Hippocampal volume influences the correlations between white matter disruption and Tau protein in aMCI and mild AD

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Abstract

**Background:** Alzheimer’s disease (AD) is classically considered a grey matter (GM) disease that starts in transentorhinal cortex and spread to limbic and neocortical regions. However, white matter (WM) damage could be more severe and widespread than expected cortical atrophy. It is not clear the role of AD biomarkers and WM integrity throughout the brain, especially including amnestic Mild Cognitive Impairment (aMCI) patients, a possible prodromal AD dementia stage, if WM damage can be detected even before the development of cortical atrophy and overt dementia and in AD process, Aβ42 Tau (and its phosphorylated form) could directly affect WM.

**Methods:** We analyzed in this study 183 individuals - 48 aMCI in the AD continuum (altered CSF Aβ42), 30 patients with very mild or mild AD dementia and 105 normal controls. All subjects underwent neuropsychological evaluation and MRI exam. aMCI and mild AD individuals were also submitted to CSF puncture to evaluate AD biomarkers.

**Results:** We observed several significant differences in WM integrity regarding the DTI measures between individuals and we found significant correlations between fornix and right cingulum hippocampal tracts and Tau and p-Tau proteins.

**Conclusions:** We hypothesize that significant correlations with tracts anatomically far from more well-established GM atrophic regions, like medial temporal lobes, would support a more direct effect of pathological proteins on WM, whereas medial temporal lobe (MTL) correlations would favor WD and/or a direct spreading of pathology from hippocampus.

**Keywords:** Alzheimer’s disease pathology, withe matter damage, DTI measures, CSF proteins, spreading

1. Background

Alzheimer’s disease (AD), the most common neurodegenerative disease, is pathologically characterized by an excessive extracellular deposition of Amyloid-beta (Aβ42) peptide and by intracellular accumulation of the phosphorylated Tau (p-Tau) protein, among other causes, leading to cognitive and neuropsychiatric problems that impair functional independence. Its prevalence is increasing mainly due to the aging of the population. According to United Nations, the number of older persons in the less developed regions grew from 376 million in 2000 to 602 million in 2015 - an increase of 60% - and it is projected to grow by 71% between 2015 and 2030 (United Nations, 2015). Projections indicate that 1.7 billion people aged 60 years or over will live in the less developed regions by 2050 (1, 2).
The causes of the neurodegenerative processes are not completely known. Although remarkable pathological characteristics have already been identified and used as biomarkers, especially regarding cerebrospinal fluid (CSF) Aβ42, p-Tau and total Tau (t-Tau) and molecular neuroimaging (Amyloid and Tau Positron Emission Tomography (PET)), other mechanisms are certainly involved in the pathogenesis. One of those mechanisms is the contribution of white matter (WM) to the development and spreading of neurodegeneration.

AD is classically considered a grey matter (GM) disease that starts in transentorhinal cortex and spreads to limbic and neocortical regions (3). However, recent studies showed that WM damage is more severe and widespread than expected cortical atrophy (4). Also, some authors state that in Mild Cognitive Impairment (MCI), a possible prodromal AD dementia stage, and even in healthy aging, WM damage can be detected even before the development of cortical atrophy and overt dementia, which makes improbable the hypothesis of classical Wallerian degeneration (WD) as the only causal factor (5-7).

In this context, retrogenesis, which assumes primary WM atrophy through myelin breakdown and axonal damage leading to secondary neuronal body death, emerges as an interesting hypothesis (8). There are anatomical regions where the retrogenesis hypothesis might better explain WM atrophy. For example, in the corpus callosum (CC), depending on anatomical localization, WM changes would be associated either with retrogenesis or WD (5).

In AD process, both Amyloid and Tau could directly affect WM. For example, the hyperphosphorylation of Tau protein leads to the destabilization of the cytoskeletal microtubules that are critical for axonal transport, resulting in the retrograde degradation of axonal cytoskeletal proteins and succeeding loss of the axon fibers (9, 10). Another
possibility of direct WM damage is the propagation pattern of misfolded proteins via interconnected neural networks in a prion-like fashion. The Aβ peptide and/or Tau protein might spread across brain networks through WM tracts that connect the remote regions of these networks (11). This spread could lead to the progressive neuronal death characteristic of AD (12). Another pathophysiological mechanism is subcortical vascular WM alterations, especially in patients with cardiovascular risk factors. However, there is no consensus regarding the role of WM subcortical vascular alterations in the pathogenesis of “pure” AD, which could be considered comorbidities rather than causal agent.

Diffusion tensor imaging (DTI) is a well-established technique used to evaluate the microstructural integrity of the WM in vivo. This technique is based on the probabilistic determination of the diffusion of the water molecule in the tissue, helping to quantify the integrity of the brain tracts (13). The main diffusion indices used to analyze WM integrity are fractional anisotropy (FA), mean diffusivity (MD), radial diffusivity (RD) and axial diffusivity (AxD). The decrease in FA values and increase in diffusivity are associated with WM degeneration (13). RD is more sensitive to changes in axon diameter and density: high RD values indicate a smaller thickness of the myelin sheath. AxD represents the maximum direction of diffusion and gives a primary estimate of the direction of the fiber (12).

Because it is not clear the association of AD biomarkers and WM integrity throughout the brain, especially considering amnestic MCI (aMCI) in the Alzheimer’s continuum (at least CSF amyloid alteration), we aimed to evaluate: 1) differences in WM integrity in mild AD and aMCI due to AD in relation to controls; 2) possible correlations between CSF measures of Aβ42, p-Tau and t-Tau with WM DTI measures (FA, MD, RD, AxD) in aMCI due to AD and mild AD.
We hypothesized that significant correlations with tracts anatomically far from more well-established GM early atrophic regions, like medial temporal lobes (MTL), would support a more direct effect of pathological proteins on WM, possibly leading to retrogenesis, whereas MTL correlations would favor WD and/or a direct spreading of pathology from hippocampus. Should this be the case, we also aimed to verify the influence of hippocampal volumes (HV) on these correlations, hypothesizing that loss of the significance would imply a direct influence of hippocampal degeneration in the associations.

2. Methods

2.1 Subjects

One hundred eighty-three individuals were enrolled in this study: 48 aMCI in the AD continuum (altered CSF Aβ42), 30 patients with very mild or mild AD dementia and 105 normal controls. All subjects underwent neuropsychological evaluation and MRI exam. aMCI and mild AD individuals were also submitted to CSF puncture to evaluate AD biomarkers.

The diagnosis of probable dementia due to AD fulfilled the criteria defined by the National Institute of Aging and Alzheimer's Association (NIA/AA) (14) and all AD patients had a Clinical Dementia Rating (CDR) (15) score of 0.5 or 1. aMCI patients were diagnosed using the core criteria of the NIA/AA for MCI (16) and had pathophysiological evidence of AD, characterized by low CSF Aβ42 (< 540pg/mL).

Controls were identified as cognitively normal: they did not exhibit any neurological or psychiatric disorders or require psychoactive medication. They demonstrated normal Mini Mental State Examination (MMSE) scores, considering age and education (17), and their structural images were characterized by absence of
abnormalities. The control subjects had neither memory complaints nor neurological deficits, and they presented a CDR score of zero.

Exclusion criteria for all subjects included: other neurological or psychiatric diseases or having suffered a head trauma that resulted in a loss of consciousness, drug or alcohol addiction, use of sedative drugs 24 hours before neuropsychological evaluation, prior chronic exposure to neurotoxic substances, Fazekas score > 1 and a Hachinski ischemic score > 4 (18).

Pre-diagnostic procedures also comprised of laboratory tests including Vitamin B12, folate, syphilis serology, and thyroid hormones.

2.2 Neuropsychological, functional and neuropsychiatric assessment

An experienced neuropsychologist that was blinded to the MRI and CSF data performed the neuropsychological evaluations. Global cognitive status was measured using the MMSE (19), and episodic memory was evaluated by the Rey Auditory Verbal Learning Test (RAVLT) (subitems encoding, delayed recall - A7, and recognition - RC-FP) (20). For visual perception, we used the following tests: subtests of Luria’s Neuropsychological Investigation (LNI) (21), using items G12, G13, G14, and G17 (item from Raven’s test), one item for mental rotation of figures (22), and a copy of the Rey-Osterrieth Complex Figure Test (23). For executive function we used the Trail Making Test A (TMT-A) and B (TMT-B) (24) and the Stroop test (congruent – Stroop C, and incongruent – Stroop I) (25). Language tests included the Boston Naming Test (BNT) (26) semantic verbal fluency (SVF) for categories (animals), and phonological fluency for letters (FAS) (27).

2.3. CSF sample collection and handling
CSF collection by lumbar puncture was performed in AD and aMCI patients. The CSF samples were centrifuged at 700 rpm for 10 minutes. Both centrifugation processes were done at 4°C and were subsequently aliquoted into 1 ml Eppendorf microtubes and stored at -80°C until the time of analysis.

2.3.1. Quantification of AD biomarkers

Measurements of Aβ42, t-Tau and p-Tau were obtained using amyloid-β INNOTEST® kits (1-42), h-TAU INNOTEST® Ag and INNOTEST® Phospho-tau (181P) (Fujirebio), respectively, a multiplex microsphere-based xMAP platform that allows the simultaneous analysis of the three biomarkers. After prewetting the filter plate with a wash buffer, a suspension of microsphere carrying the corresponding capturing antibodies (AT120, AT270, and 4D7A3 for t-Tau, p-Tau, and Aβ40-42, respectively) was added to the plate.

A mixture of biotinylated detection monoclonal antibodies, designed to detect specifically one of the capturing antibodies, and 75 mL of CSF or standards, was added to the plate and incubated overnight in the dark. Next, the plate was washed and a detection conjugate (phycoerythrin-labeled streptavidin) was added and incubated for 1 hour at room temperature. The plate was washed and after the addition of a reading solution (phosphate buffer saline), the assay was analyzed on a Luminex 100IS platform (Luminex, Austin, TX, USA).

Standard curves were constructed for each biomarker using a sigmoidal curve fitting method and the mean fluorescence values for the duplicate CSF samples were used to determine the concentration of Aβ42, t-Tau, and p-Tau.

2.4. MRI acquisition
All Magnetic Resonance images (MRI) were acquired on a Philips® Achieva 3.0T scanner. The following protocol was applied to each subject: a) for the hippocampal volume analyses: sagittal high-resolution T1-weighted (isotropic voxels of 1x1x1 mm³, TR/TE = 7/3.2 ms, FOV = 240×240 mm, 180 slices); b) for the WM microstructural analysis, a standard DTI protocol was performed (acquired voxel size=2x2x2 mm³ reconstructed with 1x1x2 mm³, no gap, TR=8.5 s, TE=61 ms, 32 diffusion directions with b=1000 s/mm², acquisition matrix=128x128 reconstructed to 256x256).

To classify subjects according to Fazekas scale we acquired coronal and axial fluid attenuated inversion recovery (FLAIR) T2-weighted images, anatomically aligned to the hippocampus (reconstructed voxel size = 0.45x0.45x4.00 mm³, TR/TE/TI = 12000/140/2850 ms, FOV = 220x206 mm, gap=1 mm).

All subjects underwent MRI scanning before CSF puncture and in the same week that neuropsychological assessment was performed. Image processing and neuropsychological examinations were performed by professionals blinded to the clinical data.

2.4.1. DTI processing and automated segmentation

The main diffusion indices used to analyze WM integrity are FA, MD, RD and AxD. To extract these values, we used an automated segmentation based on multiple labeled atlases. All analyses were performed in native space. All steps were performed using “MRICloud” (MRICloud.org), a public web-based service for multi-contrast imaging segmentation and quantification.

DTI-weighted images were corrected for eddy currents and co-registered to remove subject motion using a 12-parameter affine transform. Next, we employed a multi-contrast algorithm to register all the atlases to the images and to perform the
parcellation (segmentation), which employs a Diffeomorphic Likelihood Fusion Algorithm (DLFA) (28, 29). Finally, we obtained the diffusion parameters for each label.

2.4.2. Selection of WM tracts

The processing of MRI described above generated information of 64 WM tracts, each tract with four measures (FA, AxD, MD, and RD). Thus, we performed a principal component analysis (PCA) to evaluate which regions of interest (ROIs) have contributed the most to WM variability in our series of patients, which guided the selection of variables to perform group comparisons.

We performed four PCAs on WM tracts data, one for each DTI metric. Both Keiser’s and Catell’s criteria were applied to extract factors. After extraction, components were rotated using the non-orthogonal direct oblimin method. Sample size and model adequacy were evaluated using Barlett’s test of sphericity and the Keiser-Meyer-Olkin (KMO) measure. We chose within components those ROIs with components loadings > 0.7 (at least 64% of variability contribution) to perform hypothesis testing.

All the PCAs showed a KMO > 0.883, suggesting an appropriate adjustment to the data. Analysis of both Kaiser’s and Catell’s criteria suggested that the variability concentrated on the first two components. We then repeated the analysis extracting only a fixed number of two components explaining a total variance of 40% for FA, 47% for AxD, 55% for MD, and 54% RD. To better detail the findings, we included ROIs with loading factors > 0.6 as a safe value to interpret, considering our sample size. However, we used a more conservative loading factor (> 0.7) to include WM ROIs in the subsequent analysis.

2.4.3. Hippocampal Analyses – FreeSurfer
FreeSurfer software v.5.3 (30) (https://surfer.nmr.mgh.harvard.edu) was used for
cortical surface reconstruction and for the anatomical segmentation of MRI brain scans.

We processed all high-resolution T1-weighted MR volumetric images through the
default FreeSurfer processing stages to perform non-linear registration (warping) from
the original space to the MNI305 space (standard space), cortical and subcortical
segmentations, and cortical thickness measurements. We visually confirmed the accuracy
of warping the T1-weighted MR volumetric images to the standard space. Macroscopic
artifacts affected none of the T1-weighted volumes of the participants.

For all analyses, a Gaussian filter with a 10-mm full width at half maximum was
used for smoothing the surface. The volume of individual structure was computed from
labeled voxels and normalized to the total intracranial volume.

2.5. Statistical analyses

All statistical analyses were carried out in SPSS package (version 22, SPSS Inc.,
Chicago, IL, USA).

We performed a univariate analysis of covariance (ANCOVA) to compare the
neuropsychological test and demographic data between the groups using the Bonferroni
post-hoc test (significant p level was chosen to be < 0.05). Chi-square test was used for
categorical analyses (sex). Because age and education were significantly different among
subjects, we included these variables as nuisance covariates in the analyses.

To compare CSF proteins levels between mild AD and aMCI we performed a t-
test. To compare the DTI measures between the groups we performed a multivariate
analysis of covariance (MANCOVA) also using the Bonferroni post-hoc test.
To verify the association between DTI data and CSF biomarkers, we performed partial correlations, including, age, and education. Afterwards, we repeated the analyses including measures of HVs, to verify their influence on previous correlations.

3. Results

3.1. Neuropsychological and demographic data

There were significant differences in age and education, but not sex, of mild AD and aMCI patients compared to controls. AD and aMCI patients performed worse than controls in almost all tests as shown in Table 1.

3.2. CSF biomarker’s levels

Comparing CSF levels between mild AD and aMCI in AD continuum, just t-Tau and Aβ42/t-Tau levels were significantly different (Table 1).

3.3. Hippocampal Volumes

As expected, aMCI and mild AD presented bilateral smaller HVs in relation to controls and AD presented smaller HVs in relation to aMCI (Table 1).

3.4. Group comparison of WM integrity

Considering the core regions of our hypothesis, we highlight a significant difference (AxD, MD and RD) in tracts like fornix, cingulum, and hippocampal-cingulum between aMCI versus controls and aMCI versus mild AD, as depicted in Figure 1.

Nevertheless, we also found other tracts with significantly different DTI metrics (including FA values) between patients with aMCI versus controls (Figure 2), and between aMCI and mild AD (Figure 3).
The largest of these effects was found in temporal WM tracts including the hippocampal-cingulum, fornix, sagittal stratum, commissural fibers, and inferior fronto-occipital fasciculus, as presented in Table 2.

3.5. Correlations between CSF proteins and DTI measures

When considered both groups together (aMCI and mild AD), we found significant partial correlations between WM tracts and Tau and p-Tau, but not with Amyloid. Partial correlations considered age and sex as covariables. Significant partial correlations with Tau were found as follows: FA of right fornix (r = -0.259; p = 0.027), MD (r=0.355; p = 0.003) and AxD (r = 0.251; p = 0.03) of right cingulum hippocampal. Concerning significant correlations with p-Tau, we only found AxD of right cingulum hippocampal (r = 0.317, p = 0.009), according Figure 4.

No significant results were found when considering the groups separately.

Next, we evaluated if these significant correlations between Tau and p-Tau with WM microstructure remained after controlling for hippocampal volumes. If so, we could speculate that these associations were independent of hippocampal integrity. However, no significant correlations survived after controlling for hippocampal volumes, what could imply that HV mediates the associations between tau and WM.

4. Discussion

The relationship between AD pathologic proteins (Tau, p-Tau and Amyloid proteins) and WM microstructural alterations is not completely known. There are different possible causes of WM damage in AD spectrum, like vascular, Wallerian degeneration and retrogenesis, but a direct Tau pathological effect is another reasonable possibility.
In the present study, we first evaluated the differences in WM microstructural integrity in the AD continuum (aMCI with altered amyloid and mild AD dementia) and controls. Then, we verified if Tau and Amyloid CSF levels could be related with WM microstructure in different tracts throughout the brain, not only in regions classically involved in early disease phases. For WM analyses, we used a multi-atlas segmentation approach, which is a more detailed and refined investigation that can assess thinner and smaller tracts, enabling the analysis of differences that may be more subtle in early stages of the disease.

We hypothesized that significant correlations with tracts anatomically and functionally far from more well-established atrophic regions would support a more direct effect of pathological proteins on WM, whereas medial temporal lobes correlations would favor WD and/or a direct spreading of pathology from hippocampus. In this case, we also verified the influence of HVs on these correlations, hypothesizing that loss of the significance would imply a direct effect of hippocampal degeneration in the associations.

We found a widespread WM disruption in aMCI and mild AD (Table 2), as demonstrated in other studies (30), but only tracts directly linked to MTL structures, like hippocampus, were significantly correlated with Tau and p-Tau, but not with Amyloid (Figure 4, Figure 1). However, when controlling for HVs, these correlations lost their statistical significance.

Although the pattern of WM disruption in early stages of AD could initially take place in limbic and commissural tracts and later on may progress to projection and association fibers (5), we found a more widespread alteration in our aMCI due to AD subjects. However, if we consider the theoretical disease progression, AD starts even before clinical symptoms appear, and axonal damage may be present in AD spectrum in
earlier clinical phases, like Subjective Cognitive Decline (SCD), and even in preclinical phase.

Interestingly, Racine et al. (2014) found DTI alterations even in cognitively normal subjects with amyloid alteration, though in an inverse pattern than that found in symptomatic individuals: increase in FA and decrease in diffusivity (31). Wolf et al. (2015) found a quadratic relationship between amyloid deposition and DTI metrics in cognitively healthy older adults, i.e., increases in FA and decreases in MD and RD with increasing amyloid load at low levels of amyloid burden, and the opposite pattern at higher amyloid burden (32). Other authors found a similar pattern than that of AD, even in the normal aging: amyloid positive individuals exhibited significantly lower FA and higher AxD in the fornix compared to individuals with higher CSF levels of Aβ42 (33, 34). Brueggen et al. (2019) found similar (though less pronounced than our aMCI) alterations in SCD subjects in the anterior corona radiata, superior and inferior longitudinal fasciculus, cingulum, and splenium of the corpus callosum (35). So, it is not surprising that our subjects, who have higher chance to convert to dementia, have presented so many alterations in different WM tracts.

Although these WM alterations in AD spectrum are well-established, much less is known regarding their etiology and relationship with Amyloid and Tau proteins. AD has long been primarily considered a GM disease, with secondary WM disruption through WD and/or vascular disruption. However, WM damage is more intense, widely distributed and appear earlier than expected on the basis of solely cortical atrophy (4).

In this way, a direct deleterious effect of Tau pathology has been proposed. Based on the fact that Tau participates in the integrity and stabilization of the axonal cytoskeleton by binding to microtubules, it has been proposed that the functional failure of Tau could yield the swelling of axonal extensions and the disruption of axonal transport
Recent studies have demonstrated the mechanism through which Tau pathology initially progresses from distal axons to proximal dendrites, leading to synaptic disconnection of late myelination fibers (6, 10). In this sense, retrogenesis, with primary disruption of WM rather than GM, could also be considered a pathological mechanism in the AD spectrum. However, our study did not disclose any significant correlations between WM and pathological proteins in other regions than MTL tracts, what makes improbable this hypothesis in this early disease phase.

Our findings concur with those of Kantarci et al. (2017), who, in a study of combined antemortem DTI and autopsy in a range of subjects with AD pathology, demonstrated that the elevation in MD and the reduction in FA in the medial temporal tracts, such as the ventral cingulum, the crus of the fornix and the entorhinal WM were associated with a higher Tau neurofibrillary tangles (12). A recent study combining Tau PET and DTI, found that Tau in anterior temporal lobes predicts WM integrity in AD (40). This is an interesting finding, since Brier et al. (2016) found that CSF measures of Tau correlated with Tau PET deposition in the temporal lobe (41).

However, in the present study, controlling for HV eliminated the statistical significance of correlations. This finding supports the predominant influence of this GM structure in the association between Tau and WM disruption. Although classical WD due to hippocampal degeneration emerges as the main hypothesis, the more recent study of the “prion-like” spreading of misfolded proteins may also play a role and directly affect WM tracts adjacent to hippocampus (42).

Abnormal Tau protein, classically present in MTL structures of AD continuum individuals, has the property to aggregate with normal tau in endosomes, where they form fibrillar seeds that subsequently induce the aggregation of endogenous tau. These fibrils, consisting of endogenous and exogenous tau, are released from neurons, and spread
pathology, resulting in an unstoppable neurodegenerative process in the brain of patients with AD (43). There are also other ways of spreading the disease, like the trans-synaptic transmission (44) or by an exosynaptic mechanism through microglia (45). These findings are in accordance with the most accepted hypothesis that AD pathology starts in GM structures and spread through interconnected regions, leading to disruption and disconnection of neurofunctional networks.

Associations between WM integrity and Aβ are less clear and have not been consistently found across the literature (41). According to Strain et al. (2018), amyloid may serve as a proxy measure for other pathologies, including Tau, which are intrinsically associated with it, rather than cause direct damage to WM (40). However, other studies have found correlations between WM and Aβ42, most commonly localized in MTL structures, including the fornix, cingulum, parahippocampal cortex, and inferior temporal gyrus (30).

Considering the full amount of these findings, it is difficult to explain that the extent of WM damage in AD and aMCI is directly associated with Amyloid and Tau. Probably, WD, direct damage by Tau spreading, retrogenesis, neuroinflammation, vascular and other factors coexist in this disease. About the influence of vascular effects, all individuals of this study were normal for age regarding WM hyperintensities on MRI, presenting Fazekas scale 0 or 1.

Our study has several limitations, such as its cross-sectional nature, its correlational approach, which does not allow to infer causal relationships, and the lack of information regarding AD biomarkers in controls. Also, CSF measures of Tau and Amyloid do not have the same anatomical precision as that of PET. More translational and longitudinal studies, including post-mortem analyses, are needed for safer conclusions.
5. Conclusions

Our study confirmed the extent of WM damage in Alzheimer’s continuum (aMCI with altered amyloid and mild AD dementia) but found significant correlations with Tau pathology only in medial temporal structures, which did not survive after controlling for hippocampal volumes. We suggest that, in this case, the association between WM damage and Tau is more probable to be due to Wallerian degeneration or direct Tau spreading from atrophic hippocampus.

6. Abbreviations

AD – Alzheimer’s disease
Aβ42 – Amyloid-beta peptide
p-Tau – phosphorylated Tau
CSF – cerebrospinal fluid
t-Tau – total Tau
PET – Positron Emission Tomography
WM – white matter
GM – grey matter
MCI – mild cognitive impairment
WD – wallerian degeneration
CC – corpus callosum
DTI – diffusion tensor imaging
FA – fractional anisotropy
MD – mean diffusivity
RD – radial diffusivity
AxD – axial diffusivity
aMCI – amnestic mild cognitive impairment
MTL – medial temporal lobes
HV – hippocampal volumes
NIA/AA – National Institute of Aging and Alzheimer's Association
CDR – Clinical Dementia Rating
MMSE – Mini Mental State Examination
RAVLT – Rey Auditory Verbal Learning Test (subitems encoding, delayed recall - A7, and recognition - RC-FP)
LNI - Luria’s Neuropsychological
TMT-A – Trail Making Test A (TMT-A) and B (TMT-B)
BNT – Boston Naming Test (BNT)
SVF – semantic verbal fluency (SVF)
FAS – phonological fluency for letters (FAS)
MRI – magnetic resonance image
FLAIR – fluid attenuated inversion recovery
DLFA – Diffeomorphic Likelihood Fusion Algorithm
PCA – principal component analysis
ROIs – regions of interest
ANCOVA – analysis of covariance
MANCOVA – multivariate analysis of covariance

7. Declarations

7.1. Ethics approval and consent to participate

The Medical Research Ethics Committee of the UNICAMP Hospital approved this study and written informed consent (either from the subjects or from their responsible
caretakers, if incapable) was obtained from all participants before the commencement of the study, in accordance with the Declaration of Helsinki.

7.2. Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

7.3. Funding

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7.4. Authors' contributions

All authors made contributions to the conception, design and analyses of the study. RFC author development of figures and supervision of statistics. The MHN and LRPS authors participated in the statistical analyzes. CVLT and AFMC authors participated in data collection. The HPGJ, LLT and OV assisted in the analysis of CSF proteins. All authors have critically revised the manuscript for intellectual content and read and approved the final manuscript for submission.

7.5. Conflict of interest

The authors have no conflict of interest to report.

7.6. Acknowledgment

Not applicable.

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Table 1. Descriptive and group comparison of demographic, main neuropsychological data, and biomarkers, p corrected for age and education

|                          | Controls (n = 105) | aMCI (n = 43) | Mild AD (n = 30) |
|--------------------------|-------------------|---------------|------------------|
| Age                      | 6 (7.29)          | 68 (7.13)     | 73 (7.56)        |
| Education (years)        | 11.66 (5.05)      | 9.39 (5.78)   | 6.5 (4.96)       |
| CDR                      | 0 (0)             | 0.5 (0)       | 0.87 (0.21)      |
| Pfeffer                  | 0.53 (0.80)       | 3.33 (3.04)   | 12.97 (5.17)     |
| MMSE                     | 28.42 (1.49)      | 25.59 (2.88)  | 20.1 (5.92)      |
| RAVLT                    | 45.16 (8.44)      | 30.54 (8.03)  | 21.77 (12.33)    |
| A7                       | 8.95 (2.81)       | 3.78 (2.24)   | 1.03 (1.58)      |

CSF biomarkers (pg/mL)

|                          |                  |               |                  |
|--------------------------|------------------|---------------|------------------|
| t-Tau                    | NR               | 102.79 (66.55)| 142.34 (79.32)   |
| p-Tau                    | NR               | 45.75 (23.66)| 58.97 (36.80)    |
| Aβ                       | NR               | 375.44 (105.81)| 333.87 (113.86) |
| Aβ/t-Tau                 | NR               | 5.06 (2.96)   | 3.22 (2.4)       |
| Aβ/p-Tau                 | NR               | 11.33 (8.5)   | 7.95 (7.19)      |

Hippocampal volume (mm³)

|                          |                  |               |                  |
|--------------------------|------------------|---------------|------------------|
| Left Hippocampus (10⁻³)  | 2.86 (0.44)      | 2.53 (0.55)   | 2.09 (0.34)      |
| Right Hippocampus (10⁻³) | 3.05 (0.49)      | 2.71 (0.56)   | 2.1 (0.42)       |

Note: mean (standard deviation). Statistical analysis: ANCOVA with Bonferroni post hoc test. Pfeffer: Pfeffer Functional Activities Questionnaire. MMSE: Mini Mental State Examination. RAVLT: Rey Auditory Verbal Learning Test. A7: Rey Auditory Verbal Learning Test-delayed recall. CDR: Clinical Dementia Rating.

T-Tau: total Tau protein. p-Tau: phosphorylated-Tau. Aβ: amyloid β peptide.
a: different from controls
b: different from aMCI
* p < 0.05
** p < 0.01
*** p < 0.001

Table 2. Group comparison of WM integrity data in significant regions.

|                          | Controls (n = 103) | aMCI (n = 44) | Mild AD (n = 29) |
|--------------------------|-------------------|---------------|------------------|
| Fractional anisotropy    |                   |               |                  |
| L Genu of CC             | 0.57 (0.03)       | 0.56 (0.04)   | 0.54 (0.03)      |
| R Genu of CC             | 0.56 (0.04)       | 0.55 (0.04)   | 0.53 (0.03)      |
| L Body of CC             | 0.56 (0.03)       | 0.55 (0.03)   | 0.54 (0.02)      |
| R Body of CC             | 0.56 (0.03)       | 0.55 (0.03)   | 0.53 (0.02)      |
| R Fornix                 | 0.51 (0.06)       | 0.48 (0.04)   | 0.43 (0.04)      |
| R Sagittal Stratum       | 0.45 (0.01)       | 0.44 (0.02)   | 0.43 (0.01)      |
| L Sagittal Stratum       | 0.45 (0.02)       | 0.44 (0.02)   | 0.43 (0.02)      |
| R Anterior Corona Radiata| 0.41 (0.02)       | 0.40 (0.02)   | 0.39 (0.02)      |
| L Posterior Thalamic Radiation | 0.48 (0.02) | 0.47 (0.02) | 0.46 (0.02) |
| R Posterior Thalamic Radiation | 0.48 (0.02) | 0.47 (0.02) | 0.46 (0.02) |

Mean diffusivity (x10⁻⁴)

Note: mean (standard deviation).
| Structure                                         | Axial diffusivity (x10^-4)                                                                 | Radial diffusivity (x10^-4)                                                                 |
|--------------------------------------------------|------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------|
| R external capsule                               | 7.2 (0.4)                                                                                 | 12.3 (0.7)                                                                                |
| R inferior fronto-occipital fasciculus           | 7.6 (0.5)                                                                                 | 12.4 (0.8)                                                                                |
| L Superior Corona Radiata                        | 7.1 (0.3)                                                                                 | 13.0 (0.6)                                                                                |
| R Superior Corona Radiata                        | 7.3 (0.3)                                                                                 | 13.9 (0.8)                                                                                |
| R superior longitudinal fasciculus               | 7.4 (0.4)                                                                                 | 13.8 (0.9)                                                                                |
| L Cingulum                                       | 7.7 (0.3)                                                                                 | 14.3 (0.9)                                                                                |
| R Cingulum                                       | 7.6 (0.3)                                                                                 | 14.6 (1.0)                                                                                |
| L Anterior Corona Radiata                        | 7.9 (0.4)                                                                                 | 14.8 (1.1)                                                                                |
| R anterior corona radiata                        | 8.0 (0.4)                                                                                 | 15.0 (1.1)                                                                                |
| R retrolenticular internal capsule               | 7.7 (0.4)                                                                                 | 15.1 (1.2)                                                                                |
| R sagittal stratum                               | 8.1 (0.4)                                                                                 | 15.4 (1.2)                                                                                |
| R posterior corona radiata                       | 7.6 (0.4)                                                                                 | 8.0 (0.5)                                                                                 |
| L Genu of CC                                     | 9.7 (0.8)                                                                                 | 10.1 (0.7)                                                                                |
| R Genu of CC                                     | 7.2 (0.8)                                                                                 | 7.5 (0.9)                                                                                 |
| L Body of CC                                     | 9.6 (0.7)                                                                                 | 10 (0.8)                                                                                 |
| R Body of CC                                     | 9.5 (0.7)                                                                                 | 10 (0.8)                                                                                 |
| R posterior Thalamic Radiation                   | 8.3 (0.4)                                                                                 | 8.5 (0.5)                                                                                 |
| R Superior Fronto-occipital Fasciculus           | 7.8 (0.7)                                                                                 | 8.1 (0.7)                                                                                 |
| R Fornix Stria Terminalis                        | 9 (0.6)                                                                                   | 9.2 (0.6)                                                                                 |
| R Cingulum Hippocampal                           | 7.8 (0.3)                                                                                 | 8 (0.3)                                                                                  |

**Axial diffusivity (x10^-4)**

| Structure                                         | Axial diffusivity (x10^-4)                                                                 | Radial diffusivity (x10^-4)                                                                 |
|--------------------------------------------------|------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------|
| L superior longitudinal fasciculus               | 11.4 (0.5)                                                                               | 11.4 (0.5)                                                                               |
| R Sagittal Stratum                               | 12.3 (0.6)                                                                               | 12.6 (0.7)                                                                               |
| R external capsule                               | 10.5 (0.5)                                                                               | 10.9 (0.7)                                                                               |
| R superior corona radiata                        | 11.2 (0.5)                                                                               | 11.5 (0.5)                                                                               |
| L Superior Corona Radiata                        | 11 (0.4)                                                                                 | 11.1 (0.4)                                                                               |
| R inferior fronto-occipital fasciculus            | 11.8 (0.7)                                                                               | 12 (0.7)                                                                                |
| R superior longitudinal fasciculus               | 10.9 (0.5)                                                                               | 11.3 (0.5)                                                                               |
| L Anterior Corona Radiata                        | 11.5 (0.4)                                                                               | 11.6 (0.6)                                                                               |
| R anterior corona radiata                        | 16.4 (0.9)                                                                               | 17 (1.0)                                                                                 |
| R posterior Thalamic Radiation                   | 16.5 (0.9)                                                                               | 17.2 (1.1)                                                                               |
| R posterior Corona Radiata                       | 11.5 (0.4)                                                                               | 11.9 (0.6)                                                                               |
| R Anterior Corona Radiata                        | 11.7 (0.4)                                                                               | 12 (0.6)                                                                                 |
| R Retrolenticular Internal Capsule               | 12.4 (0.6)                                                                               | 12.9 (0.7)                                                                               |
| R Posterior Thalamic Radiation                   | 13 (0.6)                                                                                 | 13.3 (0.6)                                                                               |
| R Fornix Stria Terminalis                        | 13.9 (0.8)                                                                               | 14.2 (0.9)                                                                               |
| R Cingulum                                       | 11.6 (0.4)                                                                               | 11.8 (0.5)                                                                               |
| R Genu of CC                                     | 17 (1.0)                                                                                 | 17.6 (1.2)                                                                               |
| R Tapatum                                        | 19 (1.7)                                                                                 | 19.7 (1.7)                                                                               |
| R Cingulum Hippocampal                           | 11.78 (0.5)                                                                              | 11.9 (0.4)                                                                               |

**Radial diffusivity (x10^-4)**

| Structure                                         | Axial diffusivity (x10^-4)                                                                 | Radial diffusivity (x10^-4)                                                                 |
|--------------------------------------------------|------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------|
| L anterior corona radiata                        | 12.3 (0.7)                                                                               | 12.7 (0.9)                                                                               |
| R anterior corona radiata                        | 12.4 (0.8)                                                                               | 12.9 (1.0)                                                                               |
| L cingulum                                       | 10.7 (0.5)                                                                               | 11.1 (0.6)                                                                               |
| R cingulum                                       | 11.2 (0.5)                                                                               | 11.6 (0.6)                                                                               |

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| Brain Region                             | Mean (SD) Control | MCI | AD |
|-----------------------------------------|-------------------|-----|----|
| R inferior fronto-occipital fasciculus  | 11 (0.8)          | 11.3 (1.0) | 11.8 (0.8) **, *** |
| R external capsule                      | 11.1 (0.8)        | 11.6 (1.1) * | 11.8 (1.1) |
| R superior longitudinal fasciculus      | 11.2 (0.7)        | 11.6 (0.8) ** | 11.8 (0.8) ** |
| L Superior Corona Radiata               | 10.4 (0.6)        | 10.7 (0.8) | 11.1 (0.8) ** |
| R superior corona radiata               | 10.7 (0.6)        | 11.2 (0.8) ** | 11.4 (0.9) ** |
| R sagittal stratum                      | 12 (0.7)          | 12.6 (0.9) *** | 13.2 (0.9) ***, b*** |
| R retrolenticular internal capsule      | 10.8 (0.7)        | 11.3 (0.8) ** | 11.5 (0.7) ** |
| R Posterior Corona Radiata              | 11.3 (0.7)        | 11.8 (0.9) *** | 12 (1.0) *** |
| R Superior Fronto-occipital Fasciculus  | 11.5 (1.2)        | 12.2 (1.4) ** | 12.6 (1.6) *** |
| R Body of CC                            | 12.1 (1.4)        | 12.9 (1.6) ** | 13.9 (1.0) *** |

Note: mean (standard deviation). Statistical analysis: MANCOVA with Bonferroni post hoc test. R: right; L: left; CC: Corpus Callosum.

a: different from controls
b: different from aMCI
* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$

**Figure 1.** Differences in DTI metrics in main regions: fornix, cingulum, and hippocampal-cingulum.
Figure 2. Differences in DTI metrics comparing aMCI and healthy controls in other brain regions.

Figure 3. Differences in DTI metrics comparing aMCI and mild AD in other brain regions.
Figure 4. Partial correlations ($r$) between white matter DTI parameters and cerebrospinal fluid levels of Amyloid β (Aβ), Tau and phosphorylated Tau (p-Tau). (A) Anatomical representation of the right hippocampal cingulum (red) and the right fornix (green), and (B) graphs of significant correlations - the background color indicates the anatomical region correlated with the DTI parameters.