Published in final edited form as:

Nat Med. 2018 December ; 24(12): 1910–1918. doi:10.1038/s41591-018-0206-4.

Neurogenetic contributions to amyloid beta and tau spreading in the human cortex

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Abstract

Tau and beta-amyloid (A β) proteins accumulate along neuronal circuits in Alzheimer's disease (AD). Unraveling the genetic background for the regional vulnerability of these proteinopathies can help in understanding the mechanisms of pathology progression. To that end, we developed a novel graph theory approach and used it to investigate the intersection of longitudinal A β and tau PET imaging of healthy adult individuals and the genetic transcriptome of the Allen Human Brain Atlas. We identified distinctive pathways for tau and A β accumulation, of which the tau pathways correlated with cognitive levels. We found that tau- and A β -propagation patterns were associated with a common genetic profile related to lipid metabolism, in which *APOE* played a central role, whereas the tau-specific genetic profile was classified as "axon-related" and the A β profile as

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Contributions: J.S. contributed to the design, analysis and interpretation of the data, and preparation of the manuscript. M.J.G. contributed to the design, analysis and interpretation of the data, and preparation of the manuscript. F.d.O.U. contributed to the analysis of the data, and preparation of the manuscript. L.O.-T. contributed to the analysis of the data, and preparation of the manuscript. I.D. contributed to the analysis and interpretation of the data, and preparation of the manuscript. H.-S.Y. contributed to the analysis of the data, and preparation of the manuscript. H.I.L.J. contributed to the interpretation of the data, and preparation of the manuscript. B.H. contributed to the interpretation of the data, and preparation of the manuscript. Q.L. contributed to the interpretation of the data, and preparation of the manuscript. G.E.-F. contributed to the interpretation of the data, and preparation of the manuscript. R.A.S. contributed to the design and interpretation of the data, and preparation of the manuscript. K.A.J. contributed to the design and interpretation of the data, and preparation of the manuscript.

Competing Financial Interests Statement: The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

“dendrite-related”. This is the first study revealing distinct genetic profiles that may confer vulnerability to tau- and A β -*in-vivo*-propagation in the human brain.

Introduction

Alzheimer’s disease (AD) is characterized by the abnormal accumulation of tau and beta-amyloid (A β) proteins, a process known to affect specific neuronal systems. In fact, it has been consistently shown that both of these pathologic hallmarks of AD affect specific large-scale circuits while other circuits remain spared or become disrupted later in the disease^{1–5}. Moreover, genetic population studies have shown that AD can be genetically determined, such as in familial forms of AD characterized by *APP*, *presenilin 1* or *presenilin 2* mutations, or genetically predisposed, such as in late-onset sporadic AD, which is highly associated with apolipoprotein E (*APOE*) ϵ 4 allele positivity (and to a lesser extent to polygenic associations of ~20 genes thus far described in the literature^{6–11}). However, it is still unknown whether and how these genetic factors confer vulnerability to the distinct spread of AD pathology across specific neuronal circuits in the aging brain. Several reasons can be speculated for this neglect. Until now, we have lacked the analytical tools to determine *in vivo* longitudinal propagation patterns of tau and A β deposits in the human brain. This has precluded the detection of pathways related to the progression of AD-pathology in cerebral tissue, as well as the investigation of genetic features potentially related with hypothesized pathways. Secondly, until the advent of microarray data covering the protein-coding transcriptome of the entire human cerebral tissue (Allen Human Brain Atlas, AHBA^{12,13}), we have lacked high-spatial-resolution data for investigating gene expression levels associated with cortical anatomy. Thirdly, we have lacked research approaches for combining findings from *in vivo* tau and A β propagation topologies with genetic profiles of cerebral circuits. Overall, the characterization of the neurogenetic basis of the cortical spreading of AD pathology would provide a critical understanding of how genes interact with AD pathology and neurodegeneration¹⁴.

AD dementia is a major public health challenge, and one that poses an even greater threat as the current population ages. Recently, preclinical stages of AD have been a major topic of investigation, motivated by the acknowledgment that the pathophysiologic process begins decades before any cognitive decline appears¹⁵. Thus, studying the pathways in which tau and A β spreads early on, as well as studying the genetic underpinnings of the respective spreading pathways, is crucial for advancing diagnostic accuracy as well as for enabling early disease-modifying interventions. Following that rationale, in this study, we hypothesized that specific gene expression profiles in brain tissue sustain the propagation selectivity of AD pathology along specific neuronal systems. To that end, we first focused on the identification of *in vivo* propagation pathways of tau and A β deposits in a longitudinal sample of healthy adult study participants (SP) from the Harvard Aging Brain Study (HABS^{16–19}). We developed graph theory metrics to detect the main cortical routes of pathology progression at the group-level using Positron Emission Tomography (PET) imaging, and built a novel staging approach to investigate subject-level pathway profiles of spreading of pathology and their relationship with neuropsychological and cognitive profiles. Lastly, using the AHBA, we characterized regional gene expression profiles

associated with tau and A β PET pathology progression patterns across the cerebral cortex to better understand at the neural systems-level the vulnerability that AD-related genetic profiles confer on AD pathology progression in the human brain.

Results

Propagation Routes from Prominent Areas of Tau and Amyloid Deposits

Using no-overlapping cross-sectional (N=69; HABS healthy adults) and longitudinal (N=19; HABS healthy adults) data from HABS participants (N=88; Supplementary Table 1), we detected distinct propagation pathways for tau and A β accumulation. We developed a graph theory approach, named *Directional Graph Theory Regression* (DGTR; Supplementary Figure 1), to predict at the cross-sectional level, and corroborate at the longitudinal level, brain tau and A β pathology changes as imaged by *in vivo* PET imaging in samples of healthy adult individuals. By doing so, the cross-sectional tau findings predicted graph-based connectivity between the temporal region of interest and the anterior and inferior part of the temporal lobe, as well as midline frontal regions such as the orbitofrontal cortex (Figure 1a). The longitudinal tau findings not only confirmed anterior and inferior temporal lobe and midline frontal regions as spatiotemporal targets for tau propagation, but additionally revealed that these pathways also extend from the temporal lobe into the PCC and precuneus (Figure 1b). Overall, we found a high spatial association between cross-sectionally predicted and longitudinally observed network propagation of tau (scatterplot in Figure 1; $r=0.599$). Analogous results were obtained with a replication data-set (N=24; HABS healthy adults), both with and without PVC (Supplementary Figure 2). We also detected propagation pathways of A β , in which the PCC related with distributed regions in the lateral fronto-parietal, midline frontal, and precuneus regions at the cross-sectional level (Figure 1c). The longitudinal findings confirmed that A β predominantly spreads toward neighboring posterior and lateral parietal lobe regions (Figure 1d). Analogous to tau propagation, we found a high spatial association between cross-sectionally predicted and longitudinally observed network propagation of A β (scatterplot in Figure 1; $r=0.623$). Similar results were obtained with the replication data-set, both with and without PVC (Supplementary Figure 2).

Hubs of Pathology Propagation of Tau and Amyloid

Next, we detected brain regions where a disproportionate number of propagation routes converged in our longitudinal sample, either as out-degree hubs [regions for which signal in *time 1* explained the signal of many other regions in *time 2* (see small network diagram in Figure 2)] or in-degree hubs [regions in which signal in *time 2* is explained by the signal of many other regions in *time 1*; (see small network diagram in Figure 2)] of pathology. On one hand, we found that tau out-degree hubs were mostly located in the medial temporal lobe including the parahippocampus and the inferior temporal neocortex (top-left cortical maps in Figure 2a), while tau in-degree hubs involved the mid/posterior cingulate cortex and some frontal dorsal areas (bottom-left cortical maps in Figure 2a). The aggregation display showed that tau out-degree hubs and in-degree hubs were distantly located without overlap (cortical overlap map in Figure 2a). On the other hand, we found that A β out-degree hubs were mostly found in angular and supramarginal gyri, PCC, and mid/superior frontal regions (top-right cortical maps in Figure 2b) while A β in-degree hubs were located in precuneus, left

inferior and superior parietal cortex, and the confluence between superior frontal gyrus and supplementary motor area (bottom-right cortical maps in Figure 2b). The aggregation display showed that A β out-degree hubs and in-degree hubs display a high degree of overlap in the angular gyrus, and some degree of overlap at the junction between PCC and precuneus (cortical overlap map in Figure 2b).

Tau and Amyloid Propagation-Based Method in Individual Subjects

Next, we used the main out-degree and in-degree propagation pathways obtained in the DGTR analysis at the group-level, to characterize individual tau and amyloid deposition profiles in the SP sample (N=64; SP with complete neuropsychological assessment), as well as in an additional sample of AD/MCI individuals (N=19), both from our cross-sectional data (Figure 3). To build this propagation-based method, we extracted the main tau propagation routes –seven in total: MidCC/PCC, bilateral dorsal frontal, bilateral temporal pole, bilateral medial/inferior temporal– and main A β propagation routes –six in total: PCC, mid/superior frontal, bilateral inferior parietal, and lateral temporal – based on the out-degree hubs and in-degree hubs maps (see arrows in the polar plots of Figure 3a). Each of these tau and A β propagation routes showed significant differences between elderly subjects and AD groups (violin plots in Figure 3a). Moreover, we found that this propagation-based approach that lower global cognition and lower memory scores were associated to specific tau pathways in this cross-sectional data, particularly, deposits involving the temporal lobe (see correlation matrix and scatterplots in Figure 3b), with no associations found for executive functions. Propagation-based analysis of A β routes was not associated with any cognitive measure.

Brain Co-localization of In Vivo Propagation Patterns and Allen Gene Expression Data

Among the 21 pre-selected genes with known AD risk association, we found that *MAPT* (star symbol in Figure 4a; scatterplot and cortical map in Figure 4b) reached the highest level of brain co-localization with the imaging propagation map of tau (tau out-degree hubs map plus tau in-degree hubs map; cortical map in Figure 4b), while *CLU* (star symbol in Figure 4a; scatterplot and cortical map in Figure 4d) reached the highest level of brain co-localization with the imaging propagation map of A β (A β out-degree hubs map plus A β in-degree hubs map; cortical map in Figure 4d). Importantly, *MAPT* ($p=0.006$) and *CLU* ($p=0.018$) were the only genes of our pre-selected genetic set that showed significance when contrasting them against the null hypothesis distribution based on the entire protein-coding transcriptome (Figure 4c and Figure 4e). Moreover, *MAPT* and *CLU* gene expression levels were not associated with raw SUVR tau and DVR A β intensity maps from the same sample (Figure 4f), indicating that the expression of these genes is associated with the regional propagation of tau and A β , respectively, and not with SUVR tau or DVR A β intensity levels of pathology.

Finally, we further conducted data-driven analyses in addition to the analysis of pre-selected AD risk genes, in order to identify genes with expression pattern that are spatially correlated with A β or tau propagation maps: 354 genes correlated with the tau imaging propagation pattern and 216 genes with the A β imaging propagation pattern. Interestingly, 123 genes were correlated with both tau and A β imaging propagation patterns (Figure 4c and 4e; see

these three lists of genes, along with their putative functions in Supplementary Tables 3 to 6).

Interactome and Gene Ontology Analyses of Imaging-Genetic Profiles

Next, we investigated the genetic interactions and biological functionality of the 123 genes with expression patterns correlated with both tau and A β propagation (Figure 5a). We found that the genetic network of these shared genes displayed a dense organization of interactions in which *lipid metabolism* annotations were the most relevant neuro-related *biological process* and *cellular component* functionality (Figure 5a; FWE corr $p < 0.05$). This interactome analysis showed that *lipid metabolism* genes – belonging to the common profile of tau and A β – interact with other genes, such as *APOE*, which exhibit a central role in genetic network relationships (see network layout and centrality graph in Figure 5a). Then, we performed further exploratory analysis of interactomes and gene ontology of genes with expression patterns correlated specifically with tau (Figure 5b) or A β propagation (Figure 5c). The network of tau-related genes seemed to yield a dense organization of interactive genes in which *neuron and axon organization* was the most relevant neuro-related *biological process* and *cellular component* functionality (Figure 5b; FWE corr $p < 0.05$). For instance, this interactome analysis showed that genes implicated in microtubule organization such as *CNTN2* (top 1), *DPYSL2* (top 3) and *MAP1B* (top 19), displayed a central role in these genetic interactions (see network layout and centrality graph in Figure 5b). *MAPT* had a less central role in this network, and it did not hold a position among the top 25 most central genes (centrality graph in Figure 5b). Moreover, we found that *BACE1* or beta-secretase 1 – a gene related to the cleaving of the A β precursor protein (*APP*) – was a relevant interacting gene in this genetic network (see network layout and centrality graph in Figure 5b). The gene network of A β -related genes showed a dense organization of interactions in which *dendrite and neuron organization* annotations arose as the most relevant neuro-related *biological process* and *cellular component* functionality (Figure 5c; FWE corr $p < 0.05$). Interestingly, this interactome analysis showed that genes related to transcription regulation of *APP*, such as *APBB1* (top 14), as well as *CLU* (top 22), interact with protein-coding genes involved in neuronal cytoskeletal organization such as *TUBA1A* (top 3) or *TUBA4A* (top 16) (see network layout and centrality graph in Figure 5c).

Propagation-Based Staging of Tau, A β , and APOE Genotype

In Figure 6a we summarize the main neuroimaging-transcriptomic findings and contextualize them with our PET approach. Based on this framework in which different AD-related genotypes are expected to differently affect the tau and/or A β molecular pathways, we predicted that genes involved in both tau and A β genetic interactome profiles (gray area in Figure 6a) would induce a concomitant tau and A β vulnerability. To estimate this assumption, we investigated the propagation-based staging and *APOE* genotyping in SP individuals of our cross-sectional sample for which *APOE* $\epsilon 4$ carrier status was available ($N=68$; 21 *APOE* $\epsilon 4+$ and 47 *APOE* $\epsilon 4-$). We averaged all propagation-based staging scores from Figure 3 and obtained global scores for individual tau and A β burden. Importantly, we found that *APOE* $\epsilon 4+$ participants displayed a linear relationship between tau and A β load in the respective propagation pathways, while *APOE* $\epsilon 4-$ participants did not show any such association (Figure 6b).

Discussion

What makes specific neuronal systems vulnerable to the accumulation of AD pathology remains a field of intense research. Recent advances in multi-tracer PET neuroimaging, particularly those targeting tau and A β , as well as advances in genetic biomarker research, have provided new, exciting opportunities for studying AD-specific neuronal degeneration and the genetic risk factors that may underlie it. In this study, we used two unique datasets, a HABS sample with Flortaucipir-PET and PiB-PET imaging data, and a sample from the AHBA with data of cortical gene expression levels of the human protein-coding transcriptome, to characterize the topological distributions and co-localizations of *in vivo* tau and A β propagation with the expression of certain genes. We found that both tau and A β propagation associate to a common genetic profile related to “lipid-metabolism”, while tau propagation related to the *MAPT* gene and an “axon-related” genetic profile, and A β propagation related to the *CLU* gene and a “dendrite-related” genetic profile. Although we estimated detailed progression patterns using *in vivo* PET, it is intriguing that a previous study that matched AHBA anatomical annotations to AD regions, found an association between MAPT expression and potential vulnerability to tau accumulation as defined by Braak and Braak staging²⁰. In addition to the main findings of our study, here we also developed a novel quantitative approach for the *in vivo* staging of AD pathology in humans. Thus, overall, we provide an integrative framework for investigating the neurobiological basis of imaging phenotypes in preclinical and clinical AD as well as phenotype-genotype associations.

Although PET imaging has been able to identify and visualize A β protein deposits in the brain for over a decade, high affinity radiolabels for tau protein have only recently been successfully developed^{21–25}. Flortaucipir, a newly developed tracer for the imaging of tau pathology with PET, binds with high affinity to hyperphosphorylated tau pathology, such as in regions like the medial temporal lobe and associative cortex, and shows potential for the detection of preclinical and clinical AD populations following the classical Braak and Braak neuropathological staging of neurofibrillary tangles^{17,25,26}. Hence, it is now possible to design comprehensive side-by-side comparisons of tau and A β , and to study the initial stages and progression of these proteinopathies. Moreover, as tau and A β protein depositions are not randomly distributed, but rather exhibit characteristic spatial patterns^{27–31} that follow large-scale connectivity networks^{19,32–39}, the study of AD pathology networks has become essential for the understanding of the spatial distribution and potential propagation of the disease.

Previous PET neuroimaging studies have typically overlooked the temporal dimension and network nature of AD-related pathology. Conventional PET imaging analysis techniques are only sensitive to gross changes in signal intensity, ignoring the spatiotemporal or large-scale network relationships that distributed regions may exhibit in their molecular binding affinity. In this study, we provide new *in vivo* insights regarding network-wise longitudinal changes of tau and A β pathology. We developed a novel graph theory approach for detecting propagation-pathways of AD pathology, and found distinct propagation pathways for tau and A β accumulation. Particularly, medial/inferior temporal lobe areas projected pathways of tau-propagation toward the anterior pole, lateral and postero-medial temporal cortex, and

orbitofrontal cortex, while the posterior cingulate cortex projected A β toward surrounding areas and the lateral parietal lobe. These findings support the idea that accumulation patterns of pathology evolve beyond local neighborhoods. For instance, the cross-sectional and longitudinal tau data show that temporal lobe pathology progresses distantly toward the orbitofrontal cortex and limbic areas, such as the posterior cingulate cortex. Moreover, our results are in accordance with postmortem histopathology characterizations^{27,28,31,35,40–42}, as well as recent neuroimaging descriptions⁴³, and open new avenues for improving current staging methods and interpreting AD-related pathology findings by accounting for observed propagation routes of pathology progression. For instance, out-degree and in-degree hubs in the cerebral cortex can be seen as regions that act as “givers” or “receivers” of signal over time, respectively. Here we found that temporal cortex areas are the main “giver” regions for tau signal in this regard. The hubness of the temporal lobe out-degree pattern explains the sequential progression of signal from this area toward locally and distantly connected areas such as the cingulate cortex. Thus, we speculate that the “giving” signal property, at least for this temporal region, implies long distance connectivity toward locations that are the “receivers” of signal, such as the previously mentioned cingulate area (as seen in the in-degree maps). In this investigation, we developed new approaches to understand how AD-related pathology advances across specific brain systems in the living human brain. We posit that the human brain behaves as a dynamic network, even while, or even when, it undergoes neurodegeneration. The existence of hub cortical areas that drive the time-based or sequential patterns of tau and A β accumulation opens up new possibilities for developing more accurate staging methods that may be able to capture the network spreading nature of AD-related pathology.

Pathology studies have shown that precursor proteins such as APP, alpha-secretase, beta-secretase, and gamma secretase behave discordantly in AD patients, leading to the accumulation of self-aggregating A β ⁴⁴. Moreover, the *APOE* ϵ 4 allele genotype, as well as other single-nucleotide-polymorphisms in ~20 genes – although with less risk intensity – have been associated with late-onset sporadic AD in genome-wide association studies^{6–9}. However, it remains unknown how genetic factors relate to the concrete progression of pathology across neuronal circuits. Thus, in this study we focused on integrating information from *in vivo* propagation patterns of tau and A β deposits with putative genetic biomarkers expressed in the human cortex, to investigate whether the identified genetic biomarkers support susceptibility for network accumulation of AD pathology. We found that group-level imaging phenotypes of tau and A β propagation are associated with a rich set of genes that are expressed in analogous brain regions in the AHBA. These co-localizations suggest that a common background of genes devoted to lipid metabolism may possibly underlie the evolution of both tau and A β across specific neural systems¹⁴. Although the amyloid hypothesis, centered in the triggered and deleterious effects of A β deposits, has been the prevailing model used to explain the pathophysiology of AD, A β deposits do not correlate well with the clinical course, neuronal dysfunction, or with cognitive performance, and anti-A β treatments have failed or have shown inconsistencies in altering the course of the disease⁴⁵. Alternatively, others have emphasized the abnormal intra-neuronal accumulation of hyper-phosphorylated tau as a critical component in explaining the cause of AD. Specifically, post-mortem studies have shown better correlation between pre-mortem

cognition and tau than A β ⁴⁶. The inter-relationships between A β and tau are far from being understood however, and some neuropathological work points towards tau inducing A β ⁴⁷. Here, our findings partially support the notion of an interaction between these proteinopathies. For example, *BACE1* appeared as one of the central genes in the tau-related interactome network. However, there may also be other mechanisms at higher levels of integration beyond direct spatial interactions between tau and A β . For instance, the set of genes common to both hallmarks of the disease are implicated in lipid metabolism, in which APOE appears to have a central role. Although APOE gene expression was not identified as directly spatially related to neither tau nor A β PET progression phenotypes, *APOE* displayed a critical place in the common interactome network observed in this study. Therefore, it is plausible that APOE may influence tau and A β accumulation by third parties, generating a functionality that goes beyond its spatial constraints. If we consider that tau and A β are end-products of AD neurodegeneration, it is plausible to postulate that genetic variants along different levels of their common pathways (e.g., *APOE*) may lead to the aggregation and co-occurrence of both AD pathology hallmarks, tau and A β . In fact, recent findings postulate that ApoE binding sites include 1,700 gene promoter regions, implicating genes associated with trophic support, programmed cell death, microtubule disassembly, synaptic function, and aging⁴⁸, processes in which tau and A β have been both extensively implicated. Additionally, it is also likely that genetic variants of genes specifically associated with either tau or A β molecular pathways may confer susceptibility for a specific or predominant accumulation of one or the other. Our final findings partially support this interpretation, for example, while *APOE* ϵ 4+ individuals display concomitant tau and A β accumulation, *APOE* ϵ 4- individuals may showcase elevated tau or A β accumulation but in a way where they may be unrelated with one another, suggesting that perhaps other genes considered to be less specific to the common pathway of AD dementia could potentially also be related to the spatially different accumulation patterns of tau or A β , and account for the *APOE* ϵ 4- AD cases.

Over the last few decades, an intense debate has been sustained between A β - and tau-centrist views of AD. Although our study was not designed to validate neither the A β - nor the tau-centrist view, it does highlight the existence of specific genetic profiles associated with either tau or A β PET phenotypes. More importantly, and away from these two centrist viewpoints, we observed that a common genetic background seems to embrace propagation patterns, both for tau and A β . In other words, our findings support that common biological sources may underlie tau and A β protein malfunctioning, in which *APOE* ϵ 4+ could play a critical role (Figure 6). Therefore, we believe that now, more than ever, AD research is in need of novel approaches that integrate *in vivo* propagation patterns with individual genetic data in order to unveil idiosyncratic vulnerabilities associated with the development and trajectory of late-onset sporadic AD.

The detection and combination of *in vivo* patterns of tau and A β propagation with genetic profiles of AD risk, will aid the future development of comprehensive approaches for better diagnosis and monitoring of AD, as well as for evaluation of personalized risk to AD at earlier stages of the disease spectrum. In this study, we characterized the cortical propagation pathways of tau and A β pathology in a longitudinal cohort of cognitively normal adult participants, and identified some of the genetic underpinnings using *in situ*

information from a transcriptome atlas of cerebral tissue. It is important to note that our investigation only examined a fraction of all possible neuropathological events in AD-related neurodegeneration, and focuses on PET data from early stages in which small sample sizes and low PET intensity values may be limitations. However, we believe that our findings offer novel avenues for enhancing detectability of AD trajectories, for interpreting imaging and genetic information in a more integrated manner, and for the future monitoring of novel therapeutic strategies.

Online Methods

Participants

We included 88 clinically normal participants from the HABS ([mean age (SD): 72.24 (11.63), M/F: 43/45]; see Supplementary Table 1 for detailed demographics and clinical characteristics), out of which 19 also had a two-year follow-up assessment [mean age (SD): 75.63 (5.95), M/F: 11/8]. Apart from the main longitudinal sample, we included an additional longitudinal sample of 24 HABS individuals ([mean age (SD): 76.09 (5.66), M/F: 9/15]; see Supplementary Table 2 for detailed demographics and clinical characteristics) for validation and replication of our propagation method and findings. This replication sample was also used to investigate partial volume correction (PVC) effects on PET images. We also included 19 participants previously classified as cognitively impaired by memory clinics of Harvard School of Medicine-affiliated hospitals, including diagnoses of mild cognitive impairment (MCI) or AD dementia [mean age (SD): 67.45 (9.12), M/F: 14/5; mean CDR-SB (SD): 3.82 (2.91); mean Mini-Mental State Examination (MMSE): 24.47 (4.95)]. As part of HABS, participants undergo multiple neuroimaging sessions, *APOE* genotyping, and an annual neuropsychological testing that includes the Preclinical Alzheimer's Cognitive Composite (PACC). The PACC combines Logical Memory Delayed Recall, MMSE Total score, Wechsler Adult Intelligence Scale-Revised Digit Symbol coding, and the Free and Cue Selective Reminding Test⁴⁹. Direct genotyping of *APOE* rs7412 and rs429358 is performed at the Harvard Neurodiscovery Center to derive *APOE* haplotypes. Participants are originally included if they have a score of 0 on the Clinical Dementia Rating (CDR) scale, a MMSE score ≥ 25 , and if they perform within education-adjusted norms on the Logical Memory delayed recall test (>10 for ≥ 16 years of education, >6 for 8–15 years of education and >4 for <8 years of education). All participants undergo at least one comprehensive medical and neurological evaluation, and must not have any medical or neurological disorders at enrollment that could contribute to their cognitive abilities. Presence of clinical depression (Geriatric Depression Scale above 11/30) or other psychiatric illnesses, history of alcoholism, drug abuse, head trauma, or a family history of autosomal dominant AD dementia were exclusionary criteria. All participants took part in the study using protocols and informed consent procedures approved by the Partners Human Research Committee at the Massachusetts General Hospital. Further information on study design is available in the Life Sciences Reporting Summary.

MRI and PET Acquisition and Preprocessing Procedures

All participants underwent an MRI image acquisition of the whole head on a Siemens 3 Tesla Tim Trio system using a 12-channel phased-array head coil that included a T1-

weighted MPRAGE scan using the following parameters: TR = 6400ms, TE = 2.8ms, flip angle = 8°, inversion time (TI) = 900ms, and a voxel size of 1.0×1.0×1.2mm. We used SPM12 (Wellcome Department of Cognitive Neurology, University College of London, London, UK, <http://www.fil.ion.ucl.ac.uk/spm>), running under Matlab v8.0 (Mathworks Inc., Natick, MA), for image preprocessing and normalization of anatomical T1-weighted MRI volumes. We used the VBM8 toolbox for voxel-based morphometry analysis of the anatomical T1-weighted MR images⁵⁰. Briefly, after spatial normalization of all images from native to normalized MNI/ICBM space, images were segmented into gray matter (GM), white matter, and CSF. Then, we selected GM modulated and 10 mm full-width at half-maximum Gaussian kernel smoothed images for further analysis to control for GM volume intensity. All participants had two PET imaging acquisitions at the Massachusetts General Hospital PET facility: 1) Flortaucipir (also known as ¹⁸F-T807 or ¹⁸F-AV-1451) PET that binds to tau in neurofibrillary tangles and neurites²¹, and 2) a ¹¹C-labeled Pittsburgh Compound-B, N-methyl ¹¹C-2-(4-methylaminophenyl)-6-hydroxybenzothiazole (¹¹C-PiB) PET that binds to fibrillary Aβ plaques⁵¹. Flortaucipir PET parameters were as follows: 10 mCi of ¹⁸F-T807, 3-D mode static protocol of 20 min acquisition from 80–100 min; 63 image planes, 15.2 cm axial field of view, 5.6 mm transaxial resolution and 2.4 mm slice interval; 4×300sec frames at 80 minute post injection followed by a 6 minute transmission¹⁷. The final intensity ¹⁸F-T807 maps were obtained calculating the standardized uptake value ratio (SUVR) using a cerebellar gray reference region. On the other hand, ¹¹C-PiB PET parameters were as follows: following a transmission scan, 10–15 mCi ¹¹C-PiB was injected intravenously as a bolus and followed immediately by a 60-min dynamic PET scan in 3-D mode (63 image planes, 15.2 cm axial field of view, 5.6 mm transaxial resolution and 2.4 mm slice interval; 69 frames: 12 × 15s, 57 × 60s)^{17,51}. PiB retention intensity was expressed as the distribution volume ratio (DVR) at each voxel, and was calculated using Logan's graphical method, using a cerebellar gray reference region. Corrections for normalization, dead time, random coincidences, scattered radiation, and attenuation were performed and each frame was evaluated to verify adequate count statistics and absence of head motion. Using SPM12, all PET data in native space were co-registered with their corresponding anatomical T1-weighted MRI images and spatially normalized into MNI standard space using the normalization parameters obtained from the T1-weighted MRI normalization. Our processing pipeline also included an outlier detection approach based on the assessment of normally distributed values of each voxel within our sample to ensure that only voxels with a normal continuum range of PET values were entered in the regression analysis⁵² (Supplementary Figure 2). All PET data was down-sampled from the normalized space to 8mm isotropic voxel to study the high dimensional data without computational limitations.

Directional Graph Theory Regression and Statistics

In contrast to conventional analysis approaches in PET imaging where only regional intensity is investigated, here we studied PET signal changes using a novel DGTR approach in order to investigate network-based changes between regions of the human brain (Supplementary Figure 1a and 1b; association matrices and network diagrams). Furthermore, conventional analysis approaches in PET imaging fail to infer network changes and network temporal directionality, as they are not designed to utilize the spatiotemporal

PET patterns across different brain systems. DGTR may thus provide novel information about network-level stages of accumulation of pathology, as well as information about potential pathways of pathology spread associated with progression of the disease through brain tissue.

In DGTR, we assume that PET imaging data are in proximity to a submanifold, and are Gaussian smooth signals on a weighted graph in a discretized approximation, as verified in many relevant works^{53–55}. To that end, we first computed PET-based association matrices (or PET-based connectivity matrices) between all pairs of voxels across the brain's GM, and built group-level network graphs for the cross-sectional (Supplementary Figure 1a; and *within time 1* connectivity in 1c) and longitudinal study samples (Supplementary Figure 1b; and *between time 1* and *time 2* connectivity in 1c). We used a partial correlation approach to calculate inter-subject associations of DVR or SUVR values between all pairs of voxels of the brain controlled for MRI atrophy by regressing out the influence of GM volume intensities. We then designated seeds based on specific *a priori* regions. We selected the inferior temporal cortex and posterior cingulate cortex (PCC) due to their early high intensity values in PET *in vivo* studies. These two regions of interest were obtained from our previous studies based on PET neuroimaging independent datasets^{17,26}. We used the PCC and inferior temporal seed-based analysis to compare our PET data with and without PVC adjustment using the extended Müller-Gartner method as previously implemented for FreeSurfer^{43,56,57}. As other seeds may also be relevant in this context, particularly if early histological data are taken into account^{46,47,50}, we included additional seeds in the analysis, such as the entorhinal cortex and the medial prefrontal cortex (Supplementary Figure 3). The entorhinal cortex and medial prefrontal cortex (medial orbitofrontal cortex, rostral anterior cingulate cortex) regions of interest were based on the Desikan-Killiany atlas⁵⁸. In the particular case of tau data, we controlled our partial regression analysis not just by regressing out the voxel-level GM volume information, but also by excluding the signal intensity of the *plexus choroideus*, which may strongly correlate with the signal intensity of medial temporal lobe structures due to spatial proximity¹⁷. Signal from the choroid plexus was removed using the region of interest from the Desikan-Killiany subcortical atlas⁵⁸. In the cross-sectional propagation model, the partial correlation coefficient between each brain voxel and the *a priori* seed region was interpreted as a predictor of pathology propagation¹⁸. In the longitudinal propagation model the partial correlation coefficient between each brain voxel and the *a priori* seed region was interpreted as an observation of pathology propagation. For the particular analysis of the longitudinal study sample, we computed connectivity matrices between *time 1* and *time 2* (red connectivity in network diagram; Supplementary Figure 1b and 1c). Following the Supplementary Figure 1c example, if we were assessing connectivity profiles between voxel *b* and voxel *d*, we would compute the partial correlations of the PET values across the sample between voxel *b* in *time 1* and voxel *d* in *time 2*, as well as, between voxel *b* in *time 2* and voxel *d* in *time 1*. The partial correlation (*PC*) can be solved with the *t* statistic studying the effect of the two variables and removing the effect of atrophy by using multiple regression as shown in the equation 1:

$$PC(b, d) = \frac{t_{b,d}}{\sqrt{t_{b,d}^2 + resdf}} \quad (1)$$

where $PC(b, d)$ represents the partial correlation of node b in *time 1* with node d in *time 2* removing the effects of atrophy; $t_{b,d}$ is the t statistic looking at the effect of node d in *time 2* on our dependent node b in *time 1* (in the multiple regression model the dependent variable y are the values of node b in *time 1*, and the design matrix X is defined by the intercept, values of node d in *time 2* and atrophy values in b and d); and $resdf$ is the residual degrees of freedom.

Once all possible interactions between pairs of voxels in the brain have been calculated, we performed statistical comparisons between connectivity profiles of voxels. Again, given the previous example, we can compare the connectivity profiles of voxel b to determine if differences exist between the distributions of its partial correlation values across the two temporal conditions: 1) voxel b in *time 1* against the rest of the voxels in *time 2*; and 2) voxel b in *time 2* against the rest of the voxels in *time 1*. Next, we used the two-tailed paired t statistic to assess the significance (p-value) of this comparison per node (equation 2 and 3):

$$s^2 = \frac{\sum_{i=1}^n (PC(b, i) - PC(i, b))^2}{n-1} - \frac{(\sum_{i=1}^n (PC(b, i) - PC(i, b)))^2}{n} \quad (2)$$

$$pvalue(b) = 2 \left(1 - cdf \left| \frac{\frac{\sum_{i=1}^n (PC(b, i) - PC(i, b))}{n}}{\sqrt{\frac{s^2}{n}}} \right| \right) \quad (3)$$

where s^2 represents the variance of the data; n is the number of voxels; i represents the voxels of the brain from 1 to n ; and cdf is the cumulative distribution function to compute the t statistic.

Next, all within-voxel statistical comparisons between paired conditions were corrected for multiple comparisons to eliminate connectivity profiles that represent false positives by using an FDR correction⁵⁹ at q level of 0.05. Later, all connectivity profiles of FDR-surviving voxels were entered in the next calculation (dark nodes in Supplementary Figure 1c), in which we computed the final connectivity scores by subtracting the partial correlation values between conditions (equation 4):

$$D(b, d) = PC(b, d) - PC(d, b) \quad (4)$$

This approach provided the directional (time-based) connectivity relationships between voxels. For instance, if voxel b signal at time 1 explained voxel d signal at time 2 with a statistically significant greater magnitude than how voxel d signal at time 1 explained voxel b signal at time 2, then a directional connection from voxel b to voxel d was established (out-connectivity from b to d ; Supplementary Figure 1c). The resulting matrices D of this analysis served as inputs for the subsequent weighted degree computations. We evaluated the overall degree of each voxel to explain hub-based directional relationships in the longitudinal data -independent from *a priori* selection of specific seed regions- by calculating the out- (WD_{out}) and in-weighted-degree (WD_{in}) according to equations 5 and 6, where i and j represent the voxels of the brain:

$$WD_{out}(i) = \sum_{j=1}^n \max(D(i, j), 0) \quad (5)$$

$$WD_{in}(i) = \sum_{j=1}^n \max(-D(i, j), 0) \quad (6)$$

Of note, seed-based analysis was performed by extracting the connections (cross-sectional) or out-connections (longitudinal) from *a priori* regions of interest. We investigated the spatial similarity between the cross-sectional predicted and the longitudinal observed maps using a voxel-level linear correlation approach.

Cortical space visualizations of the seed-based and weighted-degree results were generated using Caret software [PALS surface (PALS-B12)⁶⁰; interpolated algorithm and multi-fiducial mapping]. In order to increase spatial comparability across PET modalities, the result maps were z-score normalized before visualization.

Data and Code Availability

All neuroimaging and clinical data that support the findings of this study are available from <https://nmr.mgh.harvard.edu/lab/harvard-aging-brain-study/public-data-releases>. HABS data curation is overseen by Aaron P. Schultz (aschultz@nmr.mgh.harvard.edu) at the Martinos Center for Biomedical Imaging, Massachusetts General Hospital, Harvard Medical School, Boston, MA. All codes related to PET imaging analysis are available for the research community from the corresponding author (J.S.) upon request for the purpose of scientific investigation, teaching, or the planning of clinical research studies.

Subject-Level Analysis Using Propagation-Based Method

Based on the out- and in-degree propagation patterns determined by the DGTR approach we developed a framework to characterize individualized pathological levels for tau and A β in participants. We further hypothesized that subject-level tau and A β levels determined by our approach would relate to specific rates of cognitive decline and other clinical features across the clinical spectrum of AD. Despite extensive research, it remains difficult to provide prognoses toward AD progression. There are several reasons for this. Current *in vivo* staging approaches for tau and amyloid are based on qualitative approximations of *a priori* regions

derived from postmortem histological studies that suffer from sampling limitations, low spatial resolution level, and a lack of temporal information. Moreover, standard classification approaches for A β PET imaging rely on broad cortical composite regions of interest, such as the frontal, lateral parietal, lateral temporal, and retrosplenial cortices – also known as FLR regions – that help dichotomize individuals into simple A β -positive or -negative categories. However, this approach simplifies the underlying nature of AD pathology, and makes it difficult to investigate the complex relationships between imaging features and clinical or cognitive scores. Recent work has shown that consistent regional staging models for both tau and A β can be extrapolated directly from cross-sectional PET imaging data^{61,62}. Here, we expand upon these *in vivo* staging approaches by developing a high spatiotemporal resolution and longitudinal propagation-based method to quantitatively characterize, understand, and monitor AD-pathology in early phases. This is especially relevant for tau PET imaging which has been associated with spreading tracer retention in samples ranging from cognitively normal to MCI and to AD individuals, and has been strongly related with cognitive decline^{26,42,63}.

While we cannot build directional graphs from a subject-level tau or A β PET image, we can assume that data from individuals are embedded at the group-level directional graph. Thus, we extracted tau and A β PET SUVR and DVR intensities at the individual level using masks from our previous section of *out*-degree and *in*-degree propagation pathways [templates created from group-level neuroimaging patterns above two standard deviations (2σ)]. We built a propagation-based staging score per each propagation pathway from the *in*-degree template by extracting their tau or A β intensity and multiplying it by the sum of all intensities from the *out*-degree template. We evaluated the clinical relevance of our method and assessed individual patterns of propagation pathways derived from DGTR by analyzing its relationship with clinical diagnosis (i.e., SP vs. AD/MCI) as well as with individual neuropsychological profiles of SP individuals, including memory and executive function scores. We used a memory z-scores composite that includes the delayed recall scores of the selective reminding test, the free recall of the free and cued selective reminding test, and the delayed recall of the Logical memory test⁶⁴. The statistical assessment was carried out using linear regression, and two-tailed unpaired t-test corrected by multiple comparisons.

Brain Co-Localization Analysis Between Propagation Patterns and Genetic Transcriptome

In this section, we used aprioristic knowledge of AD risk genes, as well as data-driven approaches based on the full genome-wide (protein-coding) transcriptome of the AHBA to delineate the cortical genetic profiles associated with the spread of AD pathology, and to investigate the neurobiological role of the genetic-imaging associations. In particular, we used a surface anatomical transformation of the cortical transcription profiles of 20,737 protein-coding genes, based on 58,692 measurements of gene expression in 3,702 brain samples obtained from 6 adult human participants of the AHBA⁶⁵ (see Supplementary Table 3 for demographic and brain sample details). This anatomical transformation used a mapping of the 6 individual matrices of regional gene expression (gene x MNI coordinate) to 68 pre-specified cortical brain regions defined in the Desikan-Killiany atlas⁵⁸ covering the entire cortex, which enabled inter-subject averaging (median values) of the regional gene expression levels. We also converted the hubs maps (sum of out-degree and in-degree hubs

maps) obtained from the DGTR section into the Desikan-Killiany atlas space, and vectorized both imaging and AHBA transcriptome genetic data. Then, we computed the cortical spatial similarity among imaging and genetic maps using Pearson's correlation of the magnitude of cortical profiles across the Desikan-Killiany areas (Supplementary Figure 1d). As a first step, we identified from the literature previously described genes that are associated with tau and A β processing or that confer risk to clinical AD^{6–11}. These selected candidate genes were: *MAPT*, *APOE*, *PICALM*, *BIN1*, *CLU*, *CR1*, *ABCA7*, *SORL1*, *PLEKHCI*, *CD2AP*, *CD33*, *APP*, *PSEN1*, *PSEN2*, *CASS4*, *EPHA1*, *PTK2B*, *INPPSD*, *MEF2C*, *CUGBPI*, and *MADD*. We obtained the spatial cortical similarity (or co-localizations) between our AD-related imaging maps (*in vivo* tau and A β longitudinal propagation maps) and expression maps of each of these 21 AD-related genes. Then, we assessed the statistical meaning of these co-localizations by building null hypothesis distributions with the co-localizations between the observed propagation maps and the entire protein-coding transcriptome (cortical gene expression levels of 20,737 genes). The exact p-value for specific co-localizations between imaging patterns and genes were obtained from the corresponding z-scores based on the null distribution. We delineated the cortical genetic profiles associated with the propagation of AD pathology in a data-driven approach, by determining for each PET modality all genes of the protein-coding transcriptome that met the statistical significance threshold. We considered 2 standard deviations above the transcriptome mean as the statistically significance level.

Interactome and Gene Ontology Analysis

Our first analysis focused on investigating spatial similarity between PET pathology propagation patterns and cortical gene expression of select AD risk genes, providing information about brain pathology-gene expression co-localizations. Next, we investigated whether the data-driven genetic-imaging profiles, obtained from the similarity analysis between propagation patterns and the full protein-coding transcriptome, display specific biological functionality. Using interactome and over-representation analysis, we studied genetic interactions among the identified set of genes beyond their spatial co-localizations in the cortex. We identified the role of each gene in the interactome analysis using node-level closeness centrality, and relied on knowledge-guided insight to guide our understanding of the biological processes and cellular component functions of genetic profiles, using annotations from Gene Ontology (GO)⁶⁶. We used Genemania (<http://www.genemania.org>)⁶⁷ and Cytoscape (www.cytoscape.org)⁶⁸ software for the interactome analysis and centrality assessment of our gene query lists, in which the weight of genetic associations were based on a composite gene-gene interaction profile from co-expressions, co-localizations, genetic interactions, pathways, predicted physical interactions, and shared protein domains⁶⁷. In order to avoid any arbitrary threshold when selecting neighborhoods associated to gene sets, we obtained 10 interactome networks per query list using a range of neighborhoods from 10 to 100. We obtained a single interactome network per list by calculating the mean weights of the 10 interactome networks. Finally, we used binomial test (FWE correction at $p < 0.05$ level) and select parent terms > 3 fold in the GO over-representation analysis to describe genetic annotation-based functionality⁶⁶.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments:

We thank the investigators and staff of the Harvard Aging Brain Study, Massachusetts Alzheimer's Disease Research Center, the individual research participants, and their families and caregivers. We would also like to thank the PET Core of the MGH, the Harvard Center for Brain Science Neuroimaging Core and the Athinoula A. Martinos Center for Biomedical Imaging support. This research was supported by grants from the National Institutes of Health (NIH) (K23-EB019023 to J. Sepulcre; T32EB013180 to L. Ortiz-Terán; R01HL137230 and P41-EB022544 to G. El Fakhri; R01-AG027435-S1 to R. Sperling and K. Johnson; P50-AG00513421 and R01AG046396 to K. Johnson and R. Sperling; P01-AG036694 to R. Sperling and K. Johnson, RF1AG052653 to Q. Li); Massachusetts ADRC; Alzheimer's Association (NIRG-11-205690 to J. Sepulcre; IIRG-06-32444 to R. Sperling and K. Johnson; ZEN-10-174210 to K. Johnson); and the Alzheimer Forschung Initiative e.V. (to M. Grothe). The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Figure 1. Propagation Routes from Prominent Areas of Tau and Amyloid Deposits.

Cortical connectivity maps of a priori selected areas, namely inferior temporal for tau (left) and posterior cingulate cortex (PCC) for A β (right), from cross-sectional (**a** and **c**, N=69; HABS healthy adults) and longitudinal (**b** and **d**, N=19; HABS healthy adults) data. Scatterplots of spatial similarity between cross-sectionally predicted and longitudinally observed network connectivity profiles (green dots). X- and Y-axis show the z-scores of the connectivity values. Network diagrams illustrate theoretical examples of seed-based connectivity in cross-sectional (no arrows) and longitudinal (arrows) samples.

Hubs of Pathology Propagation

Figure 2. Hubs of Pathology Propagation of Tau and Amyloid.

Cortical maps of longitudinal weighted-degree connectivity (directional relationships) of tau (a; Out-degree maps in top-left; In-degree maps in middle-left, N=19; HABS healthy adults) or A β pathways (b; Out-degree maps in top-right; In-degree maps in middle-right, N=19; HABS healthy adults). Network diagrams illustrate high and low theoretical examples of out-degree and in-degree. Overlap maps with an arbitrary threshold of z-score 3SD across the cortical mantle of tau and A β are presented at bottom left and right.

Figure 3. Tau and Amyloid Propagation-Based Staging.

Characterization of propagation pathways of tau and A β deposits in individuals of the cross-sectional data (healthy HABS study participants (SP) with complete neuropsychological assessment, N=64) and Alzheimer's disease (AD/MCI, N=19) subjects; polar and violin plots; **a** and **b**). Mean is measure of center in violin plots (dark line; **b**). Smooth histograms of SP (light gray dots and histograms) and AD/MCI (dark gray dots and histograms) data in violin plots are obtained by using the cumulative histogram, a smoothing spline and the analytical derivative. Characterization of the relationship between propagation pathways of tau and A β and cognitive levels in SP (**c** and **d**). X-axis shows the targeted regions of interest in **b**, and cognitive scores in **d**. Y-axis shows Propagation-based Staging scores in **b** and **d**. MMSE=Mini-Mental State Examination. Two-tailed unpaired t-test corrected by multiple comparisons was used in **b** and Pearson's correlation (and related p-value from one-tailed t-test non-corrected by multiple comparisons) was used in **c** and **d**.

Figure 4. Brain Co-localization of In Vivo Propagation Patterns and Allen Gene Expression Data.

Matrix of similarity scores (hot scale) between *in vivo* propagation patterns of tau and A β (columns) and Allen gene expression levels of 21 previously related AD genes (**a**; star symbols show highest similarity scores). Scatterplots between *in vivo* propagation patterns of tau and A β and *MAPT* expression (top; red dots; **b**) and *CLU* expression (top; blue dots; **d**) genes, as well as their cortical distributions (bottom; hot color scale; **b** and **d**). Null hypothesis distributions of similarity scores between *in vivo* propagation patterns of tau (**c**) and A β (**e**) and the entire protein-coding transcriptome. Similarity scores were converted to z-scores, and corresponding one-tailed p-values were obtained for *MAPT* and *CLU* z-scores. Red areas show similarity scores above 2 σ . Scatterplots are shown between *in vivo* regional intensity of tau or A β and *MAPT* (top; red dots; **f**) and *CLU* (bottom; blue dots; **f**) genes. Note that scatterplot in **f** shows SUVR and DVR values that represent linearly transformed values from the original maps, including down-sampling, conversion to 68-regions Desikan space, and whole sample averaging of the data. Pearson's correlation was used in **a** to **f** to determine linear similarity scores between cortical maps of 68-regions (dots in **b**, **d** and **f**).

Propagation Pathology and Genetic Signatures (II)

Figure 5. Interactome and Gene Ontology Analyses of Imaging-Genetic Profiles.

Interactome network showing significant Gene Ontology (GO) overrepresented functionality (FWE $corr$ $p < 0.05$) and node centrality of common (a), or only tau (b), or only A β (c), genetic sets obtained from the neuroimaging-genetics similarity approach (Figure 4). Blue color scale in the interactome networks (left) shows types of genetic interactions. Labels in interactome networks (left) and in centrality bar graphs (right) show genes associated to the main GO overrepresented domains (middle). Top 25 most central genes of the interactome networks are shown in green in centrality bar graphs (right). Of note, Supplementary Tables 7, 9 and 11 show the GO functional labels and values, and Supplementary Tables 8, 10 and 12 show the list of genes and node centrality values for each interactome network.

Imaging Phenotype and Genetic Vulnerability

Figure 6. Propagation-Based Staging of Tau and A β and APOE Genotype in Individuals.

Diagram of neuroimaging-genetic relationships between *in vivo* PET phenotypes, cortical gene expression levels, and interactome profiles, aimed to identify biomarkers conferring vulnerability for tau and A β accumulation. Scatterplots show a linear relationship between tau and A β individual staging only in *APOE* ϵ 4+ individuals (N=68; HABS healthy adults; 21 *APOE* ϵ 4+ and 47 *APOE* ϵ 4–, **b**). X- and Y-axis show propagation-based staging scores for tau and A β in **b**. Pearson's correlation (and related p-value from one-tailed t-test non-corrected by multiple comparisons) was used in **b**.