Genetically identical twins show comparable tau PET load and spatial distribution

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Running title: Tau-PET in genetically identical twins

Abstract

Tau accumulation starts during the preclinical phase of Alzheimer's disease and is closely associated with cognitive decline. For preventive purposes, it is important to identify factors associated with tau accumulation and spread. Studying genetically identical twin-pairs may give insight into genetic and environmental contributions to tau pathology, as similarities in identical twin-pairs largely result from genetic factors, while differences in identical twin-pairs can largely be attributed to non-shared, environmental factors. This study aimed to examine similarities and dissimilarities in a cohort of genetically identical older twin-pairs in 1) tau load and 2) spatial distribution of tau, measured with [^{18}F]flortaucipir PET.

We selected 78 genetically identical twins (39 pairs; average age 73±6), enriched for amyloid-β pathology and *APOE* ε4 carriership, who underwent dynamic [^{18}F]flortaucipir PET. We extracted binding potentials (BP_{ND}) in entorhinal, temporal, widespread neocortical and global regions, and examined within-pair similarities in BP_{ND} using age and sex corrected intra-class correlations. Furthermore, we tested whether twin-pairs showed a more similar spatial [^{18}F]flortaucipir distribution compared to non-twin pairs, and whether the participant's co-twin could be identified solely based on the spatial [^{18}F]flortaucipir distribution. Last, we explored whether environmental (e.g. physical activity, obesity) factors could explain observed differences in twins of a pair in [^{18}F]flortaucipir BP_{ND}.

On visual inspection, Alzheimer's disease-like [^{18}F]flortaucipir PET patterns were observed, and although we mainly identified similarities in twin-pairs, some pairs showed strong dissimilarities. [^{18}F]flortaucipir BP_{ND} was correlated in twins in the entorhinal (r=0.40; p=0.01), neocortical (r=0.59; p<0.01) and global (r=0.56; p<0.01) regions, but not in the temporal region (r=0.20; p=0.10). The [^{18}F]flortaucipir distribution pattern was significantly more similar between twins of the same pair (mean r=0.27; SD=0.09) than between non-twin pairings of participants (mean r=0.01; SD=0.10) (p<0.01), also after correcting for proxies of off-target binding. Based on the spatial [^{18}F]flortaucipir distribution, we could identify with an accuracy of 86% which twins belonged to the same pair. Finally, within-pair differences in [^{18}F]flortaucipir BP_{ND} were associated with within-pair differences in depressive symptoms ($0.37<\beta<0.56$), physical activity ($-0.41<\beta<-0.42$) and social activity ($-0.32<\beta<-0.36$) (all p<0.05).

Overall, identical twin-pairs were comparable in tau load and spatial distribution, highlighting the important role of genetic factors in the accumulation and spreading of tau pathology. Considering also the presence of dissimilarities in tau pathology in identical twin-pairs, our results additionally support a role for (potentially modifiable) environmental factors in the onset of Alzheimer's disease pathological processes, which may be of interest for future prevention strategies.

Keywords: Alzheimer's disease; tau; PET; twins; genetics

Abbreviations: BMI = body mass index; BP_{ND} = Binding Potential; DIN = Digit-in-Noise; GDS = Geriatric Depression Scale; MAP = mean arterial pressure; MNI = Montreal Neurological Institute; PASE = Physical Activity Scale for the Elderly; ROI = region-of-interest; SPM = Statistical Parametric Mapping; SRT = speech reception threshold

Introduction

The pathophysiological course of Alzheimer's disease lasts decades and includes a long preclinical phase.[1] Within this preclinical phase, the brain gradually accumulates amyloid-β and tau pathology, while cognitive functioning is still within normal limits.[2-4] Whereas amyloid-β plaques are detectable widespread throughout the brain relatively early in the disease course,[5] the distribution of neurofibrillary tau tangles starts focally and spreads throughout the brain followed by clinical disease progression.[6, 7] The neuropathological Braak staging system describes how cortical neurofibrillary tau tangles are first observed in the (trans)entorhinal region of the medial temporal lobe, followed by adjacent limbic and lateral temporal regions and finally the neocortex.[8, 9] This stereotypical tau spreading pattern has also been observed in *in vivo* positron emission tomography (PET) studies at group-level,[10-12] although deviations from this pattern at the individual-level may exist, including patterns of hemispheric asymmetry.[7, 13] Studies have shown that both the load and location of neurofibrillary tau tangles are closely associated with cognitive functioning and decline.[14-18] More specifically, the load of tau pathology correlates with symptom severity or disease severity,[16] and the regional localization of tau pathology corresponds to the type of symptoms or disease phenotype.[14, 19, 20] Also in cognitively unimpaired elderly, subtle declines in cognitive functioning have been associated with tau pathology in mainly medial temporal regions.[15, 21-23] For preventive purposes, it is therefore critical to understand which factors contribute to the accumulation onset and regional distribution of tau pathology in the earliest stages of the disease.

Studying genetically identical twins provides an excellent approach to study the contribution of genetic and environmental factors to a trait. As identical twin pairs share the same genetic background, similarities in twins from a pair result from either shared genetic or shared environmental factors, although the influence of shared environmental factors (e.g. being raised in the same family) on brain measures in older twins is typically minimal.[24, 25] Dissimilarities in twins from a genetically identical pair will largely result from non-shared environmental factors that are unique to one of the twins. Examining tau pathology in the preclinical Alzheimer's disease phase in identical twins may thereby provide valuable information for clinical trials aimed at prevention strategies.

The aim of this study is to assess to what extent genetic and environmental factors contribute to tau pathology, as measured with [^{18}F]flortaucipir PET, using a cohort of genetically

identical older twin pairs enriched for preclinical Alzheimer's disease pathology. In this study, we assessed similarities in twins in two characteristics of tau PET: 1) tau load and 2) spatial distribution of tau including hemispheric lateralization. Further, we explored which environmental (e.g. physical activity, smoking or obesity) risk factors were associated with observed differences in tau pathology, using a within twin-pair differences regression model. As the heritability for Alzheimer's disease dementia is relatively high (i.e., genetic factors explain up to 80% of the variance in Alzheimer's disease dementia)[26], and tau pathology is a key factor in Alzheimer's disease, we hypothesize that large similarities in tau pathology in genetically identical twins will be observed.

Materials and methods

Participants

We selected 80 genetically identical twins from the longitudinal Amsterdam sub-study of the EMIF-AD PreclinAD cohort to undergo tau-PET imaging.[27] We selected twin-pairs of whom both twins or either one of the twins were amyloid-β positive (based on baseline [^{18}F]flutemetamol PET visual read[28]) or were classified into a high amyloid-β stage (based on a previously applied staging model to the baseline [^{18}F]flutemetamol PET scans[29]), twin-pairs that carried an *APOE* ε4 allele, as well as age and sex matched amyloid-β negative twin-pairs. The number of participants per amyloid-β stage are shown in **Supplementary Table 1**. Tau-PET was performed during the EMIF-AD PreclinAD 4-year follow-up visit which took place 4.6 ± 0.6 years after the baseline visit. All participants had normal cognition at EMIF-AD PreclinAD study enrolment. Baseline inclusion criteria were age ≥60 years, global Clinical Dementia Rating score of 0, with a score of 0 on the memory sub domain,[30] delayed recall score >-1.5 SD of demographics-adjusted normative data on the Consortium to Establish a Registry for Alzheimer's Disease 10-word list,[31] Telephone Interview for Cognitive Status modified score of ≥23, and a 15-item Geriatric Depression Scale score of <11.[32] Baseline exclusion criteria were any significant neurologic, systemic or psychiatric disorder that could cause cognitive impairment. Additional exclusion criteria for tau-PET imaging were significant cerebrovascular disease on MRI (e.g. territorial infarcts) or a history of major traumatic brain injury. Twin zygosity was confirmed at baseline by buccal cell DNA analysis.

For the analyses, we only included complete twin pairs. Singletons (of which the co-twin deceased prior to the 4-year follow-up visit and thus did not undergo tau-PET imaging, n=2 participants) were excluded, resulting in a total of 78 genetically identical twins (39 pairs) included in the analyses.

The Medical Ethics Review Committee of the VU University Medical Center (Amsterdam, The Netherlands) approved this study. All subjects provided written informed consent.

MRI acquisition

Participants underwent three-dimensional T1 weighted MRI on a 3.0T Ingenuity TF PET/MR (Philips Medical Systems, Best, The Netherlands). All MR scans were acquired within a maximum of 12 months from the tau-PET scan (median time lag: 0.0 ± 5.9 months, IQR: 3.0 months).

Tau-PET acquisition

Participants underwent dual time-point dynamic [^{18}F]flortaucipir (tau) PET on an Ingenuity TF PET/CT (Philips Medical Systems, Best, The Netherlands), which was validated in a previous study.[33] [^{18}F]flortaucipir PET data were acquired during the 0-30min and 80-100min time interval immediately following injection of 227±56 MBq (injected mass 1.8±1.2 μg). A low-dose CT scan for attenuation correction was made prior to both the first and the second scan. PET data were 3D RAMLA reconstructed with a matrix size of 128x128x90 and a final voxel size of 2x2x2 mm^3, and corrected for attenuation, scatter, randoms, decay and dead time.

Tau-PET BP_{ND}

Both parts of the dynamic scan were co-registered using Vinci software.[33] The high-resolution T1 weighted MRI was co-registered to the corresponding PET images in native space using Vinci software. Gray matter regions-of-interest (ROIs) from the Hammers template[34] and Svarer template[35] were automatically delineated on the co-registered MR images and superimposed on the PET scan using PVElab to extract time activity curves. Voxel-wise parametric images of binding potential (BP_{ND}) were generated using receptor parametric mapping with whole cerebellar gray matter from the Hammers template as the reference region.[33, 36] PET images were analysed with and without partial volume correction using Van Cittert iterative deconvolution

methods combined with highly constrained back-projection.[37] Results from partial volume corrected data were similar and are shown in **Supplementary Table 2**.

Tau-PET ROI analyses

For ROI analyses, subject-space bilateral ROIs were a priori selected, in line with previous studies and corresponding to Braak & Braak stages.[8, 10, 38] We included the entorhinal cortex as an early-stage tau region (Braak I) obtained from the Svarer template, as this region is not available as a separate ROI in the Hammers template. We created a temporal composite ROI as an intermediate-stage tau region (Braak III/IV), which included the amygdala, fusiform, parahippocampal and ambient gyrus and middle and inferior temporal gyrus from the Hammers template. We included a widespread neocortical composite ROI as a late-stage tau region (Braak V/VI), which included the superior temporal gyrus, insula, lateral occipital lobe, cinguli anterior, cinguli posterior, middle frontal, posterior temporal, inferolateral parietal, gyrus rectus, orbitofrontal gyrus, inferior frontal gyrus, superior frontal, superior parietal, lingual gyrus, cuneus, precentral and postcentral gyrus from the Hammers template. Last, a global brain region was created including all ROIs from the early, intermediate and late tau stage regions combined (i.e., Braak I-VI). The hippocampus was not included in the ROIs because of possible off-target [^{18}F]flortaucipir spill-in from the adjacent choroid plexus.[39]

For each ROI, we also calculated a hemispheric lateralization measure (the laterality quotient[40]) using the formula $\text{Laterality quotient}\,(\%) = 100 \times (\text{R} - \text{L})/(\text{R} + \text{L})$.[14] This quotient was used to examine how similar twins were in lateralized (asymmetric) [^{18}F]flortaucipir uptake, as a measure for spatial distribution. A negative laterality quotient indicates more tau in the left hemisphere, while a positive laterality quotient indicates more tau in the right hemisphere.

Tau-PET voxel-wise analyses

For voxel-wise analyses, we spatially normalized the parametric PET images to Montreal Neurological Institute (MNI) space using Statistical Parametric Mapping (SPM) version 8 software (Wellcome Trust Center for Neuroimaging, University College London, UK) by using the transformation matrixes derived from warping the co-registered T1 weighted MRI scans to MNI space. All warped images were visually checked for transformation errors. We created a group-average image of [^{18}F]flortaucipir BP_{ND} for visualisation purposes. Prior to voxel-wise

statistical analyses, a cerebral gray matter mask (excluding the basal ganglia because of potential off-target [^{18}F]flortaucipir binding) was applied to the MNI-transformed images. These images were used to assess within-pair similarities in voxel-by-voxel [^{18}F]flortaucipir spatial distribution.

Amyloid-β PET visual read and global BP_{ND}

Participants underwent dynamic [^{18}F]flutemetamol (amyloid-β) PET during the EMIF-AD PreclinAD baseline visit (i.e. 4 years prior to tau-PET) as previously described[28, 41]. For stratification analyses, we used baseline amyloid-β status (positive/negative) based on majority visual read of the dynamic [^{18}F]flutemetamol BP_{ND} images.[28] Baseline [^{18}F]flutemetamol BP_{ND} visual read was missing for three participants, for which we used baseline [^{18}F]flutemetamol SUVr visual read (n=2) or [^{18}F]flutemetamol SUVr visual read obtained at time of tau-PET (n=1). Global baseline [^{18}F]flutemetamol $BP_{ND,}$ calculated as previously described,[41] was used as a covariate in further exploratory statistical analyses (missing for n=4 participants).

Environmental risk factors

As differences in identical twin pairs can largely be attributed to non-shared environmental (e.g. physical activity and smoking) factors, we exploratory examined whether within-pair differences in [^{18}F]flortaucipir BP_{ND} were associated with within-pair differences in environmental factors. In line with the Lancet Commission 2020 update on dementia prevention, intervention, and care on potentially modifiable risk factors for late-life cognitive decline,[42] we included proxy variables for 1) less education (years of education); 2) hearing loss (speech reception threshold (SRT) on the Digit-in-Noise (DIN) test,[43] measuring auditory speech recognition abilities in noise); 3) hypertension (average mean arterial pressure (MAP) from three subsequent measurements); 4) alcohol (current number of glasses consumed per day); 5) obesity (body mass index (BMI)); 6) smoking (number of pack years); 7) depression (Geriatric Depression Scale (GDS) total score)[32]; 8) social isolation (The Health Related Quality of Life Short Form 12 sub-question: "Did your health limited your social activities?"); and 9) physical inactivity (Physical Activity Scale for the Elderly (PASE) total score)[44]. Risk factor variables were assessed within the EMIF-AD PreclinAD study at time of tau-PET imaging. Although listed as potentially modifiable risk factors,[42] we did not include traumatic brain injury (due to it being an exclusion criteria in the

current study), air pollution and diabetes (no suitable measures available). Details regarding these questionnaires and measurements are specified in **Supplementary Table 3**.

Statistical analyses

All statistical analyses were performed in R (R, version 3.6.1, www://R-project.org). A *p*-value < 0.05 was considered statistically significant.

Similarities in twins in [^{18}F]flortaucipir BP$_{ND}$

To examine similarities in twins in [^{18}F]flortaucipir BP$_{ND}$ (tau load), we performed one-way single-measure intra-class correlations across twin pairs for each ROI (i.e. a correlation between Twin 1 and Twin 2 across the group) (**Fig. 3A**). Because identical twins share the same genetic background, these intra-class correlations provide an estimate of the upper limit of genetic contribution to a trait. We repeated these correlation analyses for 20 different sets (each set consisting of 39 pairs) of random (non-twin) pairs using Pearson correlations. We then determined whether the twin pair correlation coefficient was significantly higher than the average correlation coefficient observed for random pairs using the Fisher-Z-transformation and *z*-test statistic. This reflects whether twin-pairs are more similar in tau load than what would be observed by chance (i.e. in randomly paired participants). Both the twin pair correlation analyses and the random pair correlation analyses were adjusted for age and sex. We explored the effect of baseline amyloid-β status on the within-pair similarities in [^{18}F]flortaucipir BP$_{ND}$ by stratifying the sample based on twins concordant on amyloid-β status (i.e. both twins of the same pair amyloid-β negative or both twins of the same pair amyloid-β positive) and twins discordant on amyloid-β status (i.e. one twin of the same pair amyloid-β negative and the co-twin amyloid-β positive).

Similarities in twins in spatial distribution of [^{18}F]flortaucipir BP$_{ND}$

We examined similarities in spatial distribution of [^{18}F]flortaucipir BP$_{ND}$ in twins using three different approaches (all adjusted for age and sex). First, we assessed within-pair similarities in regional hemispheric laterality quotients for [^{18}F]flortaucipir BP$_{ND}$ by performing one-way single-measure intra-class correlations across twin pairs. This reflects whether twins show similar hemispheric lateralization patterns of [^{18}F]flortaucipir BP$_{ND}$.

Second, for every participant, we extracted [^{18}F]flortaucipir BP$_{ND}$ in each voxel from the MNI-space images with a GM mask overlayed. We correlated each participant's voxel-by-voxel

[^{18}F]flortaucipir spatial distribution to that of every other participant using Spearman correlation models (**Fig. 5A**). This yielded 77 correlation coefficients for every participant (resulting in a 78x78 matrix of correlation coefficients) indicating how similar a participant's [^{18}F]flortaucipir spatial distribution is to that of every other participant. An independent t-test was performed to assess whether the average correlation coefficient obtained for twin pairs was significantly higher than the average correlation coefficient obtained for non-twin pairings of participants. This yields an estimate of whether twin pairs show a more similar [^{18}F]flortaucipir spatial distribution than non-twin pairs. To ensure that within-pair similarities in spatial distribution could not solely be explained by similarities in MNI-space warping parameters, we repeated the analysis with subject-space brain regions from the Hammers template (including the same regions as included in the Braak III-IV and Braak V-VI ROIs).

Last, we assessed whether we could successfully identify a participant's co-twin based on the strength of the previously established spatial correlation. If the highest correlation was observed for a participant's co-twin (instead of for a random other participant), it was considered a match. Otherwise, it was considered a miss. The success rate for twin pair identification was defined as the ratio between the number of matches and the total number of participants ($success\ rate\ (\%) = \frac{\#\ matches}{\#\ participants} * 100$).

As previous studies have postulated that cortical [^{18}F]flortaucipir signal in cognitively unimpaired elderly may be affected by off-target binding,[45] we performed sensitivity analyses in which we, for each cortical voxel, regressed out part of the signal that could be explained by off-target binding in the thalamus and putamen[45] using SPM12, to ensure that these results could not solely be explained by within-pair similarities in off-target binding.

Within twin-pair differences in BP_{ND} vs. within twin-pair differences in environmental risk factors

We explored whether within-pair differences in [^{18}F]flortaucipir BP_{ND} were associated with within-pair differences in environmental risk factors using the within-identical twin pair difference model.[46] Specifically, we regressed the difference in regional [^{18}F]flortaucipir BP_{ND} in identical twin pairs on the difference in environmental risk factors in the same pair of twins corrected for age and sex. In a separate model, we additionally corrected for within-pair

differences in global [^{18}F]flutemetamol BP_{ND} to explore whether observed associations were explained by within-pair differences in global amyloid-β load. Within-pair difference models with the DIN SRT score, a measure of hearing ability, were additionally corrected for hearing aid.

Data availability

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

Results

Demographic characteristics and [^{18}F]flortaucipir BP_{ND} in each ROI are summarized in **Table 1**. Seventy-eight participants (39 twin pairs) with an age of 73.4 ± 5.9 years were included in the analyses. Of the 78 participants, 40 (51.3%) carried an *APOE* ε4 allele and 15 (19.2%) were visually assessed as amyloid-β positive (4 years prior to tau-PET).

Mean [^{18}F]flortaucipir BP_{ND} was highest in the temporal (Braak III-IV) ROI (**Table 1**). This was also observed in the group-average image of [^{18}F]flortaucipir BP_{ND}, revealing a predominant temporal binding pattern (**Fig. 1**). High [^{18}F]flortaucipir BP_{ND} was also observed in regions in the basal ganglia, typically known to be affected by off-target [^{18}F]flortaucipir binding.

Visual inspection of [^{18}F]flortaucipir PET

On visual inspection, we mostly observed similarities in twins in both the intensity as well as the distribution of [^{18}F]flortaucipir binding. However, dissimilarities in twins in [^{18}F]flortaucipir PET were also observed. **Fig. 2** shows [^{18}F]flortaucipir PET scans from four identical twin pairs showing similarities and two pairs showing dissimilarities in [^{18}F]flortaucipir PET, that were selected for illustration purposes.

Twin pair similarities in global and regional [^{18}F]flortaucipir BP_{ND}

In twins (corrected for effects of age and sex), global (Braak I-VI) [^{18}F]flortaucipir BP_{ND} correlated significantly (r = 0.56; p < 0.01) (**Fig. 3B**). Regionally, [^{18}F]flortaucipir BP_{ND} moderately correlated in twins in the entorhinal (Braak I) (r = 0.40; p = 0.01) and neocortical composite (Braak V-VI) (r = 0.59; p < 0.01) regions (**Fig. 3B**). The within-pair correlation for [^{18}F]flortaucipir BP_{ND} in the temporal composite (Braak III-IV) region, however, was non-

significant (r = 0.20; p = 0.10) and fell within the range of what was found for random pairs (**Fig. 3B and 3C**). The correlation coefficient observed for twin pairs was significantly higher than the average correlation coefficient observed for random pairs for the entorhinal (r twin pairs: 0.40 vs. average r random pairs: 0.01), neocortical composite (r twin pairs: 0.59 vs. average r random pairs: 0.00) and global (r twin pairs: 0.56 vs. average r random pairs: 0.01) regions, but not for the temporal composite region (r twin pairs: 0.20 vs. average r random pairs: 0.06) (**Fig. 3C**).

We next explored the influence of baseline amyloid-β status on the observed within-pair correlations for [^{18}F]flortaucipir BP_{ND}. We observed that twin-pairs discordant on amyloid-β status 4 years prior to tau-PET (i.e. one twin being amyloid-β positive and the co-twin amyloid-β negative) often acted as outliers in the scatterplots (**Fig. 3B**). We therefore repeated the within-pair correlations after stratifying the sample based on discordant amyloid-β status (n=7 pairs) or concordant amyloid-β status (n=32 pairs). In amyloid-β concordant twins (corrected for effects of age and sex), [^{18}F]flortaucipir BP_{ND} was moderate-to-strongly correlated in all ROIs, with strongest correlations observed in the neocortical composite (r = 0.77; p < 0.01) and global (r = 0.75; p < 0.01) regions, followed by the temporal composite (r = 0.33; p = 0.03) and entorhinal (r = 0.48; p < 0.01) regions (**Supplementary Table 4**). In contrast, in amyloid-β discordant twins, [^{18}F]flortaucipir BP_{ND} was not significantly correlated in any of the ROIs (range r: 0.04-0.25; range p: 0.26-0.45) (**Supplementary Table 4**).

Twin pair similarities in spatial distribution of [^{18}F]flortaucipir BP_{ND}

Next, we examined similarities in identical twin pairs in the spatial distribution of [^{18}F]flortaucipir BP_{ND}. First, within-pair similarities in spatial distribution were examined regionally with the laterality quotient. Hemispheric lateralization in regional [^{18}F]flortaucipir BP_{ND} was significantly correlated in twins in the temporal (r = 0.58; p < 0.01), neocortical (r = 0.69; p < 0.01) and global (r = 0.69; p < 0.01) regions. No correlation in twins was observed in entorhinal hemispheric lateralization of [^{18}F]flortaucipir (r = 0.09; p = 0.28) (**Fig. 4**).

Second, we correlated each participant's voxel-by-voxel [^{18}F]flortaucipir spatial distribution to that of every other participant (**Fig. 5A**). Correlations of [^{18}F]flortaucipir BP_{ND} across voxels were on average significantly higher for twin pairs (mean r = 0.27; SD = 0.09) than

for non-twin pairings (mean r = -0.01; SD = 0.10) ($p < 0.01$) (**Fig. 5B**). These results were not affected when correcting for proxies of cortical off-target binding (mean r for twin pairs: 0.27 ± 0.09 vs. mean r for non-twin pairs: -0.01 ± 0.09) ($p < 0.01$) or when correlating [^{18}F]flortaucipir BP_{ND} across subject-space ROIs instead of MNI-space voxels (mean r for twin pairs: 0.57 ± 0.23 vs. mean r for non-twin pairs: -0.01 ± 0.33) ($p < 0.01$).

Last, based on the strength of the spatial voxel-wise correlation between participants, we assessed the success rate in identifying a participant's co-twin. For 85.9% of the subjects, the highest spatial correlation was observed for a participant's co-twin instead of for a random other participant (67/78 = 85.9%). Upon adjusting for proxies of cortical off-target binding, the success rate remained high at 83.3%.

Environmental risk factors associated with within-pair differences in BP_{ND}

As we also observed differences in twins in [^{18}F]flortaucipir PET (**Fig. 2**), which can largely be attributed to non-shared, environmental (e.g. lifestyle) factors, we explored whether within-pair differences in global and regional [^{18}F]flortaucipir BP_{ND} were associated with within-pair differences in environmental risk factors. Corrected for age and sex, within-pair differences in [^{18}F]flortaucipir BP_{ND} were associated with within-pair differences in GDS total score ($0.37<\beta<0.56$; $p < 0.05$, depending on the ROI), within-pair differences in social activity ($-0.32<\beta<-0.36$; $p < 0.05$, depending on the ROI) and within-pair differences in PASE total score ($-0.41<\beta<-0.42$; $p < 0.05$ depending on the ROI) (**Fig. 6 and Supplementary Table 5**). Specifically, the twin with a higher [^{18}F]flortaucipir BP_{ND} also had more depressive symptoms, more strongly affected social activity and less physical activity. Upon additionally correcting the within-pair difference regression models for within-pair differences in global [^{18}F]flutemetamol BP_{ND}, within-pair differences in entorhinal [^{18}F]flortaucipir BP_{ND} remained associated with within-pair differences in GDS total score (β = 0.39; p = 0.02), and within-pair differences in entorhinal and temporal [^{18}F]flortaucipir BP_{ND} remained associated with within-pair differences in PASE total score (β = 0.40; p = 0.01 and β = 0.33; p = 0.02, respectively). Effect sizes and corresponding p-values are shown in **Supplementary Table 5.**

Discussion

In this study, we examined similarities and dissimilarities in genetically identical cognitively unimpaired older twins in load and spatial distribution of tau pathology measured with [^{18}F]flortaucipir PET. We observed substantial within-pair similarities in tau pathology load, voxel-wise spatial distribution and asymmetry measures. Based on the spatial distribution of tau pathology, we could identify which twins belonged to the same pair with an accuracy of 86%. These results suggest genetic factors to play an important role in the earliest increases and spatial patterns of tau pathology. However, dissimilarities in twins in tau PET were also observed, indicating a potential role for (possibly modifiable) environmental factors in secondary prevention.

One of the main findings in the present study was that global and regional tau PET load were significantly correlated in twins, and twins of the same twin pair were significantly more alike in their tau PET load than random paired twin individuals, also after adjusting for potential age- and sex-effects. This is in line with our earlier study,[41] in which we observed within-pair similarities in cerebrospinal fluid tau pathology of similar strength as to what we currently observed using PET imaging. Observations from post-mortem human brains of monozygotic twins with and without diagnosis of clinical Alzheimer's disease dementia have indicated both within-pair concordances[47] as well as within-pair discordances[48] in neurofibrillary tau tangle Braak staging. These results suggest that the susceptibility to and initial development of pathology may be genetically determined, but genetics alone may not be sufficient to determine the complete pathological and clinical course of the disease.

We observed moderate-to-strong within-pair correlations for regional tau pathology in the entorhinal and neocortical composite region, but a substantial weaker and non-significant correlation in the temporal composite region. The moderate within-pair correlation in the entorhinal cortex, one of the brain sites in the medial temporal lobe in which neurofibrillary tangles appear first,[8, 9] suggests genetic factors to influence the earliest increases of tau pathology. Tau tangles in the medial temporal lobe in cognitively unimpaired individuals have previously been associated with subtle cognitive deficits and longitudinal cognitive decline[15, 21-23], even when tau-tracer binding is lower than observed in clinical Alzheimer's disease, and therefore do not seem to be benign. Whether or not a genetic predisposition to the earliest increases in entorhinal tau translates to a risk for future clinical progression needs further

investigation. The within-pair correlation in the temporal composite region (Braak III-IV) was affected by some twin pairs acting as outliers in the analysis. Interestingly, upon further inspection, we noted that the twin pairs that acted as outliers – and thus were dissimilar in their temporal tau-PET BP_{ND} – were often also discordant on their baseline amyloid-β status, meaning one twin being amyloid-β positive and the co-twin being amyloid-β negative based on PET visual read. Repeating the analysis while excluding the amyloid-β discordant twin pairs indeed led to a strengthening of the within-pair correlation in temporal BP_{ND}. Studies have indicated that tau pathology beyond the medial temporal lobe is typically accompanied with the presence of cortical amyloid-β pathology,[49] which has led to the hypothesis that amyloid-β plays a key factor in the spread of tau outside of the medial temporal lobe. Our results are in line with this hypothesis, as within-pair similarities in twins in the temporal composite region (including lateral temporal regions) became stronger and significant when amyloid-β discordant twins were excluded, although it is notable that the within-pair correlation in the temporal region remained considerably weaker than that of the other brain regions, suggesting a role for other factors (or mechanisms) as well. Following the same hypothesis, it would have been expected that this would also affect the within-pair correlation in neocortical tau pathology. Instead, we observed a strong within-pair correlation for neocortical tau, exceeding levels of similarity that could have been obtained by chance. However, cautious interpretation is needed for the within-pair correlation in neocortical tau, as the BP_{ND} are all in the very low range. A cohort also consisting of twin pairs with clinically manifest Alzheimer's disease might be preferred to infer conclusions on similarities in tau pathology in late-stage tau.

Our data suggests that not only early tau load is under genetic influence, but also the regional localization of tau. Although the amount of preclinical Alzheimer's disease tau pathology is overall low (for comparison, in the current study we observed a Braak III-IV BP_{ND} of 0.1±0.1, whereas we previously observed a BP_{ND} of 0.4±0.2 in clinical Alzheimer's disease[50]), we observed strong similarities in pairs in asymmetric deposition of tau within larger cortical regions. Asymmetric tau deposition is a characteristic feature in atypical Alzheimer's disease phenotypes, but some degree of lateralization also exists in typical Alzheimer's disease.[7, 13, 14] The absence of a strong within-pair correlation in temporal tau BP_{ND} combined with the presence of a strong within-pair correlation in temporal tau laterality quotient may indicate that genetic factors influence the brain's regional vulnerability to tau pathology, but external factors (among

which amyloid-β, vascular components or lifestyle factors) that are unique to a twin may influence the actual accumulation of tau. This is corroborated by our voxel-wise analyses, indicating that the voxel-wise pattern of tau pathology was significantly more similar between twins of the same pair compared to twins of random pairs, and that even though some twin pairs were discordant for amyloid-β status, we could identify a twin solely based on the spatial correlation with its co-twin in approximately 9 out of 10 pairs. The pathway through which genetic factors may influence the regional localization of tau pathology remains to be further examined, though recent *in vivo* PET studies support a role of brain connectivity patterns in the spread of tau throughout the brain.[13, 51-54] Whether the association between brain connectivity and tau spread throughout the brain is influenced by shared underlying genetic mechanisms, for example via the influence of genetics on cortical patterning and hub connectivity,[55, 56] would be of interest for future twin projects. Although the results of this study mainly reveal similarities in tau PET characteristics in twin pairs, there were also twin pairs that showed strong dissimilarities in tau PET of which two are highlighted in **Fig. 2**. A major question remains which factors can explain the differences in tau pathology that we observed in twin pairs that are genetically identical. Our data suggest that differences in amyloid-β pathology are likely associated with the observed differences in tau, and this should be examined in more detail. We found that within-pair differences in tau pathology were associated with within-pair differences in depressive symptoms, social activity and physical activity. Interestingly, the observed associations between within-pair differences in tau pathology and within-pair differences in depressive symptoms and physical activity were partly independent of within-pair differences in global amyloid-β load. Although unable to establish causality due to the cross-sectional design of the current study, these findings are interesting given that previous studies have also linked resistance to Alzheimer's disease pathology (lower levels of pathology than expected)[57] with lifestyle factors including physical activity.[58, 59]. However, as the environmental risk factor variables in the current study were measured at time of tau-PET, it is not possible to conclude whether these are upstream or downstream effects of Alzheimer's disease pathology.

Future longitudinal twin studies will be of interest for several reasons. First, longitudinal data may enable investigation of possible causal relationships between environmental (e.g. lifestyle) risk factors, which may be potentially modifiable, Alzheimer's disease pathology and cognitive decline. Second, longitudinal twin data is needed to clarify whether twins who are

currently different for Alzheimer's disease pathology (i.e. one twin having more pathology than the co-twin), may over time become similar, or whether they remain different – and thus whether it is a matter of a difference in disease development or a difference in the timing of disease onset. Understanding which, potentially modifiable, environmental factors are associated with either the complete absence or a delay in the onset and spread of tau pathology would be of great importance for prevention strategies.

This study had some limitations. Firstly, we only included genetically identical twin pairs, while classic twin studies include both genetically identical and fraternal twins, that share on average half of their segregating genes. By not including fraternal twins, we could not disentangle genetic from *shared* environmental influences. Secondly, our study was limited by a relatively small sample size. Thirdly, a general limitation when studying preclinical Alzheimer's disease pathology is that the overall levels of tau pathology are relatively low. Fourthly, second-generation tau tracers may be preferred to examine tau pathology in the earliest stages of the Alzheimer's disease continuum because of less off-target binding in the hippocampus, a region in which tau accumulation occurs early in the disease process.[8, 9] Last, results are based on cross-sectional data only, and longitudinal data will be needed to examine whether the within-pair similarities may differ over time.

Conclusion

Our results indicate that genetically identical twins show substantial similarities in load, spatial distribution and asymmetry measures of tau pathology, supportive of an important role for genetic factors in the earliest stages of Alzheimer's disease. However, considering also the presence of dissimilarities in tau pathology in twin pairs, genetics alone are likely insufficient to explain the complete pathological course of the disease.

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Competing interests

EMC, JT, RO, SSVG, MH, LEC, EW, SL, EEW, EJCG, AB report no competing interests.

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Supplementary material

Supplementary material is available at *Brain* online.

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Figure Legends

Figure 1 Group-average image of [^{18}F]flortaucipir BP_{ND}.

Figure 2 Examples of [^{18}F]flortaucipir PET scans from six genetically identical twin pairs. Shown are the [^{18}F]flortaucipir PET scans from six genetically identical twin pairs. For illustration purposes, we selected pairs that show within-pair similarities in [^{18}F]flortaucipir BP_{ND} and distribution (top and middle row), as well as within-pair dissimilarities (bottom row).

Figure 3 Identical twin pair correlations for global and regional [^{18}F]flortaucipir BP_{ND}. (**A**) For each ROI, we performed age- and sex-adjusted one-way single-measure intra-class correlations across twin pairs (i.e. a correlation between Twin 1 and Twin 2 across the group) to examine within-pair similarities in [^{18}F]flortaucipir BP_{ND} (tau load); (**B**) Scatterplots illustrating twin-pair correlations in [^{18}F]flortaucipir BP_{ND} for each ROI. Each dot represents a twin-pair. The values presented in the plots are the residuals of [^{18}F]flortaucipir BP_{ND} in Twin 1 (y-axis) and Twin 2 (x-axis) after regressing out the effects of age and sex. (**C**) The correlation coefficient observed for twin pairs (see panel B) is plotted against the distribution of correlation coefficients observed for random pairs. * $p < 0.05$; ** $p < 0.01$

Figure 4 Identical twin pair correlations for hemispheric lateralization in global and regional [^{18}F]flortaucipir BP_{ND}. Shown are the scatterplots illustrating the twin-pair

associations in regional laterality quotients for [^{18}F]flortaucipir BP_{ND}. Each dot represents a twin-pair. The values presented in the plots are the residuals of the laterality quotient in regional and global [^{18}F]flortaucipir BP_{ND} for Twin 1 (y-axis) and Twin 2 (x-axis) after regressing out the effects of age and sex. * $p < 0.05$; ** $p < 0.01$

Figure 5 Spatial correlations for [^{18}F]flortaucipir PET between each participant and every other participant. (**A**) We correlated each participant's voxel-by-voxel [^{18}F]flortaucipir spatial distribution to that of every other participant using Spearman correlation models; (**B**) The distribution of spatial correlations (spearman's rho coefficient, corrected for age and sex) observed for twin pairs is compared against the distribution of spatial correlations observed for non-twin pairs.

Figure 6 Within twin-pair differences regression models between within-pair differences in [^{18}F]flortaucipir BP_{ND} and within-pair differences in GDS total score, social activity and PASE total score.

Each dot represents a twin-pair. Values presented in the plots represent the raw difference in temporal [^{18}F]flortaucipir BP_{ND} between Twin 1 and Twin 2 for each pair (y-axis), and the raw differences in GDS total score, social activity and PASE total score between Twin 1 and Twin 2 for each pair (x-axis). Reported β and p values are from age- and sex-corrected regression models. * $p < 0.05$

Table 1 Demographics

	Total sample
N	78 (39 pairs)
Age, y	73.4 ± 5.9
Female, n (%)	40 (51.3%)
Education, y	12.3 ± 2.9
MMSE score	28.7 ± 1.3
APOE ε 4 positivity, n (%)	40 (51.3%)
Amyloid-β status, n positive (%)	15 (19.2%)
Amyloid-β twin-pair status, n pairs	
Amyloid-β concordant negative	28 pairs
Amyloid-β discordant	7 pairs
Amyloid-β concordant positive	4 pairs
Global [^{18}F]flutemetamol BP_{ND}	0.18 ± 0.14[a]
[^{18}F]flortaucipir BP_{ND}	
Entorhinal (Braak I)	0.02 ± 0.14
Temporal (Braak III-IV)	0.10 ± 0.08

Neocortical (Braak V-VI)	0.04 ± 0.05
Global (Braak I-VI)	0.04 ± 0.05

Mean ± SD are reported unless stated otherwise. Age and MMSE are measured at time of tau-PET; amyloid-β variables are from 4 years prior to tau-PET.

[a]Global [^{18}F]flutemetamol BP_{ND} was missing for 4 participants.

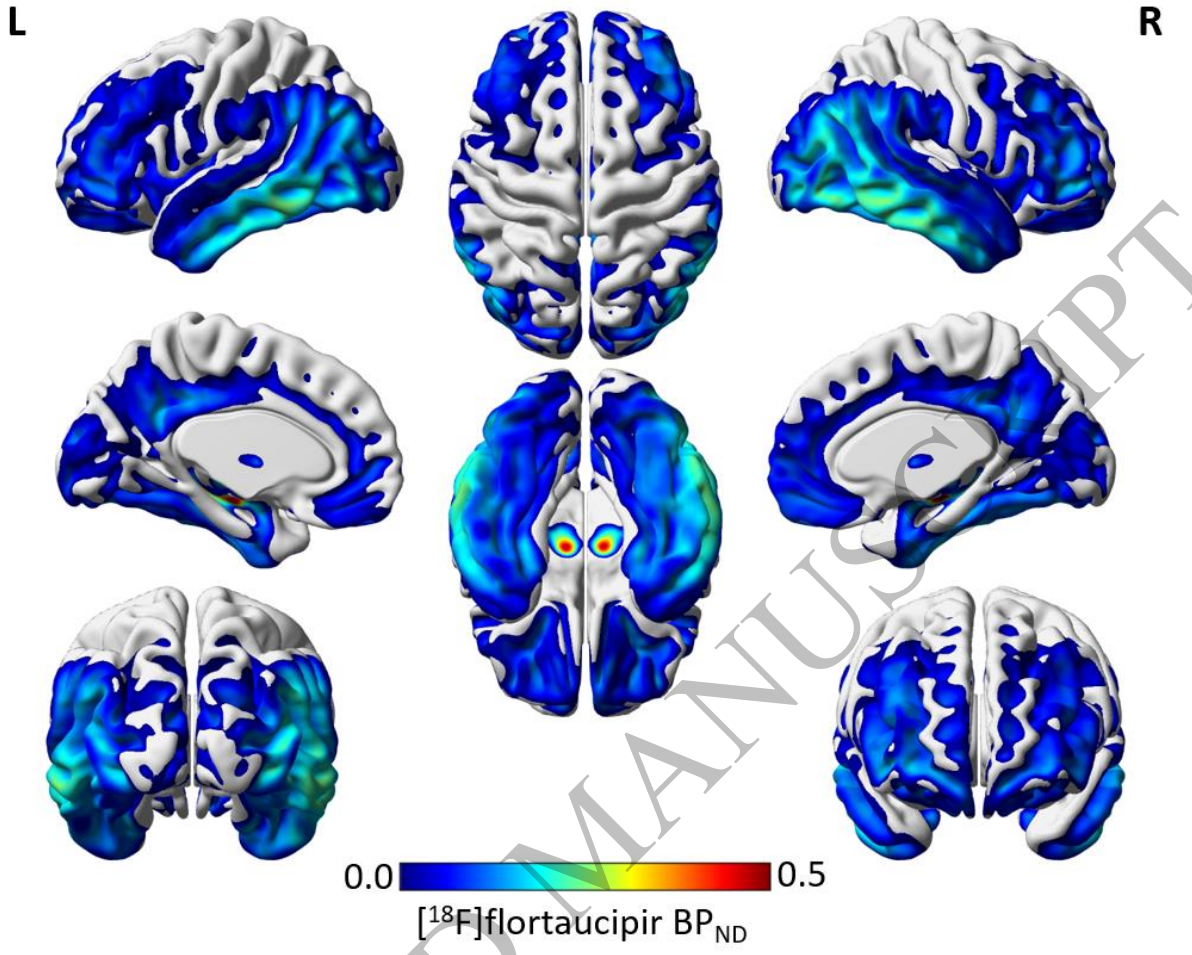

Figure 1
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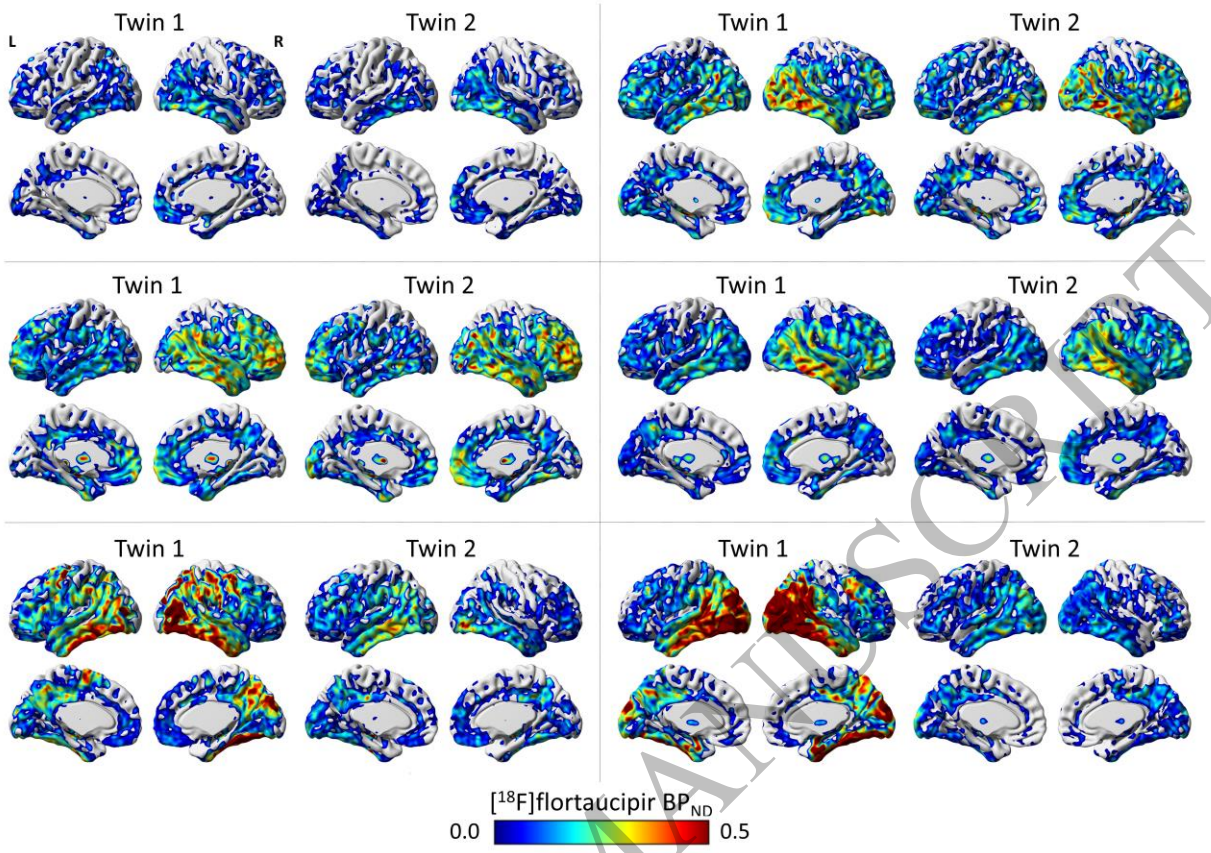

Figure 2
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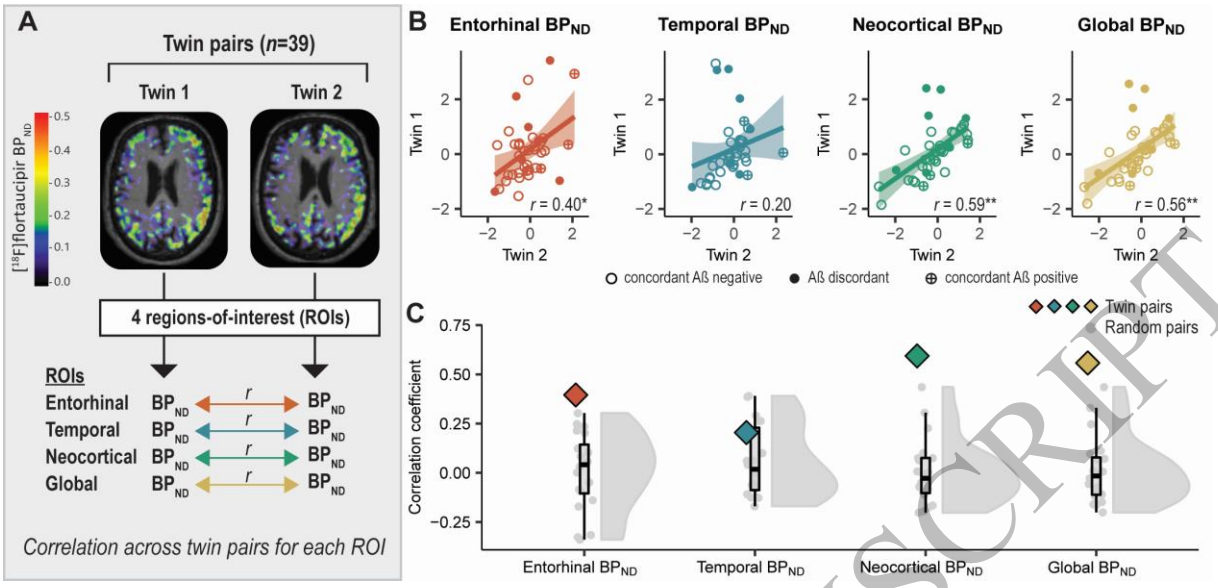

Figure 3
160x76 mm (.80 x DPI)

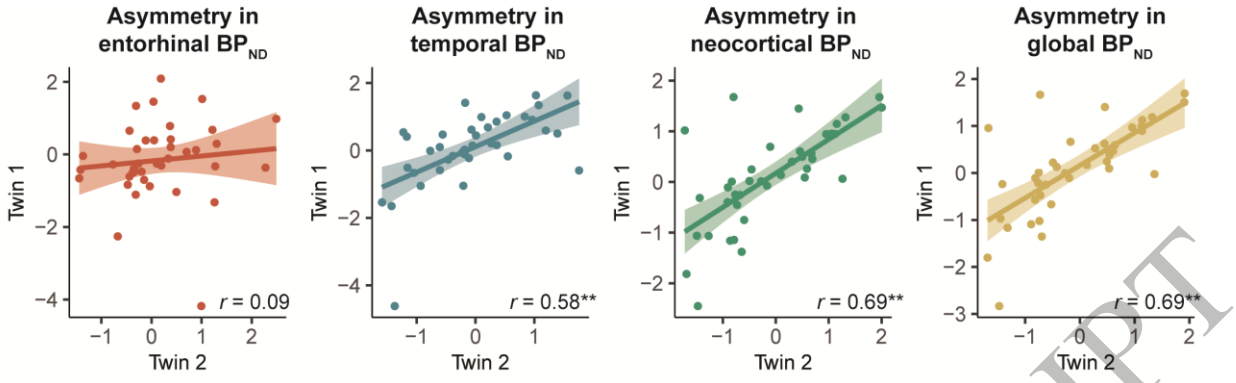

Figure 4
160x51 mm (.80 x DPI)

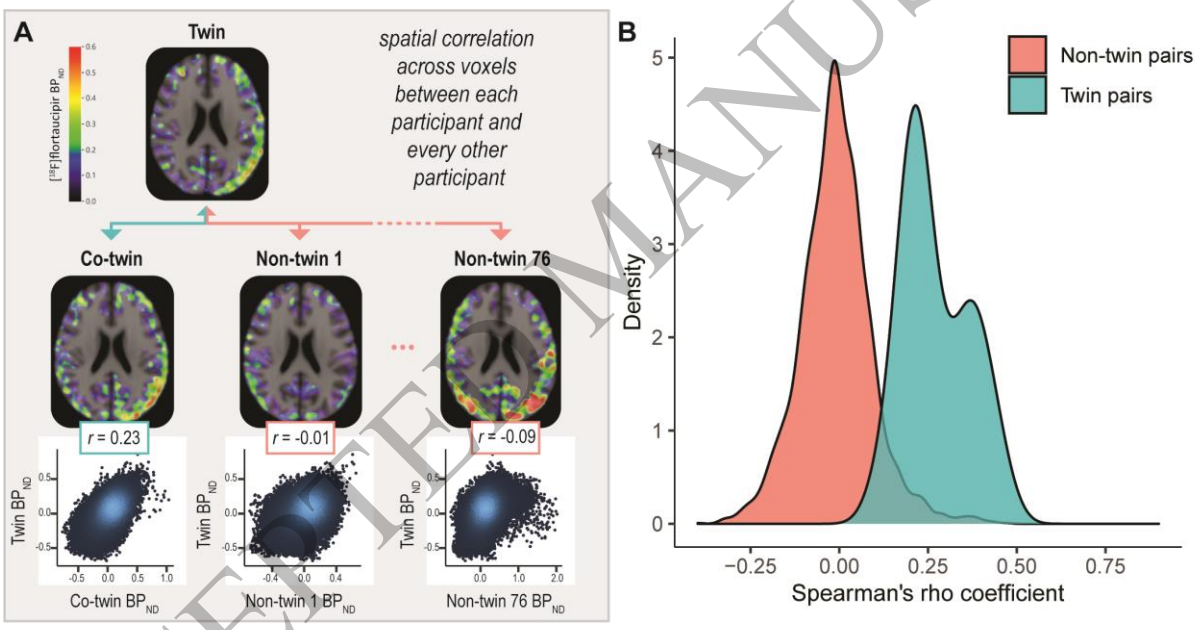

Figure 5
160x81 mm (.80 x DPI)

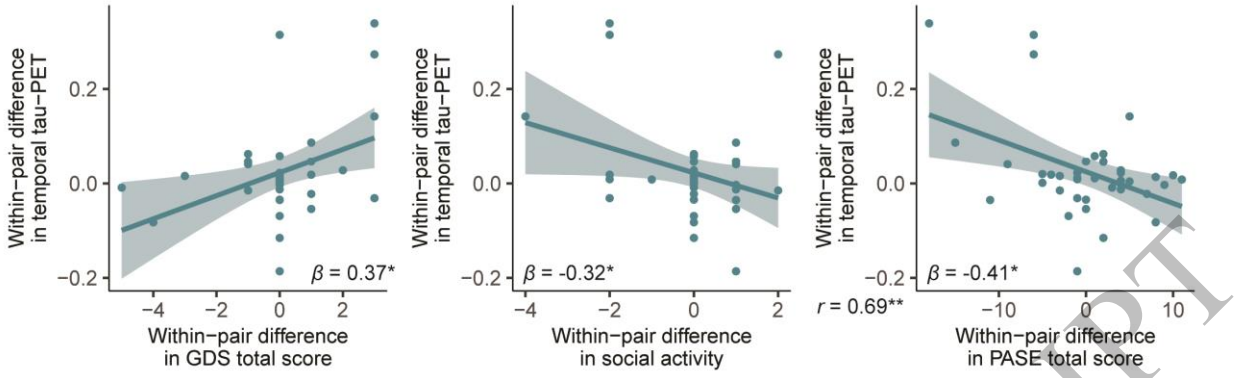

Figure 6
160x52 mm (.80 x DPI)

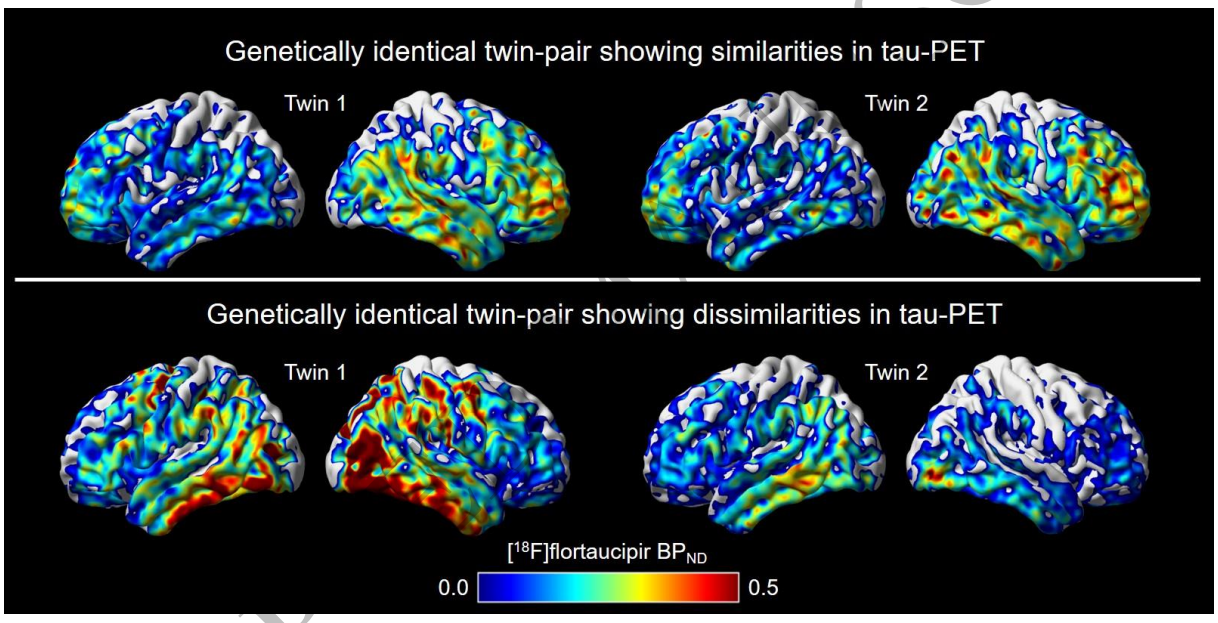

Figure 7
160x80 mm (.80 x DPI)

Coomans *et al.* reveal substantial similarities in tau load and spatial distribution in identical twins, and show that they can identify pairs of twins based on tau spatial distribution. However, within-pair differences – particularly in tau load – also suggest a role for (modifiable) environmental factors in tau accumulation.