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Tau Platelets Correlate with Regional Brain Atrophy in Patients with Alzheimer's Disease

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Abstract.
Background: Intracellular neurofibrillary tangles are part of the core pathology of Alzheimer's disease (AD), which are mainly composed of hyperphosphorylated tau protein.
Objectives: The purpose of this study is to determine whether high molecular weight (HMW) or low molecular weight (LMW) tau protein levels, as well as the ratio HMW/LMW, present in platelets correlates with brain magnetic resonance imaging (MRI) structural changes in normal and cognitively impaired subjects.
Methods: We examined 53 AD patients and 37 cognitively normal subjects recruited from two Memory Clinics at the Universidad de Chile. Tau levels in platelets were determined by immunoreactivity and the MRI scans were analyzed using voxel-based morphometry in 41 AD patients.
Results: The HMW/LMW tau ratio was statistically different between controls and AD patients, and no associations were noted between HMW or LMW tau and MRI structures. In a multivariate analysis controlled for age and education level, the HMW/LMW tau ratio was associated with reduced volume in the left medial and right anterior cingulate gyri, right cerebellum, right thalamus (pulvinar), left frontal cortex, and right parahippocampal region.
Conclusions: This exploratory study showed that HMW/LMW tau ratio is significantly higher in AD patients than control subjects, and that it is associated with specific brain regions atrophy. Determination of peripheral markers of AD pathology can help understanding the pathophysiology of neurodegeneration in AD.

Keywords: Alzheimer's disease, medial temporal lobe atrophy, non-invasive biomarkers, tau variants

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INTRODUCTION

Alzheimer's disease (AD) is the major cause of dementia and disability in people over the age of 65 [1]. The etiology of AD is unknown, although there is agreement among researchers that the most salient neuropathological lesions associated with AD are amyloid-β (Aβ) plaques and neurofibrillary tangles (NFT) formed by hyperphosphorylated abnormal tau [2]. The NFT are used to grade severity of the neuropathology of AD, and this process appears to start in the mesial temporal lobe [3]. Clinical-pathological studies have shown that the severity of the clinical symptoms tends to correlate with NFTs and synaptic loss but not with Aβ plaques [4]. Aβ plaques and NFT are also present in normal subjects and in those with mild cognitive impairment (MCI), a pre-dementia state [5].

Markers of central (i.e., cerebrospinal fluid (CSF), structural and functional neuroimaging) and peripheral (e.g., Aβ in plasma) AD pathology have been examined as biomarkers of the disease, and more recently, studies of peripheral levels of tau have started to emerge [6, 7]. In CSF, $A\beta_{42}$ levels are decreased in AD patients, while tau or phospho-tau proteins are increased [8], reflecting the nature of the neurodegenerative process; amyloid deposition in the brain parenchyma reflects low CSF $A\beta_{42}$ and neuronal death is reflected as increased CSF levels of intracellular tau proteins.

Brain MRI studies have shown a steeper decline in the insula, superior parietal cortex, and cingulate gyrus, and relative stability of mesial temporal lobe structures in cognitively normal individuals as they age [9]. By contrast, atrophy in multiple brain regions is seen in AD patients, especially in the hippocampal formation [10]. Indeed, low hippocampal volumes have been consistently found to be predictors of dementia conversion in subjects with MCI [11, 12], and hippocampal volumes have shown a correlation with CSF tau levels [13].

Although levels of Aβ in plasma have been considered as biomarkers for AD [14], plasma amyloid studies have shown inconsistent results. Some studies found that high or low $A\beta_{42}$ levels or low $A\beta_{42}/A\beta_{40}$ ratio were associated with AD [14–16]. Plasma and platelet tau proteins have been reported to be higher in AD patients compared with non-demented individuals [6, 7, 17–19]. Previously, we have described that the high molecular weight (HMW)/ low molecular weight (LMW) tau ratio differentiated AD from cognitively normal (CN) controls, and it was associated with the severity of cognitive impairment [7, 20].

Chiu and colleagues examined the relationship between tau levels in plasma and cognitive and MRI measures in AD, MCI, and normal control subjects. The authors found that plasma tau levels were increased in AD and MCI patients compared to controls, and they were negatively associated with memory and verbal fluency measures, and with gray matter volumes, especially the hippocampus and frontal lobe regions [18]. Therefore, the identification of peripheral AD biomarkers is one of the critical steps in the understanding of the physiopathology of the disease, and has the potential for the use in clinical practice as diagnostic tools. The present study is designed to further examine the association between HMW/LMW tau ratio and MRI structural measures using voxel-based morphometry (VBM) in a group of AD and normal controls subjects.

MATERIAL AND METHODS

Participants

The study participants were recruited from the Memory Clinics that belongs to the Cognitive Neurology and Dementia Unit (Unidad de Neurología Cognitiva y Demencias) of the Neurology Service at the Hospital del Salvador and Hospital Clínico Universidad de Chile, Santiago, Chile. For the purpose of this study, we examined 53 AD (41 had MRI exams) and 37 CN subjects (35 had MRI exams). AD subjects met the NINCDS-ADRDA criteria for probable AD [21]. Diagnosis was made by consensus between senior neurologists (AS and CD) based on extensive clinical investigations, interviews with a reliable proxy, laboratory tests, and global cognitive functioning. Briefly, AD patients displayed a history of episodic memory loss and impairment in activities of daily life within the context of a preserved behavior and personality. All had a Clinical Dementia Rating (CDR) score of 0.5 or above (12 patients had a CDR = 0.5; 26 patients a CDR = 1; 12 patients a CDR = 2, and 3 patients a CDR = 3) [22]. Cognitively impaired subjects were excluded when they 1) did not have a reliable informant who could provide adequate information for the evaluation; 2) had a history of strokes or silent infarcts in brain MRI larger than 3 mm diameter; 3) had severe cognitive impairment that would not allow the patient to complete the neuropsychological assessment; or 4) had sensory disturbances that could interfere with

neuropsychological testing. The inclusion criteria for CN subjects were: 1) CDR score of 0 and 2) normal cognition based on local normative data for the Mini-Mental State Examination (MMSE) [23]. Exclusion criteria were the same as for the patients. All participants signed an informed consent prior to inclusion in the study. The Ethical and Scientific Committee of Servicio de Salud Metropolitano Oriente and Hospital Clínico de la Universidad de Chile approved this study.

Neurological and neuropsychological assessment

All the subjects were evaluated by a neurologist (AS or CD) who performed a neurological exam, interviewed the patients' families, and completed a questionnaire on activities of daily living. The methodology used in our Memory Clinics has been described previously [24]. Briefly, the assessment included global cognitive efficiency tests [MMSE [23], Addenbrooke's Cognitive Examination Revised (ACE-R) [24], the Montreal Cognitive Assessment (MoCA) [25]], language tests (Boston Naming Test) [26], and verbal fluency [Phonemic verbal fluency tests (i.e., FAS) and semantic fluency tasks (i.e., animals in 1 minute)]; visuospatial-constructional function [27], working memory test (digit-span task), verbal episodic memory test [Free and Cued Selective Reminding Test (FCSRT)] [28], visual memory (Short Recognition Memory Test for Faces of the Camden Memory Tests) [29]; executive functions tests [the Frontal Assessment Battery (FAB)] [30], the Modified version of the Wisconsin Card Sorting Test (MCST), a brief version of the Wiscon-sin Card Sorting Test [31]; and the Trail Making Test A and B [32]. Activities of daily living were examined with the CDR sum of boxes [22] and with the Technology-Activities of Daily Living Questionnaire (T-ADLQ) during an interview with a knowledgeable collateral source [33].

MRI data acquisition

MRI acquisition was performed on two 1.5 Tesla MRI scanners: a Philips Intera Nova Dual gradient system (45 mT/m) and a Siemens Symphony Maestro Class (Ernlagen, Germany) with 20 mT/m gradient system. High resolution anatomical scans were obtained using a T1-weighted three dimensional gradient recalled echo acquisition: 3D T1 fast field echo sequence on Philips scanner and 3D T1 fast low angle shot on Siemens scanner both with the same acquisition parameters (TE = 4.6 ms, TR = 25 ms, flip angle = 30°, field of view on frequency = 250 mm, 256 × 256 matrix, isotropic voxel size 1 × 1 × 1 mm).

Data processing for VBM

We used VBM in order to evaluate the correlation between tau values and the grey matter density on the anatomical MRI scans. Data processing and analysis was performed using statistical parametric mapping software SPM5 (Welcome Department of Imaging Neuroscience Group. London. UK; http://www.fil.ion.ucl.ac.uk/spm/) with MATLAB version 7.3 (The Mathworks, Inc., USA) and VBM toolbox (http://dbm.neuro.uni-jena.de/vbm/). To analyze brain volumes, we used an optimized VBM procedure [9]. Images were normalized to MNI space and segmented with SPM5 and DARTEL (Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra) toolbox (http://brainmap.wisc.edu/pages/8-Normalizing-DARTEL-Templates-to-MNI-Space) following the standard procedure described by Ashburner [34]. After normalization, gray matter segments were modulated and smoothed with an 8-mm FWHM isotropic Gaussian kernel. After segmentation the images were inspected to check data quality.

Platelet tau analyses

Peripheral blood samples (5 mL) were obtained from each participant, maintained at room temperature, and processed in laboratory according to Neumann and colleagues no more than 2 h after extraction [7]. Briefly samples were centrifuged at 200 × *g* for 10 min at room temperature in a Rotina 35R Hettichzentrifugen in order to obtain platelet rich plasma. The platelet fractions were then isolated by centrifugation at 1600 × *g* for 10 min, resuspended in 150 mM NH_4Cl at room temperature for 5 min, and then centrifuged at 1600 × *g* for 10 min at 4°C to avoid activation of these cells. The plasma obtained was stored at –80°C for subsequent analyses. Platelet pellets were washed with 1 mM EGTA in phosphate buffered saline (1.4 mM NaCl, 0.02 mM KCl, 0.1 mM Na_2HPO_4, 0.017 mM KH_2PO_4). Platelets were lysed in 100 μL RIPA lysis buffer (5.0 mM Tris-HCl pH 7.5, 1.5 mM NaCl, 10% NP-40, 10% deoxycholate, 20 mM EDTA pH 8.0, 500 mM NaF, 1% SDS) and 15 μl of a protease inhibitor cocktail (Complete Mini, EDTA-free, Roche). 50 μg of

total platelet protein were loaded per well in 10% acrylamide gels and SDS-PAGE was performed for 90 min at 100 mV. Proteins were then transferred to nitrocellulose membranes for 90 min at 330 mA by tank transfer. Membranes were blocked with 5% BSA overnight at 4°C and probed with 2 g/ml Tau-5 antibody (Calbiochem) against tau protein. Tau-5 monoclonal antibody recognizes every form of phosphorylated and non-phosphorylated tau; the epitope of anti-tau (Tau5) was mapped to the human tau sequence 218–225, which is not phosphorylated *in vivo*. Immunoreactive bands were detected by using Pierce ECL Plus Substrate chemioluminescent substrate (Pierce, Rockford, IL) in Amersham Hyperfilm ECL (GE Healthcare). After developing the western blots, the bands are scanned and quantified by using the software ImageJ 1.46r (Wayne Rasband, National Institutes of Health, USA). For this purpose, the low molecular weight tau (LMWtau) species are considered those with molecular weight between 55 and 70 kDa. On the other hand, high molecular weight tau (HMW) species are considered as those with molecular weight in the range 80–240 kDa and probably correspond to tau oligomers (see Fig. 1).

Statistical analysis

Descriptive and comparative analyses were conducted with the Student's *t*-test for non-categorical variables and chi squared test for categorical variables to compare the two groups. In addition, the effect sizes (Cohen's d statistic) were calculated to determine the magnitude of the group differences. The association between HMW/LMW tau ratio and demographic variables and cognitive results were evaluated with Pearson's correlation. The analyses were conducted at $p < 0.05$ (two-tailed) using the program PASW 18 for Microsoft Windows (SPSS Inc., Chicago, IL, USA).

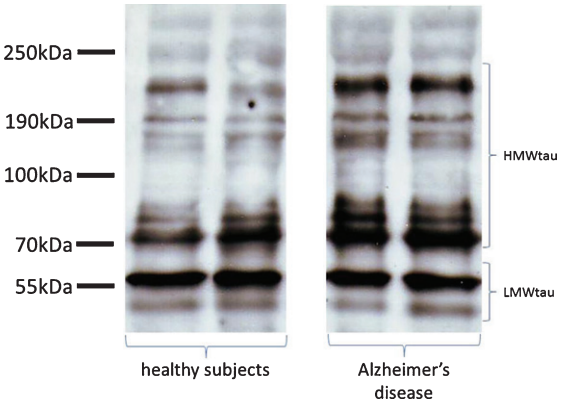


Fig. 1. Representative immunoblots of platelet tau with tau-5 antibody. High molecular weight tau bands (HMWtau) (molecular weight in the range 80–240 kDa) presented greater immunoreactivity in patients with AD as compared with healthy subjects. Low molecular weight tau bands (LMWtau) (molecular weight of about 65 kDa) presented similar immunoreactivity in patients with AD as healthy subjects.

VBM analysis

We correlated the HMW/LMW tau ratio for 41 AD patient with signal intensity in each voxel across all of the scans. Patient age and years of education were included in the analysis as a confounding covariate. We used multiple regression models to test regional effects for HMW/LMW tau ratio by using T-statistics. Results from analyses were threshold at a p value of less than 0.05 corrected for multiple comparisons using Familywise error rate (FWE).

RESULTS

Table 1 shows the characteristics of AD and CN subjects. The two groups differed significantly in education level and severity of dementia measured with the CDR and the three general cognitive functions tools (MMSE, MoCA, ACE-R). The details of the neuropsychological battery in CN and AD subjects are shown in Supplementary Table 2. The ratio of HMW/LMW tau was higher in AD patients compared to CN subjects ($p < 0.001$) (see Table 1 and Fig. 2). However, the levels of LMW tau ($p = 0.427$) and HMW tau ($p = 0.134$) were not statistically different between groups.

Clinical correlations

The HMW/LMW tau ratio correlated with the severity of dementia rated with the CDR (CDR $r = 0.318$, $p = 0.002$ and CDR–SB $r = 0.357$; $p < 0.001$) and performances in cognitive tests in the total sample (ACE-R $r = -0.405$, MOCA $r = -0.392$, and MMSE $r = -0.380$; $p < 0.001$) (see Table 2). Among all 90 study participants, no correlations were found between HMW/LMW and age or educational level ($p > 0.05$). No significant correlation was found with any of these variables in the CN group. In the AD group, only ACE-R was significantly correlated with HMW/LMW tau ratio ($r = -0.270$; $p < 0.05$).

Table 1
Demographic characteristics of normal controls and Alzheimer's disease patients

	Normal control	AD Patients	t-test/χ^2	Effect Size[1]
Number of cases	37	53		
Male/female	13 (35.1%)/24 (65.1%)	21 (39.6%)/32 (60.4%)	0.329	0.89
Age	71.3 ± 5.18 (64–83)	73.62 ± 6.64 (60–88)	–1.783	–0.38
Education level	13 ± 4.06 (6–22)	10.96 ± 4.73 (3–20)	2.129*	0.41
CDR	0 ± 0 (0–0)	1.23 ± 0.68 (0.5–3)	–13.065**	–2.56
CDR Sum of boxes	0 ± 0 (0–0)	5.9 ± 3.37 (1–14)	–10.558**	–2.47
MMSE	28 ± 2.19 (21–30)	21.7 ± 5.57 (7–30)	7.452**	1.49
MoCA	24.58 ± 3.93 (16–30)	15.56 ± 6.56 (1–29)	7.946**	1.67
ACE-R	91.22 ± 7.82 (61–100)	66.19 ± 19.19 (66.19–100)	8.534**	1.71
tau LMW	22217.81 ± 9604.67 (4866.25–41435.39)	20852.84 ± 10903.46 (1355.36–45511.87)	0.611	0.13
tau HMW	30589.47 ± 13992.49 (3543.27–56776.47)	35343.84 ± 15554.91 (6758.47–69989.64)	–1.486	–0.32
HMW/LMWtau	1.4 ± 0.43 (0.42–2.27)	2.17 ± 1.21(0.5–6.93)	–4.260**	–0.85

AD, Alzheimer's disease; CDR, Clinical Dementia Rating; MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment; ACE-R, Addenbrooke's Cognitive Examination Revised. Data are presented in mean ± standard deviation (minimum – maximum). [1]Cohen's d except for gender (Relative Risk) *$p = 0.036$; **$p < 0.001$.

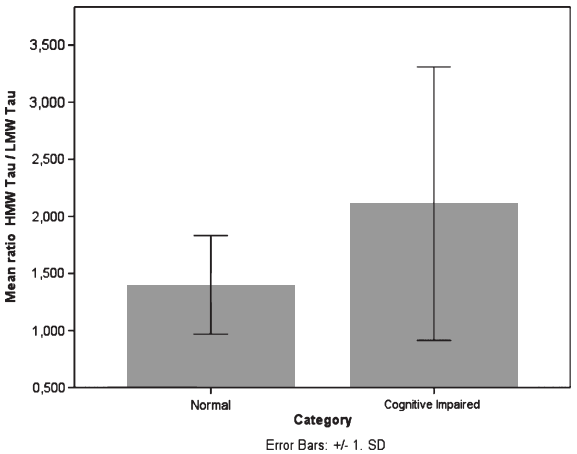


Fig. 2. Mean HMW/LMW tau ratio in AD patients MCI and control subjects. There is a significant difference between AD and control group ($p = 0.002$).

MRI analyses

There were no associations between HML and LMW tau and MRI structures. The HMW/LMW tau ratio controlled for age and education level was significantly correlated with six clusters: one of 529 voxels in the left cingulate gyrus Brodmann's area (BA) 24 with peak at Talairach coordinates –12, 0, 43; one of 364 voxels in the right cerebellum with peak at Talairach coordinates 24, –81, –26; one of 230 voxels in the right anterior cingulate (BA 32) with peak at Talairach coordinates 12, 33, 9; one of 190 voxels in the right thalamus (pulvinar) with peak at Talairach coordinates –15, –30, 15; one of 161 voxels in the left frontal lobe (BA 9) with peak at Talairach coordinates 14, 29, 30; and one of 187 voxels in the right parahippocampal (BA 36) with peak at Talairach coordinates 28, –21, –24 (Fig. 3). All the anatomical locations exceed a voxelwise statistical FEW corrected $p < 0.05$ threshold level (see Table 3).

DISCUSSION

This exploratory study showed that the HMW/LMW tau ratio was 1) significantly higher in AD subjects compared to those with normal cognition, 2) associated with decreased brain volume in left middle and right anterior cingulate gyri, right cerebellum, right thalamus (pulvinar), left frontal cortex, and right parahippocampal in AD patients, and 3) correlated with global measures of cognitive and functional performance in all subjects.

We have described an increase in HMW tau and a decrease in the normal LMW tau species in the blood samples of AD patients [20]. The molecular features of the HMW species appear to correspond to oligomeric forms of tau and not to the expression of a different gene as described for "heavy tau" of 130 kDa [35, 36]. These tau oligomers can be visualized in western blots even at denaturing conditions, as described in patients with frontotemporal dementia [37]. Accumulation of tau oligomers appears to correlate with the pathophysiology of AD that occurs in the brain, as well as the peripheral blood cells [38]. As noted previously, HMW/LMW ratio does not depend on age.

We found an association between the HMW/LMW ratio and brain volume in the mesial temporal lobe region, the cingulate cortex, the pulvinar nucleus, the frontal cortex, and the cerebellum. These findings are consistent with those from Chiu and colleagues [18]

Table 2
Pearson's correlation coefficient between HMW/LMW tau ratio and demographic and neuropsychological variables

	All subjects ($n = 90$)	Normal controls ($n = 37$)	AD Patients ($n = 53$)	
			All patients with cognitive testing ($n = 53$)	Patients with MRI ($n = 41$)
Age	–0.012	0.006	–0.111	–0.175
Education level	–0.104	0.021	–0.036	–0.034
ACE-R	–0.405*	0.125	–0.270***	–0.089
MMSE	–0.380**	0.061	–0.250	–0.109
MOCA	–0.392*	0.039	–0.266	–0.068
CDR	0.318**		0.068	–0.012
CDR-SB	0.357**	–0.175	0.138	–0.014

*$p < 0.001$; **$p = 0.002$; ***$p < 0.05$. See Table 1 for abbreviations.

Table 3
Results of VBM analysis, dependent variable: HMW/LMW tau ratio with age and education as covariable

Set-level		Cluster-level				Peak-level					MNI * Coordinates		
P	C	$p_{FWE\text{-}Corr}$	$p_{FDR\ Corr}$	K_E	p^{**}	$p_{FWE\text{-}Corr}$	$p_{FDR\ Corr}$	T	(Z_E)	p^{**}	x	y	z
0.000	6	0.001	0.073	230	0.013	0.000	0.160	7.05	5.58	0.000	12	33	9
		0.000	0.009	520	0.001	0.001	0.160	6.84	5.47	0.000	–12	0	43
		0.000	0.023	364	0.003	0.001	0.160	6.73	5.41	0.000	24	–81	–26
		0.001	0.077	190	0.022	0.002	0.221	6.34	5.18	0.000	15	–30	15
		0.002	0.091	161	0.032	0.003	0.221	6.30	5.16	0.000	–14	29	30
		0.001	0.077	187	0.023	0.006	0.390	5.99	4.98	0.000	28	–21	–24

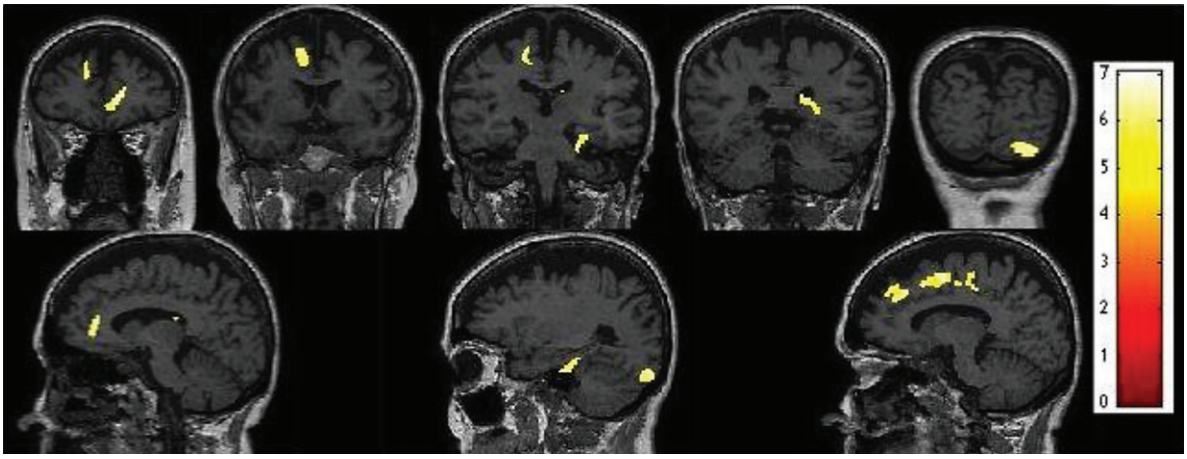
*Montreal Neurological Institute, **uncorrected.

who reported increased tau plasma levels in AD and MCI subjects compared to controls. In addition, the authors found an association between increased tau plasma levels and decreased temporal (including mesial temporal regions) and frontal cortices, thalamus, and precuneus volumes in a group of normal, MCI, and AD individuals. However, in the multivariate analysis, education and hippocampal and superior frontal cortex volumes were associated with tau plasma levels. Studies conducted with $^{[18]}$F-T807 PET, a tau ligand, showed an increased retention in the inferolateral temporal lobe and parietal and frontal cortices in MCI/AD individuals [39]. Interestingly, there was no retention in the hippocampal region, and there was increased retention in the substantia nigra. The standardized uptake value ratio of the $^{[18]}$F-T807 PET retention correlated with that of $^{[11]}$C PiB-PET, an amyloid ligand. Taken together, these studies indicated that peripheral and central tau markers are associated with the expected distribution of NFT in the neocortex as described by Braak and Braak [3]. Our data suggest that peripheral changes in platelet proteins (HMW/LMW tau) are associated with distribution of NFT in the cerebral cortex.

Finally, it is important to note that amyloid deposition in the brain detected with amyloid ligands is predominantly present in the dorsolateral prefrontal, anterior and middle cingulate gyri, superior temporal and parietal cortices, as well as in the precuneus [40], and the amyloid-associated volume loss tends to occur in the presence of phosphor-tau [41].

The association between HMW/LMW tau ratio and subcortical structures is consistent with the presence of AD pathology in multiple brain regions. AD pathology in thalamic [42], and cerebellar structures [43, 44], possibly reflect a complex cognitive-behavioral network dysfunction in AD [43]. The dorsolateral thalamic nucleus is part of the Papez circuit [42], and the pulvinar nucleus could be part of visuospatial-visual attention networks [45]. MRI studies have shown smaller cerebellar volumes in AD patients compared to controls [46–48], and Thal and colleagues showed AD pathology in the cerebellum of AD patients [44]. However, the cerebellar involvement seems to reflect more advanced disease.

The HMW/LMW ratio is significantly increased in AD patients and it was associated with measures of global cognition in the whole sample, and there was an association between a measure of frontal lobe



Brodman Area (BA)	x	y	z	nº voxels	T
Right anterior cingulate (32)	12	33	9	230	7.05
Left cingulate gyrus (24)	-12	0	43	520	6.84
Right cerebellum	24	-81	-26	364	6.73
Right thalamus (pulvinar)	15	-30	15	190	6.34
Left frontal lobe (9)	-14	29	30	161	6.30
Right parahippocampal (36)	28	-21	-24	187	5.99

Fig. 3. VBM analyses showing brain regions in which gray matter intensity correlates significantly with HMW/LMW tau ratio in AD patients. Colored voxels show regions that were significant in the analysis using a statistical threshold of $p < 0.05$ corrected for multiple comparisons (FWE) with a cluster threshold of 100 contiguous voxels. Clusters are displayed using anatomical images (radiological convention) over a normalized T1-weighted image. The regression model included age and education level as covariate parameters. Each reported anatomical location exceeds a voxelwise statistical FWE corrected $p < 0.05$ threshold level. The number of voxels denotes the extent of the cluster of significant voxels in cubic millimeters. The Talairach coordinate refers to the location of the most statistically significant voxel in the cluster.

dysfunction and HMW/LMW ratio in the AD patients. It is possible that the small number of subjects precluded to further investigate cognitive associations in this sample. Nevertheless, this is consistent with previous observations that showed that tau levels in plasma were associated with verbal memory and verbal fluency in MCI/AD patients [18].

As reported in previous study [20], neither HMW nor LMW differentiated AD subjects from CN subjects. The absence of correlation of HMW and LMW could be explained by the fact that neither HMW nor LMW platelet tau constitute biomarkers for AD.

It is important to point out that the nature of the high molecular weight species of tau in human platelets remains elusive. Berger et al. [37] found two forms of tau (140 and 170 kDa). They described the same electrophoretic pattern of tau proteins, i.e., a high molecular weight tau, in both mouse models and in tissue from patients with AD and frontotemporal dementia linked to chromosome 17. The levels of this form of tau correlated with memory loss at different ages in transgenic mice, suggesting that this high weight form of tau may correspond to a variant of high molecular weight or oligomeric of protein tau [49]. This form of the protein was called "heavy tau" due to its molecular weight (110–130 kDa) [37, 49–52].

In summary, the results of our study suggest that HMW/LMW tau ratio could constitute a valuable biomarker for AD due to its association with measure of severity of the disease and measure of disease pathology (i.e., brain volume). Compared to CSF biomarkers, plasma biomarkers could be more suitable for a broad application and repeated measures due to their low cost and low risk. Our findings need independent confirmation by other group and if replicated, longitudinal studies are needed to examine their utility as markers of disease progression.

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SUPPLEMENTARY MATERIAL

The supplementary material is available in the electronic version of this article: http://dx.doi.org/10.3233/JAD-160652.

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