

# Plasma Tau as a Window to the Brain—Negative Associations With Brain Volume and Memory Function in Mild Cognitive Impairment and Early Alzheimer’s Disease

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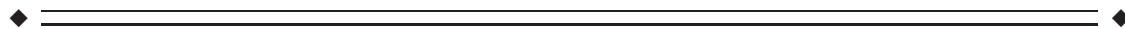
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**Abstract:** Neurofibrillary tangles are associated with cognitive dysfunction, and hippocampal atrophy with increased CSF tau markers. However, the plasma tau levels of Alzheimer’s disease (AD) have not been well studied. We investigated plasma tau by using an immunomagnetic reduction assay in 20 patients with mild cognitive impairment (MCI) due to AD, 10 early AD dementia, and 30 healthy elders (HE). All received a 3D-brain MRI scan and a set of cognitive function test. We explored their relationships with both brain structure and cognitive functions. Images were analyzed to determine the brain volumes and gray matter densities. Patients with MCI or early AD had significantly increased plasma tau levels compared with HE. Plasma tau levels were negatively associated with the performance of logical memory, visual reproduction, and verbal fluency; also negatively associated with volume of total gray matter, hippocampus, amygdala; and gray matter densities of various regions.

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Regression analyses indicated that logical memory explained 0.394 and hippocampus volume predicted .608 of the variance of plasma tau levels, both  $P < 0.001$ . Education years were negatively associated with the gray matter densities of the supramarginal ( $r = -0.407$ ), middle temporal gyrus ( $r = -0.40$ ) and precuneus ( $r = -0.377$ ; all  $P < 0.05$ ) in HE; and negatively associated with plasma tau levels in patients ( $r = -0.626$ ). We propose that plasma tau may serve as a window to both structure and function of the brain. Higher education is a protective factor against AD and is associated with lower plasma tau levels in patients. *Hum Brain Mapp* 35:3132–3142, 2014. © 2013 Wiley Periodicals, Inc.

**Key words:** Alzheimer's disease; plasma total tau; MRI volumetry; voxel-based morphometry; hippocampus; immunomagnetic reduction; biomarker

## INTRODUCTION

Recent studies have provided new insights into the pathological processes associated with Alzheimer's disease that precede the onset of clinical dementia by many years (Perrin et al., 2009; Sperling et al., 2011). Although beta amyloid (A $\beta$ ) proteins are believed to play a central role in the pathogenesis of Alzheimer's disease [Ingelsson et al., 2004], their significance as a primary causative factor remains a subject of debate [Nelson et al., 2009]. The weak correlation between the accumulation of fibrillary A $\beta$  in the brain and cognitive function that has been observed is a good example [Guannalopoulos et al., 2003]. However, the number of neurofibrillary tangles is significantly correlated with both cognitive function and clinical staging [Bennet et al., 2004; Guannalopoulos et al., 2003]. Published reports have also correlated hippocampal atrophy, a characteristic symptom of Alzheimer's disease, with tau markers in the cerebrospinal fluid (CSF) [Desikan et al., 2011; de Souza et al., 2012]. Recent studies have reached a general consensus that decreased levels of A $\beta$ 42 and increased total levels of tau in the CSF are potential biomarkers for AD [Vemuri et al., 2010]. Low levels of A $\beta$ 42 in the CSF reflect the deposition and entrapment of A $\beta$  in amyloid plaques, whereas elevated levels of tau reflect abnormal tau accumulation in neurofibrillary tangles and neural threads. These findings are both indicative of a process of active axonal and neuronal destruction [Tapiola et al., 2009].

Biofunctionalized magnetic nanoparticles are useful tools for detecting biological molecules [Kötitz et al., 1999]. In particular, these nanoparticles may be used to label various types of target biological molecules; by measuring the magnetic signals from these nanoparticles, the concentrations of the target molecules can then be determined through the use of a magnetic field. This technique is referred to as a magnetically labeled immunoassay. Magnetically labeled immunoassays are able to quantitatively detect proteins, viruses, carcinogens, chemicals, and nucleic acids.

In our previous work, we developed an approach that is capable of quantifying the plasma levels of A $\beta$ 42 and A $\beta$ 40 [Chiu et al., 2012]. In these studies, we used an immunomagnetic reduction (IMR) method to investigate the association between A $\beta$  and magnetic nanoparticles [Chiu et al., 2011, 2012; Yang et al., 2011].

The hypothesis that the total tau levels in the CSF may reflect axonal damage and neuronal degeneration is supported by previously published results. However, the plasma tau levels of patients with AD have not been thoroughly characterized. Thus, in this study, we sought to use the aforementioned IMR method to measure the plasma levels of tau in control subjects, subjects with mild cognitive impairment (MCI) and subjects with AD. We further explored the relationships between the plasma levels of tau and both the anatomical brain structures and the cognitive functions of the study participants.

## METHODS

### Participants

We recruited 20 subjects with MCI (mean age  $71.2 \pm 9.7$ ; 11 women, 9 men; mean MMSE =  $26.3 \pm 2.7$ ), 10 subjects with early AD (mean age  $69.3 \pm 9.4$  years; 6 women, 4 men; mean MMSE =  $22.7 \pm 3.6$ ), and 30 control subjects (mean age  $64.4 \pm 9.5$ ; 17 women, 13 men; mean MMSE =  $28.8 \pm 1.6$ ; Table I). There were no significant group differences in the ages ( $F = 3.038$ ,  $P = 0.056$ ), gender (Pearson *chi-square* = 0.068,  $P = 0.967$ ) or APOE  $\epsilon 4$  distributions (Pearson *chi-square* = 2.125,  $P = 0.346$ ). The subjects with AD were recruited from the memory clinic at the National Taiwan University Hospital. After undergoing routine tests at the memory clinic, each participant received a comprehensive clinical checkup that included a review of his or her medical history, physical, and neurological examinations, laboratory tests, and neuroimaging studies. All of the patients with dementia met diagnostic guidelines for probable AD dementia proposed by the National Institute on Aging-Alzheimer's Association (NIA-AA) workgroups in 2011 [McKhann et al., 2011]. The diagnosis of MCI due to AD also followed the recommendations from the NIA-AA on diagnostic guidelines [Albert et al., 2011]. For the diagnosis of MCI due to AD we used formal cognitive test with the cut-off value at or below the 4<sup>th</sup> percentile (lower than 1.5 SD) of scale score of the age and education matched control. To increase the certainty level of probable AD dementia we included evidence of the AD pathophysiological process with amyloid deposition by

**TABLE I. Demographic data, cognitive functions, plasma tau levels of the subjects**

Group	Control (30)	MCI (20)	Early AD (10)
Age (years)	64.4 ± 9.5	71.2 ± 9.7	69.3 ± 9.4
Gender female/male	17/13	11/9	6/4
APOEε4	8 (1ε4/4)	8 (1ε4/4)	5 (3ε4/4)
Education (years)	13.1 ± 3.0	12.0 ± 3.1	7.6 ± 3.2*†
CDR	0	.5	.5/one with CDR1
MMSE	28.8 ± 1.6	26.3 ± 2.7*	22.7 ± 3.0*†
Plasma tau level (pg/ml)	15.6 ± 6.9	32.7 ± 5.8*	53.9 ± 11.7*†

Mean ± SD; \*: significant group difference between controls and MCI or early AD by using ANOVA at a significance level of  $P < 0.01$ ; †: significant group difference between MCI and Early AD at a significance level of  $P < 0.01$ ; no significant between-group difference in age, gender and APOEε4 distribution.

PIB-PET, temporoparietal hypometabolism by FDG-PET or decreased hippocampal volume (either by volumetry of lower than 95% of the healthy elderly controls or by Visual Rating System) [Scheltens et al., 1995]. Image biomarkers used in probable AD dementia were also incorporated in the research criteria for MCI due to AD group. The control subjects were selected from a group of healthy volunteers in an MCI project [Chen et al., 2009]. In this project, the volunteers were given a medical checklist to identify any major systemic diseases, operations, and/or hospitalizations. Volunteers who reported experiencing certain uncontrolled medical conditions including heart failure, recent myocardial infarction (within the past 6 months), malignancy (during the past 2 years), or poorly controlled diabetes (Hb A1C > 8.5) were excluded from the present study. The volunteers also received physical and neurological examinations, and they were scored on a short-form Geriatric Depression Scale (GDS-S). Volunteers, who had GDS-S scores greater than 9, were also excluded from participation. Control subjects possessed normal cognitive function, as confirmed by a battery of neuropsychological tests. In addition to the MMSE, CDR and GDS that were used as screening tools to include subjects, all of the study participants including patients with probable AD dementia, MCI due to AD and healthy elders experienced the same neuropsychological test battery, which included the Wisconsin Card Sorting Test (WCST), two Trail Making Tests (A and B), two subtests of the WMS-III [logical memory and visual reproduction; Hua et al., 2005], selected subtests of the WAIS-III (digit span, matrix reasoning, block design, and digit symbol substitution), a test of verbal fluency; and a 3D T1-weighted brain MRI scan which was obtained within one month of both the neuropsychological examination and the acquisition of a blood sample. At last, fluid-attenuated inversion recovery (FLAIR) images were used to identify lacunar infarcts and diffusely confluent white matter hyperintensities (WMH). We excluded those subjects with the image findings of subcortical ischemic vascular dementia such as Fazekas WMH scale greater than 2 or multiple deep gray matter lacunes [Chen et al., 2009; Fazekas et al., 1987; Román et al., 2002].

All of the study subjects or their primary caregivers provided informed consent prior to participating in this investigation. The study was approved by the ethics committee and institute review board of the university hospital.

### Immunomagnetic Reduction Technique and Assessment of Plasma Tau

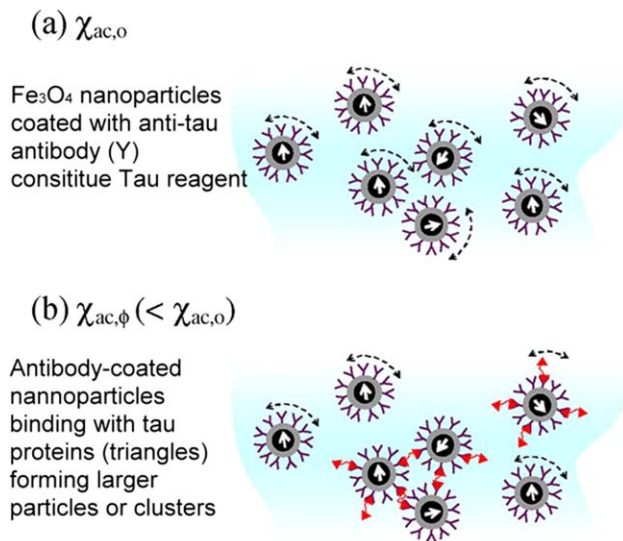
#### Immunomagnetic reduction technique

Immunomagnetic reduction (IMR) is a method to assay target molecules via measuring the reduction in the mixed frequency magnetic susceptibility of magnetic reagent due to binding of the target proteins to magnetic nanoparticles, in this study the tau protein (Fig. 1a,b). Under external multiple alternating current (ac) magnetic fields, magnetic nanoparticles oscillate with the multiple ac magnetic fields via magnetic interaction. Thus, the reagent under external multiple ac magnetic fields shows a magnetic property, called mixed-frequency ac magnetosusceptibility  $\chi_{ac}$ , (Fig. 1a). Through the anti-tau antibody on the outer shell, magnetic nanoparticles bind with and magnetically label tau proteins. With the binding, magnetic nanoparticles become larger or clustered (Fig. 1b). The oscillation response of these larger magnetic nanoparticles to external multiple ac magnetic fields becomes much less than that of the originally unbound individual magnetic nanoparticles. Thus, the  $\chi_{ac}$  of the magnetic reagent is reduced due to the association between magnetic nanoparticles and tau proteins. The reduction percentage in  $\chi_{ac}$  of the magnetic reagent is referred to as IMR signals (Eq. 1). If the amount of tau proteins is increased, more magnetic nanoparticles become larger or clustered. The reduction in  $\chi_{ac}$  of the reagent is then increased.

$$\text{IMR (\%)} = (\chi_{ac,0} - \chi_{ac,\phi}) / \chi_{ac,0} \times 100\% \quad (1)$$

#### Preparation of magnetic-nanoparticle tau reagent

The magnetic nanoparticles used in this study were composed of Fe<sub>3</sub>O<sub>4</sub> that was coated with dextran and dispersed in a PBS solution at a pH of 7.4 (MF-DEX-0060, MagQu). Antibodies against the tau protein (anti-tau)



**Figure 1.**

(a) The reagent under external multiple ac magnetic fields shows a magnetic property, called mixed-frequency ac magnetosusceptibility  $\chi_{ac,o}$ . (b) With the binding, magnetic nanoparticles become larger or clustered. The oscillation response of these larger magnetic nanoparticles to external multiple ac magnetic fields becomes much less than that of the originally unbound individual magnetic nanoparticles. Thus, the magnetic reagent is reduced due to the association between magnetic nanoparticles and tau proteins  $\chi_{ac,\phi}$ . [Color figure can be viewed in the online issue, which is available at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).]

(Tau-441, Sigma) were covalently bound to the dextran coatings of the magnetic nanoparticles. A magnetic separation technique was used to separate the unbound antibodies from the magnetic solution. The magnetic solution that contained magnetic nanoparticles that had been biofunctionalized with anti-tau is referred to as the tau reagent (MF-TAU-0060, MagQu).

### Specimen collection and preparation

Participants were asked to provide a 10-ml non-fasting venous blood sample (K3 EDTA, lavender-top tube). Each sample was assigned a registry number following the sampling sequence; hence, colleagues in the laboratory were blind to the clinical status and the demographic data of the subjects. In consideration of the possible circadian changes and food effects, all blood samples were collected between 10 AM and 2 PM and were postprandial. The blood samples were centrifuged (2500g for 15 min) within 1 h of collection, and plasma was aliquoted into cryotubes and stored at  $-80^{\circ}\text{C}$  for less than three months until thawed for measurement. Cellular part was further used to extract DNA from WBC for gene analyses. Genotyping of the APOE epsilon alleles ( $\epsilon 2$ ,  $\epsilon 3$ , and  $\epsilon 4$ ) was conducted using sequence-specific primer (SSR)-PCR methodologies.

### Measurement of plasma tau through IMR signal change

The percent reduction in the  $\chi_{ac}$  of the tau reagent was measured using an ac magnetosusceptometer (XacPro-S, MagQu) that was equipped with a high- $T_c$  superconducting quantum interference device (SQUID) magnetometer, which functioned as a magnetic sensor. The degree of binding between plasma tau and biofunctionalized magnetic nanoparticles was assessed by mixing 60  $\mu\text{l}$  of tau reagent with 60  $\mu\text{l}$  of a solution containing a plasma sample and subsequently recording the time-dependent  $\chi_{ac}$  signal of the mixture with the SQUID-based ac magnetosusceptometer. The  $\chi_{ac}$  signal of the mixture decreases upon the binding of the nanoparticles to the tau proteins in the mixture; thus, the percent reduction in the  $\chi_{ac}$  signal for the sample can be determined from the starting and ending  $\chi_{ac}$  values. The percent reduction in the  $\chi_{ac}$  signal is referred to as the IMR signal. Once the tau concentration-dependent IMR signal has been obtained, the level of the plasma tau can be determined using a logistic function (for technique details, please refer to our previously published work) [Chiu et al., 2011, 2012; Yang et al., 2011, Yang, 2013].

### Test of the coefficient variance of the IMR signals

We used three concentration levels of tau protein to test the coefficient variance (CV) of our IMR method, i.e., 0.1 pg/ml, 10 pg/ml, and 1000 pg/ml. For each concentration, the IMR signals were measured in triplicate. Both the intra-assay and inter-assay CV are less than 5%.

### The acquisition and analysis of magnetic resonance images.

#### Image acquisition and preprocessing

High-resolution structural brain MRI scans were acquired using a 1.5 T MRI scanner (EXCITE, General Electric, Milwaukee, USA). A whole-brain T1-weighted 3D spoiled gradient recovery (SPGR) sequence was used (TE = 9.3 ms, TR = 3.9 ms, TI = 600 ms, flip angle =  $12^{\circ}$ , matrix size =  $192 \times 192$ , FOV = 25 cm), and a total of 170 contiguous sagittal slices that were 1.3 mm in thickness were acquired. The analyzed datasets include the T1-weighted structural MRI scans from all available subjects ( $n = 60$ ). The MRI images were corrected for intensity inhomogeneities and reoriented; then registered against MNI 152 space using FSL (FMRIB Software Library) tools [Smith et al., 2004].

#### Segmentation and volumetry

The converted T1-weighted images were then processed; in particular, the FIRST and SIENAX FSL tools were used to extract the relevant regions of interest and calculate the total brain volume [Patenaude et al., 2011]. The FIRST tool



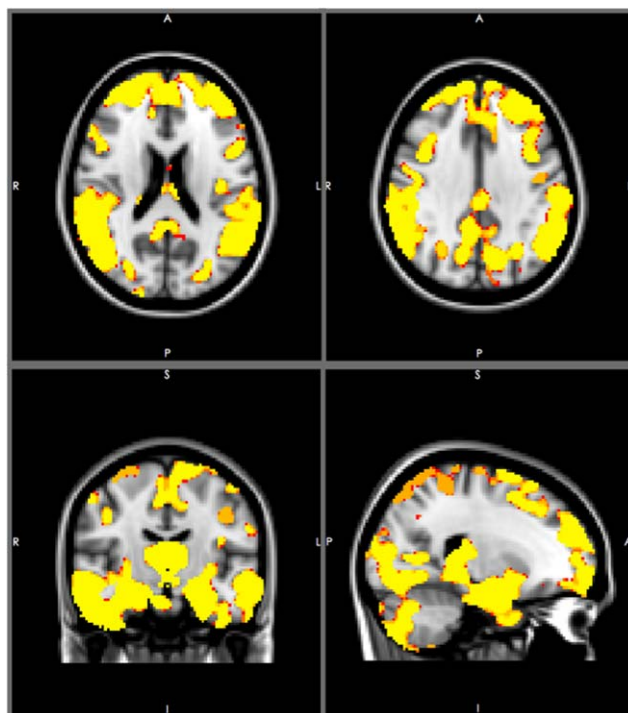
is a model-based segmentation/registration tool. The shape/appearance models used in FIRST are constructed from manually segmented images that are provided by the Center for Morphometric Analysis (CMA) at MGH, Boston. The shape and appearance models are based on multivariate Gaussian assumptions that allow the shape parameter can be expressed as a mean with certain modes of variation (principal components). FIRST then searches through several linear combinations of the modes of shape variation to determine the most probable shape given the observed intensities in the T1 images (Smith et al., 2004). We used this method to obtain the regional brain volumes of both the right and left hippocampi and the amygdala. Brain tissue volumes were normalized to the head sizes of the subjects and were estimated using the SIENAX [Smith et al., 2002], tool of the FSL. Tissue-type segmentation with partial volume estimation was then performed to calculate the total volume of brain tissue for each study subject, including separate estimates for the gray matter, white matter, peripheral gray matter and ventricular CSF volumes [Jenkinson et al., 2001].

### Voxel-based morphometry

The structural data were further analyzed using the FSL-VBM tool for a voxel-based morphometry (VBM) analysis. In its simplest form, VBM involves a voxel-wise comparison of the local gray matter concentrations of two groups of subjects [Good et al., 2001; Smith et al., 2004]. Tissue-type segmentation was conducted using the FAST4 methodology [Zhang et al., 2001]. The resulting gray-matter partial volume images were then aligned using the FLIRT affine registration tool [Jenkinson et al., 2001]; this alignment was followed by nonlinear registration using FNIRT [Andersson et al., 2007]. The modulated segmented images were then smoothed through the use of an isotropic Gaussian kernel with a sigma of 3 mm. The gray matter density images were then utilized for additional regional comparisons between the groups that were examined in this study (Fig. 2).

### Statistical Analysis

Pearson chi-square tests were used to examine the between group difference of the gender and the APOE $\epsilon$ 4 distributions. The significance levels of differences in ages, years of education, MMSE scores and plasma tau levels between groups was assessed through analyses of variance (ANOVAs). Pearson correlations were used to examine the pairwise associations between ages, years of education, plasma tau levels, global or regional brain volumes and neuropsychological test scores of the healthy elder group to identify possible confounding effect of age and years of education. A multivariate analysis of covariance (MANCOVA) with a Bonferroni correction was used to examine the between-group differences in global and regional brain volume, gray matter density and cognitive test scores; in



**Figure 2.**

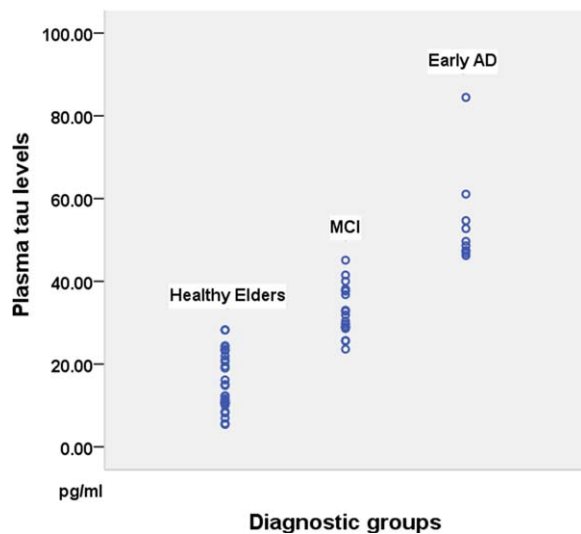
A comparison of voxel-based morphometry (VBM) between 30 healthy elders and patients (20 MCI and 10 patients). The yellow color indicates a significance level of  $p < 0.001$ . [Color figure can be viewed in the online issue, which is available at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).]

this analysis, age and years of education were considered to be covariates. Partial correlation using the same covariates was performed to identify factors to be included in the regression analysis. Finally, linear regression analyses (stepwise) were performed to evaluate the proportion of variance that was explained by each factor.

## RESULTS

### Demographic Data and Plasma Tau Levels

There were significant between-group differences in MMSE scores ( $F = 27.58$ ,  $P < 0.001$ ), years of education ( $F = 11.975$ ,  $P < 0.001$ ) and plasma tau levels ( $F = 103.07$ ,  $P < 0.001$ , Fig. 3). The AD group had the lowest average number of years of education ( $7.6 \pm 3.2$ ) and the lowest mean MMSE score ( $22.7 \pm 3.1$ ); the patients in the AD group also had the highest levels of plasma tau ( $53.9 \pm 11.7$  pg/ml; Table I). Pearson correlations demonstrated significant negative associations between age and various aspects of brain structure in the healthy elder group, including the total gray matter volume ( $r = -0.430$ ,  $P < 0.05$ ), the total hippocampal volume ( $r = -0.591$ ,  $P < 0.01$ ) and the gray matter densities of various regions such as ( $r$



**Figure 3.**

The plasma tau levels of different groups of subjects. The mean ( $\pm$ standard deviation) tau concentrations were  $15.6 \pm 6.9$  pg/mL for the healthy elder group,  $33.2 \pm 5.4$  pg/mL for the MCI group and  $53.9 \pm 11.7$  pg/mL for the early AD group ( $F = 57.7$ ,  $p < 0.01$ ). Post hoc analyses identified significant differences between the control and early AD groups and between the MCI and early AD groups (both  $p < 0.01$ ). [Color figure can be viewed in the online issue, which is available at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).]

$= -0.0707$  for superior temporal gyrus;  $r = -0.704$  for middle temporal gyrus, both  $P < 0.01$ ). Similarly, for the healthy elder group, the number of years of education was also significantly correlated with the performance on various cognitive tests, such as the delayed recall of logical memory ( $r = 0.405$ ,  $P < 0.05$ ) and Trail Making Tests A and B ( $r = -0.586$  for A,  $r = -0.529$  for B, both  $P < 0.01$ ) and so on. Because of these correlations, we controlled for the effects of both ages and years of education in all between-group comparisons of both brain structures and cognitive test scores.

### Between-Group Comparison of Cognitive Functions and Brain Structures

#### Cognitive functions

MANCOVA tests that controlled for the effects of age and education indicated significant between-group differences in all of the aspects of cognitive functioning that we assessed (all  $P < 0.001$ ) with the exception of number of perseverative errors of WCST ( $P < 0.05$ ) and digit span ( $p > .05$ ). However, post-hoc analyses revealed that only certain cognitive score were statistically different; in particular, these significant results included differences with respect to the delayed recall of both logical memory and visual reproduction; and semantic verbal fluency

(Table II). The statistically significant differences in cognitive test scores were typically between the healthy elder group and either AD or MCI patients; significant differences between AD and MCI patients were otherwise scanty.

#### Brain Structures

The analyses of brain structures also revealed significant between-group differences in the total volume of gray matter ( $P < 0.01$ ) and the volumes of the hippocampus and the amygdala ( $P < 0.001$ ). In terms of gray matter densities, almost all of the regions of interest had significant between-group differences (all  $P < 0.01$ ) except VBM of hippocampus ( $P > 0.05$ ).

#### Genetic factor

Carriers of APOE $\epsilon$ 4 had higher plasma tau levels than non-carriers of APOE $\epsilon$ 4, but the differences between the plasma tau levels of carriers and noncarriers did not reach statistical significance in the patient group (AD and MCI,  $43.1 \pm 16.6$  vs.  $37.3 \pm 9.1$  pg/mL;  $P > 0.1$ ) or the healthy elder group ( $16.4 \pm 5.9$  vs.  $15.3 \pm 7.3$  pg/mL;  $P > 0.1$ ) but a marginal difference ( $32.9 \pm 18.8$  vs.  $24.9 \pm 13.6$  pg/mL;  $P = 0.062$ ) for all subjects.

#### Regression analysis

Partial correlation analysis controlling age and education between plasma tau levels and brain volumes revealed significant negative associations between plasma tau levels and total gray matter ( $r = -0.393$ ,  $P < 0.01$ ), total hippocampus ( $r = -0.570$ ,  $P < 0.01$ ) and total amygdala ( $r = -0.282$ ,  $P < 0.05$ ). Partial correlation analysis controlling age and education between plasma tau levels and gray matter densities (VBM) revealed significant negative associations between plasma tau levels and superior, middle and inferior frontal gyrus ( $r = -0.432$ ,  $-0.411$ ,  $-0.351$  respectively, all  $P < 0.01$ ), superior and middle temporal gyrus ( $r = -0.377$ ,  $-0.445$  respectively, both  $P < 0.01$ ), supramarginal gyrus ( $r = -0.401$ ,  $P < 0.01$ ), thalamus ( $r = -0.397$ ,  $P < 0.01$ ) and precuneus ( $r = -0.314$ ,  $P < 0.05$ ). Partial correlation analysis controlling age and education between plasma tau levels and cognitive functions revealed significant negative associations between plasma tau levels and delayed recall of logical memory ( $r = -0.679$ ,  $P < 0.01$ ), delayed recall of visual reproduction ( $r = -0.576$ ,  $P < 0.01$ ) and verbal fluency ( $r = -0.588$ ,  $P < 0.01$ ).

The aforementioned independent variables obtained from the partial correlation analyses were then selected for linear regression analysis.

Stepwise linear regression analysis on brain structures demonstrated that the value of  $R^2$  change of the total hippocampal volume was 0.394 ( $P < 0.001$ ), followed by education 0.154 ( $P < 0.05$ ) and superior frontal gyrus 0.045 ( $P < 0.05$ ; Table III). Stepwise linear regression analysis on cognitive functions demonstrated that the value of  $R^2$

**TABLE II. Cognitive functions, brain volume, and gray matter density of the subjects**

Group	Control (30)	MCI (20)	Early AD (10)
WMS-III			
DLM	78.7 ± 20.6	24.0 ± 22.1**	2.6 ± 3.5**
DVR	61.70 ± 22.6	30.6 ± 24.7**	6.8 ± 5.5**
WCST			
Categories completed	5.1 ± 1.9	3.4 ± 2.2	1.6 ± 1.3**†
Perseverative errors	3.7 ± 4.8	9.3 ± 10.5	10.0 ± 4.6
Trail Making-A	46.1 ± 16.0	60.1 ± 32.1	86.0 ± 54.4
Trail Making-B	95.5 ± 41.3	201.3 ± 195.2	241.7 ± 88.0
WAIS-III			
Digit Span F:6 B:4	12.3 ± 2.4	12.3 ± 3.2	11.2 ± 2.8
Matrix Reasoning	11.4 ± 3.1	10.3 ± 2.4	7.8 ± 3.2
Block Design	11.1 ± 2.4	10.4 ± 2.2	7.8 ± 2.9
DSS	12.6 ± 2.7	10.6 ± 2.9	8.9 ± 3.1
Semantic verbal fluency	41.3 ± 7.5	27.2 ± 1.6**	21.1 ± 6.7**†
Volumetry in cm <sup>3</sup>			
Gray matter	753.56 ± 43.3	702.4 ± 47.1*	699.49 ± 64.3*
Hippocampus	7.66 ± .69	5.78 ± .65**	6.05 ± .67**
Amygdala	2.69 ± .29	2.13 ± .38**	2.47 ± .23
Voxel-based morphometry			
Superior frontal gyrus	.5398 ± .0158	.5405 ± .0159*	.4968 ± .0258**
Middle frontal gyrus	.5390 ± .0167	.5127 ± .0407*	.4894 ± .0291**†
Inferior frontal gyrus	.5595 ± .0166	.5325 ± .0439*	.5258 ± .0222**
Sup temporal gyrus	.5313 ± .0199	.4953 ± .0413**	.4914 ± .0339**
Middle temporal gyrus	.5888 ± .0260	.5379 ± .0444**	.5352 ± .0422**
Hippocampus	.6531 ± .0341	.6182 ± .0480	.6242 ± .0395
Precuneus	.5160 ± .0277	.5013 ± .0297	.4691 ± .0352**††
Supramarginal gyrus	.5045 ± .0379	.4644 ± .0441*	.4367 ± .0489**

Mean ± SD; WMS-III, Wechsler Memory Scale-3rd edition; DLM, delayed recall of logistic memory scaled score; DVR, delayed recall of visual reproduction scaled score; WCST, Wisconsin Card Sorting Test; WAIS-III, Wechsler Adult Intelligence Scale-3rd edition; DSS: Digit Symbol-Substitution; \*: significant group difference between controls and MCI or AD by using MANCOVA controlling age and years of education effects at a significance level of  $P < 0.05$ ; \*\*:  $P < 0.01$ ; †: significant group difference between MCI and Early AD at a significance level of  $P < 0.05$ ; ††:  $P < 0.01$ .

change of the delayed recall of logical memory 0.608 ( $P < 0.001$ ), followed by education 0.031 ( $P < 0.05$ ) and verbal fluency 0.026 ( $P < 0.05$ ; Table IV).

Education predicts 0.154 of the variance of plasma tau levels in the association with brain structures. Partial correlations of education year association with brain

**TABLE III. Regression coefficients of brain structures association with plasma tau**

Model	B [95% CI]	$\beta$	$P$	$R^2$
Model I				
Hippocampal volume	-0.009 [-0.012~-0.006]	-0.628	.000	.394
Model II				
Hippocampal volume	-0.007 [-0.01~-0.005]	-0.519	.000	.548
Education	-1.806 [-2.626~-0.985]	-0.407	.000	
Model III				
Hippocampal volume	-0.006 [-0.009~-0.003]	-0.424	.000	.593
Education	-1.727 [-2.516~-0.939]	-0.389	.000	
Superior frontal VBM	-119.908 [-216.632~-23.183]	-0.235	.016	

VBM: voxel-based morphometry; CI: confidence interval; excluded variables in Model III (ExV-Model III): age, gender, total gray matter volume, total amygdala volume, middle and inferior frontal gyrus, superior and middle temporal gyrus, supramarginal gyrus and precuneus. In Model I, all the ExV-Model III and hippocampal volume were entered into stepwise regression analysis; in Model II, all the ExV-Model III, hippocampal volume and education were entered; in Model III, all the EV-Model III, hippocampal volume, education and superior frontal VBM were entered.

**TABLE IV. Regression coefficients of cognitive functions associations with plasma tau**

Model	B [95% CI]	$\beta$	<i>P</i>	<i>R</i> <sup>2</sup>
Model I				
Logical memory	-0.336 [-0.407~-0.265]	-0.78	0.000	0.608
Model II				
Logical memory	-0.292 [-0.371~-0.212]	-0.677	0.000	0.639
Education	-0.900 [-1.719~-0.080]	-0.203	0.032	
Model III				
Logical memory	-0.217 [-0.322~0.111]	-0.503	0.000	0.665
Education	-0.936 [-1.734~-0.139]	-0.211	0.022	
Verbal fluency	-0.342 [-0.670~-0.013]	-0.235	0.042	

CI, confidence interval; excluded variables in Model III (ExV-Model III): age, gender, visual reproduction. In Model I, all the ExV-Model III and logical memory were entered into stepwise regression analysis; in Model II, all the ExV-Model III, logical memory and education were entered; in Model III, all EV-Model III, logical memory, education and verbal fluency were entered.

structures or plasma tau levels controlling age in both control and patient groups were further examined. The results showed that education was negatively associated with VBM of the middle temporal gyrus ( $r = -0.440$ ), precuneus ( $r = -0.377$ ) and supramarginal gyrus ( $r = -0.407$ ; all  $P < 0.05$ ) in the healthy elder group while in the patient group education was negatively associated with the plasma tau levels ( $r = -0.626$ ,  $P < 0.01$ ) both controlling the effect of age.

## DISCUSSION

The hypothesis that total tau levels in the CSF may reflect axonal damage and neuronal degeneration is supported by previous studies, which have shown marked increases in the total tau levels in the CSF of patients with certain neurological conditions, such as Creutzfeldt-Jakob disease, acute stroke, and traumatic brain injury [Hesse et al., 2000; Kapaki et al., 2001; Zemlan et al., 1999]. Moreover, the results of approximately 50 studies that included a total of more than 5000 subjects have demonstrated that compared with controls, patients with AD have a threefold increase in CSF tau levels [Hampel et al., 2008]. As discussed above, the relationship between the tau level in the CSF and hippocampal atrophy has recently been studied [Desikan et al., 2011; de Souza et al., 2012; Hampel et al., 2005]. CSF tau levels may be regarded as an injury marker that may predict a rapid progression from MCI to AD-associated dementia [van Rossum et al., 2012].

By contrast, the plasma tau levels in neurological diseases have not been well studied. One early study of plasma tau levels in small groups of patients with dementia produced discouraging results; in particular, the researchers reported a lack of any obvious increase in plasma tau levels in dementia patients [Ingelson et al., 1999]. Although the results of this previous study may have been limited by the relatively low sensitivity of the ELISA technology that it used, a more recent study that employed an ELISA technique with greater sensitivity to detect tau levels reported reduced plasma tau levels in

patients with AD [Sparks et al., 2012]. Other studies that have used these more sensitive ELISA approaches have reported that plasma tau levels were increased in the late phase of comatose patients who had experienced cardiac arrest [Mörtberg et al., 2011] and in patients with Creutzfeldt-Jakob disease, acute stroke, and traumatic brain injury [Bielewicz et al., 2011; Liliang et al., 2010; Noguchi-Shinohara et al., 2011].

In this study, we found significantly increased plasma tau levels in subjects with MCI or early AD compared with the plasma tau levels of controls. A threefold increase in plasma tau levels in subjects with early AD is observed in this study and is similar to its CSF counterpart [Hampel et al., 2008]. This finding was supported by our experiment in this study that the CSF tau levels correlate well with the plasma tau levels (Spearman  $\rho = 0.682$ ,  $P < 0.01$ ). A study of experimental brain injury in rats found that serum tau levels increased by more than fourfold in the hour following the insult [Liliang et al., 2010], indicating that the tau protein from the brain is readily excreted into the peripheral blood shortly after it is produced. Therefore, the elevated CSF tau levels that are observed in subjects with AD should result in elevated plasma tau levels that are similar to the levels that have been reported in the other aforementioned clinical studies [Bielewicz et al., 2011; Liliang et al., 2010; Mörtberg et al., 2011; Noguchi-Shinohara et al., 2011]. Plasma tau can therefore be considered to be a window that reveals brain structure in terms of both total brain volume and the gray matter density of the hippocampus.

A regression analysis indicates that plasma tau levels were negatively associated with total hippocampus volume (which predicted 0.394 of the variance in the plasma tau level) and the gray matter density of the superior frontal (predicting 0.054 of the variance in the plasma tau level). In summary, the total hippocampus volume and the gray matter density of the superior frontal gyrus were the most valuable imaging factors for explaining the observed variance in the plasma tau levels of the study participants.

Furthermore, our regression analysis also identified significant negative associations between plasma tau levels



and cognitive functions that scores of delayed recall of logical memory (predicting 0.608 of the variance in the plasma tau levels;  $P < 0.01$ ) and verbal fluency contribute additional 0.026 ( $P < 0.05$ ) of the variance in the plasma tau levels. This finding implies that plasma tau levels can also be regarded as a window that provides insights regarding the brain functions that are represented by verbal memory and executive function.

Education has long been known to be a protective factor against AD [Karp et al., 2004; Sattler et al., 2012]. The primary explanation of this phenomenon is the cognitive reserve hypothesis, which proposes that individuals with higher IQs, levels of education or levels of occupational attainment have lower risks of developing Alzheimer's disease [Meng et al., 2012]. The cognitive reserve hypothesis considers both protective and compensatory mechanisms. The compensatory mechanism claims that given the same clinical staging, patients with higher levels of education are likely to demonstrate greater pathological severity. In this study, we found that education years were negatively associated with gray matter densities of the precuneus, supramarginal gyrus and middle temporal gyrus ( $r = -0.377, -0.407, -0.400$ ; all  $P < 0.05$ ); while education years were negatively associated with the plasma tau levels of the patient group ( $r = -0.626, P < 0.01$ ). The finding of negative associations between education years and brain structures supports the compensatory mechanism of cognitive reserve from education against the onset of dementia. However, education may have a further protection effect; even after the onset of cognitive impairment tau level remains lower in those people with higher education. This required further study to elucidate and validate this observation.

However, the study is not without limitations. The first limitation is that we measured the total tau of the plasma but not the phosphorylated tau (p-tau) proteins. Indeed, total tau is not specific for degenerative disease as mentioned in our manuscript. However, we had excluded patients with those neurological conditions such as Creutzfeldt-Jakob disease, acute stroke, and traumatic brain injury [Hesse et al., 2000; Kapaki et al., 2001; Zemlan et al., 1999]. Although p-tau is more specific for degenerative dementia, it also has two problems. First, p-tau has much lower concentration in both CSF and plasma (1/8~1/5), it is much more difficult to measure the plasma levels of p-tau (even lower than in CSF). Second, p-tau proteins have different formats (e.g., p-tau 181, 199, 231) and each has different clinical implication. For example, p-tau 231 is useful for differentiating between AD and frontotemporal dementia, while p-tau 181 is useful for differentiating AD from dementia of Lewy bodies [Hampel et al., 2010]. Still the task of measuring plasma p-tau is our future work. The second limitation is that we do not have plasma-CSF sample pairs from healthy controls for comparison. In our society, it is difficult to obtain CSF samples even from AD patients and we were not able to obtain CSF samples from healthy volunteers for measuring tau

levels. The permeability of blood brain barrier may be changed by AD process (Matsumoto et al., 2007) and the relation of the plasma tau protein to its CSF counterparts in healthy controls remains unsolved in our study. Third, we did not have investigate the A $\beta$ 40 and 42 simultaneously and explore the A $\beta$ 42/tau ratios which can provide comparison with the findings from CSF studies. A final limitation is that we have quite a few different measures for cognitive functions and brain structures, although we have applied a strict statistical correction for multiple comparisons by using Bonferroni correction, in the generalization of our results it is better that readers refer only to those with higher significance (e.g.,  $P < 0.01$ ) to avoid possible false positives.

In conclusion, plasma tau levels are capable of differentiating between healthy elderly individuals and elderly patients with MCI or early AD. This study establishes associations that support its suggestion that plasma tau may serve as a window that reveals both the structure and the function of the brain.

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