Published in final edited form as:

Neurobiol Aging. 2011 December ; 32(12): 2131–2141. doi:10.1016/j.neurobiolaging.2009.12.026.

Phosphorylated tau 231, memory decline and medial temporal atrophy in normal elders

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Abstract

Little is known whether cerebrospinal fluid (CSF) biomarkers of Alzheimer's disease (AD) can predict both memory decline and associated longitudinal medial temporal lobe (MTL) gray matter (GM) reductions in cognitively healthy individuals. 57 normal elderly subjects received comprehensive evaluation at baseline and 2 years later. The baseline phosphorylated tau₂₃₁ (p-tau₂₃₁), total tau, the amyloid beta (Aβ) Aβ₄₂/Aβ₄₀, t-tau/Aβ₄₂ and p-tau₂₃₁/Aβ₄₂ ratios were examined as predictors of memory change and reductions in the global and MTL GM, determined from T1-weighted MRI. Twenty out of 57 participants experienced reduced memory performance at follow-up. The group with decreased memory performance showed higher baseline p-tau₂₃₁ ($Z=-2.2$, $p=.03$), lower Aβ₄₂/Aβ₄₀ ($t=-2.2$ [55], $p=.04$) and greater longitudinal MTL GM reductions ($t_{[52]}=-2.70$, $p=.01$). Higher baseline p-tau₂₃₁ was also associated with the absolute decrease in memory scores ($\rho=-.30$, $p=.02$) and with longitudinal MTL GM reduction ($F_{[2, 52]}=4.4$, $p=.04$, age corrected). Our results indicate that in normal individuals, elevated p-tau₂₃₁, a marker of neurofibrillary pathology is related to both a decrease in declarative memory and progressive atrophy of the MTL, suggesting its diagnostic potential in preclinical stage.

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Keywords

Alzheimer's disease; aging; memory performance; prediction; biomarkers; phosphorylated tau 231; cerebrospinal fluid; medial temporal lobe

Introduction

Identifying individuals at risk for future Alzheimer's disease (AD) is fundamental to developing preventative therapies. "A silent phase" of AD where tissue damage precedes the clinical expression has been noted, whereby patients with mild cognitive impairment (MCI) meet criteria for a pathological diagnosis of AD (Bennet *et al.*, 2005). Cerebrospinal fluid (CSF) AD biomarkers (total tau: t-tau, phosphorylated tau: p-tau, amyloid A β 42), reflecting key pathological processes like neurofibrillary tangles (NFT) and amyloid accumulation predict AD at the MCI stage (Blennow *et al.*, 2006). Moreover, recent evidence indicates that these biomarkers can predict conversion to MCI (Li *et al.*, 2007), AD (Fagan *et al.*, 2007; Skoog *et al.*, 2003) or other untoward cognitive outcomes (Stomrud *et al.*, 2007) already in cognitively normal elderly (Gustafson *et al.*, 2006). Furthermore, in this group, biomarkers are related to brain atrophy measures at cross-section (Fagan *et al.*, 2009). Much less is known about CSF biomarkers predicting longitudinal gray matter (GM) reductions concomitant with cognitive decline in normal subjects.

The medial temporal lobes (MTL) are an early site of brain pathology in AD (Braak and Braak, 1991). Their accelerated atrophy (Rusinek *et al.*, 2003) forecasts cognitive deterioration in normal individuals, and has been specifically linked to declarative memory decline (Golomb *et al.*, 1993; Mortimer *et al.*, 2004).

In this longitudinal study of cognitively healthy subjects, we examined the relationships between baseline CSF biomarkers, a decline in memory performance, and progressive reductions in MTL GM.

Materials and methods

Participants

Fifty seven cognitively normal individuals (mean age 62.4 $\pm$ 8.7, range 46–86 years) from the New York University School of Medicine, Alzheimer's Disease Center and the Center for Brain Health were studied. All subjects received medical, neurological, psychiatric, radiological and laboratory examinations. Subjects with significant medical conditions (e.g. uncontrolled metabolic disease), neurological abnormalities (e.g. cortical stroke, head trauma), or psychiatric disorders (e.g. depression), deemed to influence their cognitive status were excluded. The diagnosis of 'normal' was based on a semi-structured clinical interview (Reisberg *et al.*, 1983) and a Global Deterioration Scale (GDS) rating (Reisberg *et al.*, 1993), a measure of cognitive and functional capacity. GDS=1 or 2 represent normal functioning; where GDS=1 indicates no subjective memory complaint, and GDS=2 indicates awareness of memory change, in the absence of objective evidence of memory or functional problems on clinical interview. A neuropsychological test battery (see below) was administered but not used for diagnostic classification. The clinical diagnosis was made at a consensus case conference. At baseline, all subjects were normal; all underwent high resolution MRI and lumbar puncture. A second comprehensive clinical evaluation was repeated 2.1 $\pm$.46 years later.

Since our longitudinal studies are set up in a way allowing periodical examination of participants every 2 years, the mean time to follow was very similar for all the participants

and was the same both for clinical (memory assessment) and MRI examinations. They are both routinely performed during follow-up visits

Cardiovascular risk was determined with the Framingham risk equation (NIH Publication No.02-5125 and US Department of Health and Human Services, 2002), based on age, gender, systolic blood pressure, anti-hypertensive treatment, smoking, total cholesterol and HDL levels. Each subject was given a score representing the percentage risk for coronary heart disease (CHD) in the next 10 years. Because patients with preexistent CHD or diabetes have a high 10-year risk for myocardial infarction or death (>20%) (NIH Publication No. 02-5125 and US Department of Health and Human Services, 2002), in this study such individuals (n=4) were given a risk score of 25%.

All participants were research volunteers and signed an NYU Institutional Review Board approved informed consent form for the longitudinal aging study conducted at the Center for Brain Health, prior to study initiation. Lumbar puncture was always a part of a research evaluation and was never performed as a part of a diagnostic evaluation elicited by a complaint or medical condition.

Cognitive outcome measures

The delayed paragraph recall subtest (PARD) from the Guild Memory Test (Gilbert, 1970), also known as the NYU Paragraph Recall Test, was used to assess declarative memory change. A paragraph is read aloud and the participant is asked to give a verbatim recall of the text immediately following presentation and after a 10 minute delay. During the delay period the subjects perform other cognitive measures. Two paragraphs are administered, and an average score is derived. For both the baseline and the follow-up, scores were converted into a standardized score (z-score) based on a normative reference group, accounting for age, education and gender (De Santi *et al.*, 2008). PARD has been routinely administered at NYU since 1975. This measure was the single best predictor of decline to dementia in non demented individuals among both cognitive and demographic measures (Kluger *et al.*, 1999). Moreover, it is related to hippocampal atrophy (Golomb *et al.*, 1993).

Two PARD outcomes were defined: 1) a dichotomous outcome with either **memory maintained** (MEM+; PARD z-score improved) or **memory declined** (MEM-; PARD z-score decreased); 2) the PARD change score: the difference between follow-up and baseline z-scores.

Other cognitive measures were administered including immediate paragraph recall, immediate and delayed verbal paired associate recall, and immediate visual paired associate recall (Gilbert, 1970), digits forward and backward, and Digit Symbol Substitution (Wechsler Adult Intelligence Scale (Wechsler, 1981)). The last three measures were obtained on the subset of individuals (n=48, n=48, n=54, respectively).

Lumbar puncture, CSF collection and assays

Fifteen ml of clear CSF was collected into three polypropylene tubes using a fine 25G needle guided by fluoroscopy. CSF samples were kept on ice for a maximum of 1 hour until being centrifuged for 10 min at 1500g at 4°C. Samples were frozen and stored in polypropylene tubes at -80°C. All samples were blindly analyzed in batches that did not differ in age or gender distributions.

Total tau (t-tau) measurements were determined using the commercially available INNOTEST hTAU Antigen kit from Innogenetics (Blennow *et al.*, 1995). The detection limit is 60pg/ml and the coefficients of variability are 5.5% (intra-assay) and 11.6% (inter-assay).

Tau phosphorylated at threonine 231 (p-tau₂₃₁) was measured with a sandwich ELISA assay (Applied NeuroSolutions Vernon Hills, IL). In this assay, tau is captured with two backbone-directed antibodies, tau-1 and CP-27. The captured tau is then detected by CP9, which is specific for p-tau₂₃₁ (Kohnken *et al.*, 2000). The detection limits for this assay was 4pg/ml; the limit of quantification was 4–9pg/ml. The coefficients of variation ranged 6.0–10.3% (intra-assay) and 11.6–14.4% (inter-assay).

Amyloid β A β 40 and A β 42 levels were measured using ELISA with the monoclonal antibody 6E10 (specific to an epitope present on Ab-16) and rabbit antisera to A β 1–40 and A β 1–42, respectively, in a double antibody sandwich ELISA (Mehta *et al.*, 2000). The detection limit for A β 40 and A β 42 was 10pg/mL. The coefficients of variability ranged from 8–14% (intra-assay) and 10–18% (inter-assay). For final analyses, A β 42/40 ratio was used (Wiltfang *et al.*, 2007). We also analyzed ratios of t-tau/A β 42 and p-tau₂₃₁/A β 42.

MRI and image analysis

MRI scans were acquired in the coronal orientation (slice thickness: 1.6mm FOV=25cm, NEX=1, matrix=256×192, TR=35ms, TE=9ms, FA=60°), using a 1.5T GE scanner. We used MATLAB7.1 and Statistical Parametric Mapping (SPM'2, Wellcome Department of Cognitive Neurology, London, UK) procedures (Ashburner and Friston, 2000; Good *et al.*, 2002). Baseline and follow-up scans were realigned and spatially normalized to the common anatomical space of Montreal Neurological Institute template (Talairach and Tournoux, 1988). The initial normalization step was done by estimating the optimal 12-parameter affine transformation (Freeborough *et al.*, 1996). The spatially normalized images were resliced using sinc interpolation to a final voxel size of 1.5×1.5×1.5mm, and segmented into GM (gray matter), WM (white matter), and CSF images (Ashburner and Friston, 2000). GM images were re-normalized to an *a priori* GM template (Good *et al.*, 2002), by using high-dimensional normalization (optimal 12-parameter affine transformation, followed by an iterative estimate of local alignment based on a family of 7×8×7 discrete cosine functions, using the residual sum of squared differences as the matching criterion (Good *et al.*, 2002)) and smoothed with an 8-mm FWHM isotropic Gaussian kernel. To preserve the volume of a particular tissue compartment within each voxel, during the second normalization the images were modulated by the Jacobian determinants of the transformation matrix (Good *et al.*, 2002). The normalization, segmentation and smoothing procedures were carried out in an identical way for baseline and follow-up scans. SPM was used to derive a measure of global gray matter density for each subject at both time points. MarsBaR (Brett *et al.*, 2002) was used to create spherical regions of interest with a radius of 10mm, centered at the left and right pes hippocampi (Talairach–Tournoux coordinates: -27, -17, -12 and 27, -17, -12, respectively) (Fig. 1). The ROIs were applied to the baseline and follow-up GM images, to extract the GM density in these regions. The use of a predefined, larger ROI placed in the MTL region is somewhat similar to the usage of ROI in regional boundary shift method. This technique has been previously successfully developed and used by our group both to predict decline to AD (Rusinek *et al.*, 2003) and track disease progression (Rusinek *et al.*, 2004). For comparison: previously used MTL box ROI averaged 33.2 cc ± 3.1 cc (Rusinek *et al.*, 2004); in this study ROI's volume was 4.2 cc. The coefficients of variation for extracted GM estimates indicated that the values were derived from low-variance distributions. They specifically were: Left MTL baseline: 0.08; Right MTL baseline: 0.07; Left MTL follow-up: 0.08; Right MTL follow-up: 0.07.

For global and MTL GM we calculated percentage change from baseline to follow-up: ((FU-Bas)/Bas)*100. For the MTL this was done separately for each hemisphere, and the average computed.

Statistical analysis

Demographic measures were examined using t-test for continuous variables and χ^2 for categorical variables. Between-group comparisons of CSF biomarkers, GM reductions and cognitive variables were performed using t-tests. When demographic variables were significantly different between groups, these variables were used as covariates in analysis of covariance (ANCOVA) models. Correlations between CSF biomarkers, cognitive change and GM reductions were examined with Pearson coefficient (r).

Normality was checked using the Shapiro-Wilk test. When the data did not meet the assumptions of normality, the Mann Whitney U test was used to compare groups where covariates were not necessary. For the ANCOVA models dependent variables were converted to ranks. Correlations were examined with Spearman coefficient (rho).

Linear regression models were used to examine the relationship between change in cognition and CSF biomarkers accounting for the number of test exposures, since this variable was significantly correlated with cognitive change. Similarly, linear regression models were used to examine the relationship between change in cognition and GM reduction, after accounting for the number of cognitive test exposures. All analyses were performed with SPSS 16, Chicago IL, with p values declared statistically significant when <0.05 .

Results

General description of the outcome groups

Twenty out of 57 participants had reduced memory performance at follow-up and constituted the MEM- group. Two of these subjects received the diagnosis of MCI, none were demented. The remaining subjects were classified as MEM+. The MEM+ and MEM- groups did not differ in age, gender, education, time to follow-up, baseline PARD score, baseline and follow-up MMSE score, cardiovascular risk, subjective memory complaints or ApoE genotype (Table 1). The groups differed in the number of exposures to the cognitive test battery (U Mann Whitney test $Z=-2.1$, $p=.04$).

Predicting memory outcome with CSF biomarkers

1. Memory outcome groups—The MEM- group had higher baseline p-tau₂₃₁ ($Z=-2.2$, $p=.03$) and lower A β _{42/40} ($t_{155}=-2.1$, $p=.04$) than the MEM+ group (Table 1, Fig. 2). For p-tau₂₃₁/A β ₄₂ the difference was of borderline significance ($Z=-1.9$, $p=.06$).

2. PARD changes score—Only p-tau₂₃₁ correlated with the change in the PARD z-score ($\rho=-.30$, $p=.02$) (Fig. 3). In a linear regression model controlling for the number of test exposures, a higher p-tau₂₃₁ was associated with a decline in memory performance at a trend level ($\beta=-.22$, $p=.09$) (for the whole model $F_{[2, 54]}=3.8$, $p=.03$). Time to follow-up was not related to the change in PARD z-score ($r=.08$, $p=.50$), thus it was not necessary to control for it. At baseline, no correlations were found between CSF biomarkers and PARD z-score. No correlations were found between the change in the z-score of other cognitive tests and CSF biomarkers.

To further eliminate the exposure- related bias, the analyses were repeated after exclusion of subjects with more than 2 exposures to the test. In the smaller subset ($n=31$) MEM+ ($n=18$) and MEM- ($n=13$) group did not differ in age, education, gender, ApoE genotype, subjective memory complaints or race distribution, time to follow-up, baseline and follow-up MMSE scores or baseline PARD score. The biomarkers results remained very similar to those obtained from a bigger sample. The MEM- group had higher baseline p-tau₂₃₁ ($Z=-$

-2.2, $p=.03$) than the MEM+ group. The MEM- as compared to the MEM+ subjects also tended to have lower A β 42/40 ($t_{[29]}=-1.8$, $p=.08$), higher p-tau₂₃₁/A β 42 ($Z=-2.0$, $p=.05$) and higher t-tau/A β 42 ($Z=-1.8$, $p=.07$). The correlation between p-tau₂₃₁ and the change in the PARD z-score was at a trend level ($\rho=-.32$, $p=.08$).

GM reductions in the MTL and CSF biomarkers

Fifty four subjects had good quality longitudinal MRI studies. Two of the subjects with missing scans were in the MEM+ group and one was in the MEM-. For subjects with MRI data, the levels of t-tau, p-tau₂₃₁, A β 42/40, t-tau/A β 42 and p-tau₂₃₁/A β 42 were each split into high and low groups at their respective medians. Both p-tau₂₃₁ and t-tau/A β 42 were related to brain measures.

Controlling for age (the high p-tau₂₃₁ group was older), the high p-tau₂₃₁ group had significantly greater GM reduction in the left MTL ($F_{[2, 52]}=4.5$, $p=.04$) and averaged MTL ($F_{[2, 52]}=4.4$, $p=.04$) compared to the low p-tau₂₃₁ group. Differences in the right MTL were at a trend level ($F_{[2, 52]}=3.1$, $p=.08$). No effects were seen for the global GM changes (Fig. 4a, Table 2). The time to follow-up was similar for both groups ($t_{[52]}=-1.7$, $p=.10$), and was not controlled for.

The high t-tau/A β 42 group had significantly greater GM reduction in the left MTL ($t_{[52]}=2.0$, $p=.046$), right MTL ($t_{[52]}=4.7$, $p<.001$) and in the global GM ($t_{[52]}=2.4$, $p=.02$) as compared to the low t-tau/A β 42 group (Fig. 4b, Table 2). The time to follow-up was not different between groups ($t_{[52]}=.47$, $p=.60$), and was not controlled for.

T-tau/A β 42 correlated negatively with GM change in the right MTL ($\rho=-.46$, $p<.001$) and the averaged MTL ($\rho=-.33$, $p=.02$).

Memory outcome and associated longitudinal changes in MTL GM

1. Memory outcome groups—Longitudinal MTL GM reductions (but not global GM reductions) differed between MEM+ and MEM- groups (Fig. 5, Table 1). The MEM- group showed greater GM reductions in the left MTL ($t_{[52]}=-3.1$, $p=.004$), right MTL (trend: $t_{[52]}=-1.9$, $p=.06$), and averaged MTL ($t_{[52]}=-2.70$, $p=.01$) compared to the MEM+ group. No group differences were found for the baseline GM measures.

The analyses repeated after exclusion of subjects with more than 2 exposures to the test yielded similar results. The MEM- ($n=12$) as compared to the MEM+ ($n=16$) group showed greater GM reductions in the left MTL ($t_{[26]}=-2.7$, $p=.01$) and averaged MTL ($t_{[26]}=-2.0$, $p=.06$).

2. PARD change score—Reductions in left MTL GM were positively correlated with the decrease in PARD z-score ($r=.27$, $p=.048$). Trends for similar relationships were observed for the right MTL ($r=.22$, $p=.1$) and the averaged MTL ($r=.26$, $p=.06$). Neither global GM changes nor baseline GM measurements were related to PARD z-score change.

After accounting for the number of test exposures, greater MTL GM reductions continued to be associated with a decline in memory. MTL GM reductions were accounted for in one model as the average of both sides ($F_{[2, 51]}=4.8$, $p=.01$, the averaged MTL $\beta=.29$, $p=.03$), and in another model both left and right MTL changes were entered simultaneously ($F_{[2, 51]}=5.0$, $p=.01$, only left MTL was significant $\beta=.30$, $p=.03$).

Other cognitive tests—The decrease in the z-score of delayed verbal paired associate recall was related to more severe GM reduction in the right MTL ($\rho=.28$, $p=.04$).

Discussion

The diagnosis of AD at the pre-symptomatic stage is challenging due to the lower expression of pathology and the time to full clinical presentation. Little is known about how well CSF biomarkers predict cognitive performance at pre-symptomatic stages, because few reports exist (Fagan *et al.*, 2007;Stomrud *et al.*, 2007;Gustafson *et al.*, 2006;Li *et al.*, 2007;Skoog *et al.*, 2003). Far less is known about the relationships between CSF biomarkers and longitudinal atrophy measures in normal population. This study examined the value of CSF biomarkers as predictors of memory changes in normal elderly and verified their association with GM reductions in the MTL, a site vulnerable to early AD pathology (Braak and Braak, 1991). We observed that in cognitively healthy individuals, p-tau₂₃₁ and A β 42/40 discriminated groups defined longitudinally as having either reduced or maintained memory performance. An elevated baseline p-tau₂₃₁ correlated with declining memory performance. In contrast, previous reports showed t-tau/A β 42 and p-tau/A β (Fagan *et al.*, 2007;Li *et al.*, 2007) or A β 42 alone (Skoog *et al.*, 2003) were better indicators of decline to MCI or dementia. We did not find these measures superior to p-tau₂₃₁ alone or A β 42/40. Second, we have consistently found that the A β 42/40 ratio is a better predictor of decline than A β 42 alone (Brys *et al.*, 2009). We believe that the differences between cohorts or analytical methods, contributed to this discrepancy.

Conversion to AD in normal populations aged 75 or older have been estimated at a yearly rate of 1–5% (Fitzpatrick *et al.*, 2004;Boyle *et al.*, 2006;Fischer *et al.*, 2007). We believe our 0% conversion rate over 2-years is consistent with these low figures which is further explained by the fact that our group was young (mean age 62). As a surrogate outcome of clinical conversion to AD, we used a measure of declarative verbal memory (Gilbert, 1970). Reduced performance in delayed paragraph recall is a recognized early feature of AD (Welsh *et al.*, 1991;Petersen *et al.*, 1994) and known to predict decline (Kluger *et al.*, 1999;De Santi *et al.*, 2008).

Since change on PARD score was opposite for memory outcome groups, the possibility that this finding reflects a regression to the mean must be addressed. The memory outcome groups differed in longitudinal MTL GM reductions, which constituted a second independent confirmation of an ongoing pathological process. Moreover, change in PARD z-score correlated with longitudinal MTL change. Of the additional cognitive tests administered, only change in paired associate delayed recall was related to MTL GM reductions, further supporting relationships between delayed recall and MTL atrophy (Mortimer *et al.*, 2004).

During the study period, our patients were performing within acceptable ranges for normal elderly. At both time-points, global cognitive functioning, measured with MMSE, was high (29) and did not differ between the two groups. The observed decline in memory performance could not be attributed to the risk conferred by the presence of subjective memory complaints (GDS=2) or ApoE4 genotype, since their prevalence was similar in both groups.

It is well established that normal subjects improve their cognitive performance at repeated examination as learning effects occur. The lack of learning effects may in and of itself be an indicator of pathology (Zehnder *et al.*, 2007). Since the MEM+ subjects had on average one additional test exposure prior to this study, we controlled for this confound and observed similar results after the correction, that is p-tau₂₃₁ tended to predict the change in delayed memory performance ($p=.09$). Also repeated analyses within a smaller subset of subjects with only 2 exposures confirmed the associations seen in a bigger group.

The baseline MTL GM measurements did not predict memory performance change, but rather the longitudinal MTL GM decreases were associated with memory outcome. Progressive GM reductions were greater in the MEM– group. MTL atrophy is consistently found in AD and MCI patients (for review (Glodzik-Sobanska *et al.*, 2005)) and in normal individuals at risk for AD (Jessen *et al.*, 2006; Saykin *et al.*, 2006). Our current findings add to the previous report that MTL atrophy rates accurately predicted decline from normal to MCI or AD (Rusinek *et al.*, 2003).

Previously, an association was reported between baseline CSF biomarkers and temporal lobe atrophy across all diagnostic categories (NL, MCI, AD) or in AD group only but not in cognitively intact individuals (Loew *et al.*, 2009). Similarly, in another report, baseline p-tau₁₈₁ was related to the whole brain atrophy rate in AD, but not in MCI or controls (Sluimer *et al.*, 2008). We also did not observe global GM effects for p-tau. Local effects were, however, present. Global changes are small early in the course of AD, and the MTL is typically most informative. Contrary, to a recent cross-sectional study of normal subjects (Fagan *et al.*, 2009), we found baseline elevations of p-tau₂₃₁, but not A β reductions associated with longitudinal MTL GM changes. The MTL is the earliest site of neurofibrillary pathology and neuronal loss (Braak and Braak, 1991), thus a restricted relationship between a marker of NFT load and a measure of MTL GM is not surprising. The relationship between amyloid plaques and brain atrophy is still debated as A β depositions are not strong correlates of atrophy (Silbert *et al.*, 2003). Even though in our study t-tau/A β ₄₂ was not associated with memory decline, it was related to both MTL and global GM reductions. First, it substantiates the notion that t-tau reflects general neuronal damage and might be less region specific than p-tau. Second, it corroborates predictive value of t-tau/A β ₄₂ previously reported by others (Fagan *et al.*, 2007).

While assessing biomarkers-MRI relationships for consistency we used median split in all calculations. Even though for t-tau, p-tau₂₃₁ or A β ₄₂/40 diagnostic cut-off thresholds are better agreed upon, much less is known about t-tau/A β ₄₂ and p-tau/A β ₄₂. Considerable discrepancies exist between reported values (Fagan *et al.*, 2007; Vemuri *et al.*, 2009; Li *et al.*, 2007). Moreover, we are not aware of any publications where p-tau/A β ₄₂ was calculated with p-tau₂₃₁. In addition, the cutoffs are established for AD patients and the proportion of normal subjects with values exceeding these thresholds would be much smaller. Consequently our groups would have been less balanced in size, making analysis more difficult.

Our study has several limitations. First, since there is no neuropathological confirmation, it remains unknown if subjects in MEM– group really harbor AD pathology or will develop clinical disease. Second, our group was not racially diverse and with relatively high educational achievements. Thus, the generalizability is uncertain. Third, the time to follow-up was on average two years which was not long enough to observe clinically defined cognitive decline. Finally, we did not correct for multiple comparisons, nonetheless, convergent findings of p-tau – cognition, p-tau – MTL atrophy and cognition – MTL atrophy relationships speak against the spuriousness of our results.

In conclusion, these data are the first to show that in normal individuals, p-tau₂₃₁, an NFT marker, is related both to a decrease in declarative memory and progressive atrophy of the MTL, the site of the earliest brain tangle accumulation. Moreover, the interrelationships among declarative memory and MTL integrity are present. These results suggest the recognition of AD pathology in preclinical stages is possible and await follow-up study for clinical validation.

Acknowledgments

We thank Drs. Pauline McHugh M.D. and Martin Sadowski M.D. for patients' evaluation, and Ms. Schantel Williams and Ms. Rachel Mistur for their help with study coordination and cognitive testing. This study was supported by the following grants: National Institute of Health-National Institute of Aging: AG12101, AG08051, and AG022374. Dr. Zinkowski is an employee of Applied NeuroSolutions, Inc and has stock options and owns stock in Applied NeuroSolutions, Inc. Other authors have nothing to disclose in connection with this manuscript.

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Figure 1.
Positioning of the spherical ROI (radius 10 mm) on the T1 template.

Figure 2.

Scatter plots of p-tau₂₃₁ (A), t-tau (B), Aβ₄₂/40 (C), Aβ₄₂ (D), t-tau/Aβ₄₂ (E) and p-tau₂₃₁/Aβ₄₂ (F) ratios in MEM- (red circles) and MEM+ (black circles) groups. Units for p-tau₂₃₁, t-tau and Aβ₄₂ are pg/mL.

Figure 3. Correlation between baseline p-tau₂₃₁ and the continuous change in PARD z-score from baseline to follow-up. Units for p-tau₂₃₁ are pg/mL.

Figure 4.

Longitudinal (2-year) changes in the averaged MTL (upper row), and global GM density (center row) in the high and low (median split) biomarker groups. Lower row represents scatter plots of p-tau₂₃₁ and t-tau/ Aβ₄₂ in respective high and low biomarker groups. First column (A) presents high (red lines, circles) and low (black lines, squares) p-tau₂₃₁ groups; second column (B) presents high (red lines, circles) and low (black lines, squares) t-tau/ Aβ₄₂ groups. Error bars represent 95% confidence interval for the mean.

Figure 5. Baseline and follow-up GM density in two outcome groups: green lines represent MEM+ group, blue lines MEM- group. (A) averaged MTL, (B) global GM. Error bars represent 95% confidence interval for the mean.

Table 1

Patients' demographics and biomarkers' levels. MEM+: group with maintained memory performance, MEM-: group with reduced memory performance.

	MEM + (n=37)	MEM - (n=20)
AGE (years)	61.0 ± 7.5	64.9 ± 10.2
% Caucasian	92	95
% with subjective memory complaint (GDS 2)	68	60
EDUCATION (years)	17.2 ± 1.6	16.5 ± 2.4
TIME TO FOLLOW -UP (years)	2.1 ± 0.5	2.1 ± 0.4
% FEMALE	65	55
% Apo E4+ (n=52)	48	26
BASELINE PARD SCORE	8.9 ± 2.9	10.3 ± 2.9
FOLLOW-UP PARD SCORE	11.0 ± 3.2 ^a	7.8 ± 3.6 ^a
NUMBER OF EXPOSURES	3.4 ± 2.2 ^a	2.5 ± .9 ^a
BASELINE MMSE	29.5 ± .9	29.3 ± 1.0
FOLLOW-UP MMSE	29.4 ± .8	29.4 ± .7
Average CHD risk (% risk in the next 10 years)	5.9 ± 6.8	6.9 ± 7.3
Aβ42/40	.13 ± .04 ^a	.11 ± .03 ^a
Aβ42 (pg/mL)	1080.2 ± 508.0	1023.3 ± 528.5
t-tau (pg/mL)	299.7 ± 137.3	355.1 ± 158.1
p-tau ₂₃₁ (pg/mL)	7.3 ± 9.4 ^a	14.3 ± 14.3 ^a
t-tau/ Aβ42	.35 ± .39	.44 ± .34
p-tau ₂₃₁ / Aβ42	.01 ± .03 ^b	.02 ± .03 ^b
% change in the center MTL GM (n=54)	.12 ± 2.9% ^a	-2.2 ± 2.2% ^a
% change in the right MTL GM (n=54)	.28 ± 2.9% ^b	-1.26 ± 2.6% ^b
Averaged % change in the MTL GM (n=54)	.20 ± 2.7% ^a	-1.72 ± 2.2% ^a
% change in global GM (n=54)	-.67 ± 2.4%	-1.52 ± 1.7%

^aSignificant differences are marked at p < .05

^bp = .06

The number of participants with available data is indicated by 'n'.

CHD risk: risk for coronary heart disease, MMSE: Mini Mental State Examination, PARD: Guild Delayed Paragraph recall test, MTL: medial temporal lobe, GM: gray matter

Table 2

Percentage change in MTL and global GM in the high and low p-tau₂₃₁ groups (split at the median), and high and low t-tau/Aβ42 groups (split at the median). P-tau₂₃₁ high group is compared with p-tau₂₃₁ low group; t-tau/Aβ42 high group is compared with t-tau/Aβ42 low group.

	p-tau ₂₃₁ groups		t-tau/Aβ42 groups	
	low	high	low	high
% change in the center MTL GM	.16±2.7% ^a	-1.54±2.8% ^a	.08±2.6% ^a	-1.46±3.0% ^a
% change in the right MTL GM	.46±2.8% ^b	-.97±2.8% ^b	1.31±2.2% ^a	-1.82±2.7% ^a
Averaged % change in the MTL GM	.31±2.5% ^a	-1.26±2.7% ^a	.70±2.2% ^a	-1.65±2.7% ^a
% change in global GM	-.74±2.2%	-1.20±2.3%	-.27±1.5% ^a	-1.67±2.6% ^a

^a Significant differences between high and low groups are marked at p<.05

^b p=.08.