

Synergistic Association between Plasma $A\beta_{1-42}$ and p-tau in Alzheimer's Disease but Not in Parkinson's Disease or Frontotemporal Dementia

Ming-Jang Chiu,* Shieh-Yueh Yang, Ta-Fu Chen, Chin-Hsien Lin, Fu-Chi Yang, Wen-Ping Chen, Henrik Zetterberg, and Kaj Blennow,

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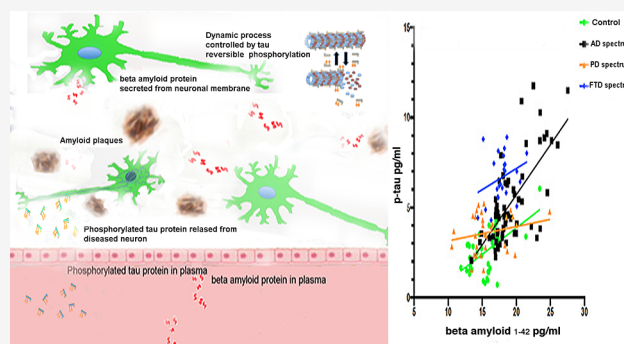
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ABSTRACT: Beta-amyloid ($A\beta_{1-42}$) triggers the phosphorylation of tau protein in Alzheimer's disease (AD), but the relationship between phosphorylated tau (p-tau) and $A\beta_{1-42}$ in the blood is not elucidated. We investigated the association in individuals with AD ($n = 62$, including amnesic mild cognitive impairment and dementia), Parkinson's disease ($n = 30$), frontotemporal dementia ($n = 25$), and cognitively unimpaired controls ($n = 41$) using immunomagnetic reduction assays to measure plasma $A\beta_{1-42}$ and p-tau181 concentrations. Correlation and regression analyses were performed to examine the relation between plasma levels, demographic factors, and clinical severity. Both plasma $A\beta_{1-42}$ and p-tau concentrations were significantly higher in AD and frontotemporal dementia than in the controls and Parkinson's disease. A significant positive association was found between plasma p-tau and $A\beta_{1-42}$ in controls ($r = 0.579$, $P < 0.001$) and AD ($r = 0.699$, $P < 0.001$) but not in frontotemporal dementia or Parkinson's disease. Plasma p-tau was significantly associated with clinical severity in the AD in terms of scores of clinical dementia rating ($r = 0.288$, $P = 0.025$) and mini-mental state examination ($r = -0.253$, $P = 0.049$). Regression analysis showed that plasma $A\beta_{1-42}$ levels explain approximately 47.7% of the plasma p-tau levels in the AD after controlling age, gender, and clinical severity. While in non-AD participants, the clinical dementia rating explained about 47.5% of the plasma p-tau levels. The disease-specific association between plasma $A\beta_{1-42}$ and p-tau levels in AD implies a possible synergistic effect in mechanisms involving these two pathological proteins' genesis.

KEYWORDS: Plasma biomarkers, β -amyloid, phosphorylated-tau protein, Alzheimer's disease, frontotemporal dementia, Parkinson's disease



INTRODUCTION

The key pathological features of Alzheimer's disease (AD) include extracellular accumulation of amyloid β ($A\beta$) peptides and intracellular hyperphosphorylation of tau protein and consequent neurofibrillary tangle (NFT) formation.^{1,2} The amyloid cascade hypothesis has dominated research and therapeutic drug development of AD since it was proposed three decades ago;³ this hypothesis states that the imbalance of $A\beta$ production and clearance is an early or initiating event.⁴⁻⁶ However, recent failures of clinical trials of disease-modifying therapies targeting amyloid deposition and formation have not supported the amyloid cascade hypothesis. On the other hand, although the amyloid cascade hypothesis also endorses the hyperphosphorylation of intracellular tau protein to be triggered by soluble $A\beta$ oligomers in the interstitial fluid, its mechanism has not been fully elucidated.⁴⁻⁶ Glycogen synthase kinase-3 β has been proposed as a mediator of tau phosphorylation in neurons;⁷ insulin-like growth factor-bind-

ing protein 3 may also be an essential tau kinase.⁸ The coexistence of $A\beta$ and phosphorylated tau (p-tau) was demonstrated pathologically in human post-mortem studies⁸ and AD animal models.⁴⁻⁶ Biomarker studies in cerebrospinal fluid (CSF) of individuals with AD show concomitant reduction of $A\beta_{1-42}$ and an increase in p-tau levels,^{9,10} which was suggested to reflect the deposition of $A\beta$ into plaques and the increased phosphorylation state of tau protein.¹¹ However, the interrelation or effect size of the two abnormal proteins remains to be explored.

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Table 1. Demographic, Clinical Information, and Measured Levels of Plasma $A\beta_{1-42}$ and p-tau181 for Enrolled Subjects^a

group	CON	AD spectrum			PD spectrum			FTD
		aMCI	ADD	combined	PD-NC	PD-IC	combined	
<i>N</i> (female%)	41 (75.6%)	36 (83.3%)	26 (57.7%)	62 (72.6%)	17 (35.3%)	13 (46.2%)	30 (40%)	25 (72%)
age (yr.)	65.1 ± 6.8	72.7 ± 7.8	76.7 ± 7.5	74.4 ± 7.8	66.3 ± 13.1	69.8 ± 9.5	67.8 ± 11.6	63.8 ± 7.4
MMSE	29.2 ± 0.7	25.9 ± 2.6	19.0 ± 4.0	23.0 ± 4.7	29.5 ± 0.9	27.1 ± 1.0	28.5 ± 1.5	18.4 ± 9.1
CDR (0/0.5/1/2/3)	41/0/0/0/0	0/36/0/0/0	0/6/17/3/0	0/42/17/3/0	17/0/0/0/0	0/13/0/0/0	17/13/0/0/0	0/8/11/3/3
$A\beta_{1-42}$ (pg/mL)	15.6 ± 2.3	18.2 ± 1.9	20.4 ± 3.5	19.2 ± 2.9	15.4 ± 2.4	16.4 ± 3.6	15.9 ± 2.9	18.3 ± 2.7
p-tau181(pg/mL)	2.6 ± 1.2	4.3 ± 1.5	6.6 ± 2.6	5.3 ± 2.4	3.9 ± 1.0	3.3 ± 1.1	3.6 ± 1.1	6.6 ± 1.3

^a*N*: number of subjects; CON: control group; aMCI: amnesic mild cognitive impairment due to AD; ADD: Alzheimer's disease dementia; AD spectrum: aMCI plus AD; PD: Parkinson's disease; PD-NC: Parkinson's disease with normal cognition; PD-IC: Parkinson's disease with impaired cognition including Parkinson's disease dementia and Parkinson's disease mild cognitive impairment; PD spectrum: PD-NC plus PD-IC; FTD: frontotemporal dementia; MMSE: mini-mental state examination; CDR: clinical dementia rating.

With the developments of ultrasensitive technologies in the past decade, it has been shown that $A\beta_{1-42}$ and p-tau181 are present at low concentrations in human plasma.^{12–14} These technological advances enable the investigation of the interrelation between p-tau181 and $A\beta_{1-42}$ in human plasma, which may help to elucidate the pathogenic mechanism of proteinopathies. For instance, in individuals with prodromal or clinical AD, extracellular $A\beta$ deposition precedes and precipitates aggregation of pathological intracellular p-tau protein. This report aims to use immunomagnetic reduction (IMR) to measure $A\beta_{1-42}$ and p-tau181 in cognitively unimpaired individuals and participants with mild cognitive impairment (MCI) due to AD and AD dementia (ADD). In addition to AD, individuals with Parkinson's disease (PD) and frontotemporal dementia (FTD) were enrolled as positive controls for assessing these two plasma protein biomarkers' disease-specific association.

RESULTS AND DISCUSSION

The enrolled subjects' demographic information is listed in Table 1 with the information on the AD spectrum subgroups, including MCI due to AD and ADD, and PD spectrum subgroups, including PD with normal cognition (PD-NC) and PD with impaired cognition (PD-IC). There were 41 individuals (65.1 ± 6.8 years, 75.6% women) in the control group. There were 62 (74.4 ± 7.8 years, 72.6% women) in the AD spectrum group, 36 with MCI due to AD, and 26 with ADD. There were 30 patients in the PD spectrum group (67.8 ± 11.6 years, 40% women), 17 with PD-NC, and 13 with PD-IC. There were 25 patients in the FTD (age: 63.8 ± 7.4 years, 72% women). Individuals in the FTD group were further classified according to their clinical features, including 12 with semantic-type primary progressive aphasia (PPA), 3 with nonfluent PPA, 5 with behavior-variant FTD, 2 with behavior-variant FTD-amyotrophic lateral sclerosis, 2 with FTD-progressive supranuclear palsy, and, finally, 1 with FTD corticobasal degeneration. One subject with logopenic variant PPA was included in the AD spectrum group for analysis. Around 90% of the subjects with logopenic variant PPA showed amyloid positivity in brain image, and about 80% showed AD pathology.^{15,16} Individuals in the AD spectrum group were significantly older than those in the other groups (all $P < 0.005$); therefore, subsequent statistical analyses were performed by adjusting for the age effect. Significant between-group differences in gender distribution (Pearson's $\chi = 17.327$, $P = 0.004$) and clinical severity in terms of global clinical dementia rating (CDR) scores (Pearson's $\chi = 259.678$, $P <$

0.001) were also observed. We used regression analyses to examine gender and clinical severity effects.

The measured levels of plasma $A\beta_{1-42}$ in the four groups are plotted in Figure 1a. Group differences in plasma $A\beta_{1-42}$ and p-

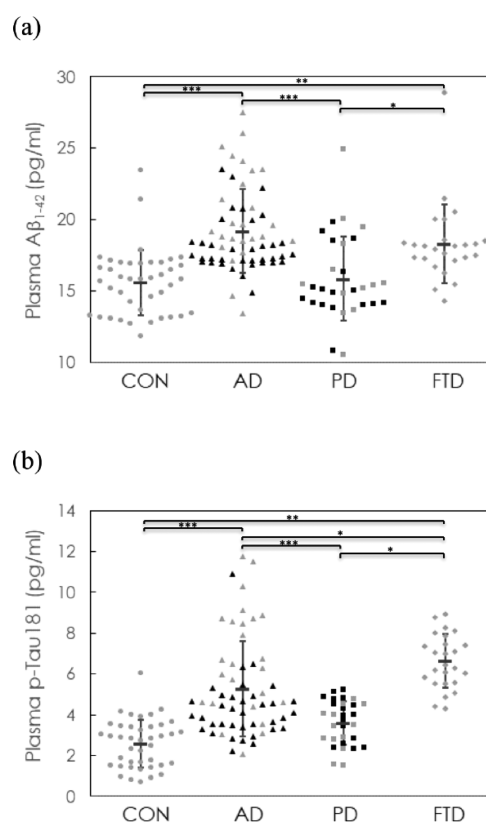


Figure 1. Concentrations of (a) plasma $A\beta_{1-42}$ and (b) plasma p-tau181 for subjects in the controls (CON), AD spectrum (AD), PD spectrum (PD), and FTD spectrum (FTD). The black triangles denote MCI due to AD, and gray triangles represent ADD. The black rectangles are for PD-NC, and the gray rectangles indicate PD-IC (PDMCI and PDD). The symbols * indicate MANCOVA $p = 0.01$, ** indicate $p = 0.001$, and *** indicate $p < 0.001$ with Bonferroni correction for confidence intervals.

tau181 were compared using multivariate analysis of covariance (MANCOVA), controlling for the age effect. The controls had the mean plasma $A\beta_{1-42}$ level of 15.6 ± 2.3 pg/mL. The mean level of the MCI group was 18.2 ± 1.9 pg/mL, and the ADD was 20.6 ± 3.5 pg/mL for plasma $A\beta_{1-42}$. The MCI group had higher levels of plasma $A\beta_{1-42}$ than the

controls ($P < 0.001$); the levels of plasma $A\beta_{1-42}$ in the ADD group were higher than those in the MCI group ($P = 0.007$).

In the PD group, both PD-NC (15.4 ± 2.4 pg/mL) and PD-IC (16.4 ± 3.6 pg/mL) did not show significantly different between-group levels of plasma $A\beta_{1-42}$ from each other or the controls ($P > 0.5$). However, the FTD group had a mean level of plasma $A\beta_{1-42}$ of 18.3 ± 2.7 pg/mL, which was significantly higher than the controls ($P = 0.002$). We removed one outlier by the 3-standard deviation rule in the FTD group due to a plasma level of $A\beta_{1-42}$ 28.9 before further correlation and regression analyses.

Figure 1b shows plasma p-tau181 concentrations for all four groups. The controls had a mean level of 2.6 ± 1.2 pg/mL for plasma p-tau181. The MCI and ADD groups had mean levels of 4.3 ± 1.5 and 6.6 ± 2.6 pg/mL, respectively. The concentrations of plasma p-tau181 in both aMCI and mild AD were found to be significantly higher than the controls ($P < 0.001$); similarly, the concentrations of plasma p-tau181 in the ADD group were significantly higher than those in the MCI group ($P < 0.001$). Compared to the controls, the concentrations of plasma p-tau181 in the PD-NC and PDD groups were not significantly different (PD-NC: 3.56 ± 1.16 pg/mL; PDD: 3.70 ± 0.96 pg/mL, both $P > 0.05$). The mean plasma p-tau181 concentration in the FTD group (6.67 ± 1.34 pg/mL) was significantly higher than the controls ($P < 0.001$).

In addition to the comparison with the controls, we also examined the between-group differences of various neurodegenerative diseases. For plasma $A\beta_{1-42}$, there were significant between-group differences between AD and PD spectrum ($p < 0.001$) and PD and FTD ($p = 0.01$). For plasma p-tau, there were significant between-group differences between AD and FTD and between PD and FTD (both $p = 0.01$) and AD and PD ($p < 0.001$). However, for clinical classification of various neurodegenerative diseases, multiplex plasma biomarkers with the help of a machine-learning model are helpful.¹⁷

We further examined the subgroup difference in various FTD subtypes but found no significant difference, which was probably due to small sample size (both ANCOVA $p > 0.4$). However, some premature yet interesting findings could be presented. The highest plasma p-tau was in the PSP subgroup (7.6 ± 0.8 pg/mL, $n = 2$) and the lowest in the ALS subgroup (5.2 ± 0.5 pg/mL, $n = 2$). On the other hand the highest plasma $A\beta_{1-42}$ was in the behavioral variant (20.5 ± 4.9 pg/mL, $n = 5$) and the lowest in the ALS subgroup (16.4 ± 1.3 pg/mL, $n = 2$). Further exploration with a large sample size should be warranted.

The relationships between p-tau181 and $A\beta_{1-42}$ concentrations in plasma for various types of dementia are plotted in Figure 2. A partial correlation controlling the age effect was performed to examine the interrelation between plasma $A\beta_{1-42}$ and p-tau181 concentrations. The correlation coefficients between plasma p-tau181 and $A\beta_{1-42}$ concentrations reached significant levels for the controls ($r = 0.579$, $P < 0.001$) and for the AD spectrum ($r = 0.699$, $P < 0.001$) but not for the PD spectrum ($r = 0.208$, $P = 0.280$) or for FTD ($r = 0.052$, $P = 0.811$).

For the correlation of plasma biomarkers to clinical severity, the only significant findings were between plasma p-tau levels and both global CDR scores ($r = 0.288$, $P = 0.025$) and mini-mental state examination (MMSE) scores ($r = -0.253$, $P = 0.049$) but not between plasma $A\beta_{1-42}$ and global CDR scores ($r = 0.173$, $P = 0.184$) or MMSE scores ($r = -0.246$, $P = 0.056$) in the AD spectrum group.

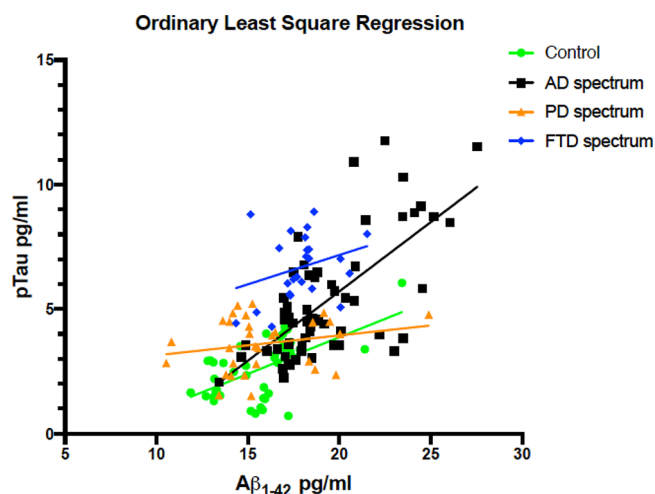


Figure 2. Concentration relationships displayed by ordinary least-squares regression between plasma p-tau and $A\beta_{1-42}$ in the control (green), AD spectrum (black), PD spectrum (orange), and FTD spectrum (blue). AD denotes AD spectrum, including aMCI due to AD and ADD groups. PD spectrum includes PD-NC and PD-IC groups. Only the control and AD spectrum reach significant positive associations.

We further performed group-wise (in terms of the spectrum) regression analyses using plasma p-tau levels as the dependent variable. The results showed that plasma $A\beta_{1-42}$ levels indicated an r square (R^2) change of 0.324 ($P < 0.001$) in the controls, excluding age, gender, MMSE, and CDR. In the AD spectrum, plasma $A\beta_{1-42}$ levels indicated an R^2 change of 0.477, while gender indicated an additional R^2 change of 0.064, and CDR further indicated an R^2 change of 0.031, excluding age and MMSE (Table 2). No significant effect of the independent variables could be found in the PD spectrum or FTD group.

Finally, we also performed a regression analysis with non-AD participants, all subjects excluding the AD spectrum. The result

Table 2. Regression Analyses Using Plasma p-tau Levels as Dependent Variable and Plasma $A\beta_{1-42}$ Levels and Demographic and Clinical Information as Independent Variables^a

group-wise	R^2/R^2 change	F change	P-value	excluding variables
Control ($n = 41$)				
model I: $A\beta_{1-42}$	0.324/0.324	18.659	<0.001	age, gender, MMSE
AD Spectrum ($n = 62$)				
model I: $A\beta_{1-42}$	0.477/0.477	54.676	<0.001	Age, gender, CDR, MMSE
model II: $A\beta_{1-42}$, gender	0.540/0.064	8.155	0.006	age, CDR, MMSE
model III: $A\beta_{1-42}$, gender, CDR	0.572/0.031	4.254	0.044	age, MMSE
All Excluding AD Spectrum ($n = 96$)				
model I: CDR	0.473/0.473	84.333	<0.001	age, gender, $A\beta_{1-42}$, MMSE
model II: CDR, $A\beta_{1-42}$	0.095/0.558	20.344	<0.001	age, gender, MMSE

^aMMSE: mini-mental state examination; CDR: clinical dementia rating; no significant results found in all independent variables of both PD spectrum and FTD groups.

showed that p-tau was mainly explained by CDR with an R^2 change of 0.477, excluding age, gender, MMSE, and $A\beta_{1-42}$ levels, and $A\beta_{1-42}$ levels further added an R^2 change of 0.095 excluding age, gender, and MMSE (Table 2).

In tau positron-emission tomography (PET) studies, tracer retention increases modestly with age throughout the brain in cognitively unimpaired individuals, while elevated tau is seen more often when amyloid brain accumulation is present.¹⁸ In cognitively unimpaired individuals, total tau concentrations in CSF increase with age, and CSF p-tau concentrations increase with age in ApoE4 carriers.¹⁹ In our previous study, age explained approximately 13% of the variance in plasma total tau levels in a group of 126 cognitively unimpaired individuals aged 45–95 years.²⁰

On the other hand, the age-related deposition of brain amyloid protein in cognitively unimpaired individuals is less well studied, and most relevant studies have reported that approximately 15–30% of cognitively unimpaired aging adults showed positive amyloid deposition, with ApoE4 carriers having a higher risk.^{21,22} The Australian Imaging, Biomarkers and Lifestyle study of aging showed that amyloid burden increased with age most strongly in ApoE4 carriers.²³ However, the impact of ApoE4 on cognitive function has not been determined.²⁴ For plasma biomarkers, our previous studies showed that ApoE4 carriers have higher plasma $A\beta_{1-42}$ / $A\beta_{1-40}$ ratios¹² and have higher plasma total tau levels than their noncarrier counterparts.²⁵

In contrast to CSF tau levels, the CSF- $A\beta_{1-42}$ or $A\beta_{1-42}$ / $A\beta_{1-40}$ ratios did not correlate with age.¹⁹ In our previous study, the plasma $A\beta_{1-42}$ levels showed a modest but significant negative relation with age ($r = -0.126$, $p = 0.0128$) in a population of 391 cognitively unimpaired adults aged 23–91 years.²⁶

According to the results in Figure 1a, subjects in the AD spectrum showed the most noticeable difference in plasma $A\beta_{1-42}$ levels compared with the other diagnostic groups. This finding is in keeping with the results of our previous studies of plasma $A\beta_{1-42}$ using IMR measurements,^{12,27} which was found to be positively correlated with brain amyloid deposition in terms of the $A\beta_{1-42}$ / $A\beta_{1-40}$ ratio²⁸ but moderately negatively correlated with CSF $A\beta_{1-42}$ levels.¹³

In Figure 1b, both the AD spectrum and FTD groups showed increased levels of p-tau in plasma. AD pathology is a dual proteinopathy characterized by the coexistence of extracellular aggregates of mainly $A\beta_{1-42}$ forming neuritic $A\beta$ plaques and intracellular aggregates of p-tau forming NFT.²⁹ Despite the potential synergistic relationship between these two proteinopathies, the observation of tau pathology early in the disease course³⁰ and relatively good association with clinical severity suggest that AD is a dual proteinopathy consisting of both $A\beta_{1-42}$ and p-tau. The latter finding was supported by this study that only plasma p-tau levels but not $A\beta_{1-42}$ were associated with clinical severity in the AD spectrum groups, compatible with a recent study which also reported that plasma p-tau181 was associated with clinical severity and tau-PET.¹⁴

On the other hand, FTD consists of a spectrum of clinical syndromes associated with several underlying neurodegenerative diseases characterized by frontotemporal lobar degeneration (FTLD).³¹ From a pathological point of view, most (90–95%) FTLDs are caused by intracellular aggregates of p-tau or TAR DNA-binding protein 43 (TDP-43).^{32,33} Mixed FTLD

pathologies or unclassifiable tauopathies are also not infrequently observed.

A recent study assessing $A\beta$ in 98 individuals with pathologically confirmed frontotemporal dementia syndromes showed that in individuals with various types of frontotemporal dementias, 8%–29% of individuals showed $A\beta$ deposition in the frontotemporal cortex, and the prevalence increased to 29%–50% if the basal ganglia or substantia nigra were included.³⁴ Amyloid molecular imaging studies using Pittsburgh Compound B (PiB)-PET scans also revealed amyloid depositions in cortex and subcortical areas in individuals with FTD.^{34,35} The coexistence of $A\beta$ and p-tau or other proteinopathies in FTD syndrome, such as TDP-43, fused in sarcoma can be expected; thus, it is unsurprising to observe elevated plasma $A\beta_{1-42}$ levels in individuals with FTD. The mean level of plasma $A\beta_{1-42}$ is approximately at the level of MCI due to the AD in this study.

The main pathological hallmark for PD is Lewy bodies, which mainly consist of α -synuclein. Although amyloid molecular imaging studies showed amyloid deposition in individuals with α -synucleinopathy, those with amyloid positivity mostly have dementia with Lewy bodies (DLB) and PDD. In a meta-analysis study including 233 individuals with DLB, PD, and PD-MCI receiving PiB-PET scans, the prevalence rates were 0.68 (95% CI 0.55–0.82) in the DLB group, 0.34 (95% CI 0.13–0.56) in the PDD group, and 0.05 (95% CI –0.07–0.17) in the PD-MCI group. In our PD-IC group (combined PD-MCI and PDD), most of the cases were PD-MCI (with a mean MMSE score of 27.1 ± 0.1), and the mean plasma level was 3.6 ± 1.1 pg/mL, which, although elevated, did not reach significant levels compared to the controls.

Although we observed elevation of both levels of plasma $A\beta_{1-42}$ and p-tau181 concentrations in both the AD spectrum and FTD groups, their interrelations are notably different and might have different implications. In Figure 2, a significant linear correlation between p-tau181 and $A\beta_{1-42}$ in plasma was observed in the AD spectrum group ($r = 0.699$, $P < 0.001$) but not in the FTD group ($r = 0.052$, $P = 0.811$). Groupwise regression analysis showed that in the AD spectrum, plasma $A\beta_{1-42}$ levels explained approximately 47.7% ($P < 0.001$) of the plasma p-tau levels, followed by gender (6.4%, $P = 0.006$) and severity in terms of CDR (3.1%, $P = 0.044$). Amyloid hypothesis obtained supporting mechanistic evidence, at least in parts, from those observations that soluble oligomers of $A\beta_{1-42}$ decrease synapse number, inhibit long-term potentiation, and enhance long-term depression hippocampal neurons. The $A\beta_{1-42}$ oligomers also increase abnormal phosphorylation of tau, driving vicious cycles leading to AD pathology.³⁶ The amyloid hypothesis finds its support mainly from early onset AD when dominant mutations involving amyloid- β -related pathogenesis. However, recent evidence from observation of aging human brains and an animal model of late-onset AD, aging rhesus macaque,³⁷ leads to a hypothesis that tau pathology is probably an initiating factor for sporadic late-onset AD.³⁸ Pathological tau may also drive $A\beta$ cleavage, consequently increasing $A\beta$ production by p-tau in the microtubules trapping amyloid precursor protein-containing endosomes in dendrites, propelling a vicious cycle of tau and amyloid pathology over a long lifetime.³⁸

Regarding the positive association between plasma $A\beta_{1-42}$ and p-tau181 concentrations in the controls, we proposed that some individuals of the controls might be at the preclinical

stage of AD, the assumption is reasonable considering the high prevalence of AD in the general population, especially in individuals with subjective memory decline and in ApoE4 carriers, or it also reflects a brain aging process with both proteinopathies.^{18,23,26} These findings warrant further exploration with a longitudinal study.

There are two limitations to this study. First, most participants did not have molecular imaging, such as PiB PET or tau PET, to support their diagnoses. However, with a careful clinical diagnosis and structural brain imaging (mostly magnetic resonance imaging), we can still achieve a degree of clinical confidence.

Second, tau has over 40 sites for phosphorylation, but we measured only plasma p-tau181 in this study. Although CSF p-tau181 was considered capable of discriminating between AD and other neurodegenerative diseases, p-tau181 changes not entirely specific to AD. CSF p-tau181 elevations could be found in FTL, especially late-onset types.⁴⁰ Recent studies showed that p-tau217 outperforms p-tau181 in the differential diagnosis between AD and non-AD dementia.⁴¹ In the future, the inclusion of the plasma TDP-43, α -synuclein, and p-tau217 is warranted.

In conclusion, by using immunomagnetic reduction to assay plasma $A\beta_{1-42}$ and p-tau181 concentrations, we observed a positive correlation between $A\beta_{1-42}$ and p-tau181 in plasma in AD but not in PD or FTD. The disease-specific association between plasma $A\beta_{1-42}$ and p-tau levels in AD warrants further exploration of a possible bidirectional synergic effect involving the genesis of these two pathological proteins.

SUMMARY

Alzheimer's disease's (AD) key pathological features include extracellular aggregates of amyloid-beta ($A\beta$) forming neuritic plaques and intracellular aggregates of hyperphosphorylated tau (p-tau) forming neural fibrillary tangles. $A\beta$ triggers the hyperphosphorylation of tau in AD, but the relationship between blood p-tau and $A\beta$ is not elucidated. We investigated the relation in AD, Parkinson's disease, frontotemporal dementia, and controls using immunomagnetic reduction assays to measure plasma $A\beta$ and p-tau. We found a positive association between plasma p-tau and $A\beta$ in controls and AD but not in frontotemporal dementia or Parkinson's disease. Plasma p-tau but not $A\beta$ was associated with clinical severity in the AD. Regression analysis showed that plasma $A\beta$ explains approximately 47.7% of the plasma p-tau levels in the AD. In comparison, CDR explains about 47.5% of plasma p-tau in the non-AD participants. The disease-specific association in AD implies a possible synergic effect involving the genesis of these two pathological proteins.

METHODS

Recruitment of Subjects. The 158 subjects were enrolled at National Taiwan University Hospital (NTUH), Taiwan, Triservice General Hospital (TSGH), Taiwan, and Sahlgrenska University Hospital (SUH), Sweden. Individuals with either MCI due to AD or ADD were diagnosed according to Naitonal Institute on Aging and Alzheimer's Association (NIA-AA) diagnostic guidelines.^{42,43} Individuals with Parkinson's disease (PD) were diagnosed using the United Kingdom PD Society Brain Bank clinical diagnostic criteria.⁴⁴ PD with dementia (PDD) or mild cognitive impairment (PD-MCI) was further diagnosed according to the diagnostic guidelines suggested by the Movement Disorder Society Task Force to separate them from PD-NC.^{45,46} We used MMSE scores of ≤ 25 as the cutoff value for significant cognitive dysfunction in PDD as well as impairment of

instrumental activities of daily living (IADL) and MMSE scores of 26–28 with normal informant-based IADL for PD-MCI.⁴⁷ The two groups, PDD and PD-MCI, were combined to constitute PD-IC for further analysis. Individuals with FTD were enrolled and classified according to two diagnostic criteria consensus.^{48,49} In SUH, AD patients were further confirmed by CSF $A\beta_{1-42} \leq 530$ pg/mL and total tau protein ≥ 350 pg/mL.⁵⁰ The SUH cohort recruited 29 participants, including 14 ADD patients and 15 control subjects (Supplementary Table 1). For both the controls and ADD groups, there is no significant difference in both plasma levels of $A\beta_{1-42}$ and p-tau between the Taiwanese and Swedish cohorts (Supplementary Table 2).

Most participants received a CDR and an MMSE to evaluate their clinical severity.

All study participants or their primary caregivers provided informed consent before participation in this investigation. The study was approved by the Institutional Research Ethics Committee of NTUH, Institutional Review Board of TSGH, National Defense Medical Center, and Central Ethical Review Board, University of Gothenburg for SUH. The study was carried out following the Helsinki Declaration of 1975.

Preparation of Plasma. An EDTA blood collection tube was used for blood collection, followed by centrifugation with speeds ranging from 1500–2500 g for 15 min at room temperature. The plasma in the EDTA tube was transferred and aliquoted into 0.5 mL microcentrifuge tubes and stored at -80 °C until IMR measurements were performed. Plasma was frozen no later than 3 h after blood collection. Collected plasma samples were delivered to MagQu Co., Ltd., Taiwan, by a dry ice package for blindly assaying plasma $A\beta_{1-42}$ and p-tau181. The protocols for plasma preparation were identical in both Taiwanese and Swedish cohorts.

Assay of $A\beta_{1-42}$ and p-tau181. IMR was utilized to assay $A\beta_{1-42}$ and p-tau181 in collected plasma samples. Briefly, IMR, using the reduction in the ac magnetic susceptibility, that is, the IMR signal, of the reagent after being mixed with a sample, was measured using a superconducting quantum interference device-based ac magnetic susceptometer (XacPro-S, MagQu).^{12,51}

For assaying $A\beta_{1-42}$, 60 μ L of plasma was mixed with 60 μ L of reagent (MF-AB2-0060, MagQu), which involves the use of an antibody (ab34376, Abcam) against $A\beta_{1-42}$ immobilized on dextran-coated magnetic Fe_3O_4 nanoparticles (MF-0060-DEX, MagQu). The epitope of the $A\beta_{1-42}$ antibody is amino acid region 37–42 aa. For assaying p-tau181, 40 μ L of plasma was mixed with 80 μ L of reagent (MF-PT1-0060, MagQu), involving the use of an antibody (MN1050, Thermo), which is the same as the commonly used AT270 antibody, against p-tau181 immobilized on dextran-coated magnetic Fe_3O_4 nanoparticles (MF-0060-DEX, MagQu), and an IMR analyzer (XacPro-S, MagQu) was used to analyze the concentrations of $A\beta_{1-42}$ and p-tau181 in each plasma. Duplicate measurements were conducted for each biomarker of every sample. The reported concentration of $A\beta_{1-42}$ or p-tau181 of a plasma sample was the averaged value of duplicate measurements. The variation in the duplicate measurements' levels was lower than 20% for both $A\beta_{1-42}$ and p-tau181.

Before performing the analysis of $A\beta_{1-42}$ and p-tau181, two calibrators consisting of magnetic fluid (CA-DEX-0060, CA-DEX-0080, MagQu) were applied to calibrate the IMR analyzer readings. The details of the preparation of the magnetic fluid are described in our previous work. For every batch of analysis of $A\beta_{1-42}$ and p-tau181, control solutions were used for quality control. The control solutions for $A\beta_{1-42}$ are pure PBS solution (negative control) (CL-AB2-00T, MagQu) and 20 pg/mL $A\beta_{1-42}$ solution (CL-AB2-020T, MagQu). The control solutions for p-tau181 were pure PBS solution (negative control) (CL-PT1-00T, MagQu), and 5 pg/mL p-tau181 solution (CL-PT1-005T, MagQu). The acceptable measured concentrations for the 20 pg/mL $A\beta_{1-42}$ control solution are 17–23 pg/mL and 4.25–5.75 pg/mL for the 5 pg/mL p-tau181 control solution.

Statistical Analyses. Continuous variables for each measurement were presented as the mean \pm standard deviation, and group differences were compared using analysis of variance for continuous

variables and Pearson's χ^2 test for noncontinuous variables. Missing data were handled by using subgroup means. Group differences of plasma biomarkers were compared using MANCOVA, controlling for the age effect. Partial correlation controlling for the age effect was performed to examine the interrelation of plasma biomarkers or their relation to clinical data. Ordinary least square regression was also used to explore relationship of $A\beta_{1-42}$ levels to plasma p-tau levels. We further performed a stepwise linear regression analysis to estimate the independent variables' potential contribution to the dependent variables. We examined and removed possible outliers in further correlation and regression analyses. The statistical analyses were performed with IBM SPSS Statistics version 20 (Armonk, NY), and graphs were produced using GraphPad Prism version 8 (San Diego, California).

■ ASSOCIATED CONTENT

SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acscchemneuro.1c00010>.

Supplementary Table 1 shows numbers of enrolled subjects and Supplementary Table 2 shows levels of plasma $A\beta_{1-42}$ and p-tau in Taiwanese and Swedish cohorts (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Ming-Jang Chiu, MD – Department of Neurology, National Taiwan University Hospital, College of Medicine, Graduate Institute of Brain and Mind Sciences, College of Medicine, Department of Psychology, and Graduate Institute of Biomedical Electronics and Bioinformatics, National Taiwan University, Taipei 100, Taiwan; orcid.org/0000-0002-4158-4423; Email: mjchiu@ntu.edu.tw

Authors

Shieh-Yueh Yang, PhD – MagQu Co., Ltd., New Taipei City 231, Taiwan; MagQu LLC, Surprise, Arizona 85378, United States

Ta-Fu Chen, MD – Department of Neurology, National Taiwan University Hospital, College of Medicine, National Taiwan University, Taipei 100, Taiwan

Chin-Hsien Lin, MD – Department of Neurology, National Taiwan University Hospital, College of Medicine, National Taiwan University, Taipei 100, Taiwan

Fu-Chi Yang, MD – Department of Neurology, Tri-Service General Hospital, National Defense Medical Center, Taipei 114, Taiwan

Wen-Ping Chen, PhD – MagQu LLC, Surprise, Arizona 85378, United States

Henrik Zetterberg, MD – Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, The Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal 405 30, Sweden; Department of Neurodegenerative Disease, UCL Queen Square Institute of Neurology, London WC1N 3BG, United Kingdom; UK Dementia Research Institute at UCL, London WC1E 6BT, United Kingdom

Kaj Blennow, MD – Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, The Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal 405 30, Sweden; Department of Neurodegenerative Disease, UCL Queen

Square Institute of Neurology, London WC1N 3BG, United Kingdom

Complete contact information is available at:

<https://pubs.acs.org/doi/10.1021/acscchemneuro.1c00010>

Author Contributions

M.J.C.: Concept formation, statistical analysis, data interpretation, and manuscript drafting. S.Y.Y.: Assisted with manuscript drafting and data analysis. T.F.C., C.H., and F.J.Y.: Assisted with participants recruitment, sample collection, and clinical information interpretation. W.P.: IMR measurement, quality control, and trouble shooting. H.Z. and K.B.: Sample collection, data interpretation, and manuscript comments. All authors read and approved the final manuscript.

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Notes

The data of this study are available from each hospital under the approval of the individual university's institutional review board or ethics committee; thus, restrictions apply to the availability of these data, which were used under license for the current study and so are not publicly available. However, data are available from the authors upon reasonable request and with permission of the individual university's institutional review board or ethics committee.

The authors declare the following competing financial interest(s): S.Y.Y. is an employee of MagQu Co., Ltd. and MagQu LLC. S.Y.Y. is a shareholder of MagQu Co., Ltd. W.P.C. is an employee of MagQu LLC. H.Z. has served on scientific advisory boards for Roche Diagnostics, Wave, Samumed, and CogRx, has given lectures in symposia sponsored by Alzecure and Biogen, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-based platform company at the University of Gothenburg. K.B. has served as a consultant or on advisory boards for Alector, Biogen, CogRx, Lilly, MagQu, Novartis, and Roche Diagnostics and is a co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-based platform company at the University of Gothenburg.

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■ ABBREVIATIONS

$A\beta$, amyloid beta; AD, Alzheimer's disease; ADD, Alzheimer's disease dementia; CDR, clinical dementia rating; CSF, cerebrospinal fluid; DLB, dementia with Lewy bodies; FTD, frontotemporal dementia; FTLD, frontotemporal lobar degeneration; IMR, immunomagnetic reduction; MANCOVA, multivariate analysis of covariance; MCI, mild cognitive

impairment; MMSE, mini-mental state examination; NFT, neural fibrillary tangles; NIA-AA, National Institutes on Aging and Alzheimer's Association; NTUH, National Taiwan University Hospital; PD, Parkinson's disease; PDD, Parkinson's disease dementia; PD-IC, Parkinson's disease with impaired cognition; PD-MCI, Parkinson's disease with mild cognitive impairment; PD-NC, Parkinson's disease with normal cognition; PET, positive emission tomography; PiB, Pittsburgh compound B; PPA, primary progressive aphasia; p-tau, phosphorylated tau; TDP-43, TAR DNA-binding protein; TSGH, Triservice General Hospital; SUH, Sahlgrenska University Hospital

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