BRAIN
ORIGINAL ARTICLE

Genetically identical twin-pair difference models support the amyloid cascade hypothesis

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The amyloid cascade hypothesis has strongly impacted the Alzheimer's disease research agenda and clinical trial designs over the past decades, but precisely how amyloid-β pathology initiates the aggregation of neocortical tau remains unclear. We cannot exclude the possibility of a shared upstream process driving both amyloid-β and tau in an independent manner instead of there being a causal relationship between amyloid-β and tau. Here, we tested the premise that if a causal relationship exists, then exposure should be associated with outcome both at the individual level as well as within identical twin-pairs, who are strongly matched on genetic, demographic and shared environmental background. Specifically, we tested associations between longitudinal amyloid-β PET and cross-sectional tau PET, neurodegeneration and cognitive decline using genetically identical twin-pair difference models, which provide the unique opportunity of ruling out genetic and shared environmental effects as potential confounders in an association. We included 78 cognitively unimpaired identical twins with [^{18}F]flutemetamol (amyloid-β)-PET, [^{18}F]flortaucipir (tau)-PET, MRI (hippocampal volume) and cognitive data (composite memory). Associations between each modality were tested at the individual level using generalized estimating equation models, and within identical twin-pairs using within-pair difference models. Mediation analyses were performed to test for directionality in the associations as suggested by the amyloid cascade hypothesis.

At the individual level, we observed moderate-to-strong associations between amyloid-β, tau, neurodegeneration and cognition. The within-pair difference models replicated results observed at the individual level with comparably strong effect sizes. Within-pair differences in amyloid-β were strongly associated with within-pair differences in tau ($\beta = 0.68$, $P < 0.001$), and moderately associated with within-pair differences in hippocampal volume ($\beta = -0.37$, $P = 0.03$) and memory functioning ($\beta = -0.57$, $P < 0.001$). Within-pair differences in tau were moderately associated with within-pair differences in hippocampal volume ($\beta = -0.53$, $P < 0.001$) and strongly associated with within-pair differences in memory functioning ($\beta = -0.68$, $P < 0.001$). Mediation analyses showed that of the total twin-difference effect of amyloid-β on memory functioning, the proportion mediated through pathways including tau and hippocampal volume was 69.9%, which was largely attributable to the pathway leading from amyloid-β to tau to memory functioning (proportion mediated, 51.6%).

Our results indicate that associations between amyloid-β, tau, neurodegeneration and cognition are unbiased by (genetic) confounding. Furthermore, effects of amyloid-β on neurodegeneration and cognitive decline were fully mediated

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by tau. These novel findings in this unique sample of identical twins are compatible with the amyloid cascade hypothesis and thereby provide important new knowledge for clinical trial designs.

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Introduction

The amyloid cascade hypothesis has provided the leading framework for investigating and understanding Alzheimer's disease pathophysiology.[1,2] This hypothesis has been modified several times but the basic premise is that the deposition of amyloid-β in the brain initiates a cascade of downstream events, which includes the aggregation of hyper-phosphorylated tau in the neocortex, synaptic dysfunction, neuronal loss and eventually cognitive impairment and dementia. *In vivo* support for this hypothesis has come from studies on participants with autosomal dominant mutations in genes involved in amyloid-β metabolism leading to familial Alzheimer's disease,[3,4] as well as from prospective cohort studies on sporadic Alzheimer's disease, showing a temporal emergence of biomarker abnormality in line with the amyloid cascade sequence.[5–7] For example, whereas it is relatively common to observe amyloid-β pathology in the absence of neocortical tau pathology, it is rare to observe neocortical tau pathology in the absence of amyloid-β pathology.[8] However, although the amyloid cascade hypothesis has strongly impacted the research agenda and clinical trial designs over the past decades, precisely how amyloid-β deposition initiates hyperphosphorylation of neocortical tau pathology remains unclear. The association between amyloid-β and tau is further complicated by both a spatial and temporal paradox. Whereas amyloid-β is often observed first in posterior cingulate and orbitofrontal regions ~20 years before symptom onset,[9–11] tau neurofibrillary tangles are first observed in the (trans)entorhinal cortex and the spread of tau outside of the medial temporal lobe occurs in close proximity to symptom onset.[12,13] Therefore, we cannot exclude the possibility of a shared upstream process simultaneously driving amyloid-β and tau in an independent (yet correlated) manner instead of there being a causal relationship between amyloid-β and tau. Previous studies have suggested several biological pathways, including cholesterol metabolism, microglial immune activation and apolipoprotein E (*APOE*), which may drive both amyloid-β and tau pathology through shared but independent pathways.[14] Moreover, we have previously shown that genetically identical twins show substantial similarities in both amyloid-β load and tau load,[15,16] and therefore some genetic factors that influence amyloid-β may overlap with the genetic factors that also, independently, influence tau, which could potentially result in an association between amyloid-β and tau that is actually confounded by genetic variation. Gaining a better understanding into whether the relationships between key pathological hallmarks of Alzheimer's disease may actually be causal (as suggested by the amyloid cascade hypothesis) is of utmost importance for clinical trial designs.

Studying genetically identical twins provide the unique opportunity of ruling out genetic and shared environmental effects as potential confounders in an association, which is typically a concern for causal inferences in observational studies. The genetically identical within-pair difference design is a strong non-experimental case-control design in which individuals are strongly matched on genetic, demographic and shared environmental background. If a

causal relationship exists, then we would expect exposure to be associated with outcome both at the individual level and within identical twin-pairs, in which potential shared confounding effects are excluded. Although the within-pair difference design cannot rule out all possible alternatives to prove causality, it allows testing associations beyond what is possible in typical cohort studies. Therefore, the primary aim of the current study was to use genetically identical within-pair difference models to investigate the relationship between amyloid-β accumulation and aggregated tau while taking potential confounding by, among others, genetic factors into account. Secondary aims included to investigate associations with neurodegeneration and cognitive decline. To this end, we used longitudinal amyloid-β PET and cross-sectional tau PET, MRI and cognitive data from genetically identical twins with initially normal cognition. Inspired by previous twin studies,[17] we tested the following predictions generated by the amyloid cascade hypothesis:

First, if there is a causal relationship between amyloid-β and tau, then the twin that shows higher levels of amyloid-β should also have higher levels of tau compared to the genetically identical co-twin. Second, if changes in amyloid-β are the driving force behind tau accumulation, then the twin that shows larger longitudinal changes in amyloid-β should also have higher levels of tau compared to the genetically identical co-twin. We tested these predictions using within-pair difference models, in which within-pair differences in amyloid-β were regressed on within-pair differences in tau. The resulting within-pair difference effect represents an association that is free of confounding due to factors that are shared between the two twins of the same pair, which includes genetic factors (the twins are genetically identical), but also shared demographic (e.g. sex, age) and shared environmental (e.g. growing up in the same environment) factors. Finally, if tau pathology mediates the associations between amyloid-β pathology and neuronal loss and cognitive impairment (as predicted by the directionality in the amyloid cascade hypothesis) this should be supported by serial mediation models between within-pair differences in amyloid-β, tau, neurodegeneration and cognitive decline.

Materials and methods

Participants

We included genetically identical twins from the ongoing longitudinal Amsterdam sub-study of the EMIF-AD PreclinAD cohort.[18] At study entry, all participants were ≥60 years old and had normal cognition based on the performance on several neuropsychological tests.[18] Twin zygosity was confirmed by DNA analysis. Exclusion criteria included any significant neurologic, systemic or psychiatric disorder that could cause cognitive impairment. The study protocol for the total cohort ($n = 204$) included an amyloid-β PET scan at baseline and at 4-year follow-up. A subset of the cohort ($n = 80$) was selected to additionally undergo tau PET at 4-year follow-up.[16] This subset included twin-pairs of whom both twins or either one of the twins were amyloid-β positive or were classified into a high amyloid-β stage,[9] twin-pairs that carried an *APOE* ε4 allele, as well as age and sex matched amyloid-β negative twin-pairs (details described previously[16]). Structural MRI and cognitive data were also acquired at 4-year follow-up.

For the analyses in the current study, we included twins who completed amyloid-β PET, tau PET, structural MRI and neuropsychological assessment at the 4-year follow-up visit to enable cross-sectional associations between each modality. We therefore refer to the 4-year follow-up data as 'cross-sectional data'. Participants with incomplete cross-sectional data ($n = 125$) or participants with data of insufficient quality ($n = 1$ of whom cross-sectional amyloid-β PET was not suitable for quantification due to movement) were excluded, resulting in a total of 78 twins (37 genetically identical pairs and four singletons) included in the analyses. Twins' amyloid-β status (positive/negative) was defined by consensus visual read of cross-sectional [^{18}F]flutemetamol PET according to the manufacturer's guidelines.

This study was approved by the Medical Ethics Review Committee of the VU University Medical Center (Amsterdam, The Netherlands). All participants provided written informed consent.

Amyloid-β PET, tau PET and MR acquisition and processing

[^{18}F]flutemetamol (amyloid-β) PET was performed on an Ingenuity TF PET/MRI (Philips Medical Systems) (time difference between longitudinal scans: 4.1 ± 0.4 years). [^{18}F]flortaucipir (tau) PET was performed on an Ingenuity TF PET/CT (Philips Medical Systems). Both [^{18}F]flutemetamol and [^{18}F]flortaucipir PET images were acquired using a dynamic dual time-point acquisition protocol (0–30 and 90–110 min post-injection for [^{18}F]flutemetamol, and 0–30 and 80–100 min post-injection for [^{18}F]flortaucipir). Before both parts of the dynamic scan, for attenuation correction purposes, a T_1-weighted gradient echo pulse MRI was acquired for [^{18}F]flutemetamol and a low-dose CT scan for [^{18}F]flortaucipir. Details on [^{18}F]flutemetamol and [^{18}F]flortaucipir acquisition are described elsewhere.[16,19] For both tracers, we coregistered the two parts of the dynamic scan to each other using Vinci software.[20] Subsequently, 3D isotropic T_1-weighted MR images were coregistered to the corresponding native-space PET images. Grey matter regions of interest (ROI) from the Hammers atlas[21] were automatically delineated on the coregistered MR images and superimposed on both [^{18}F]flutemetamol and [^{18}F]flortaucipir PET scans to extract time activity curves using PVElab. For [^{18}F]flortaucipir, we additionally superimposed grey matter ROI from the Svarer atlas[22] onto the PET scan, to extract time activity curves in the entorhinal cortex. Voxel-wise parametric images of binding potential (BP_{ND}) were generated using SRTM2 for [^{18}F]flutemetamol and using RPM for [^{18}F]flortaucipir, validated in previous studies.[20,23–26] For both tracers, whole cerebellar grey matter was used as the reference region.

For [^{18}F]flutemetamol PET, we additionally calculated (retrospective) annual change in BP_{ND} ($BP_{ND}/y = (BP_{ND[4y\text{-}follow\text{-}up]} - BP_{ND[baseline]})$/time difference in years) to test our hypothesis that longitudinal changes in amyloid-β pathology are associated with tau pathology, neurodegeneration and cognitive decline. [^{18}F]flutemetamol BP_{ND}/y was missing for $n = 4$ twins due to missing baseline [^{18}F]flutemetamol PET.

For voxel-wise [^{18}F]flortaucipir analyses, BP_{ND} images were spatially normalized to Montreal Neurological Institute space (using the transformation matrixes derived from warping the coregistered T_1-weighted MRI), followed by smoothing using an 8 mm isotropic Gaussian kernel, using Statistical Parametric Mapping v.8 software (Wellcome Trust Center for Neuroimaging, University College London, UK) in line with our previous studies.[27,28] All warped images were visually checked for transformation errors.

Participants underwent three-dimensional T_1-weighted MRI on a 3.0 T Ingenuity TF PET/MR (Philips Medical Systems) with an eight-channel head coil. T_1-weighted sequences were acquired using sagittal turbo field echo sequence (1.00 mm^3 isotropic voxels,

repetition time/echo time = 7.9 ms/4.5 ms and flip angle = 8°).[29] Cortical reconstruction and volumetric segmentation was performed with Freesurfer v.7.1.1, which is documented and freely available online (http://surfer.nmr.mgh.harvard.edu). The technical details of these procedures are described previously.[30]

Amyloid-β PET, tau PET and MRI regions of interest

We computed two ROIs for each imaging modality (amyloid-β PET, tau PET and MRI) on the basis of previous literature: (i) a modality-specific early-Alzheimer's disease ROI; and (ii) a modality-specific Alzheimer's disease-signature ROI.

For amyloid-β PET, we created a volume-weighted average of BP_{ND} in the bilateral posterior cingulate cortex (PCC) and orbitofrontal gyrus (OFG) as the early amyloid-β PET ROI.[9,10] A global ROI was created as the Alzheimer's disease-signature amyloid-β ROI, which was a volume-weighted average of BP_{ND} in the bilateral parahippocampal gyrus, ambient gyrus, superior temporal gyrus, middle temporal gyrus, inferior temporal gyrus, fusiform gyrus, insula, anterior cingulate cortex, PCC, superior parietal gyrus, lateral parietal lobe, lateral occipital lobe, lingual gyrus, cuneus, middle frontal gyrus, gyrus rectus, OFG, inferior frontal gyrus and superior frontal gyrus.[9,10]

For tau PET, we created a volume-weighted average of BP_{ND} in the bilateral entorhinal cortex as the early tau ROI.[13,16] A temporal meta-ROI was created as the Alzheimer's disease-signature tau ROI, which was a volume-weighted average of BP_{ND} in the bilateral entorhinal cortex, amygdala, parahippocampal gyrus, fusiform gyrus and middle and inferior temporal gyrus.[16]

For MRI, we calculated bilateral mean hippocampal volume (adjusted for intracranial volume) as an early neurodegeneration ROI. We calculated cortical thickness in a temporal meta-ROI as the Alzheimer's disease-signature neurodegeneration ROI, which was an average of the bilateral entorhinal, inferior temporal, middle temporal and fusiform cortices.[31]

Neuropsychological assessment

We created two neuropsychological composite scores for capturing early cognitive decline: (i) a memory composite score, since this cognitive domain is often affected first in Alzheimer's disease; and (ii) a modified Preclinical Alzheimer's Cognitive Composite (mPACC) score, which is designed to be sensitive to cognitive changes in the preclinical stage of Alzheimer's disease.[32,33] In the main text, we report on results with the composite memory score, whereas results for the mPACC are shown in the Supplementary material to have an alternate measure of cognitive functioning.

The memory composite score included the total immediate and delayed recall of the Dutch version of the Rey Auditory Verbal Learning Test,[34,35] the 3- and 20-min recall of the Rey Complex Figure Test[36] and the delayed recall of names and occupations of the Face-Name Associated Memory Exam.[37] The mPACC in the current study consisted of the Mini Mental State Examination,[38] the delayed recall of the Dutch version of the Rey Auditory Verbal Learning Test, the Digit Symbol Substitution Test score,[39] the total score of the Face-Name Associated Memory Exam and the one-minute Animal Fluency test.[40] Missing data were imputed (for two participants, one cognitive test was missing) using predictive mean matching as method,[41] after which individual neuropsychological test scores were standardized using the mean and standard deviation from baseline neuropsychological test scores from the entire Amsterdam EMIF-AD PreclinAD cohort ($n = 204$) and subsequently averaged into the composite scores.[18]

Statistical analyses

We used R version 4.0.3 for statistical analyses unless specified otherwise. A P-value < 0.05 was considered significant.

First, we tested associations between amyloid-β PET (BP_{ND} and BP_{ND}/y), tau PET BP_{ND}, neurodegeneration and cognitive functioning at the individual level. We used generalized estimating equation (GEE) models between each modality, corrected for age and sex, and taking potential clustering within families into account. GEE models with cognitive functioning as the outcome measure additionally included years of education as a covariate. GEE models with amyloid-β PET annual change (BP_{ND}/y) as predictor were performed with and without correcting for initial amyloid-β PET BP_{ND}. As our cohort consisted of a relatively large number of *APOE* ε4 carriers, sensitivity analyses additionally correcting for *APOE* ε4 status are shown in the Supplementary material.

Subsequently, we tested associations between amyloid-β PET (BP_{ND} and BP_{ND}/y), tau PET BP_{ND}, neurodegeneration and cognitive functioning using genetically identical within-pair difference models, by regressing the within-pair difference of the predictor onto the within-pair difference of the outcome variable. The resulting effect represents an association that can be interpreted similarly to the GEE effect, but the twin-difference effect is free of confounding due to factors that are shared between the two twins of the same pair, which includes genetic factors (the twins are genetically identical), but also shared demographic (e.g. sex, age) and shared environmental (e.g. growing up in the same environment) factors. Age and sex were therefore not included as covariates in these analyses. Since twins of the same pair could differ on education, linear regression twin-difference models with within-pair difference in cognitive functioning as outcome measure were corrected for within-pair difference in years of education. Linear regression twin-difference models with within-pair difference in amyloid-β PET annual change (BP_{ND}/y) as predictor were performed with and without correcting for within-pair difference in initial amyloid-β PET BP_{ND}.

We further explored the association between amyloid-β and tau in more detail, by examining the association between ROI-level (within-pair differences in) amyloid-β PET (BP_{ND} and BP_{ND}/y) and (within-pair differences in) voxel-wise tau PET BP_{ND} using linear regression models in Statistical Parametric Mapping12. Analyses at the individual level were corrected for age and sex. Results are displayed at more liberal (i.e. $P < 0.001$, uncorrected) and more stringent ($P < 0.05$, family-wise error corrected) thresholds for visualization purposes.

Finally, following the cascade of events according to the amyloid cascade hypothesis, we tested the mediating effect of tau in the association between amyloid-β and neurodegeneration, and the mediating effects of both tau and neurodegeneration in the association between amyloid-β and cognitive decline (separate mediation models). These single mediation models were performed using the 'Mediation' package in R. In a final serial mediation model, we tested a four-factor mediation model including direct and indirect pathways between amyloid-β, tau, neurodegeneration and cognition, and using a bootstrap method (1000 iterations) for the mediation effects, using the 'Lavaan' package in R.[42] All mediation analyses were performed on within-pair difference variables only.

Data availability

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Results

Participant characteristics

Participant characteristics are shown in Table 1. In total, 78 twins with an average age of 73.7 ± 6.2 years were included. The cohort consisted of 37 complete pairs and four singletons of which the co-twins were not included in the current study due to incomplete cross-sectional data. Of the 78 twins, 39 (50.0%) carried an *APOE* ε4 allele and 30 (38.5%) were visually read as amyloid-β PET positive.

Associations between amyloid-β and tau

Table 2 shows all GEE and twin-difference estimates and *P*-values for both early-Alzheimer's disease and Alzheimer's disease-signature ROI. In the text and figures, we report results for early-Alzheimer's disease amyloid-β PET (OFG/OCC), tau PET (entorhinal cortex) and neurodegeneration (hippocampal volume) ROIs, but similar results were observed for Alzheimer's disease-signature ROIs (Table 2).

We first tested the association between cross-sectional amyloid-β PET BP_{ND} and tau PET BP_{ND}. At the individual level, higher amyloid-β PET BP_{ND} was associated with higher tau PET BP_{ND} (β = 0.64, $P < 0.001$). The twin-difference model replicated this. Across pairs, within-pair differences in amyloid-β PET BP_{ND} were associated with within-pair differences in tau PET BP_{ND} (β = 0.68, $P < 0.001$) (Fig. 1A). We then tested this association using voxel-wise tau PET as the outcome measure. At the individual level, voxel-wise analyses showed that amyloid-β PET BP_{ND} in the early-Alzheimer's disease ROI was associated with tau PET BP_{ND} in medial and inferior lateral temporal brain regions. When regressing within-pair differences in early-Alzheimer's disease ROI amyloid-β PET BP_{ND} on within-pair differences in voxel-wise tau PET BP_{ND}, we observed an overlapping pattern, slightly extending into the parietal lobe (Fig. 1A). Sensitivity analyses additionally correcting for *APOE* ε4 status yielded similar results (Supplementary Table 1).

We then repeated the analyses using annual change in amyloid-β PET BP_{ND} (BP_{ND}/y, corrected for initial BP_{ND}) as predictor for tau PET BP_{ND} (Fig. 1B). At the individual level, higher amyloid-β PET BP_{ND}/y was associated with higher tau PET BP_{ND} (β = 0.36, $P < 0.001$). The twin-difference model showed a similar result, with within-pair differences in amyloid-β PET BP_{ND}/y being associated with within-pair differences in tau PET BP_{ND} (β = 0.41, $P = 0.02$). We observed similar results without correcting for initial amyloid-β PET BP_{ND} (Supplementary Table 2). Voxel-wise analyses showed effects of amyloid-β PET BP_{ND}/y predominantly on medial temporal tau PET BP_{ND} lateralized to the left hemisphere at both the individual level and within twin-pairs (Fig. 1B).

Associations between amyloid-β, tau and neurodegeneration

Next, we tested associations of amyloid-β PET (BP_{ND} and BP_{ND}/y) and tau PET BP_{ND} with neurodegeneration. In the text, we report results for early-Alzheimer's disease ROI, whereas a complete overview of results obtained for both early-Alzheimer's disease and Alzheimer's disease-signature ROIs are shown in Table 2. At the individual level, higher amyloid-β PET BP_{ND} was associated with lower hippocampal volume (β = −0.19, $P = 0.02$), however amyloid-β PET BP_{ND}/y (retrospective annual change) was not (β = −0.03, $P = 0.75$). Similarly, within-pair differences in cross-sectional amyloid-β PET BP_{ND}, but not annual change, were associated with within-pair differences in hippocampal volume (β = −0.37, $P = 0.03$ and β = −0.04, $P = 0.82$ respectively) (Fig. 2A).

At the individual level, higher tau PET BP_{ND} was associated with lower hippocampal volume (β = −0.22, $P = 0.02$). In addition, within-pair differences in tau PET BP_{ND} were associated with within-pair differences in hippocampal volume (β = −0.53, $P < 0.001$) (Fig. 2C).

We performed mediation analyses to test for the potential mediating effect of tau in the within-pair difference association between amyloid-β and neurodegeneration. Mediation analyses were performed for early-Alzheimer's disease ROI and only on cross-sectional data, since twin-difference results for annual change in amyloid-β PET BP_{ND} were less pronounced and/or non-significant (Table 2). Upon testing whether the effect of within-pair differences in amyloid-β PET BP_{ND} on within-pair differences in hippocampal volume was mediated by within-pair differences in tau PET BP_{ND} (A→T→N), we observed a full mediation effect of tau (proportion mediated: 91%, $P < 0.05$) (Fig. 2B and D). We also tested whether the association between within-pair differences in tau PET BP_{ND} and within-pair differences in hippocampal volume was mediated by within-pair differences in amyloid-β PET BP_{ND} (T→A→N), but we did not observe such a mediation effect (proportion mediated: 0.05%, $P > 0.05$) (Supplementary Fig. 1A).

Associations between amyloid-β, tau, neurodegeneration and cognitive functioning

Next, we tested associations of amyloid-β PET (BP_{ND} and BP_{ND}/y), tau PET BP_{ND} and neurodegeneration with cognitive functioning.

Table 1 Demographics

	Total sample
n (%)	78
Age, years	73.7 ± 6.2
Sex, *n* female (%)	40 (51.3)
Education, years	12.3 ± 2.9
MMSE	28.7 ± 1.3
APOE ε4 status, *n* carrier (%)	39 (50.0)
Amyloid-β status, *n* positive (%)	30 (38.5)
Amyloid-β twin-pair status, *n* pairs concordant negative/discordant/concordant positive (%)[a]	15 (38.5)/15 (38.5)/ 7 (17.9)
[^{18}F]flutemetamol (amyloid-β)-PET	
OFG/PCC (early-Alzheimer's disease) BP_{ND}	0.33 ± 0.22
Global (Alzheimer's disease-signature) BP_{ND}	0.24 ± 0.18
[^{18}F]flortaucipir (tau)-PET	
Entorhinal (early-Alzheimer's disease) BP_{ND}	0.02 ± 0.14
Temporal meta-ROI (Alzheimer's disease-signature) BP_{ND}	0.10 ± 0.09
MRI	
Hippocampal volume (early-Alzheimer's disease)	3700 ± 480
Temporal cortical thickness (Alzheimer's disease-signature)	2.70 ± 0.12
Cognition	
Composite memory (z-score)	0.13 ± 0.88
mPACC (z-score)	−0.02 ± 0.72

Shown are mean ± SD unless specified otherwise. All variables are derived from the cross-sectional visit (at time of tau PET). MMSE = Mini Mental State Examination.
[a]The cohort consisted of 37 complete twin-pairs and four singletons.

Table 2 Associations between amyloid-β, tau, neurodegeneration and memory

	Early-Alzheimer's disease ROI		Alzheimer's disease-signature ROI	
	GEE	Twin-difference	GEE	Twin-difference
Aβ-PET BP_{ND} versus tau PET BP_{ND}	**β = 0.64, P < 0.001 n = 78**	**β = 0.68, P < 0.001 n = 37**	**β = 0.57, P < 0.001 n = 78**	**β = 0.68, P < 0.001 n = 37**
Aβ-PET BP_{ND}/y versus tau PET BP_{ND}	**β = 0.36, P < 0.001 n = 74**	**β = 0.41, P = 0.02 n = 33**	**β = 0.25, P = 0.02 n = 74**	β = 0.21, P = 0.24 n = 33
Aβ-PET BP_{ND} versus neurodegeneration	**β = −0.19, P = 0.02 n = 78**	**β = −0.37, P = 0.03 n = 37**	β = −0.17, P = 0.11 n = 78	β = −0.28, P = 0.09 n = 37
Aβ-PET BP_{ND}/y versus neurodegeneration	β = −0.03, P = 0.75 n = 74	β = −0.04, P = 0.82 n = 33	β = 0.02, P = 0.84 n = 74	β = 0.21, P = 0.25 n = 33
Tau PET BP_{ND} versus neurodegeneration	**β = −0.22, P = 0.02 n = 78**	**β = −0.53, P < 0.001 n = 37**	**β = −0.22, P = 0.01 n = 78**	**β = −0.53, P < 0.001 n = 37**
Aβ-PET BP_{ND} versus composite memory	**β = −0.47, P < 0.001 n = 78**	**β = −0.57, P < 0.001 n = 37**	**β = −0.45, P < 0.001 n = 78**	**β = −0.54, P < 0.001 n = 37**
Aβ-PET BP_{ND}/y versus composite memory	**β = −0.25, P = 0.01 n = 74**	β = −0.22, P = 0.22 n = 33	**β = −0.23, P = 0.01 n = 74**	β = −0.23, P = 0.22 n = 33
Tau PET BP_{ND} versus composite memory	**β = −0.63, P < 0.001 n = 78**	**β = −0.68, P < 0.001 n = 37**	**β = −0.55, P < 0.001 n = 78**	**β = −0.72, P < 0.001 n = 37**
Neurodegeneration versus composite memory	**β = 0.48, P < 0.001 n = 78**	**β = 0.56, P < 0.001 n = 37**	β = 0.19, P = 0.10 n = 78	**β = 0.42, P = 0.01 n = 37**

All GEE models are corrected for age and sex. GEE and twin-difference models with Aβ-PET BP_{ND}/y as predictor are additionally corrected for (within-pair difference in) initial Aβ-PET BP_{ND}. Aβ-PET BP_{ND}/y was missing for n = 4 twins (from four pairs) due to missing initial Aβ-PET. GEE and twin-difference models with memory functioning as outcome are additionally corrected for (within-pair difference in) education. We scaled predictor and outcome variables within each GEE to enable comparison of effect sizes (except for hippocampal volume, which was a standardized residual corrected for intracranial volume). Significant associations (at P < 0.05) are highlighted in bold.

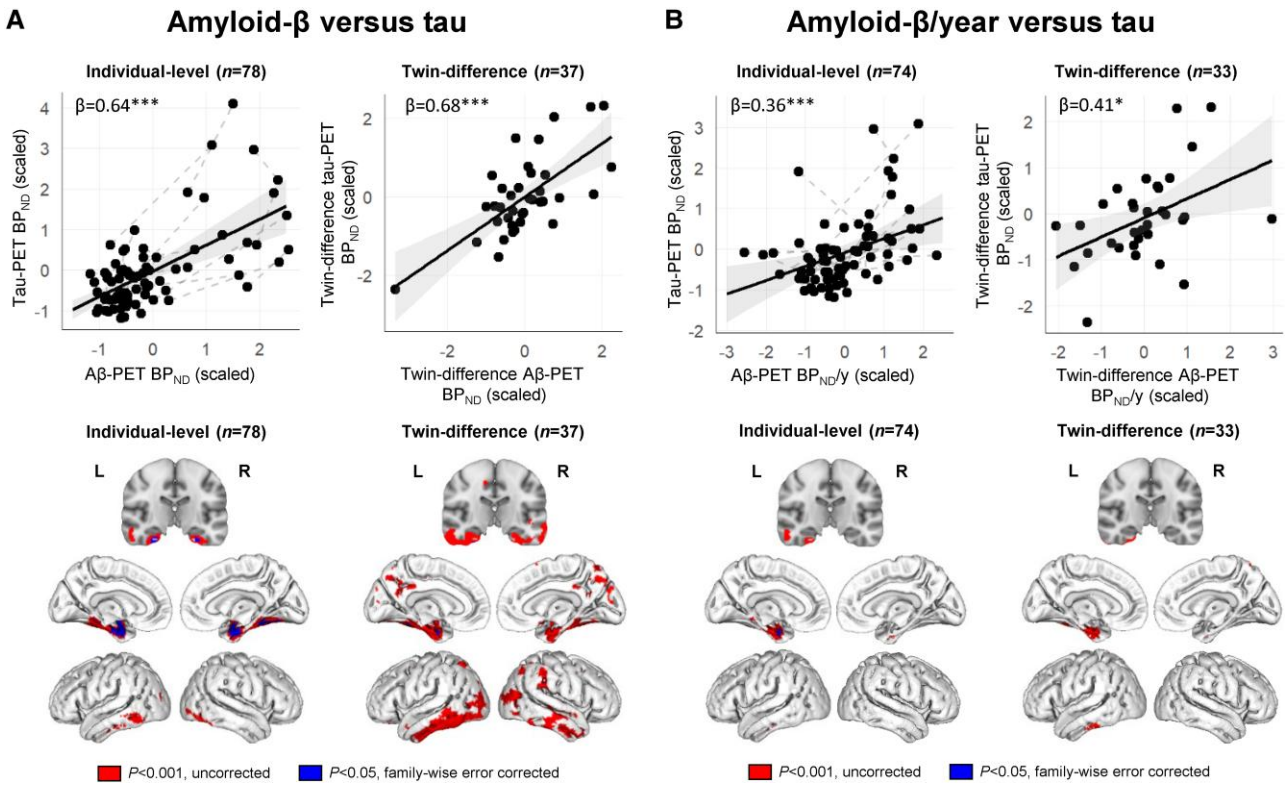

Figure 1 Individual-level and twin-difference associations between amyloid-β and tau. Shown are individual-level and twin-difference associations between (**A**) cross-sectional amyloid-β versus tau, and (**B**) annual change in amyloid-β versus tau. For both plots, amyloid-β PET and tau PET BP_{ND} in the early-AD ROI is shown (OFG/PCC for amyloid-β PET and entorhinal cortex for tau PET). In the individual-level scatter plots, each dot reflects a twin, and twins that belong to the same pair are connected with the dashed line. In the twin-difference scatter plots, each dot reflects a twin-pair. Voxel-wise results reflect the association between voxel-wise tau PET BP_{ND} as the outcome variable, and amyloid-β PET BP_{ND} in the early-Alzheimer's disease ROI (OFG/PCC) as the predictor variable at (**A**) the individual level and (**B**) within identical twin-pairs. Voxel-wise results at the individual level are corrected for age and sex. *P < 0.05; **P < 0.01; ***P < 0.001.

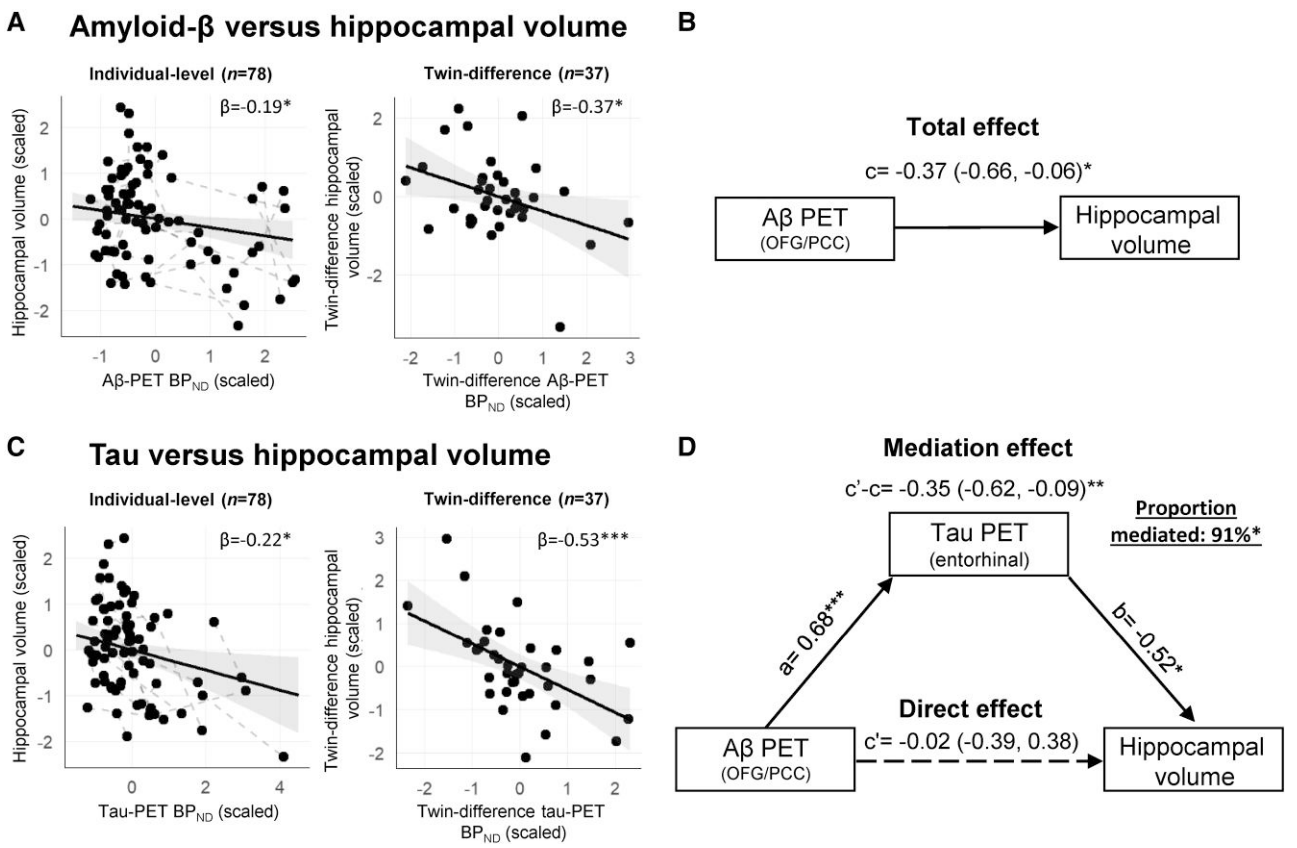

Figure 2 Individual-level and twin-difference associations between amyloid-β, tau and hippocampal volume. Shown are individual-level and twin-difference associations between (**A**) amyloid-β and hippocampal volume, and (**C**) tau and hippocampal volume. For both plots, amyloid-β PET and tau PET BP_{ND} in the early-Alzheimer's disease ROI is shown (OFG/PCC for amyloid-β PET and entorhinal cortex for tau PET). In the individual-level scatter plots, each dot reflects a twin, and twins that belong to the same pair are connected with the dashed line. In the twin-difference scatter plots, each dot reflects a twin-pair. In **B**, the direct effect of within-pair differences in amyloid-β PET on within-pair differences in hippocampal volume is shown. In **D**, the mediating effect of within-pair differences in tau PET in the association between within-pair differences in amyloid-β PET and within-pair differences in hippocampal volume is shown. Dashed lines indicate pathways that were not significant. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

In the text, we report results for early-Alzheimer's disease ROI, whereas a complete overview of results obtained for both early-Alzheimer's disease and Alzheimer's disease-signature ROI are shown in Table 2. At the individual level, higher amyloid-β PET BP_{ND} and higher amyloid-β PET BP_{ND}/y were associated with lower composite memory scores ($\beta = -0.47$, $P < 0.001$ and $\beta = -0.25$, $P = 0.01$, respectively). Furthermore, higher tau PET BP_{ND} and lower hippocampal volume were also associated with lower composite memory scores ($\beta = -0.63$, $P < 0.001$ and $\beta = 0.48$, $P < 0.001$, respectively). Twin-difference models showed largely similar results. Within-pair differences in cross-sectional amyloid-β PET BP_{ND} ($\beta = -0.57$, $P < 0.001$), tau PET BP_{ND} ($\beta = -0.68$, $P < 0.001$) and hippocampal volume ($\beta = 0.56$, $P < 0.001$) were all associated to within-pair differences in composite memory scores (Fig. 3A, C and E and Table 2). However, we did not observe a significant association between within-pair differences in amyloid-β PET BP_{ND}/y and within-pair differences in composite memory scores ($\beta = -0.22$, $P = 0.22$). We performed sensitivity analyses with mPACC as a measure of cognitive functioning instead of composite memory and found very similar results for both GEE models and twin-difference models (results shown in Supplementary Table 3).

We then investigated whether within-pair differences in tau PET BP_{ND} and within-pair differences in hippocampal volume mediated the association between within-pair differences in amyloid-β PET BP_{ND} and within-pair differences in composite memory scores (A→T→C and A→N→C, respectively). In line with the previous mediation model, these analyses were only performed on cross-sectional data and for early-Alzheimer's disease ROI. The association between within-pair differences in amyloid-β PET BP_{ND} and within-pair differences in composite memory scores was fully mediated by within-pair differences in tau PET BP_{ND} (proportion mediated: 70%, $P < 0.001$) and partially mediated by within-pair differences in hippocampal volume (proportion mediated: 27%; $P < 0.05$) (Fig. 3B, D and F). We again tested the possibility of within-pair differences in amyloid-β PET BP_{ND} mediating the association between within-pair differences in tau PET BP_{ND} and within-pair differences in composite memory scores (T→A→C), but we did not observe such a mediation effect (proportion mediated: 14%, $P > 0.05$) (Supplementary Fig. 1B).

Serial mediation model between amyloid-β, tau, neurodegeneration and cognitive functioning

Finally, to test the full amyloid cascade hypothesis, we tested a four-factor serial mediation model including pathways between amyloid-β, tau, neurodegeneration and cognitive functioning. In line with the simpler mediation models above (Figs. 2 and 3), this serial mediation model was performed on within-pair difference scores, cross-sectional data and early-Alzheimer's disease ROI. For the total effect of amyloid-β (A) on memory functioning (C),

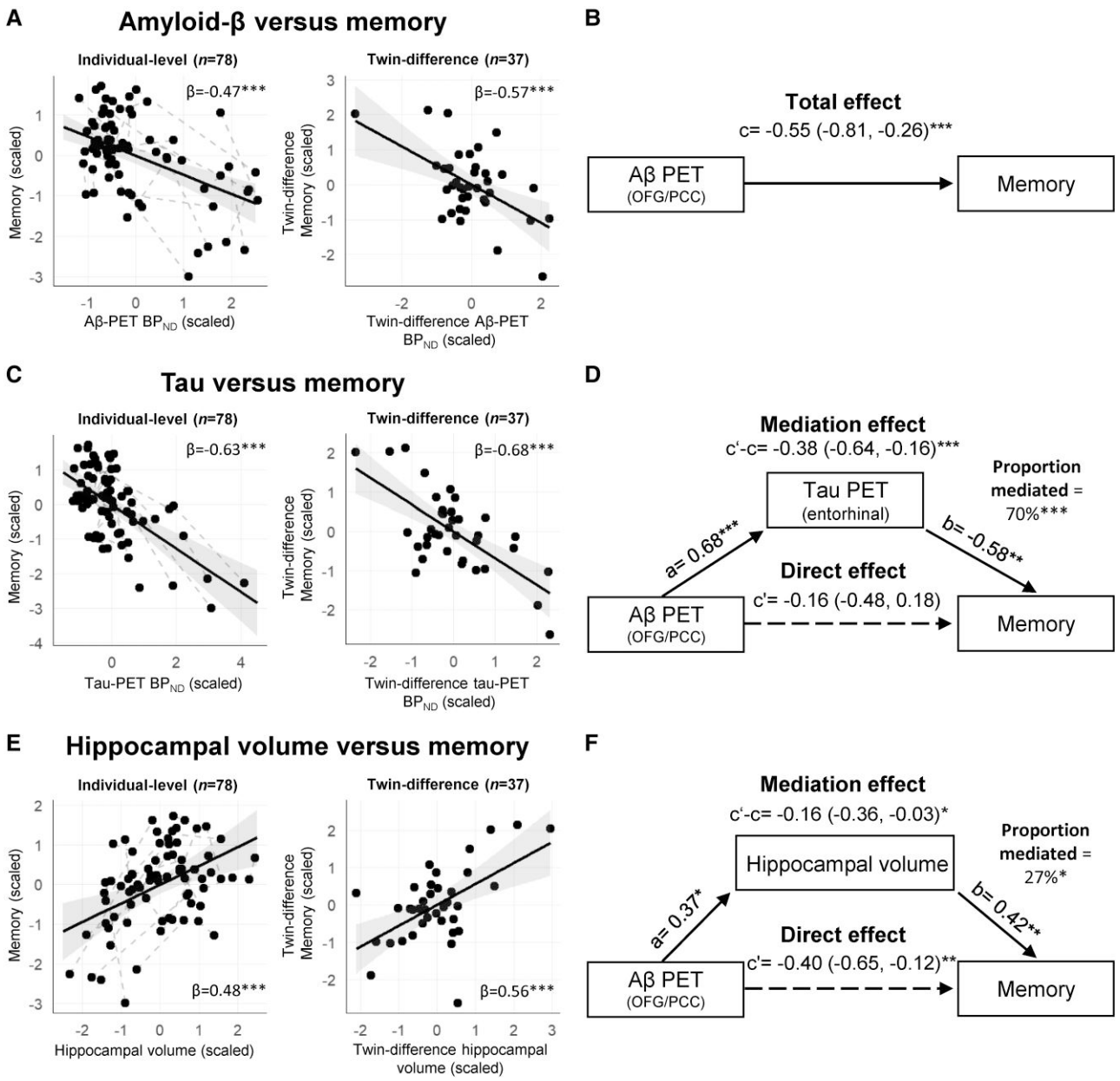

Figure 3 Individual-level and twin-difference associations between amyloid-β, tau, hippocampal volume and memory. Shown are individual-level and twin-difference associations between (**A**) amyloid-β and composite memory, (**C**) tau and composite memory, and (**E**) hippocampal volume and composite memory. For all plots, amyloid-β PET and tau PET BP_{ND} in the early-Alzheimer's disease ROI is shown (OFG/PCC for amyloid-β PET and entorhinal cortex for tau PET). On the individual level, each dot reflects a twin, and twins that belong to the same pair are connected with the dashed line. In the twin-difference scatter plots, each dot reflects a twin-pair. In **B**, the direct effect of within-pair differences in amyloid-β PET on within-pair differences in composite memory is shown. In **D**, the mediating effect of within-pair differences in tau PET is shown, and in **F**, the mediating effect of within-pair differences in hippocampal volume is shown. Dashed lines indicate pathways that were not significant. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

we calculated the proportion that was mediated through the following pathways: pathway 1, via tau (T) (A→T→C, while taking pathways 2 and 3 into account); pathway 2, via hippocampal volume (N) (A→N→C, while taking pathways 1 and 3 into account); and pathway 3, via tau and subsequently hippocampal volume (A→T→N→C, while taking pathways 1 and 2 into account) (Fig. 4). The total proportion mediated (pathways 1, 2 and 3 combined) was 69.9% ($P < 0.001$). Pathways 1, 2 and 3 separately indicated that this was largely attributable to pathway 1, which had the largest mediating effect (51.6%, $P = 0.03$). Pathways 2 and 3 accounted for only 0.7% ($P = 0.83$) and 17.8% ($P = 0.26$), respectively. After accounting for the different mediation pathways, there was no longer a direct effect of amyloid-β on memory functioning, amyloid-β on hippocampal volume and hippocampal volume on memory functioning (all $P > 0.05$) (Fig. 4). Sensitivity analyses with mPACC as a measure of cognitive functioning instead of composite memory showed highly similar results (Supplementary Fig. 2).

Discussion

In this study, we used amyloid-β PET, tau PET, MRI and cognitive data from cognitively unimpaired genetically identical twins to test hypotheses of causality, as suggested by the amyloid cascade

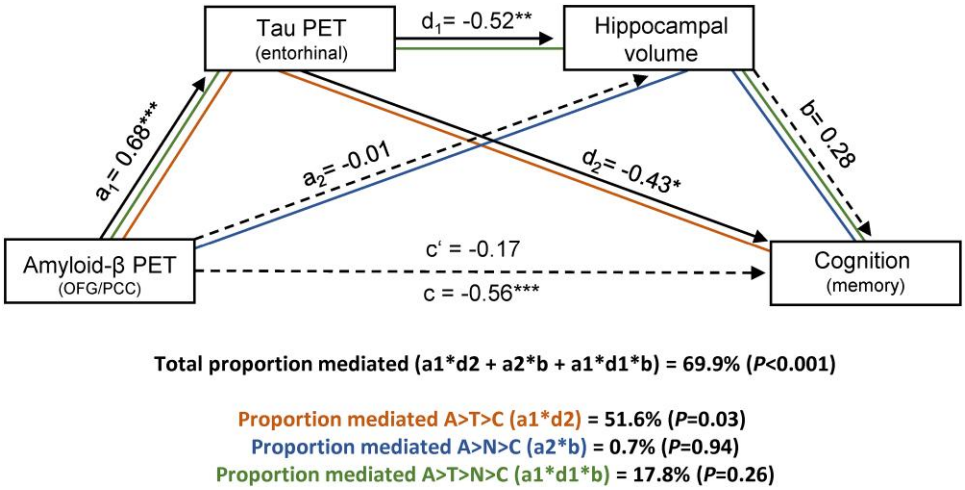

Figure 4 Serial mediation model. Serial mediation model including pathways between within-pair differences in amyloid-β, tau, hippocampal volume and memory functioning ($n = 37$). For the direct effect of amyloid-β on memory functioning, the total proportion that was mediated via pathways including tau and neurodegeneration was 69.9%. The pathway leading from amyloid-β to tau to memory functioning (highlighted in orange) revealed the largest proportion mediated (51.6%). Dashed lines indicate pathways that were not significant. *P < 0.05; **P < 0.01; ***P < 0.001.

hypothesis, beyond typical group analyses. Using genetically identical twin-difference designs, we observed that associations between amyloid-β, tau, neurodegeneration and cognition are minimally affected by (genetic) confounding. This is congruent with the causal predictions generated by the amyloid cascade hypothesis. Sequential mediation analyses revealed that within-pair difference effects of amyloid-β on neurodegeneration, and within-pair difference effects of amyloid-β on cognitive functioning, were fully mediated by tau. This underscores the important mediating role of tau pathology in the pathway leading from amyloid-β to cognitive decline, even in very early disease stages when tau load (at least when measured with PET) is still relatively low. This is of specific interest for clinical trials, which are increasingly focused on preclinical stages of the disease.

One of the most important findings of this study is the robustness of the associations between amyloid-β, tau, neurodegeneration and cognitive decline. In our study, the twin that had more amyloid-β pathology compared to the genetically identical co-twin, also had more tau pathology, lower hippocampal volume and worse performance on neuropsychological tests as underlined by the within-pair difference models. The effect sizes of the regressions of amyloid-β on tau, neurodegeneration and cognition seen at the individual level were highly comparable to the effect sizes seen in the within-pair differences regression. This means that the associations that have been reported in the population at large between these parameters[6–8,43–47] can be considered to be unbiased by genetic confounding. In addition to eliminating genetic confounding, many other forms of confounding are eliminated including confounding by shared demographic and shared environmental factors. These important new findings in this unique sample of identical twins further increase the possibility of true causal relationships between amyloid-β, tau, neurodegeneration and cognitive decline.

Our primary focus was the relationship between amyloid-β and tau. The twin-difference models replicated the GEE models, indicating that both the association between cross-sectional amyloid-β and tau and the association between annual change in amyloid-β and tau are robust and minimally affected by (genetic) confounding. Moreover, the strong similarities in voxel-wise results at the individual level and within twin-pairs particularly support a relationship between amyloid-β deposition (in a region that is affected by amyloid-β early in the disease[9,10]) and the accumulation of tau in medial temporal and inferolateral temporal regions. These regions correspond to what has been observed in previous non-twin studies.[46,48,49] It is, however, important to recognize that although within-pair difference models are more powerful compared to analyses at the individual level, we cannot rule out all possible alternatives to prove causality in this association. For example, although identical twins are strongly matched on genetic and shared environmental factors, they are not matched on non-shared environmental factors, and confounding by non-shared environmental factors can therefore not be excluded.[50] Furthermore, on the basis of the current study, we cannot make any inferences on the exact mechanism underlying the link between amyloid-β and tau, and the spatial and temporal paradox between the two pathologies remains of interest. Previous studies have suggested that amyloid-β may lead to neuronal hyperactivity,[51] which in turn may amplify the secretion and spread of tau.[52,53] Although there thus seems to be a deterministic association between amyloid-β and tau (if a twin had more amyloid-β compared to the identical co-twin, that twin also had more tau), the observation that twins of the same pair could show differences in both amyloid-β and tau also indicates that the timing of the onset of pathology could be probabilistic.[54] It is likely a complex interplay between genes and environment that determines whether and when pathology will develop.

While twin-difference models can exclude (genetic) confounding, additional use of mediation models allows further tests of directionality in the associations. We therefore performed multiple single mediation models, and one serial mediation model combining all modalities, to test the sequence of events as described by the amyloid cascade hypothesis. Our single mediation models using twin-difference scores as input variables (since these variables are free of confounding due to factors that twins share) showed that tau fully mediated the associations between amyloid-β and hippocampal volume, and between amyloid-β and cognitive decline. Importantly, amyloid-β did not mediate the associations between tau and hippocampal volume or between tau and cognitive

decline, for which only a direct effect of tau was observed. These results are compatible with a pathway leading from amyloid-β to tau, as suggested by the amyloid cascade hypothesis.[1] In a single mediation model, we observed a partial mediation effect of hippocampal volume in the association between amyloid-β and cognitive decline. However, in the serial mediation model—which also took mediation pathways of tau into account—the proportion mediated by the pathway from amyloid-β to hippocampal volume to cognitive decline was only 0.7%. In contrast, the proportion mediated directly through tau was 51.6%, and the proportion mediated through tau and subsequently hippocampal volume loss was 17.8%. These results corroborate to a wide variety of previous studies suggesting that to slow or halt cognitive decline (and neurodegeneration), one must need to prevent or slow tau accumulation—even in very early stages of the disease when tau load is still relatively low.[6,55–58]

Strengths of the study include the unique study population, the availability of multimodal imaging and the use of longitudinal amyloid-β PET data. This study also had some limitations. The main limitation of this study is the relatively small sample size. Since twin-difference models can only be performed on complete pairs with complete data, we had to exclude a substantial number of pairs from the analyses. Some results (e.g. the serial mediation model) may have been affected by limited power. Furthermore, this cohort consisted of a relatively large percentage of *APOE* e4 carriers and amyloid-β positive unimpaired individuals, which may limit generalizability to the general population. In addition, our [^{18}F]flutemetamol PET scans were acquired on a PET/MR system, which has been shown to have fewer optimal attenuation correction methods compared to PET/CT systems. Finally, a general limitation when studying preclinical Alzheimer's disease pathology is that the overall levels of pathology are relatively low.

Conclusion

The associations between amyloid-β, tau, neurodegeneration and cognition are robust and minimally affected by (genetic) confounding. These novel findings in this unique sample of identical twins are compatible with predictions generated by the amyloid cascade hypothesis and thereby provide important new knowledge for clinical trial designs.

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Competing interests

E.M.C., J.T., L.L., M.K., R.R., S.M.L., M.H., S.S.V.G., M.Y., E.J.C.G. and A.B. report no competing interests. R.O. has given a lecture in a symposium sponsored by GE Healthcare (fee paid to the institution), and is an editorial board member of the European Journal of Nuclear Medicine and Molecular Imaging and of Alzheimer's Research and Therapy. L.E.C. has received research support from GE Healthcare (paid to institution). A.D.W. is Editor-in-Chief of Nuclear Medicine and Biology. F.B. is in a steering committee or iDMC member for Biogen, Merck, Roche, EISAI and Prothena. Consultant for Roche, Biogen, Merck, IXICO, Jansen, Combinostics. Research agreements with Merck, Biogen, GE Healthcare, Roche. Co-founder and shareholder of Queen Square Analytics LTD. F.B. is supported by the NIHR biomedical research centre at UCLH. P.S. has received consultancy fees (paid to the university) from Alzheon, Brainstorm Cell and Green Valley. Within his university affiliation he is global PI of the phase 1b study of AC Immune, Phase 2b study with FUJI-film/Toyama and phase 2 study of UCB. He is chair of the EU steering committee of the phase 2b program of Vivoryon, the phase 2b study of Novartis Cardiology and co-chair of the phase 3 study with NOVO-Nordisk. He is also an employee of EQT Life Sciences (formerly LSP). B.M.T. and P.J.V. are inventors on a patent (#P122938EP10, #P1222938PC00, owner: Stichting VUmc). B.v.B. has received research support from EU-FP7, CTMM, ZonMw, NWO and Alzheimer Nederland, has performed contract research for Rodin, IONIS, AVID, Eli Lilly, UCB, DIAN-TU and Janssen, was a speaker at a symposium organized by Springer Healthcare, has a consultancy agreement with IXICO for the reading of PET scans and is a trainer for GE. B.v.B. only receives financial compensation from Amsterdam UMC.

Supplementary material

Supplementary material is available at *Brain* online.

References

1. Hardy JA, Higgins GA. Alzheimer's disease: The amyloid cascade hypothesis. *Science*. 1992;256:184-185.
2. Selkoe DJ, Hardy J. The amyloid hypothesis of Alzheimer's disease at 25 years. *EMBO Mol Med*. 2016;8:595-608.
3. Gordon BA, Blazey TM, Christensen J, *et al*. Tau PET in autosomal dominant Alzheimer's disease: Relationship with cognition, dementia and other biomarkers. *Brain*. 2019;142:1063-1076.
4. Quiroz YT, Sperling RA, Norton DJ, *et al*. Association between amyloid and tau accumulation in young adults with autosomal dominant Alzheimer disease. *JAMA Neurol*. 2018;75:548-556.
5. Jack CR Jr, Holtzman DM. Biomarker modeling of Alzheimer's disease. *Neuron*. 2013;80:1347-1358.
6. Hanseeuw BJ, Betensky RA, Jacobs HIL, *et al*. Association of amyloid and tau with cognition in preclinical Alzheimer disease: A longitudinal study. *JAMA Neurol*. 2019;76:915-924.
7. Mattsson-Carlgren N, Andersson E, Janelidze S, *et al*. Aβ deposition is associated with increases in soluble and phosphorylated

tau that precede a positive Tau PET in Alzheimer's disease. *Sci Adv*. 2020;6:eaaz2387.
8. Jack CR, Wiste HJ, Botha H, *et al*. The bivariate distribution of amyloid-beta and tau: Relationship with established neurocognitive clinical syndromes. *Brain*. 2019;142:3230-3242.
9. Collij LE, Heeman F, Salvado G, *et al*. Multitracer model for staging cortical amyloid deposition using PET imaging. *Neurology*. 2020;95:e1538-e1553.
10. Mattsson N, Palmqvist S, Stomrud E, Vogel J, Hansson O. Staging beta-amyloid pathology with amyloid positron emission tomography. *JAMA Neurol*. 2019;76:1319-1329.
11. Jansen WJ, Ossenkoppele R, Knol DL, *et al*. Prevalence of cerebral amyloid pathology in persons without dementia: A meta-analysis. *JAMA*. 2015;313:1924-1938.
12. Scholl M, Ossenkoppele R, Strandberg O, *et al*. Distinct 18F-AV-1451 tau PET retention patterns in early- and late-onset Alzheimer's disease. *Brain*. 2017;140:2286-2294.
13. Braak H, Braak E. Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathol*. 1991;82:239-259.
14. van der Kant R, Goldstein LSB, Ossenkoppele R. Amyloid-beta-independent regulators of tau pathology in Alzheimer disease. *Nat Rev Neurosci*. 2020;21:21-35.
15. Konijnenberg E, Tomassen J, den Braber A, *et al*. Onset of preclinical Alzheimer disease in monozygotic twins. *Ann Neurol*. 2021;89:987-1000.
16. Coomans EM, Tomassen J, Ossenkoppele R, *et al*. Genetically identical twins show comparable tau PET load and spatial distribution. *Brain*. 2022;145:3571-3581.
17. De Moor MH, Boomsma DI, Stubbe JH, Willemsen G, de Geus EJ. Testing causality in the association between regular exercise and symptoms of anxiety and depression. *Arch Gen Psychiatry*. 2008;65:897-905.
18. Konijnenberg E, Carter SF, Ten Kate M, *et al*. The EMIF-AD PreclinAD study: Study design and baseline cohort overview. *Alzheimers Res Ther*. 2018;10:75.
19. Collij LE, Konijnenberg E, Reimand J, *et al*. Assessing amyloid pathology in cognitively normal subjects using (18) F-flutemetamol PET: Comparing visual reads and quantitative methods. *J Nucl Med*. 2019;60:541-547.
20. Tuncel H, Visser D, Yaqub M, *et al*. Effect of shortening the scan duration on quantitative accuracy of [(18)F]flortaucipir studies. *Mol Imaging Biol*. 2021;23:604-613.
21. Hammers A, Allom R, Koepp MJ, *et al*. Three-dimensional maximum probability atlas of the human brain, with particular reference to the temporal lobe. *Hum Brain Mapp*. 2003;19: 224-247.
22. Svarer C, Madsen K, Hasselbalch SG, *et al*. MR-based automatic delineation of volumes of interest in human brain PET images using probability maps. *Neuroimage*. 2005;24:969-979.
23. Heeman F, Yaqub M, Hendriks J, *et al*. Parametric imaging of dual-time window [(18)F]flutemetamol and [(18)F]florbetaben studies. *Neuroimage*. 2021;234:117953.
24. Golla SS, Wolters EE, Timmers T, *et al*. Parametric methods for [(18)F]flortaucipir PET. *J Cereb Blood Flow Metab*. 2020;40:365-373.
25. Wu Y, Carson RE. Noise reduction in the simplified reference tissue model for neuroreceptor functional imaging. *J Cereb Blood Flow Metab*. 2002;22:1440-1452.
26. Gunn RN, Lammertsma AA, Hume SP, Cunningham VJ. Parametric imaging of ligand-receptor binding in PET using a simplified reference region model. *Neuroimage*. 1997;6:279-287.
27. Wolters EE, Ossenkoppele R, Verfaillie SCJ, *et al*. Regional [(18)F] flortaucipir PET is more closely associated with disease severity than CSF p-tau in Alzheimer's disease. *Eur J Nucl Med Mol Imaging*. 2020;47:2866-2878.
28. Visser D, Verfaillie SCJ, Wolters EE, *et al*. Differential associations between neocortical tau pathology and blood flow with cognitive deficits in early-onset vs late-onset Alzheimer's disease. *Eur J Nucl Med Mol Imaging*. 2022;49:1951-1963.
29. Ten Kate M, Redolfi A, Peira E, *et al*. MRI Predictors of amyloid pathology: Results from the EMIF-AD Multimodal Biomarker Discovery study. *Alzheimers Res Ther*. 2018;10:100.
30. Fischl B. Freesurfer. *Neuroimage*. 2012;62:774-781.
31. Jack CR Jr, Wiste HJ, Weigand SD, *et al*. Different definitions of neurodegeneration produce similar amyloid/neurodegeneration biomarker group findings. *Brain*. 2015;138(Pt 12):3747-3759.
32. Donohue MC, Sperling RA, Salmon DP, *et al*. The preclinical Alzheimer cognitive composite: Measuring amyloid-related decline. *JAMA Neurol*. 2014;71:961-970.
33. Papp KV, Rentz DM, Orlovsky I, Sperling RA, Mormino EC. Optimizing the preclinical Alzheimer's cognitive composite with semantic processing: The PACC5. *Alzheimers Dement (N Y)*. 2017;3:668-677.
34. Saan R, Deelman B. *De 15-woordentest A en B (een voorlopige handleiding)*. Afdeling Neuropsychologie, AZG; 1986.
35. Rey A. *L'examen clinique en psychologie [clinical psychological examination]*. Presses Universitaires de France; 1964.
36. Meyers JE, Bayless JD, Meyers KR. Rey complex figure: Memory error patterns and functional abilities. *Appl Neuropsychol*. 1996; 3:89-92.
37. Rentz DM, Amariglio RE, Becker JA, *et al*. Face-name associative memory performance is related to amyloid burden in normal elderly. *Neuropsychologia*. 2011;49:2776-2783.
38. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state": A practical method for grading the cognitive state of patients for the clinician. *J Psychiatric Res*. 1975;12:189-198.
39. Wechsler D. *Wechsler adult intelligence scale-revised (WAIS-R)*. Psychological Corporation; 1981.
40. Rosen WG. Verbal fluency in aging and dementia. *J Clin Exp Neuropsychol*. 1980;2:135-146.
41. Van Buuren S, Groothuis-Oudshoorn K. Mice: Multivariate imputation by chained equations in R. *J Stat Softw*. 2011;45:1-67.
42. Rosseel Y. Lavaan: An R package for structural equation modeling. *J Stat Softw*. 2012;48:1-36.
43. Jack CR, Wiste HJ, Weigand SD, *et al*. Predicting future rates of tau accumulation on PET. *Brain*. 2020;143:3136-3150.
44. Dore V, Krishnadas N, Bourgeat P, *et al*. Relationship between amyloid and tau levels and its impact on tau spreading. *Eur J Nucl Med Mol Imaging*. 2021;48:2225-2232.
45. Pontecorvo MJ, Devous MD Sr, Navitsky M, *et al*. Relationships between flortaucipir PET tau binding and amyloid burden, clinical diagnosis, age and cognition. *Brain*. 2017;140:748-763.
46. Tosun D, Landau S, Aisen PS, *et al*. Association between tau deposition and antecedent amyloid-beta accumulation rates in normal and early symptomatic individuals. *Brain*. 2017;140: 1499-1512.
47. Ossenkoppele R, Smith R, Ohlsson T, *et al*. Associations between tau, Aβ, and cortical thickness with cognition in Alzheimer disease. *Neurology*. 2019;92:e601-e612.
48. Scholl M, Lockhart SN, Schonhaut DR, *et al*. PET Imaging of tau deposition in the aging human brain. *Neuron*. 2016;89:971-982.
49. Sepulcre J, Schultz AP, Sabuncu M, *et al*. *In vivo* tau, amyloid, and gray matter profiles in the aging brain. *J Neurosci*. 2016;36:7364-7374.
50. McGue M, Osler M, Christensen K. Causal inference and observational research: The utility of twins. *Perspect Psychol Sci*. 2010;5:546-556.
51. Wu JW, Hussaini SA, Bastille IM, *et al*. Neuronal activity enhances tau propagation and tau pathology *in vivo*. *Nat Neurosci*. 2016;19:1085-1092.

52. Busche MA, Chen X, Henning HA, *et al*. Critical role of soluble amyloid-beta for early hippocampal hyperactivity in a mouse model of Alzheimer's disease. *Proc Natl Acad Sci U S A*. 2012; 109:8740-8745.
53. Yamada K, Holth JK, Liao F, *et al*. Neuronal activity regulates extracellular tau *in vivo*. *J Exp Med*. 2014;211:387-393.
54. Frisoni GB, Altomare D, Thal DR, *et al*. The probabilistic model of Alzheimer disease: The amyloid hypothesis revised. *Nat Rev Neurosci*. 2022;23:53-66.
55. La Joie R, Visani AV, Baker SL, *et al*. Prospective longitudinal atrophy in Alzheimer's disease correlates with the intensity and topography of baseline tau-PET. *Sci Transl Med*. 2020;12: eaau5732.
56. Ossenkoppele R, Smith R, Mattsson-Carlgren N, *et al*. Accuracy of tau positron emission tomography as a prognostic marker in preclinical and prodromal Alzheimer disease: A head-to-head comparison against amyloid positron emission tomography and magnetic resonance imaging. *JAMA Neurol*. 2021;78: 961-971.
57. Scott MR, Hampton OL, Buckley RF, *et al*. Inferior temporal tau is associated with accelerated prospective cortical thinning in clinically normal older adults. *Neuroimage*. 2020;220: 116991.
58. Karran E, Mercken M, De Strooper B. The amyloid cascade hypothesis for Alzheimer's disease: An appraisal for the development of therapeutics. *Nat Rev Drug Discov*. 2011;10:698-712.