Plasma tau association with brain atrophy in mild cognitive impairment and Alzheimer's disease

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RUNNING TITLE (45 characters including spaces): Plasma tau is associated with brain atrophy.

ABSTRACT (250 word max)

Background: Peripheral (plasma) and central (cerebrospinal fluid, CSF) measures of tau are increased in Alzheimer's disease (AD) relative to prodromal stages and controls. While elevated CSF tau levels have been shown to be associated with lower grey matter density (GMD) in AD-specific regions, this correlation has yet to be examined for plasma in a large study.

Methods: Cross-sectional data for 508 ADNI participants were collected for clinical, plasma tau, CSF amyloid (Aβ42) and tau, and MRI variables. The relationship between plasma tau and GMD and between CSF total-tau (t-tau) and GMD were assessed on a voxel-by-voxel basis using regression models. Age, gender, *APOE* ε4 status, diagnosis, and intracranial volume were used as covariates where appropriate. Participants were defined as amyloid positive (Aβ+) if CSF Aβ42 was <192pg/mL.

Results: Plasma tau was negatively correlated with GMD in the medial temporal lobe (MTL), precuneus, thalamus, and striatum. The associations with thalamus and striatum were independent of diagnosis. A negative correlation also existed between plasma tau and GMD in Aβ+ participants in the MTL, precuneus, and frontal lobe. When compared to CSF t-tau, plasma tau showed a notably different associated brain atrophy pattern, with only small overlapping regions in the fusiform gyrus.

Conclusions: Increased plasma tau was associated with atrophy in several AD-specific brain regions, as well as in the striatum and thalamus. These findings support plasma tau as a peripheral marker of ongoing AD-type neurodegeneration. The reduced GMD in thalamic and striatal regions associated with plasma tau suggest that this association may not be AD-specific.

KEYWORDS (4-10): plasma; tau protein; magnetic resonance imaging; mild cognitive impairment; Alzheimer disease

INTRODUCTION

Understanding the underlying pathological processes of Alzheimer's disease (AD) and developing reliable biomarkers are critical to identify the causes and pathogenesis of AD. Both *in vivo* and *post-mortem* studies have shown that pathological tau is correlated with neurodegeneration, disease severity, and cognitive impairment [1]. Likewise, *in vivo* central measures of tau in cerebrospinal fluid (CSF) also correlate with *post-mortem* tau pathology and are increased in AD patients relative to those in a prodromal stage of AD referred to as mild cognitive impairment (MCI) and cognitively normal controls (CN), aiding prediction of disease progression [2, 3]. However, CSF collection is regarded as invasive, leading researchers to search for alternative methods to monitor MCI and AD such as blood biomarkers.

A recent meta-analysis reported plasma tau as the only blood-based biomarker to delineate AD from controls [4]. Fortunately, a new ultrasensitive technique was developed capable of detecting tau at low concentrations in plasma [REF= Randall]. Similar to CSF, plasma tau levels are higher in AD relative to MCI and CN [5]. However, a large overlap was observed between the MCI and CN groups suggesting plasma tau may not be suitable as a diagnostic marker. Further, the correlation between plasma tau and CSF tau was weak [5].

Previous studies have reported conflicting results regarding CSF tau and its correlation with cortical atrophy [6]. The goal of our project was to investigate the association of plasma tau with atrophy in participants of the ADNI. We also aimed to determine if plasma and CSF tau levels were related to atrophy in similar brain regions. We hypothesize that plasma tau will be inversely correlated with grey matter density (GMD), as is seen with a majority of studies with CSF tau. Secondly, we hypothesize that plasma tau and CSF t-tau will be related to similar regions of atrophy.

MATERIALS and METHODS

Alzheimer's Disease Neuroimaging Initiative (ADNI)

Subjects used in this study were participants in the Alzheimer's Disease Neuroimaging Initiative (ADNI) (www.adni-info.org). The ADNI was launched in 2004 to help researchers and clinicians develop new treatments for MCI (mild cognitive impairment) and early AD, monitor their effectiveness, and decrease the time and cost of clinical trials. The Principal Investigator of this initiative is Michael W. Weiner, M.D., VA Medical Center and University of California, San Francisco. The goal of ADNI is to test whether serial magnetic resonance imaging (MRI), positron emission tomography (PET), other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of MCI and AD. This multi-year multi-site longitudinal study was started by the National Institute on Aging (NIA), the National Institute of Biomedical Imaging and Bioengineering (NIBIB), the Food and Drug Administration (FDA), private pharmaceutical companies, and non-profit organizations as a $60 million, 5-year public-private partnership. The ADNI participants consist of AD, MCI, and elderly healthy individuals. They were aged 55-90 years and recruited from 59 sites across the U.S. and Canada. Written informed consent was obtained from all participants and the study was conducted with prior Institutional Review Board approval.

Plasma Tau Collection and Quality Control

Peripheral plasma tau levels were measured for 581 non-Hispanic Caucasian participants using the Single Molecule array (Simoa) technique with the Human Total Tau assay (Human Total Tau 2.0 kit, Quanterix Corp, Boston, MA, USA). This assay and the plasma tau characteristics for ADNI have been previously described [5, 7]. In brief, the assay uses two monoclonal antibodies which bind to the N-terminus and mid-region of tau, and measures both normal and phosphorylated tau protein. Values are given as pg/mL. A total of 38 samples had plasma levels below the Limit of Detection (LOD) or below the Lower Limit of Quantification (LLOQ) and were removed from further analysis. An additional four samples had missing values. To reduce the possible effect of extreme outliers on statistical analysis, the mean and standard deviation (SD) of plasma tau were calculated; participants with a value more than three SDs above or below the mean value were regarded as outliers and removed from further analysis. This resulted in the removal of eight participants, leaving 508 participants for the study (166 CN, 174 MCI, 168 AD).

CSF Collection and Quality Control

CSF samples were available for 370 of the 508 ADNI plasma tau subjects with comparable demographic, clinical, and apolipoprotein (*APOE*) genotyping results to the full sample [8]. Briefly, a lumbar puncture was performed after an overnight fast and the CSF was collected into collection tubes. The CSF was transferred into polypropylene tubes, frozen on dry ice within one hour of collection, and then shipped to the ADNI Biomarker Core laboratory at the University of Pennsylvania Medical Center on dry ice. Aliquots (0.5ml) were prepared from these samples after one hour of thawing at room temperature and stored in bar code-labeled polypropylene vials at -80°C.

CSF analytes ($A\beta_{1-42}$, t-tau and p-tau_{181p}) were measured using the multiplex xMAP Luminex platform (Luminex Corp, Austin, TX) with Innogenetics (INNO-BIA AlzBio3; Ghent, Belgium; for research use-only reagents) immunoassay kit-based reagents. To reduce the possible effect of outliers on statistical analysis, the mean and standard deviation of CSF analytes were calculated and subjects with at least one analyte value more than three SD below or above the mean value of each of CSF variable were regarded outliers and removed from the analysis. This resulted in 341 valid CSF samples. For the MRI-CSF study, only participants with a CSF value for both t-tau and p-tau were included in the CSF study (91 CN, 158 MCI, 82 AD). Participants were classifies as amyloid positive (Aβ+) if CSF $A\beta_{1-42}$ <192pg/mL.

MRI Scan Processing

All participants had baseline 1.5T magnetization-prepared rapid gradient-echo (MPRAGE) images downloaded from the ADNI LONI site (http://adni.loni.usc.edu/). Scan processing with voxel-based morphometry (VBM) in Statistical Parametric Mapping 8 (SPM8; Wellcome Trust Centre for Neuroimaging, http://www.fil.ion.ucl.ac.uk/spm/software/spm8/) and quality control were done as previously described [9]. Briefly, scans were co-registered to a T1-weighted template and segmented into different tissue classes (gray matter, GM; white matter, WM; CSF). Grey matter maps were normalized to MNI space without modulation as 1 x 1 x 1 mm voxels and smoothed with an 8 mm Gaussian kernel to create GM density (GMD) images for further analysis.

Image Analysis

To evaluate the relationship between central (CSF) and peripheral (plasma) measures of tau and GMD, voxel-wise linear regression models in SPM8 were used. Covariates included in the regression models were age, gender, *APOE* status (ε4 carrier or ε4 non-carrier), and total intracranial volume, generated using Freesurfer version 5.1. Analyses were done with and without diagnosis. An explicit GM mask was applied to the MRI scans to restrict the search area for the statistical analysis. Significant results were displayed at a voxel-wise $p < 0.05$ (family-wise error (FWE) corrected for multiple comparisons) and with a minimum cluster size (k) of 100 voxels. If no brain regions survived correction for multiple comparisons, then a slightly less stringent voxel-wise p-value of 0.001 (uncorrected) was used. To determine if there were any overlapping brain regions significantly correlated to plasma tau and CSF, a composite image was created in SPM8.

Statistical Analysis

SPSS V24.0 was used to log transform the CSF t-tau and p-tau values in order to achieve normal distribution. Plasma tau values were normally distributed and thus absolute values were used for analysis; results were unchanged when a transformed plasma value was use. The association of gender, *APOE* status, and Aβ positivity with diagnostic groups was assessed using a Pearson chi-squared test. ANOVA was used to assess the relationship of age, Mini-Mental State Exam (MMSE), Clinical Dementia Rating Scale – Sum of Boxes (CDR-SB), plasma tau, and CSF analytes with diagnostic status. Post-hoc pairwise differences among diagnostic groups was assessed using a Bonferroni correction for multiple comparisons. The MarsBaR toolbox in SPM8 was used to extract mean GMD from significant clusters from the voxel-wise results for further characterization of the results.

Results

Demographic and clinical characteristics

There was a near equal number of subjects in each diagnostic group with 166 CN, 174 MCI, and 168 AD. Significant differences among diagnostic groups were observed for all demographic and clinical characteristics examined except for age (Table 1). As expected, the AD group had the highest percentage of *APOE* ε4 carriers (67.9%), followed by the MCI group consisting of 54% *APOE* ε4 carriers. Significant differences in the mean MMSE and CDR-SB among the diagnostic groups, with the AD group showing the most impairment and the MCI group showing intermediate impairment between AD and CN ($p < 0.001$). Plasma tau, CSF t-tau, and CSF p-tau were significantly different between groups, with the AD group showing significantly higher levels compared to MCI and CN ($p = 0.002$, $p < 0.001$, and $p < 0.001$, respectively). Similar to previous reports, the mean plasma tau levels in the MCI and CN groups were nearly equal. The number of Aβ+ subjects was also significantly different among diagnostic groups ($p < 0.001$).

Voxel-based MRI analysis

A significant negative association between increased plasma tau and decreased GMD in several brain regions was observed, including in the middle, inferior, and superior temporal gyrus, parahippocampus, hippocampus, fusiform, uncus, precuneus, thalamus, caudate, putamen, and middle and inferior frontal gyrus (Fig. 1A-D, voxel-wise $p<0.001$ (uncorrected), $k=100$ voxels). As would be expected, AD participants had lower mean GMD in the larger clusters identified (MTL structures,

striatum, and thalamus; Supplemental Fig.1A). When controlling for diagnosis, a significant negative association between plasma tau and GMD was still observed in the right thalamus and bilaterally in the striatum (Fig. 1C-D). No significant clusters were observed in the positive direction (data not shown).

Amyloid-positive subjects

There was no significant association between plasma tau and GMD in the Aβ- subjects, however increased plasma tau was significantly correlated with decreased GMD in the Aβ+ subjects in the fusiform, hippocampus, parahippocampus, precuneus, and premotor cortex (Fig. 2A-D, voxel-wise $p<0.001$ (uncorrected), $k=100$ voxels), as well as the frontal and parietal lobes, pre- and post-central gyri, and the globus pallidus. Notably, in the MTL cluster, Aβ+ subjects showed lower mean GMD compared to Aβ- subjects (Supplemental Fig. 1B). After diagnosis was added as a covariate, many of the same regions of reduced GMD remained significantly negatively correlated with increased plasma tau including in the precuneus, parahippocampus, and premotor cortex (Fig. 2B-D). No significant clusters were observed in the positive/unexpected direction (data not shown).

CSF-Plasma tau comparison

Peripheral measures of tau protein were of total-tau only, thus, we sought to compare the regional atrophy associated with plasma tau to that associated with CSF t-tau only. At $p < 0.05$ (FWE corrected), increased CSF t-tau was negatively associated with reduced GMD in the precuneus and temporal gyrus (Supplemental Fig. 2). However, the uncorrected results were used for comparison with the plasma tau results, as this threshold was used in the plasma tau analyses described above. Central and peripheral measures of tau protein were associated with GMD in some overlapping, but largely different brain regions. The temporal pole, fusiform, and angular gyrus were brain regions in which both increased CSF t-tau and plasma tau were associated with reduced GMD (Fig. 3A-D). As several reports have previously shown, increased CSF t-tau was associated with lower GMD in cortical structures known to be affected in persons with AD. However, as above, in the present study plasma tau was predominantly associated with subcortical structures. Within the Aβ+ subjects, no significant overlap was observed between the association of GMD with plasma tau and the association of GMD with CSF t-tau (data not shown).

Discussion

The main goal of this study was to determine if plasma tau was associated with cortical atrophy in a population at risk for AD or already manifesting signs of clinical AD. We found increased plasma tau was negatively correlated with reduced GMD in several AD-specific brain regions, including the MTL and precuneus, as well as in the thalamus and striatum. Further investigation into only Aβ+ subjects also revealed an association between increased plasma tau levels and reduced GMD in MTL structures, the precuneus, and the premotor cortex. When the pattern of GM atrophy associated with increased plasma tau was compared to that associated with increased CSF t-tau, only small regions of overlap were observed in the temporal pole, fusiform, and angular gyrus. Otherwise, the central (i.e. CSF) and peripheral (i.e. plasma levels of tau showed quite different associated patterns of atrophy. These findings suggest that plasma tau may reflect neurodegeneration in AD-specific regions and more generally.

The MTL is one of the first brain regions that shows tau pathology and the earliest to degenerate in AD patients [10]. Thus, our observation that high plasma tau is associated with cortical

atrophy in several MTL structures including the parahippocampus and hippocampus across all subjects and in Aβ+ subjects suggests that this peripheral measure of tau may be reflective of CSF and brain tau pathology as well as brain atrophy. We also observed a negative correlation between increased plasma tau and reduced GMD in the precuneus. A functional decline in the precuneus occurs early in the course of AD, but tau deposition normally occurs in later stages. Specifically, the precuneus along with the hippocampus are both core components of the default mode network, an important functional resting-state network that is impaired early in AD [11]. Thus, the atrophy in the precuneus associated with plasma tau could be reflective of structural deterioration in the default mode network early in AD.

Even more striking was the inverse association of plasma tau with GMD in the thalamus and the striatum across all subjects, especially given that these areas are affected later in the disease course. These were the only brain regions that were also independent of diagnosis, suggesting that plasma tau may reflect neurodegeneration unrelated to AD diagnosis. Alternatively, in the Aβ+ subjects, the association of plasma tau and GMD in the parahippocampus and precuneus was independent of diagnosis. These findings suggest that plasma tau may be reflecting neurodegeneration specific to AD pathology. Taken together, the results independent of diagnosis suggests that plasma tau may be reflecting disease-specific neurodegeneration in different regions, with associated atrophy in AD-relevant regions linked to AD pathology and associated atrophy in other regions potentially reflecting other pathology or a more general neurodegeneration. Studies evaluating plasma tau in other tauopathies may shed light on this hypothesis.

Our initial hypothesis was that plasma tau and CSF t-tau would be related to similar regions of atrophy, especially if the two tau measures were truly reflective of AD neurodegeneration. Unexpectedly, plasma tau and CSF t-tau had very little overlap, with plasma tau mapping more to subcortical structures and CSF t-tau mapping more to cortical structures. Only small regions of overlap were observed in the temporal pole, fusiform, and angular gyrus. These results suggest that CSF and plasma measures of tau protein may reflect related but somewhat different pathological substrates of AD. This could be due to differences in the assays, tau isoforms detected by the assays, or in the variability of the measurements. There may also be differences in how tau, released from neurons, is cleared from brain interstitial fluid to CSF and plasma. Further work is needed to further elucidate the differences between plasma and CSF tau.

One study limitation is the lack of a commensurate replicate data set. This tau quantification method is relatively new and hopefully independent data sets will become available. Additionally, accumulated p-tau is the main hallmark in AD and other tauopathies but there is not yet a technique for measuring p-tau in plasma, thus yielding a second limitation to this study. New techniques to assess p-tau in plasma could be extremely beneficial. A third limitation is that our data is cross-sectional only. Longitudinal data would provide us the information needed to better assess changes in plasma tau over time and the association between changing plasma tau measures and rate of neurodegeneration.

In conclusion, high levels of plasma tau were associated with lower grey matter density in both AD-specific and non-AD-related brain regions. Future replication and longitudinal studies will be important to fully elucidate the contribution of plasma tau as a possible biomarker in AD and other tauopathies.

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Table 1. Demographic and clinical characteristics.

	CN	MCI	AD	p-value
N	166	174	168	-
Age (years)	75.2 (5.1)	74.1 (7.6)	75.3 (7.3)	0.789
Gender (M, F)	95, 71	115, 59	87, 81	*0.025*
***APOE ε4* (% *ε4* positive)**	27.1%	54%	67.9%	*<0.001*
MMSE	29.1 (1)	26.9 (1.8)	23.2 (2)	*<0.001*
CDR-SB	0 (0.1)	1.6 (0.9)	4.3 (1.6)	*<0.001*
Plasma tau	2.7 (1)	2.8 (1.2)	3.1 (1.3)	*0.002*
CSF t-tau*	65.7 (24.7)	97.6 (48.6)	121.2 (52.4)	*<0.001*
CSF p-tau*	22.3 (10.5)	34.3 (16.2)	41.5 (18.5)	*<0.001*
Amyloid (-/+)**	59, 32	41, 116	5, 76	*<0.001*

CN = cognitively normal; MCI = mild cognitive impairment; AD = Alzheimer's disease; M = male; F = female; *APOE* = apolipoprotein; MMSE = mini-mental state exam; CDR-SB = clinical dementia rating-sum of boxes; CSF = cerebrospinal fluid. Mean +/- standard deviation. Significant p-values < 0.05 are italicized.

*n = 91 CN, 158 MCI, 82 AD

**missing 1 MCI and 1 AD

Figure 1: Increased plasma tau is negatively correlated with reduced grey matter density (GMD) in the (A) parahippocampus, (B) precuneus, (C) striatum, and (D) thalamus. C and D represent the anatomic overlap (orange) of regions of GM atrophy associated with increased plasma tau using only age, gender, *APOE* e4 status, and total intracranial volume as covariates (yellow) and with the addition of diagnosis as a covariate (red). Results are displayed at p<0.001 (uncorrected) and k=100 voxels in all figures.

Figure 2: Increased plasma tau is negatively correlated with reduced GMD in the amyloid positive subjects in the (A) hippocampus, (B) precuneus, (C) parahippocampus, and (D) BA 6 premotor cortex. B-D also represent the anatomic overlap (orange) of regions of GM atrophy associated with increased plasma tau using only age, gender, *APOE* e4 status, and total intracranial volume as covariates (yellow) and with the addition of diagnosis as a covariate (red). Results are displayed at p<0.001 (uncorrected) and k=100 voxels in all figures.

Figure 3: Anatomical overlap of GM atrophy (orange) associated with increased plasma tau (red) and that associated with increased CSF t-tau (yellow). Results are displayed at p<0.001 (uncorrected) and k=100 voxels in all figures.

Supplemental Figure 1: Mean GMD in selected regions significant in the voxel-wise analysis across (A) diagnostic and (B) amyloid status. Covariates included in both models include age, gender, *APOE* e4 status, and total intracranial volume.

Supplemental Figure 2: Increased CSF total-tau is negatively correlated with reduced GMD in the (A-B) precuneus, (C) fusiform, and (D) BA21. Covariates included in the analysis were age, gender, *APOE* e4 status, and total intracranial volume. Results are displayed at p<0.05 (FWE corrected) and at a threshold (k) of 100 voxels.

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