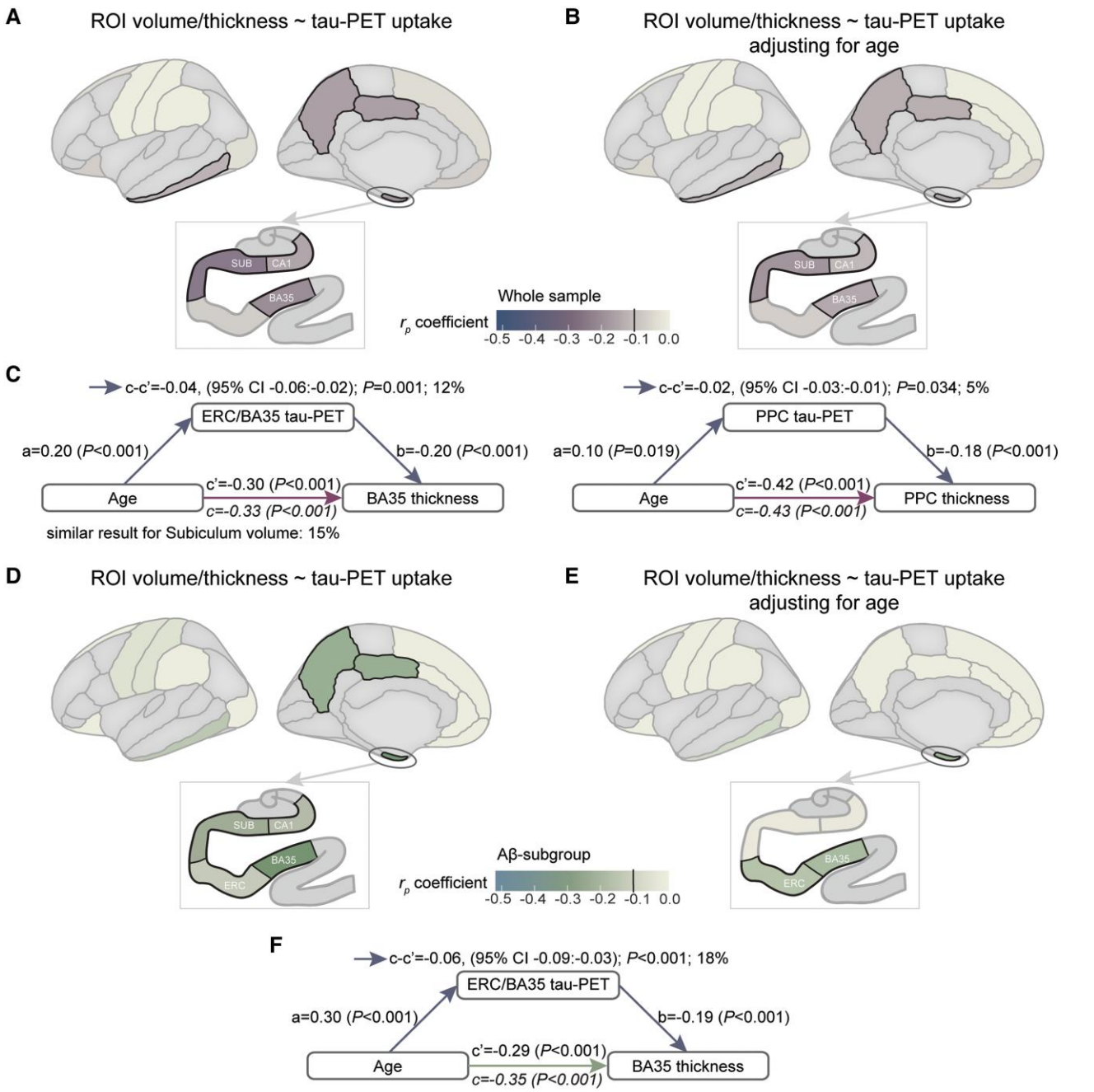

Figure 3 Age and regional tau-PET ^{18}F-RO948 uptake associations with regional volume/thickness in the whole sample and Aβ− subgroup. (**A**) Increasing regional tau-PET SUVR associated with decreased thickness/volume in temporal and parietal regions including sex and regional Aβ-PET signal as covariates. (**B**) Increasing regional tau-PET SUVR associated with decreased thickness/volume in medial temporal and parietal regions additionally adjusting for age. (**C**) There is an Aβ-independent effect of regional tau-PET signal on the age-structure associations in the whole group for MTL and parietal regions (paths a and b). The direct effect is reported below the arrow (in italics) going from age to structure. (**D**) In the Aβ− subgroup, increasing regional tau-PET SUVR is associated with decreased thickness/volume in medial temporal and parietal regions. (**E**) In the Aβ− subgroup, increasing regional tau-PET SUVR is associated with decreased thickness/volume in medial temporal and parietal regions additionally adjusting for age. (**F**) In the Aβ− subgroup, there is an Aβ-independent effect of regional tau-PET signal on the age-structure associations for BA35. Models including tau-PET are adjusted for age-independent putamen tau-PET tracer uptake. Pearson partial correlation coefficients > 0.10 are $P_{FDR} < 0.05$ (dark outline). Light grey regions were not investigated. Similar results are found using global Aβ-PET. BA = Brodmann area; CA1 = cornu ammonis 1; ERC = entorhinal cortex; MTL = medial temporal lobe; PPC = precuneus/posterior cingulate cortex; ROI = region of interest; SUB = subiculum.

temporal, and precuneus/posterior cingulate cortex thickness were associated with reduced global cognition and episodic memory scores. None of the associations remained statistically significant after adjusting for age. Also, no significant mediation effects were found.

Ex vivo: RADC cohort

In the full neuropathological cohort of cognitively unimpaired older adults, age was associated with global cognition ($rho_P = -0.17$, $P_{FDR} < 0.001$; Supplementary Table 9 and Supplementary Fig. 6). Tau

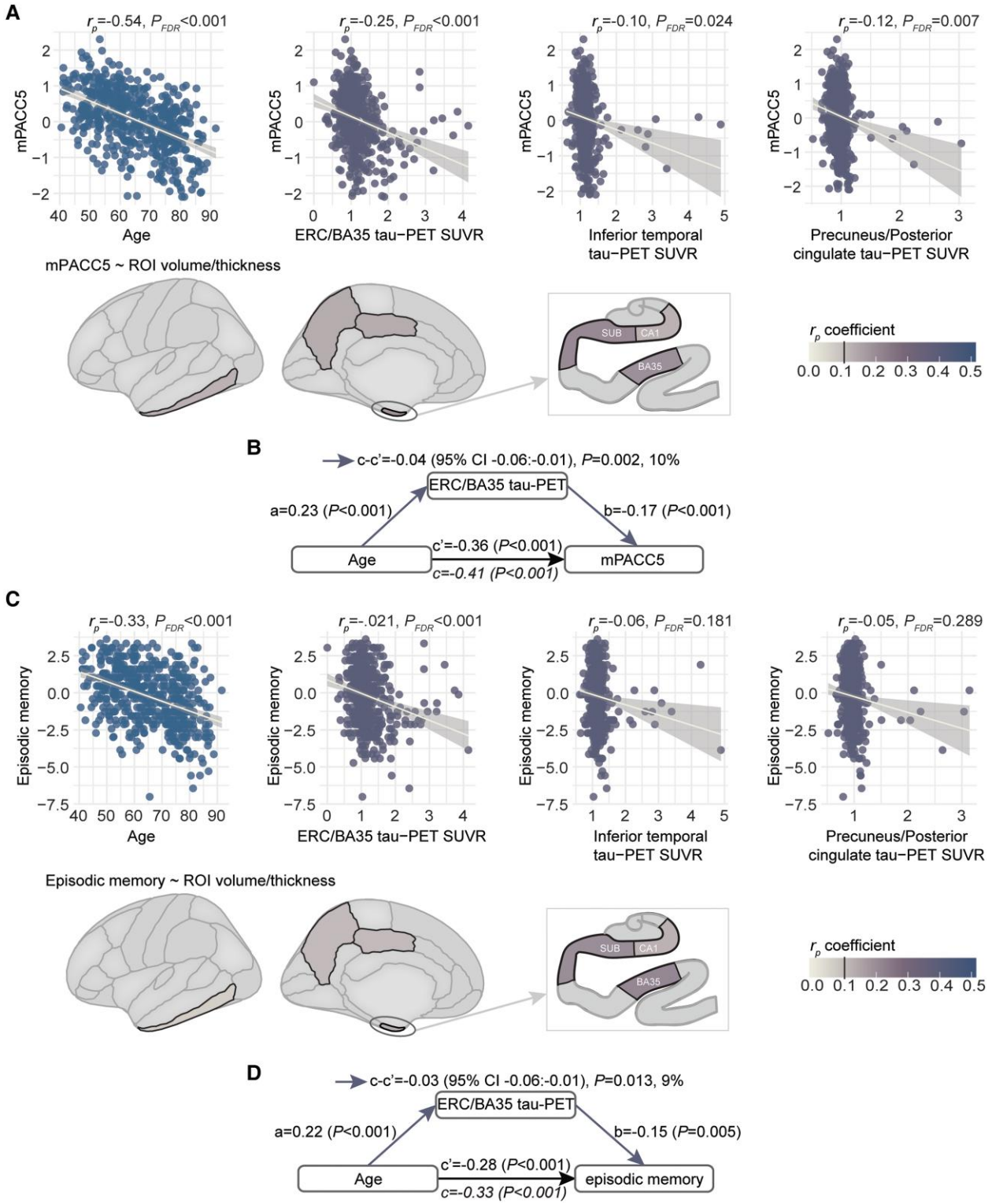

Figure 4 Age, regional tau-PET and regional volume/thickness associations with cognition in the whole BioFINDER sample. (**A**) Increasing tau-PET SUVR, age and lower volumes/thickness in selected regions are associated with reduced global cognition. Associations are adjusted for sex, education and the other predictors (and off-target binding variable if tau-PET is the main predictor). (**B**) There is an Aβ-independent mediating effect of ERC/BA35 tau-PET SUVR on age-mPACC5 association in the whole group (blue path). The direct effects are reported below the arrow (in italics) going from age to mPACC5. (**C**) Increasing ERC/BA35 tau-PET SUVR, age, and lower volumes/thickness are associated with reduced episodic memory. Associations are adjusted for sex, education, and off-target binding variable if tau-PET is the main predictor. (**D**) ERC/BA35 tau-PET SUVR partially mediates the age-episodic memory association. Similar results are found using global Aβ-PET. Pearson partial correlation coefficients > 0.10 are $P_{FDR} < 0.05$. BA = Brodmann area; CA1 = cornu ammonis 1; ERC = entorhinal cortex; mPACC5 = modified Preclinical Alzheimer's Cognitive Composite; SUB = subiculum; SUVR = standardized uptake value ratio.

tangles in frontal, parietal, and temporal regions were negatively, but weakly, associated with global cognition ($rho_P = -0.10–0.17$, $P_{FDR} = 0.046–0.002$). Tau load in temporal regions partially mediated the age-global cognition association (Supplementary Fig. 6; variance explained: 12–29%). Additionally, tau tangles in frontal and temporal regions, but not age, were weakly but significantly associated with episodic memory ($rho_P = -0.11–0.21$, $P_{FDR} = 0.030–<0.001$).

In the Aβ− subgroup, only higher ERC tau load was associated with reduced episodic memory ($rho_P = -0.20$, $P_{FDR} = 0.021$; Supplementary Table 9).

Sensitivity analyses

In vivo, the results remained consistent even when excluding individuals with high meningeal/skull tau-PET binding (Supplementary Tables 1–9), using robust regression models (data not shown) or additionally adjusting the analyses for *APOE-ε4* carriership (data not shown), and when using CSF Aβ42/Aβ40 ratio instead of regional Aβ-PET as a covariate (Supplementary Tables 1–9). Results further converged with the primary analyses when using global instead of regional Aβ-PET as a covariate for Pearson partial correlations and mediation models (age-thickness/volume associations: Supplementary Fig. 4; age-cognition associations: mPACC5: $c\text{-}c' = -0.03$, 95% CI: −0.04:−0.01, $P = 0.010$, 8%; ADAS: $c\text{-}c' = -0.03$, 95% CI: −0.05:−0.01, $P = 0.015$, 9%). Note that age-tau associations in the Aβ− subgroup yielded slightly stronger results (Supplementary Table 3). Finally, tau-PET measures were significantly associated with CSF p-tau181 for all but the motor region in the whole sample and for temporal and frontal regions in the Aβ− subgroup (Supplementary Table 5). While CSF and PET measures are both proxies of tau pathology with their own limitations, the positive correlations between the two supports the use of tau-PET as a proxy of tau pathology in the brain, even in Aβ− cases.

Ex vivo, similar results are found when defining the Aβ− subgroup with Thal stages, except for the age and anterior cingulate cortex tangle association ($rho_P = 0.09$, $P_{FDR} = 0.454$). Moreover, the ERC tau load and episodic memory associations was slightly weaker and not significant ($rho_P = -0.16$, $P_{FDR} = 0.248$) potentially due to the decreased power in this smaller sample.

Discussion

We investigated age-related tau-PET deposition patterns and potential downstream effects on brain structure and cognition in a large cohort of cognitively unimpaired older adults as well as in a subgroup with low Aβ levels. Our study highlights two main findings. First, in this cognitively unimpaired sample, the occurrence of age-related tau-PET signal was observed within the MTL but also in several neocortical areas (e.g. inferior temporal, precuneus/posterior cingulate, orbitofrontal, anterior cingulate, inferior parietal, superior frontal) and appeared to be partially independent of Aβ-PET. This was supported by the analyses in the Aβ− subgroup and in the neuropathological cohort, where positive associations between age and tangle density were found in temporal and frontal regions. Second, this partially Aβ-PET-independent age-related tau-PET retention appears to exert downstream effects on structure and cognition within the MTL and, interestingly also in the precuneus/posterior cingulate in our sample. These findings indicate that tau pathology may accumulate outside the MTL in 'normal' ageing and already exerts modest downstream effects on structure and global cognition.

We found a significant age-related increase in tau-PET retention (*in vivo*) and tau tangle density (*ex vivo*) in the MTL in cognitively unimpaired older adults with no or low amounts of Aβ. This is in line with previous *in vivo* PET studies.[21,23] We additionally find similar, albeit weaker, associations outside the MTL. The observation of age-related tau deposition within, and to some extent outside, the MTL in Aβ− individuals is in agreement with the definition of PART,[10] where tau pathology is reported in the insula and inferior temporal gyrus in Braak stages III and IV. However, our findings suggest that age-related tau pathology goes beyond these Braak stage III-IV regions and includes, among others, the anterior and posterior cingulate and the precuneus. The occurrence of tau pathology in more widespread neocortical regions is in line with recent neuropathological studies, which indicate that tau pathology may occur outside the MTL independently of Aβ and widespread tau seeding activity is not confined to the MTL in cognitively unimpaired adults.[15,18-20] In addition, also *in vivo* studies showed tau-PET retention outside the MTL in Aβ- and tau-positive older individuals[62,63] and in cognitively unimpaired individuals with low Aβ levels.[21-23,64,65] Yet, our study uses a second-generation tau-PET tracer and a larger sample size compared to prior *in vivo* studies. While our results suggest that there is tau pathology in these regions independent of Aβ pathology, it is possible that there are individuals in our study which have Aβ pathology not detectable by our methods. It is unlikely that our results were severely impacted by individuals with undetected Aβ pathology since we used a conservative cut-off, adjusted analyses for Aβ levels, and found similar results in the *in vivo* data when using CSF Aβ, which is generally thought to be more sensitive to early Aβ pathology.[66] We additionally found similar results in the neuropathological dataset. However, we likely captured individuals with low Aβ levels, rather than with no Aβ, who can therefore be categorized as possible PART rather than definite PART. We also cannot rule out that some individuals in our Aβ− subgroup will progress to an Aβ-positive status. Therefore, longitudinal investigations are necessary to examine whether these observations would fall on the Alzheimer's disease continuum. Similarly, this study does not allow us to draw conclusions about whether PART is a separate entity or lies on the Alzheimer's disease spectrum.[67,68] Nevertheless, our findings, suggestive of a partially Aβ-independent presence of tau deposition in ageing, additionally highlight the possibility of measuring PART[10] *in vivo* already in preclinical stages. The autoradiography experiment in the current study gives support of the tracer's sensitivity to tau pathology also in PART, albeit likely dependent on the severity and maturity of the tau pathology.

Interestingly, tau-PET signal in the MTL and in medial parietal regions is also associated with volume or cortical thickness measures in the same regions, independent of Aβ pathology, in this group of cognitively unimpaired individuals. The results for the MTL are in line with previous research,[69,70] but extends previous work by showing that the negative effect of ageing on MTL subfields is, independently of Aβ, partially mediated by tau-PET, and by showing that these results hold also in the Aβ− subgroup for BA35. Our results for the medial parietal regions suggest that age-related, Aβ-independent tau pathology is not only present in neocortical regions, but already has negative effects on brain structure. Tau-PET in this parietal region (precuneus/posterior cingulate) appears to partially mediate the age-thickness association. We also found an association of ERC/BA35 tau-PET with cognition in this cognitively unimpaired sample, which adds to the existing literature (e.g.[64,71-73-74]). We extended previous research by showing that tau within the MTL (ERC/BA35) additionally partially mediates

age-related differences in global cognition. No association between the tau-PET signal in the parietal regions and cognition were found, which could reflect lower levels of tau compared to ERC/BA35 tau-PET (Supplementary Table 1) or a relative recent aggregation of tau and, thus, has not exerted an effect on cognition yet. In general the effect sizes are relatively small, indicating that other factors, such as co-pathologies or non-specific age-related changes, potentially mediate the age-structure and age-cognition associations. Though, also methodological limitations of the imaging, pathology, and cognition measures and limitations of mediation models may play a role in the small effect sizes. Notably, in this study, MTL grey matter does not mediate the age-cognition relationship indicating that any structural changes that the MTL is undergoing are either too small to detect using MRI or too small to contribute to cognitive change. Perhaps tau pathology affects cognition in a different way, e.g. through synaptic changes (see Wu *et al.*[75] for an overview).

The most widely supported hypothesis is that Aβ accumulation is necessary for tau spread/accumulation outside the MTL to take off.[7,8,76] Nevertheless, our findings show that there are tau aggregates independent of Aβ outside the MTL and there are effects of tau pathology on brain structure and function even in cognitively unimpaired individuals. While we cannot make claims about spread based on this cross-sectional data, the findings provide several insights into the notion that Aβ is necessary for tau pathology to spread, albeit not challenging the critical role of Aβ in tau accumulation. First, it may be possible that the subthreshold Aβ levels in our samples are high enough to facilitate the beginning accumulation and/or potential spread of tau outside the MTL.[77] Second, it is conceivable that while there is tau seeding activity[18] and tau pathology in several regions outside the MTL, tau will only accumulate to a limited extent and spread slowly or not at all without the presence of Aβ.[78] Third, it is possible that Aβ and tau pathology accumulate separately[79] until a point where Aβ starts to facilitate tauopathy.[80] While Aβ may trigger tau pathology spread and facilitate accumulation, the findings of this study suggest that tau deposition may occur in the absence of Aβ at lower ranges already in 'healthy' ageing (see also Lowe *et al.*[22] and Groot *et al.*[72]), but future longitudinal studies are needed to explore this further.

Our results could also shed light on the so-called spatial disconnection paradox in Alzheimer's disease,[81] the question how Aβ facilitates the spread of tau if the first occurrence of these two pathologies is in different brain regions. Since we found evidence of age-related tau in regions beyond the MTL, both in the *in vivo* and *ex vivo* cohorts, with most pronounced effects in early Aβ regions[40] (precuneus/posterior cingulate) *in vivo*, there may not be a spatial disconnect but tau may start to accumulate outside the MTL independent of Aβ. Whether tau can, independently of Aβ, accumulate in larger quantities or spread across the neocortex, or whether Aβ drives the speed of tau spread, remains to be elucidated by longitudinal studies.

Strengths and limitations

Strengths of the current study include the use of *in vivo* and neuropathological cohorts, large sample sizes, fine-grained investigation of changes in MTL subfields, and the autoradiography study. The robustness of our findings and the validity of the used *in vivo* measurements are supported, given the consistency of our results when conducting sensitivity analyses and observing Aβ-independent tau *in vivo* and *ex vivo*, despite a large age difference between cohorts. Yet, the results of the study must be interpreted given its limitations. First, the cross-sectional nature of the study does not allow to conclude causal relationships. Second, the enrichment of *APOE*-ε4 allele carriership may limit generalizability. However, other studies with lower percentage of cognitively unimpaired *APOE*-ε4 allele carriers reported similar results (e.g. Lowe *et al.*[22] and Harrison *et al.*[24]). Third, not all regions investigated *in vivo* were available in the *ex vivo* cohorts (e.g. precuneus/posterior cingulate), potentially limiting the conclusions of the *in vivo* findings. Fourth, mediation models, while useful, have limitations.[82,83] Using different measures (e.g. PET, MRI, cognitive tests), the caveats of these measures are introduced into the mediation models, which may be part of the variance (un-)explained by the models and smaller effect sizes. We reduced this risk by fitting mediation models only for associations previously found significant in the partial correlation analysis. Lastly, since the investigated cognitively unimpaired older adults have low tau-PET SUVRs, it is possible that we capture other age-related changes or co-pathologies, as some uptake may be noise or that the used tau-PET tracer shows some off-target binding.[25] Yet, sensitivity analyses where individuals with high meningeal/skull binding were excluded support our results. Additionally, we adjusted all analyses with tau-PET signal as main predictor/outcome for age-independent putamen uptake, to ensure limited effects of off-target binding (also note Krishnadas *et al.*[84]), and we used a second-generation tau-PET tracer that shows less off-target binding compared to previous tracers.[25] Lastly, the finding of age-related tau within and outside the MTL in the *ex vivo* cohort, supports that the tau-PET SUVRs findings do not reflect solely age-related noise.

Conclusion

In this study comprising cognitively unimpaired people, we observe Aβ-independent age-related tau deposition (defined *in vivo* with tau-PET and *ex vivo* neuropathologically) not only in the MTL but also in neocortical regions (*in vivo*: temporal, frontal, and parietal regions; *ex vivo*: MTL, inferior temporal, anterior cingulate cortex). Further *ex vivo* validation in other neocortical regions is still needed, especially for parietal regions. This partially Aβ-PET-independent tau accumulation appears to exert downstream effects on (i) brain structure, again, within and interestingly to a limited extent also outside the MTL; and (ii) cognitive functioning. These insights aid in elucidating the progression of pathophysiology in ageing by indicating an early involvement of low levels of tau in parietal and frontal regions and thereby accumulation that appears to be at least partially Aβ independent. This has implications for understanding the spreading of tau outside the MTL, also in the context of Alzheimer's disease. The results also highlight that tau in the absence of higher levels of Aβ is relevant during aging given its association with atrophy and cognition. This, thereby, highlights the clinical relevance of PART, providing additional impetus to investigate and understand this entity further. Even in the absence of cerebral β-amyloidosis, tau pathology should be considered as a potential cause of at least modest cognitive impairment and atrophy. Thus, the results of our study support the potential benefits of tau-targeting treatments already in preclinical stages in individuals with no or relatively low Aβ levels.

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Competing interests

S.P. has served on the scientific advisory boards for Hoffman-La Roche and Geras Solutions. O.H. has acquired research support (for the institution) from ADx, AVID Radiopharmaceuticals, Biogen, Eli Lilly, Eisai, Fujirebio, GE Healthcare, Pfizer and Roche. In the past 2 years, he has received consultancy/speaker fees from AC Immune, Amylyx, Alzpath, BioArctic, Biogen, Cerveau, Fujirebio, Genentech, Merk, Novartis, Roche and Siemens. M.H. and S.M. are full-time employees of F. Hoffmann La Roche Ltd. The other authors report no competing interests.

Supplementary material

Supplementary material is available at *Brain* online.

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