


SCIENTIFIC REPORTS

OPEN

Levels of plasma neurofilament light chain and cognitive function in patients with Alzheimer or Parkinson disease

Yung-Shuan Lin^{1,2}, Wei-Ju Lee^{2,3,4,5,6}, Shuu-Jiun Wang^{1,2,6} & Jong-Ling Fuh^{1,2,6} 

Plasma neurofilament light (NFL) has been proposed as a blood-based biomarker for neurodegeneration in Alzheimer's disease (AD) and parkinsonian disorders. However, the relationship between plasma NFL and cognitive decline in dementia due to Parkinson's disease (PD) remains to be elucidated. In this research, 119 AD, 56 mild cognitive impairment (MCI), 26 non-demented PD (PDND), and 23 Parkinson's disease dementia (PDD) patients, as well as 59 cognitively healthy controls (HC) were recruited. Each subject underwent a battery of neuropsychological testing. Plasma NFL levels were measured in duplicate using an NF-Light assay and transferred onto the Simoa platform with a home-brew kit. Plasma NFL was significantly increased in the AD group, compared with the control, MCI, PDND, and PDD groups. Plasma NFL was significantly higher in the PDD group, compared with the PDND group. High plasma NFL correlated with poor cognition in AD and PD, but not with motor symptoms in PD. Plasma NFL may represent a biomarker of cognitive decline in AD and PD, with more specificity for AD.

Neurofilament light chain (NFL) is one of the three subunits of neurofilaments, which are specific cytoskeletal proteins of neurons and particularly abundant in largely myelinated axons. Axonal damage releases NFLs into cerebrospinal fluid (CSF) and eventually into blood. Higher NFL levels are believed to represent more severe cerebral axonal degeneration. NFL levels have been linked to several neurodegenerative diseases, such as Alzheimer's disease (AD)¹, Parkinson's disease (PD), atypical parkinsonian disorders (APD)², frontotemporal dementia (FTD)³, amyotrophic lateral sclerosis (ALS)⁴, Huntington disease (HD)^{5,6}, and multiple sclerosis (MS)⁷.

In mouse models, NFL levels in CSF and blood increased in association with the existence of α -synucleinopathy, tauopathy, and β -amyloidosis. In addition, blood and CSF NFL levels were strongly correlated, and NFL increases coincided with the onset and progression of proteopathic brain lesions⁸. Clinical studies also demonstrated that patients with mild cognitive impairment (MCI) or AD dementia with A β pathologic features have higher plasma NFL¹. As for parkinsonian disorders, one recent study showed blood NFL concentration can be used to distinguish PD from APD²; significantly increased levels were observed in patients with multiple system atrophy (MSA), progressive supranuclear palsy (PSP), or cortical basal syndrome (CBS), compared to patients with PD, as well as healthy controls. However, to our knowledge, no previous study has investigated blood NFL concentrations in non-demented PD (PDND) vs Parkinson disease's dementia (PDD).

Neuropsychological tests, neuroimaging, and CSF biomarkers may be used to diagnose cognitive decline in various syndromes that involve dementia. However, the use of these markers is hampered by lack of sensitivity at early stages of disease, high cost, and invasiveness. A readily available blood-based biomarker is needed for efficient monitoring of disease progress and could be used as a screening tool in primary care. When Simoa method,

¹Department of Neurology, Neurological Institute, Taipei Veterans General Hospital, Taipei, Taiwan. ²Faculty of Medicine, National Yang-Ming University School of Medicine, Taipei, Taiwan. ³Neurological Institute, Taichung Veterans General Hospital, Taichung, Taiwan. ⁴Dementia and Parkinson's Disease Integrated Center, Taichung Veterans General Hospital, Taichung, Taiwan. ⁵Center for Geriatrics and Gerontology, Taichung Veterans General Hospital, Taichung, Taiwan. ⁶Brain Research Center, National Yang-Ming University, Taipei, Taiwan. Yung-Shuan Lin and Wei-Ju Lee contributed equally. Shuu-Jiun Wang and Jong-Ling Fuh jointly supervised this work. Correspondence and requests for materials should be addressed to S.-J.W. (email: sjwang1@ym.edu.tw) or J.-L.F. (email: jlfuh@vghtpe.gov.tw)

rather than ELISA or electrochemiluminescence immunoassay, is used to measure plasma NFL, levels correlate strongly with those of CSF NFL⁹. As a potential indicator of disease severity or prognosis, serial measurements of plasma NFL may be considerably more practical than measurements of CSF NFL, which are inherently invasive.

In this study, we sought to investigate whether plasma NFL levels could be used as a reliable biomarker of disease severity and prognosis in patients with MCI, AD, PDND, or PDD. We expected that plasma NFL concentration would be elevated in patients with dementia due to AD or PD, compared with healthy controls. We hypothesized that concentrations would vary between PDND and PDD, and that increased plasma NFL levels would reflect disease severity.

Methods

Participants. We recruited normal healthy controls (HC), as well as amnesic MCI, AD, and PD patients seen at the outpatient clinics of Taipei Veterans General Hospital. HC were volunteers with normal cognitive function. AD diagnoses required multidisciplinary consensus, according to the clinical criteria for probable AD as described by the National Institute on Aging-Alzheimer's Association¹⁰. A diagnosis of MCI was made according to the revised consensus criteria from 2004¹¹. The cut-off value for diagnosis of MCI was set at 1.5 standard deviations below the age-adjusted norm for Wechsler Memory Scale III logical memory test¹². Inclusion criteria for PD adhered to those proposed by the UK Parkinson's Disease Society Brain Bank¹³. PD patients were further divided into PDND and PDD. PDD was diagnosed when patients fulfilled criteria proposed by the Movement Disorder Society Task Force¹⁴. Patients who had developed dementia within 1 year after PD onset were excluded. We also excluded patients with cognitive and behavioral symptoms presenting as a result of other conditions, including acute confusion due to systemic disease/abnormality or drug intoxication, major depressive disorder according to the Diagnostic and Statistical Manual of Mental Disorders 5th edition (DSM-V), probable vascular dementia, normal pressure hydrocephalus, progressive supranuclear palsy, or history of significant head trauma followed by persistent neurologic deficit or known structural brain abnormality. Disease duration was defined as the period between the initial onset of symptoms (as reported by the caregiver) and participation in the study. Informed consent was obtained from all patients and their caregivers before participation in the study. This research project was approved by the institutional review boards at Taipei Veterans General Hospital.

Clinical evaluation and procedures. All patients received a standardized evaluation that included physical examination, clinical interview, neuropsychological assessment, laboratory tests, and either brain magnetic resonance or computed tomographic imaging. Height and body weight were measured to calculate body mass index (BMI). Cognitive function was assessed with standard procedures. The Min-Mental State Examination (MMSE)¹⁵ was used to assess global cognition. The Clinical Dementia Rating (CDR)¹⁶ was administered to determine the severity of dementia. The 12-item memory test¹⁷, modified 15-item Boston Naming Test¹⁸, category verbal fluency test¹⁹, and forward and backward digit span test²⁰ were used to assess short-term memory, language, executive function, attention, and working memory, respectively. The UPDRS²¹ was used as a measurement of clinical PD severity.

Measurement of plasma NFL levels. Freshly drawn venous blood was collected at baseline in tubes containing ethylenediaminetetraacetic acid (EDTA). Samples were then centrifuged and stored in polypropylene tubes at -80°C until biochemical analysis. Plasma NFL levels were measured in duplicate using Quanterix SIMOA kits, according to the manufacturer's instructions and standard procedures. All sample coefficients of variance (CVs) of duplicate measurements were below 18.5%.

DNA analysis. Genomic DNA was isolated from whole blood using a Gentra Puregene kit, according to the manufacturer's protocol (Qiagen, Hilden, Germany). Presence of the $\epsilon 2$, $\epsilon 3$, and $\epsilon 4$ alleles of the Apolipoprotein E (*APOE*) gene were determined by genotyping of SNPs rs429358 and rs7412. An *APOE* $\epsilon 4$ carrier was defined as having at least one $\epsilon 4$ allele (including $\epsilon 2/\epsilon 4$, $\epsilon 3/\epsilon 4$, and $\epsilon 4/\epsilon 4$). Genotyping of rs429358 and rs7412 was performed using the TaqMan genotyping assay (Applied Biosystems, Foster City, CA, USA). Polymerase chain reaction was performed in 96-well microplates with an ABI 7500 real-time PCR machine (Applied Biosystems). Allele discrimination was achieved by detecting fluorescence using System SDS software version 1.2.3 (Applied Biosystems).

Statistical analysis. Because the skewness and kurtosis values of plasma NFL measurements exceeded 1, we log-transformed these values prior to analysis. The Chi-square test, independent two-sample *t*-test, and one-way analysis of variance with post-hoc Tukey test were used to identify differences between control and patient groups in terms of demographic, clinical, and neuropsychiatric variables. One-way analysis of covariance was used to examine differences in plasma NFL levels between controls and patient groups after adjusting for age and sex, year of education and *APOE* $\epsilon 4$ carrier status. Pearson correlation analysis was used to assess the relationship between plasma NFL levels and age, as well as correlations between plasma NFL levels, MMSE score, and UPDRS. Independent *t*-test was used to examine the difference of NFL levels between *APOE* $\epsilon 4$ carriers and non-carriers as well as between stable MCI patient and MCI conversion to AD patient during follow-up. We used Cox regression model adjusting for age, sex, years of education, disease duration, and *APOE* $\epsilon 4$ carrier status to evaluate the association of NFL with MCI conversion to AD. All statistical analyses were performed with SPSS software, version 18.0 (IBM, Inc., Armonk, NY, USA), with $p < 0.05$ used to indicate statistical significance.

Ethics approval and consent to participate. Informed consent was obtained from all patients and their spouse or offspring who are legal representatives of the patient prior to participation in the study. This

	AD (n = 119)	MCI (n = 56)	PDND (n = 26)	PDD (n = 23)	HC (n = 59)	P-value
Male patients*	56 (47.1%)	27 (48.2%)	13 (50%)	14 (60.9%)	31 (52.5%)	0.79
Age, year	77.3 (5.1)	76.0 (5.6)	69.6 (10.8)	76.3 (9.1)	77.0 (6.2)	<0.001 ^a
Years of education	9.5 (4.8)	11.0 (3.7)	10.7 (5.5)	8.0 (5.1)	12.4 (5.0)	<0.001 ^b
Body mass index	24.0 (3.2)	23.6 (2.8)	25.2 (3.5)	24.6 (3.1)	24.4 (3.0)	0.22
APOE ϵ 4 carrier*	42 (35.3%)	13 (23.2%)	4 (17.4%)	3 (14.3%)	7 (12.1%)	0.007
Clinical dementia rating scale						
0.5	14	56	—	—	—	—
1.0	74	—	—	—	—	—
2.0	26	—	—	—	—	—
3.0	5	—	—	—	—	—
UPDRS	—	—	17.6 (10.1)	30.9 (19.5)	—	0.005
MMSE	18.6 (6.2)	26.4 (2.3)	26.9 (2.6)	23.3 (4.6)	27.8 (2.1)	<0.001 ^c
Delayed-recall	1.6 (2.2)	5.3 (2.5)	7.7 (1.5)	4.0 (2.6)	7.8 (2.2)	<0.001 ^d
Verbal fluency	6.8 (3.1)	10.4 (3.2)	12.7 (2.7)	9.6 (3.0)	12.3 (2.8)	<0.001 ^e
Forward Digit Span	8.7 (3.0)	9.7 (2.6)	10.8 (2.2)	8.6 (2.4)	10.9 (2.1)	<0.001 ^f
Backward Digit Span	3.8 (2.4)	5.8 (2.1)	6.2 (2.1)	3.9 (2.1)	6.2 (2.4)	<0.001 ^g
Boston naming test	11.5 (3.0)	14.0 (1.1)	13.9 (1.0)	13.0 (1.7)	14.3 (1.1)	<0.001 ^h

Table 1. Demographic data of study participants. AD, Alzheimer's disease; MCI, mild cognitive impairment; PDD, Parkinson's disease dementia; PDND, non-demented Parkinson's disease; HC, healthy controls; APOE, apolipoprotein E; MMSE, mini-mental state examination; UPDRS, Unified Parkinson's Disease Rating Scale. Data presented as mean and standard deviation in parentheses unless noted. *Presented as number of patients and percentage in parentheses. ^aPDND vs HC, $p < 0.001$; PDND vs MCI, $p < 0.001$; PDND vs PDD, $p = 0.04$; PDND vs AD, $p < 0.001$. ^bHC vs PDD, $p = 0.02$; HC vs AD, $p = 0.01$. ^cPDD vs HC, $p = 0.01$; PDD vs MCI, $p = 0.047$; PDD vs PDND, $p = 0.048$; PDD vs AD, $p < 0.001$; AD vs HC, $p < 0.001$; AD vs MCI, $p < 0.001$; AD vs PDND, $p < 0.001$. ^dMCI vs HC, $p < 0.001$; MCI vs PDD, $p < 0.001$; MCI vs AD, $p < 0.001$; PDND vs PDD, $p < 0.001$; PDND vs AD, $p < 0.001$; PDD vs AD, $p < 0.001$. ^eHC vs MCI, $p = 0.01$; HC vs PDD, $p = 0.003$; HC vs AD, $p < 0.001$; MCI vs PDND, $p = 0.016$; MCI vs AD, $p < 0.001$; PDND vs PDD, $p = 0.004$; PDND vs AD, $p < 0.001$; PDD vs AD, $p = 0.001$. ^fHC vs PDD, $p = 0.004$; HC vs AD, $p < 0.001$; PDND vs PDD, $p = 0.029$; PDND vs AD, $p = 0.003$. ^gHC vs PDD, $p = 0.001$; HC vs AD, $p < 0.001$; MCI vs PDD, $p = 0.006$; MCI vs AD, $p < 0.001$; PDND vs PDD, $p = 0.004$; PDND vs AD, $p < 0.001$. ^hHC vs AD, $p < 0.001$; MCI vs AD, $p < 0.001$; PDND vs AD, $p < 0.001$; PDD vs AD, $p = 0.02$.

research project was approved by the institutional review boards at Taipei Veterans General Hospital (IRB number 2012-05-033B).

Consent for publication. Written informed consent was obtained from the patient for publication of this research article. All authors have approved the manuscript for submission and gave consent for publication.

Results

Subjects and demographics. Demographic features of study participants are presented in Table 1. There were no significant differences across groups (HC, MCI, AD, PDND, and PDD) in terms of sex or BMI. Age differed significantly across groups, with post-hoc comparisons revealing younger age in participants with PDND compared to controls, as well as patients with MCI, AD, or PDD ($p < 0.001$, $p < 0.001$, $p < 0.001$, $p = 0.004$, respectively). Post-hoc analysis showed that patients with PDD or AD had years of education compared to controls in the post hoc analysis ($p = 0.002$ and 0.001 respectively). UPDRS motor scores differed significantly between PDND and PDD groups, with worse motor severity in participants with PDD compared to those with PDND ($p = 0.005$).

Neuropsychological evaluation. AD patients scored significantly worse than HC, MCI, and PDND subjects across all cognitive tests. Regarding PDD and AD, AD patients had significantly worse performances on MMSE, delayed recall, and category verbal fluency than did PDD patients (MMSE, $p < 0.001$; delayed recall, $p < 0.001$; verbal fluency, $p < 0.001$). MCI patients had worse performance on delayed recall and category verbal fluency, compared to HC ($p < 0.001$ and $p = 0.01$, respectively). PDD patients scored worse than PDND patients on MMSE, delayed recall, category verbal fluency, forward and backward digit span tests (MMSE, 0.048 ; delayed recall, < 0.001 ; category verbal fluency, 0.004 ; forward digit span, 0.029 ; backward digit span, 0.004).

Plasma NFL levels. Mean plasma NFL level was 32.9 ± 25.5 pg/ml in AD patients, 20.0 ± 7.3 pg/ml in MCI patients, 15.4 ± 9.9 pg/ml in PDND patients, 23.3 ± 10.8 pg/ml in PDD patients, and 17.8 ± 6.4 pg/ml in controls. Male patients had higher plasma NFL levels than female patients ($p = 0.03$, t -test). Plasma NFL level increased with age (Pearson $r = 0.427$, $p < 0.001$). This age-related effect on plasma NFL level was seen across all sub-groups. After adjusting for age, sex, years of education, and APOE ϵ 4 carrier status, AD patients had higher plasma NFL levels, compared with patients with HC, MCI, PDND (all $p < 0.001$), or PDD ($p = 0.047$) (Fig. 1 and Table 2).

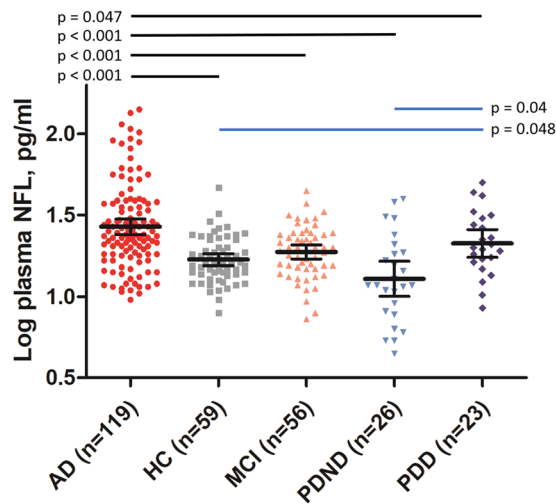


Figure 1. Comparison of plasma NFL levels among groups. Plasma NFL levels were higher in the AD group compared with controls, MCI group, PDND group, and PDD group. Plasma NFL levels were also higher in the PDD group, compared with controls and the PDND group. Abbreviations: NFL, neurofilament light chain; AD, Alzheimer's disease; MCI, mild cognitive impairment; PDND, non-demented Parkinson's disease; PDD, Parkinson's disease dementia.

Parameter	B	SE	t value	P value
Intercept	0.454	0.171	2.661	0.008
Age	0.013	0.002	6.591	<0.001
Sex	-0.039	0.026	-1.504	0.134
Years of education	0.003	0.003	1.068	0.286
<i>APOE</i> ϵ 4 carrier	0.027	0.029	0.918	0.359
Control	-0.201	0.034	-5.954	<0.001
MCI	-0.141	0.033	-4.254	<0.001
PDND	-0.225	0.048	-4.676	<0.001
PDD	-0.096	0.048	-1.996	0.047
AD	Ref			

Table 2. Results of one-way analysis of covariance in plasma NFL levels after adjusting age, sex, years of education, and *APOE* ϵ 4 carrier status. Dependent variable: plasma NFL level (log transformation). NFL, neurofilament light chain; *APOE*, apolipoprotein E; SE, standard error; MCI, mild cognitive impairment; PDND, non-demented Parkinson's disease; PDD, Parkinson's disease dementia; AD, Alzheimer's disease.

PDD patients had significantly higher plasma NFL levels, compared with PDND ($p = 0.04$) and control patients ($p = 0.048$). Plasma NFL levels were similar in MCI patients and PD patients, and also in MCI patients and PDND patients. When adjusting for age, sex, years of education, *APOE* ϵ 4 carrier status and MMSE scores, AD patients still had higher plasma NFL levels compared with patients with HC ($p = 0.0021$) and PDND ($p = 0.006$) (Table 3).

Plasma NFL level and MMSE score. Pooling all participants together, higher plasma NFL levels correlated with lower MMSE scores (Pearson $r = -0.491$, $p < 0.001$). In each diagnostic group, the correlation between plasma NFL level and MMSE score was strongly evident in AD patients (Pearson $r = -0.370$, $p < 0.001$), with a trend toward statistical significance in the MCI group (Pearson $r = -0.216$, $p = 0.055$). The correlation was also significant in all PD patients (Pearson $r = -0.323$, $p = 0.023$).

Plasma NFL levels and UPDRS. Plasma NFL level was not correlated with UPDRS, among PDND patients (Pearson $r = -0.069$, $p = 0.743$), PDD patients (Pearson $r = 0.056$, $p = 0.81$), or among all PD patients (Pearson $r = 0.177$, $p = 0.238$).

Plasma NFL level and *APOE* ϵ 4 carrier status. Across all groups, plasma NFL levels were similar between *APOE* ϵ 4 carriers and non-carriers (all participants, $p = 0.22$; AD group, $p = 0.97$, MCI group, $p = 0.60$; PDND group, $p = 0.21$; PDD group, $p = 0.95$; HC group, $p = 0.95$).

Parameter	B	SE	t value	P value
Intercept	0.877	0183	4.798	<0.001
Age	0.011	0.002	5.473	<0.001
Sex	-0.042	0.025	-1.694	0.091
Years of education	0.006	0.003	2.249	0.025
APOE ϵ 4 carrier	0.002	0.028	0.054	0.957
MMSE	-0.014	0.003	-5.085	<0.001
Control	-0.091	0.039	-2.329	0.021
MCI	-0.049	0.037	-1.309	0.192
PDND	-0.137	0.049	-2.776	0.006
PDD	-0.035	0.048	-0.739	0.461
AD	Ref			

Table 3. Results of one-way analysis of covariance in plasma NFL levels after adjusting age, sex, years of education, APOE ϵ 4 carrier status, and MMSE scores. Dependent variable: plasma NFL level (log transformation). NFL, neurofilament light chain; APOE, apolipoprotein E; SE, standard error; MMSE, Mini-mental status examination; MCI, mild cognitive impairment; PDND, non-demented Parkinson's disease; PDD, Parkinson's disease dementia; AD, Alzheimer's disease.

	HR	95% CI	P value
Age	1.04	0.87–1.24	0.69
Sex	1.29	0.28–5.89	0.74
Years of education	1.01	0.8–1.26	0.96
APOE ϵ 4 carrier status	0.97	0.12–7.66	0.98
Disease duration	1.01	0.97–1.05	0.56
NFL	1.67	0.003–1108.73	0.88

Table 4. Cox regression analysis of predictor for MCI conversion to AD. HR, hazard ratio; APOE, apolipoprotein E; NFL, neurofilament light chain.

Plasma NFL level and MCI conversion to AD. In MCI patients, we have followed 34 patients (34/45 = 60.7%), and the mean follow-up months is 19.5 ± 8 months. During follow-ups, 10 MCI patients have converted to AD. The plasma NFL levels were similar between the converter and non-converter in MCI patients ($p = 0.26$). The Cox regression model adjusting for age, sex, years of education, disease duration, and APOE ϵ 4 carrier status also showed no association between NFL levels and MCI conversion to AD (Table 4).

Discussion

Our study found higher plasma NFL in PDD, compared with PDND patients. This finding indicates that plasma NFL level is associated with the cognitive function in PD patients. Among all parkinsonian disorders, typical PD is associated with the lowest elevation in plasma NFL levels, compared to HC^{2,5}. Our results support this assertion. We also observed the greatest elevation in plasma NFL levels in patients with AD, compared to other patient groups. This finding is consistent with prior studies^{1,22} that showed elevated plasma NFL levels in AD, compared with MCI and HC. However, our results did not indicate that MCI patients had higher plasma NFL levels than HC. Analysis of the results collected for MCI and AD patients did not indicate that APOE ϵ 4 carriers had higher plasma NFL than non-carriers. These findings are different from previous studies which suggested MCI patients had higher plasma NFL levels than HC and APOE ϵ 4 carriers had higher plasma NFL than non-carriers¹, which may reflect the highly nuanced and complex clinical entity in MCI²³ and relatively low proportion of APOE ϵ 4 carriers among the Asian AD and MCI groups^{24–27}.

Compared with PDND patients, PDD patients had significantly higher levels of periventricular and deep white matter lesions (WMLs)²⁸. Previous studies have reported an association between cerebral WMLs and cognitive dysfunction²⁹. Among patients with WMLs, tasks that evaluate the speed of cognitive processes appear to be more affected by periventricular WMLs than are memory tasks²⁹. According to the consensus in the contemporary scientific literature, executive dysfunction is greater in PDD patients than in PDND patients. Disruption in the dorsolateral prefrontal circuit is known to result in executive dysfunction³⁰. The circuit consists of fibers that project from frontal cortex to deep structures in the basal ganglia. In PDD, in addition to degeneration of dopaminergic neurons in substantia nigra, periventricular WMLs may further interfere with executive function. Given that NFL may be a marker of white matter pathology³¹, PDD patients are expected to have higher plasma NFL levels than PDND patients. In addition to higher levels of periventricular and deep WML in PDD patients compared with PDND patients, the reduction of gray matter volume (GMV) over frontal-limbic-temporal regions might contribute to the higher NFL levels in PDD patients^{32,33}. Although the paper did not show the association between the WMLs and plasma NFL level in AD patients¹, some other papers reported that the NFL levels related

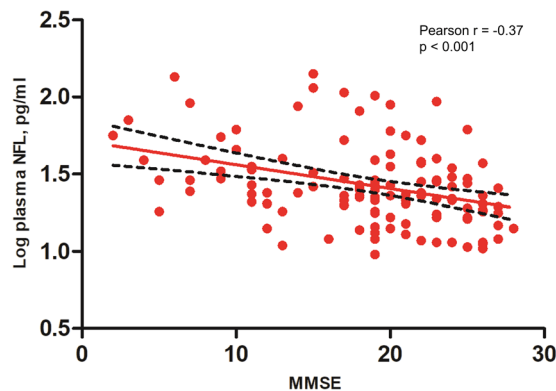


Figure 2. Correlation between plasma NFL and MMSE score in the AD group. Plasma NFL level correlates with MMSE score in the AD group (Pearson $r = -0.37$, $p < 0.001$). Abbreviations: NFL, neurofilament light chain; MMSE, mini-mental state examination; AD, Alzheimer's disease; PDD, Parkinson's disease dementia.

to WML³⁴. The reason increased NFL in cognitively impaired PD patients could be either increased WML or reduced GMV over frontal-limbic-temporal regions.

In the current study, we found that plasma NFL level was positively correlated with age, regardless of diagnostic status. This result corresponds to those reported previously^{35–37}. Nonetheless, the reason for this association between age and plasma NFL level remains to be elucidated. Presumably, it could be due to age-related atrophic changes or brain hypometabolism, leading to NFL and axonal degeneration. In the present study, plasma NFL level correlated with male gender. However, the relationship between plasma NFL level and sex has not been consistently observed. Mattson *et al.*¹ found no correlation, whereas Hansson *et al.*² reported that women have higher NFL levels than men. Additional studies are needed to clarify the relationship between sex and NFL levels.

When the overall patient population was considered, plasma NFL level showed a negative association with MMSE score (Fig. 2). Such an association was previously reported¹. In line with previous reports, the correlation was more significant in AD patients than in MCI and HC²². The reduced significance of the correlation between NFL levels and MMSE scores in the MCI group may reflect the limited range of MMSE scores allowed by the disease's clinical definition. In addition, plasma NFL level may not reflect subtle cognitive changes that are detected by the MMSE during progression of MCI. In PD patients, plasma NFL levels were also associated with MMSE scores but no correlation between NFL level and UPDRS score was observed. The results suggested that plasma NFL might be a biomarker for cognition in AD and PD patients but not a biomarker for motor symptoms in PD patients. In addition, our data showed that plasma NFL levels were still higher in AD group compared with HC and PDND groups after adjusting MMSE scores, which suggested plasma NFL was a more specific biomarker for AD than PD.

The current study has some limitations. First, diagnoses of AD and MCI were made using only clinical criteria, without the use of biomarkers of A β deposition or tau-mediated neuronal degeneration. This factor may have impeded diagnostic accuracy. It was not possible for us to analyze relationships among biomarkers of A β deposition, tau-mediated neuronal degeneration, and plasma NFL levels. Second, the relatively small sample size of each diagnostic group is a concern. We performed a cross-disease comparison of MCI, AD, PDND, and PDD, which may serve as supplemental evidence for future clinical use of plasma NFL as a diagnostic biomarker.

Conclusion

In conclusion, plasma NFL levels were significantly increased in the AD group, compared with controls and MCI, PDND, and PDD patients. Plasma NFL levels were significantly higher in the PDD group, compared with the PDND group. High plasma NFL correlated with poor cognition both in AD and PD patients, but not with motor symptoms in PD patients. Plasma NFL may represent a biomarker of cognitive decline in AD and PD, with greater specificity for AD. Future longitudinal follow-up studies are needed to validate whether plasma NFL levels may be used to predict progression of dementia in MCI and PD patients.

Availability of Data and Material

The datasets used and/or analyzed during the current study are available from the corresponding author upon request.

References

- Mattsson, N., Andreasson, U., Zetterberg, H., Blennow, K. & Alzheimer's Disease Neuroimaging, I. Association of Plasma Neurofilament Light With Neurodegeneration in Patients With Alzheimer Disease. *JAMA Neurol* **74**, 557–566, <https://doi.org/10.1001/jamaneurol.2016.6117> (2017).
- Hansson, O. *et al.* Blood-based NFL A biomarker for differential diagnosis of parkinsonian disorder. *Neurology* **88**, 930–937 (2017).
- Rohrer, J. D. *et al.* Serum neurofilament light chain protein is a measure of disease intensity in frontotemporal dementia. *Neurology* **87**, 1329–1336, <https://doi.org/10.1212/wnl.0000000000003154> (2016).
- Lu, C.-H. *et al.* Neurofilament light chain A prognostic biomarker in amyotrophic lateral sclerosis. *Neurology* **84**, 2247–2257 (2015).
- Constantinescu, R., Romer, M., Oakes, D., Rosengren, L. & Kiebertz, K. Levels of the light subunit of neurofilament triplet protein in cerebrospinal fluid in Huntington's disease. *Parkinsonism Relat. Disord.* **15**, 245–248 (2009).

6. Byrne, L. M. *et al.* Neurofilament light protein in blood as a potential biomarker of neurodegeneration in Huntington's disease: a retrospective cohort analysis. *The Lancet Neurology* **16**, 601–609 (2017).
7. Disanto, G. *et al.* Serum Neurofilament light: A biomarker of neuronal damage in multiple sclerosis. *Ann. Neurol.* (2017).
8. Bacioglu, M. *et al.* Neurofilament Light Chain in Blood and CSF as Marker of Disease Progression in Mouse Models and in Neurodegenerative Diseases. *Neuron* **91**, 56–66, <https://doi.org/10.1016/j.neuron.2016.05.018> (2016).
9. Kuhle, J. *et al.* Comparison of three analytical platforms for quantification of the neurofilament light chain in blood samples: ELISA, electrochemiluminescence immunoassay and Simoa. *Clin. Chem. Lab. Med.* **54**, 1655–1661, <https://doi.org/10.1515/cclm-2015-1195> (2016).
10. McKhann, G. M. *et al.* The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement* **7**, 263–269, <https://doi.org/10.1016/j.jalz.2011.03.005> (2011).
11. Winblad, B. *et al.* Mild cognitive impairment—beyond controversies, towards a consensus: report of the International Working Group on Mild Cognitive Impairment. *Journal of internal medicine* **256**, 240–246, <https://doi.org/10.1111/j.1365-2796.2004.01380.x> (2004).
12. Wechsler, D. Wechsler Memory Scale-III. San Antonio, Texas: The Psychological Corporation; 1998.
13. Gibb, W. R. & Lees, A. J. The relevance of the Lewy body to the pathogenesis of idiopathic Parkinson's disease. *Journal of neurology, neurosurgery, and psychiatry* **51**, 745–752 (1988).
14. Emre, M. *et al.* Clinical diagnostic criteria for dementia associated with Parkinson's disease. *Movement disorders: official journal of the Movement Disorder Society* **22**, 1689–1707; quiz 1837, <https://doi.org/10.1002/mds.21507>, (2007).
15. Folstein, M. F., Folstein, S. E. & McHugh, P. R. "Mini-mental state" A practical method for grading the cognitive state of patients for the clinician. *Journal of psychiatric research* **12**, 189–198 (1975).
16. Morris, J. C. The Clinical Dementia Rating (CDR): current version and scoring rules. *Neurology* **43**, 2412–2414 (1993).
17. Vanderploeg, R. D. *et al.* Elderly norms for the Hopkins Verbal Learning Test-Revised. *The Clinical neuropsychologist* **14**, 318–324, [https://doi.org/10.1076/1385-4046\(200008\)14:3;1-p;ft318](https://doi.org/10.1076/1385-4046(200008)14:3;1-p;ft318) (2000).
18. Mack, W. J., Freed, D. M., Williams, B. W. & Henderson, V. W. Boston Naming Test: shortened versions for use in Alzheimer's disease. *Journal of gerontology* **47**, P154–158 (1992).
19. Harrison, J. E., Buxton, P., Husain, M. & Wise, R. Short test of semantic and phonological fluency: normal performance, validity and test-retest reliability. *The British journal of clinical psychology* **39**(2), 181–191 (2000).
20. Wechsler, D. 1997. *Wechsler Memory Scale*, third ed. The Psychological Corporation, San Antonio, TX.
21. Goetz, C. G. *et al.* Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS): scale presentation and clinimetric testing results. *Movement disorders: official journal of the Movement Disorder Society* **23**, 2129–2170, <https://doi.org/10.1002/mds.22340> (2008).
22. Zhou, W. *et al.* Plasma neurofilament light chain levels in Alzheimer's disease. *Neurosci. Lett.* **650**, 60–64, <https://doi.org/10.1016/j.neulet.2017.04.027> (2017).
23. Libon, D. J. *et al.* The heterogeneity of mild cognitive impairment: A neuropsychological analysis. *J. Int. Neuropsychol. Soc.* **16**, 84–93 (2010).
24. Nishimura, A. *et al.* Characterization of APOE and TOMM40 allele frequencies in the Japanese population. *Alzheimer's & dementia (New York, N. Y.)* **3**, 524–530, <https://doi.org/10.1016/j.trci.2017.07.003> (2017).
25. Zheng, L. *et al.* Association of Apolipoprotein E (ApoE) Polymorphism with Alzheimer's Disease in Chinese Population. *Current Alzheimer research* **13**, 912–917 (2016).
26. Lee, W. J. *et al.* Plasma MCP-1 and Cognitive Decline in Patients with Alzheimer's Disease and Mild Cognitive Impairment: A Two-year Follow-up Study. *Scientific reports* **8**, 1280, <https://doi.org/10.1038/s41598-018-19807-y> (2018).
27. Chou, C. T., Liao, Y. C., Lee, W. J., Wang, S. J. & Fuh, J. L. SORL1 gene, plasma biomarkers, and the risk of Alzheimer's disease for the Han Chinese population in Taiwan. *Alzheimer's research & therapy* **8**, 53, <https://doi.org/10.1186/s13195-016-0222-x> (2016).
28. Bohnen, N. I. & Albin, R. L. White matter lesions in Parkinson disease. *Nature Reviews Neurology* **7**, 229 (2011).
29. Groot, J. C. D. *et al.* Cerebral white matter lesions and cognitive function: The Rotterdam scan study. *Ann. Neurol.* **47**, 145–151, [https://doi.org/10.1002/1531-8249\(200002\)47:2<145::AID-ANA3>3.0.CO;2-P](https://doi.org/10.1002/1531-8249(200002)47:2<145::AID-ANA3>3.0.CO;2-P) (2000).
30. Smith, E. E. *et al.* Correlations between MRI white matter lesion location and executive function and episodic memory. *Neurology* **76**, 1492–1499, <https://doi.org/10.1212/WNL.0b013e318217e7c8> (2011).
31. Mattsson, N. CSF biomarkers in neurodegenerative diseases. *Clin. Chem. Lab. Med.* **49**, 345–352 (2011).
32. Xu, Y., Yang, J., Hu, X. & Shang, H. Voxel-based meta-analysis of gray matter volume reductions associated with cognitive impairment in Parkinson's disease. *J. Neurol.* **263**, 1178–1187, <https://doi.org/10.1007/s00415-016-8122-3> (2016).
33. Lee, S. H. *et al.* Brain volumetry in Parkinson's disease with and without dementia: where are the differences? *Acta Radiol.* **54**, 581–586, <https://doi.org/10.1177/0284185113476029> (2013).
34. Osborn, K. E. *et al.* Cerebrospinal fluid beta-amyloid42 and neurofilament light relate to white matter hyperintensities. *Neurobiol. Aging* **68**, 18–25, <https://doi.org/10.1016/j.neurobiolaging.2018.03.028> (2018).
35. Gisslén, M. *et al.* Plasma Concentration of the Neurofilament Light Protein (NFL) is a Biomarker of CNS Injury in HIV Infection: A Cross-Sectional Study. *EBioMedicine* **3**, 135–140, <https://doi.org/10.1016/j.ebiom.2015.11.036>.
36. Vågberg, M. *et al.* Levels and Age Dependency of Neurofilament Light and Glial Fibrillary Acidic Protein in Healthy Individuals and Their Relation to the Brain Parenchymal Fraction. *PLoS One* **10**, e0135886, <https://doi.org/10.1371/journal.pone.0135886> (2015).
37. Idland, A.-V. *et al.* CSF neurofilament light levels predict hippocampal atrophy in cognitively healthy older adults. *Neurobiol. Aging* **49**, 138–144, <https://doi.org/10.1016/j.neurobiolaging.2016.09.012>.

Acknowledgements

This work was financially supported by the Brain Research Center, National Yang-Ming University from The Featured Areas Research Center Program within the framework of the Higher Education Sprout Project by the Ministry of Education (MOE) in Taiwan. The study was supported by grants from Academia Sinica of Taiwan (Taiwan Biobank: Biosignature Study of Alzheimer disease), the Ministry of Science and Technology of Taiwan (104-2314-B-075 -005 -MY2, 104-2745-B-075 -001 -, 107-2321-B-075 -001 -, 107-2221-E-075-006-), Taipei Veterans General Hospital (V107C-032, V105D9-001-MY2-2, V106E-006-1), the Brain Research Center, National Yang-Ming University, and the Ministry of Education Aim for the Top University Plan. The authors also thank the Taiwan Biobank and Academia Sinica of Taiwan for technical support for this work.

Author Contributions

Y.S.L.: analysis of the data and drafting of the manuscript. W.J.L. study design, acquisition of data, analysis and interpretation of the data, and critical revision of the manuscript. S.J.W.: study design, acquisition of data and obtaining funding. J.L.F.: study design, acquisition of data, analysis and interpretation of data, study coordination, obtaining funding and critical revision of the manuscript.

Additional Information

Competing Interests: The authors declare no competing interests.

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2018