Numerous clinical presentations of Parkinson’s disease (PD) are characterised by rigidity, bradykinesia, tremor and postural instability. While dopaminergic treatments provide symptomatic benefit for some of the motor symptoms of the disease, their long-term use is problematic. As the disease progresses, patients with PD deteriorate in their motor aspects and in cognitive function, which is one of the most disabling non-motor features of PD. Therefore, there is a currently unmet need to identify biomarkers that could identify these patients at earlier disease stages to better predict disease progression and possible early therapeutic interventions.

PD is characterised by the presence of Lewy bodies containing aggregated α-synuclein, which is also present in human body fluids including cerebrospinal fluid (CSF) and blood plasma. Although there was a significant increase in plasma α-synuclein levels in PD patients compared with controls (median: 1.56 pg/mL, 95% CI 1.02 to 1.98 pg/mL vs 0.02 pg/mL, 95% CI 0.01 to 0.03 pg/mL; p<0.001). However, there was a significant increase in plasma α-synuclein levels in PD patients with a higher Hoehn-Yahr (H-Y) stage, there was no correlation with motor symptom severity, as assessed by Unified Parkinson’s Disease Rating Scale part III scores, after confounders (age, gender, and disease duration) were taken into account. However, plasma α-synuclein levels were significantly higher in patients with dementia (PDD) than in PD patients with mild cognitive impairment (PD-MCI) or normal cognition (PDD-MCI) or normal cognition (0.42 pg/mL, 95% CI 0.25 to 0.93) for PD with normal cognition; 1.29 pg/mL (95% CI 0.76 to 1.93) for PD-MCI and 4.09 pg/mL (95% CI 1.99 to 6.19) for PDD, p<0.01) and were negatively correlated with Mini-Mental State Examination scores (R²-adjusted=0.3004, p<0.001), even after confounder adjustment.

Conclusions Our data suggest that plasma α-synuclein level correlates with cognitive decline but not motor severity in patients with PD. Plasma α-synuclein could serve as a surrogate biomarker for patients at risk of cognitive decline.
contributing to an ultra-high sensitivity when compared with traditional immunoassay. In this study, we aimed to utilise this IMR-based immunoassay to investigate plasma levels of α-synuclein in healthy control subjects and in patients with PD of varying motor and cognitive status. This would allow us to evaluate the plausibility of using this easily accessible plasma marker to predict both disease risk and severity of PD.

**METHODS**

**Study participants**

Patients with PD were recruited from the movement disorder clinics of the National Taiwan University Hospital, a tertiary referral centre in Taiwan. PD was diagnosed according to the United Kingdom PD Society Brain Bank clinical diagnostic criteria. We excluded subjects who had any of the following signs or symptoms, as these would suggest atypical or secondary parkinsonism syndromes: multiple system atrophy, progressive supranuclear palsy, corticobasal ganglionic degeneration, vascular parkinsonism and neuroleptic agent-related parkinsonism. None of the subjects had a parental consanguineous marriage. Muta-tions of previously reported PD causative genes were excluded, including SNCA, Parkin, PINK1, DJ-1, ATP13A2, PLA2G6, FBX07, LRRK2, HtrA2 and CHCHD2. Healthy control subjects, who were neurologically normal patients visiting our clinics, or spouses and accompanied friends of the PD patients, were recruited from the same institute. Our research protocols were reviewed by the institutional ethics board committee of the National Taiwan University Hospital, with all subjects having provided informed consent.

**Evaluation of motor and non-motor symptoms**

Motor symptom severity was evaluated using the motor subscale of the Unified Parkinson’s Disease Rating Scale (UPDRS part III) and Hoehn-and-Yahr (H-Y) staging. Cognitive evaluation was performed using the Mini-Mental State Examination (MMSE). PDD was diagnosed according to the recommended diagnostic criteria of the Movement Disorder Society (MDS) task force, with an MMSE score of 25 or less being the cut-off for identifying subjects with severe cognitive impairment. We excluded patients with severe dementia, defined as CDR scale more than 3, to make sure that the IMR assay is a sensitive and robust method to detect plasma α-synuclein level.

**Measurement of plasma α-synuclein**

Ten millilitres of venous blood was drawn from each participant at enrollment. Blood samples were centrifuged (2500 g for 15 min) within 1 hour of collection, and the plasma was aliquoted into cryotubes and stored at −80°C for less than 3 months before IMR testing. The reagent for assaying α-synuclein consists of magnetic Fe₃O₄ nanoparticles (MF-DEX-0060, MagQu) bio-functionalised with monoclonal antibodies (sc-12767, Santa Cruz Biotech) against monomers of α-synuclein. Eighty microlitres of reagent was mixed with 40 μL of plasma for the measurement of α-synuclein concentration via IMR. Details of the methodologies to immobilise antibodies onto magnetic Fe₃O₄ nanoparticles, to measure the magnetic concentration of the immunocomplex, and validation assay have been published previously. In brief, the reagent for IMR consists of magnetic nanoparticles functionalised with antibodies against α-synuclein was dispensed in pH 7.2 phosphate-buffered saline (MF-ASC-0060, MagQu). The reagent was superparamagnetic with a saturation magnetisation of 0.3 emu/g. After mixing the reagent and the tested plasma sample, each mixture was put into a superconducting-quantum-interference-device (SQUID)-based alternative current (ac) magnetosusceptometer (XacPro-S, MagQu) to determine the time-dependent ac magnetic susceptibility, which approximates the association between magnetic nanoparticles and α-synuclein molecules in the plasma. Because of the association between the antibody-functionalised magnetic nanoparticles and the target biomarkers, the ac magnetic susceptibility of the mixture was reduced. This reduction in the magnetic susceptibility due to the association between magnetic nanoparticle and α-synuclein molecule can be sensed by high-Tc SQUID magnetometer and is referred to as the IMR signal. The IMR signal is therefore a function of the concentration of α-synuclein. We previously have performed a standard curve analysis to examine the robustness of the IMR method to detect plasma α-synuclein. We tested the dynamic range of plasma α-synuclein level using the IMR signals, which were denoted by φ_{α-syn,IMR}. The correlation between φ_{α-syn,IMR} and the concentration of α-synuclein was examined. Duplicate measurements were performed for IMR signals at each concentration of α-synuclein solution. We observed that among the concentration range of α-synuclein between 0.3 fg/mL and 310 pg/mL, the slope of the correlation line is 0.93 and the coefficient of determination R² is 0.999, indicating that the IMR assay is a sensitive and robust method to detect plasma α-synuclein level.

**Statistical analyses**

Numerical variables were expressed as the mean±SD of the mean or median with 95% CI. For variables following a Gaussian distribution, data were compared using the two-tailed t-test, and multiple comparisons were performed using analysis of variance (ANOVA). To control for possible confounders (eg, age, gender and disease duration), ANOVA was used for group comparisons, including, as covariates, any demographic variable that differed significantly between groups. For variables not following a normal distribution, data was compared using the Mann-Whitney test, which is the non-parametric equivalent of the independent samples t-test, and Kruskal-Wallis test was used when there are more than two groups. Correlations between variables were graphically analysed using the slope of the regression line including the 95% CI. The correlation between variables was explored with multiple linear regression analyses, with the covariance of the model (R²) and the standardised correlation coefficient as the measures of association.
coefficient presented. We performed all analyses using the Stata software, version 9. A p value <0.05 was considered significant.

**RESULTS**

**Clinical characteristics**

A total of 114 study participants comprising 80 patients with PD and 34 normal control subjects were enrolled. Table 1 summarises the demographic and clinical information for all participants. While the mean age of the PD group was higher than that of the control (69.6±12.3 vs 61.9±9.5, p<0.01), there were no significant differences in either gender or education levels between the two groups. However, there was a larger proportion of male PD participants than male controls (52% PD; 48% controls). Patients with PD were classified into three groups according to their cognitive function: PD with normal cognition (defined as MMSE >25, n=30), PD-MCI (defined as MMSE >25 but ≤28, n=30) or PDD (defined as MMSE ≤25, n=29). Mean UPDRS part III scores and H-Y stages during the ‘on’ and ‘off’ state are summarised in Table 1.

**Plasma α-synuclein measurements**

Plasma levels of α-synuclein were significantly higher in patients with PD compared with controls (figure 1, median level of plasma α-synuclein: PD vs controls: 1.56 pg/mL (95% CI 1.02 to 1.98 pg/mL) vs 0.02 pg/mL (95% CI 0.01 to 0.03 pg/mL) (p<0.0001 by Mann-Whitney test). With regard to the possible influence of age on plasma concentrations of α-synuclein, we examined plasma α-synuclein levels in control subjects of varying age. Consistent with our previous data, we did not find any significant correlation between age and plasma α-synuclein levels in control subjects (correlation coefficient r=0.1602, p=0.195, online supplementary figure 1).

**Correlation between plasma α-synuclein levels and motor severity**

We next examined whether the plasma levels of α-synuclein correlated with disease severity, either in terms of motor or non-motor symptoms. For the motor symptom severity evaluation, we observed that plasma α-synuclein levels were higher in PD patients with a higher H-Y stage (figure 2). Specifically, the median plasma α-synuclein levels were 1.06 pg/mL (95% CI 0.52 to 1.62), 0.73 pg/mL (95% CI 0.36 to 1.84), 1.48 pg/mL (0.41–15.70) and 1.64 pg/mL (0.71–11.19) for H-Y stage I–IV, respectively (p<0.01 by Kruskal-Wallis test). However, the simultaneous inclusion of age, gender and disease duration in the multivariate linear regression model resulted in a significant correlation between motor symptom severity (as assessed by UPDRS part III scores) and disease duration only; no significant correlation was found between motor symptom severity and plasma levels of α-synuclein (table 2).

**Correlation between plasma α-synuclein levels and the severity of non-motor symptoms**

As cognitive decline is one of the most disturbing non-motor symptoms of PD, we then examined the relationship between plasma levels of α-synuclein and PD patients with various levels of cognitive ability. While there were no robust correlations between α-synuclein levels and MMSE or SCOPA-AUT data (data not shown), we observed that plasma α-synuclein levels were significantly higher in PD patients with more severe cognitive dysfunction than in PD patients with normal cognition (figure 3). The median plasma α-synuclein levels were 0.42 pg/mL (95% CI 0.25 to 0.93) for PD with normal cognition, 1.29 pg/mL (95% CI 0.76 to 1.93) for PD-MCI and 4.09 pg/mL (95% CI 1.99 to 6.19) for PDD, respectively (p<0.0001 by Kruskal-Wallis test). When simultaneously considering the effects of age, gender and disease duration in the multivariate linear regression model, plasma levels of α-synuclein were found to negatively correlate with MMSE scores (R²-adjusted=0.3004, p<0.001, table 3), suggesting that a higher plasma α-synuclein level was associated with a lower cognitive performance in patients with PD.

**DISCUSSION**

The results of this study demonstrate several major advances with regard to a plasma α-synuclein assay that exploits ultra-sensitive IMR analyses for patients with PD. Comparing patients with PD with healthy controls revealed PD-associated differences in the plasma levels of α-synuclein. We have, for the first time, demonstrated that plasma levels of α-synuclein are associated with motor dysfunction and cognitive decline in patients with PD. Additional analyses revealed a significant negative correlation between plasma α-synuclein levels and MMSE scores in patients...
Figure 1  Plasma α-synuclein levels for all participants in the study. Plasma α-synuclein levels of normal controls and patients with PD. The plasma α-synuclein level was significantly increased in patients with PD compared with normal control subjects (p<0.001). PD, Parkinson’s disease. *, p<0.05; **, p<0.01.
with PD after considering the confounders of age, gender and disease duration. Our observations suggest that plasma α-synuclein could serve as a surrogate biomarker for cognitive decline in PD patients, and our results may assist clinicians in identifying patients at risk of cognitive dysfunction in the future.

α-Synuclein is the pathognomonic protein associated with PD. Mutations or multiplications of the α-synuclein gene (SNCA) are known to be causative in some familial forms of PD, and genome-wide association studies have also confirmed that common variants of SNCA modulate the risk of sporadic PD. Experimental data have shown that α-synuclein would be released from neurons by exocytosis into body fluids, including the CSF and plasma, which contribute to cell-to-cell transmission of α-synuclein pathology in the brain.

Numerous studies have attempted to compare levels of total or oligomeric α-synuclein in CSF samples from patients with PD compared with healthy controls, although results, thus far, have been inconsistent. However, there is a trend that the level of total α-synuclein is, on average, lower in samples of CSF taken from patients with PD than in groups of normal controls.

### Table 2 Multivariate linear regression models for motor symptom severity in patients with PD as assessed by UPDRS part III scores

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>Coefficient</th>
<th>SE</th>
<th>r</th>
<th>t</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>-0.530</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disease duration (years)</td>
<td>0.361</td>
<td>0.177</td>
<td>0.303</td>
<td>2.038</td>
<td>0.048*</td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.173</td>
<td>0.107</td>
<td>0.246</td>
<td>1.625</td>
<td>0.112</td>
</tr>
<tr>
<td>Gender (male)</td>
<td>4.021</td>
<td>2.688</td>
<td>0.228</td>
<td>1.496</td>
<td>0.142</td>
</tr>
<tr>
<td>Plasma α-synuclein level</td>
<td>0.213</td>
<td>0.176</td>
<td>0.186</td>
<td>1.213</td>
<td>0.232</td>
</tr>
</tbody>
</table>

In this model, the motor score of UPDRS (part III) in the on state was set as the dependent variable, with age, gender, disease duration and plasma level of α-synuclein set as independent variables. r: correlation coefficient based on the model of multiple linear regression; t: t value for the coefficient of each parameter in the model; p: for r or t; R²-adjusted=0.1998, p=0.01 for the model. *: statistically significant, p<0.05; **: p<0.01. PD, Parkinson’s disease; UPDRS, Unified Parkinson’s Disease Rating Scale.

### Table 3 Multivariate linear regression models for cognitive symptom severity in patients with PD as assessed by MMSE scores

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>Coefficient</th>
<th>SE</th>
<th>r</th>
<th>t</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>39.526</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disease duration (years)</td>
<td>0.0612</td>
<td>0.095</td>
<td>0.097</td>
<td>0.650</td>
<td>0.519</td>
</tr>
<tr>
<td>Age (years)</td>
<td>-0.223</td>
<td>0.056</td>
<td>-0.512</td>
<td>-4.003</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Gender (male)</td>
<td>0.208</td>
<td>1.422</td>
<td>0.022</td>
<td>0.147</td>
<td>0.884</td>
</tr>
<tr>
<td>Plasma α-synuclein level</td>
<td>-0.226</td>
<td>0.096</td>
<td>-0.331</td>
<td>-2.351</td>
<td>0.023*</td>
</tr>
</tbody>
</table>

In this model, the MMSE score of patients with PD was set as the dependent variable, with age, gender, disease duration and plasma level of α-synuclein set as independent variables. r: correlation coefficient based on the model of multiple linear regression; t: t value for the coefficient of each parameter in the model; p: for r or t; R²-adjusted=0.3004, p=0.001 for the model. *: statistically significant, p<0.05; **: p<0.01. MMSE, Mini-Mental State Examination; PD, Parkinson’s disease.
large multicentre Parkinson’s Progression Markers Initiative study showed the decreased CSF \( \alpha \)-synuclein level in early-stage, drug-naive PD patients compared with healthy controls.12 With regard to the role of CSF \( \alpha \)-synuclein level in PD with cognitive decline, a recent large cohort study who participated in the deprenyl and tocopherol antioxidative therapy of parkinsonism (DATATOP) study, with an 8-year follow-up period, observed that higher CSF levels of total \( \alpha \)-synuclein predicted worse cognitive function in patients with PD.31 Similarly, another longitudinally follow-up study observed that higher CSF levels of total \( \alpha \)-synuclein were associated with progression of both motor symptoms and cognitive decline in PD over 2 years.13 The mechanisms why the concentrations of plasma \( \alpha \)-synuclein showed a biphasic pattern, decreased in early stage of PD compared with controls and then increased as disease severity deteriorated, remain elusive. One possible explanation is that the decreased level of CSF \( \alpha \)-synuclein in early-stage PD may be the result of cellular compensatory mechanisms for sequestering pathological soluble \( \alpha \)-synuclein and, therefore, resulting in lower level of CSF \( \alpha \)-synuclein compared with controls.33 As the disease severity increased with loss of cellular compensatory ability to sequester the toxic species of \( \alpha \)-synuclein with forming of Lewy bodies, the CSF levels of \( \alpha \)-synuclein increased.33 Since obtaining CSF is an invasive procedure, recent studies have instead focused on assessing plasma \( \alpha \)-synuclein levels as a potential surrogate biomarker from the much more accessible, peripheral blood. Few studies have shown an increased level of this protein in plasma samples from patients with PD versus healthy controls.34–36 Consistent with these observations, our results also demonstrate a significantly higher plasma \( \alpha \)-synuclein level in patients with PD compared with controls. One recent study that used an intracerebroventricular injection of radiolabelled \( \alpha \)-synuclein into mouse brain demonstrated that CSF \( \alpha \)-synuclein could be diverted to the blood via exosome efflux.37 Subsequent studies in patients with PD confirmed that the levels of plasma exosomal \( \alpha \)-synuclein were substantially higher in PD patients than in control subjects, suggesting an increased efflux of \( \alpha \)-synuclein to the peripheral blood in the disease state.33 Although we could not differentiate exosomal and non-exosomal \( \alpha \)-synuclein in the plasma by the IMR method used in the current study, the results of increased plasma exosomal \( \alpha \)-synuclein in patients with PD compared with controls34 are consistent with our findings. The compensatory increased clearance of excess, potentially toxic \( \alpha \)-synuclein species from the PD brain to the peripheral blood through exosomes release into the extracellular environment may partly contribute to the increased plasma levels of \( \alpha \)-synuclein. Although further studies are clearly needed to elucidate the mechanism involved in the transport of \( \alpha \)-synuclein from the central nervous system to the peripheral blood, our results suggest that plasma levels of \( \alpha \)-synuclein would be a feasible biomarker with which to predict disease progression, as this marker partly reflects the escalation of \( \alpha \)-synuclein pathology in the brains of patients with PD.

Our study also observed that plasma \( \alpha \)-synuclein levels were associated with motor severity and cognitive dysfunction in patients with PD. The correlations between \( \alpha \)-synuclein levels and motor severity (as assessed by UPDRS part III scores) and non-motor severity (as assessed by NMSS scores) were, however, found to be insignificant after confounders (age, gender and disease duration) were taken into account. However, we found that levels of plasma \( \alpha \)-synuclein negatively correlated with MMSE scores, even after considering confounder effects. These data suggest that higher levels of plasma \( \alpha \)-synuclein are associated with poor cognitive performance in patients with PD. Few studies have examined the relationships between plasma \( \alpha \)-synuclein levels and cognitive function in patients with PD. Cortical Lewy body/neuritic pathology is more extensive in PDD than in PD without dementia, which implies that the \( \alpha \)-synuclein burden in plasma is more severe in PDD than in PD with normal cognition. Our results support this hypothesis, in that plasma levels of \( \alpha \)-synuclein were significantly higher in patients with PDD than in PD-MCI and PD patients with normal cognition. Consistent with our findings, recent technically more advanced analyses show that PDD patients express a greater quantity of the oligomeric forms of \( \alpha \)-synuclein than PD patients without dementia.37 Furthermore, a higher CSF total \( \alpha \)-synuclein concentration was associated with a faster decline in cognitive performance in de novo patients with PD, with no correlation with motor progression.33 In addition to a recent study showing that a specific genetic haplotype in intron 4 of SNCA is associated with a risk of PDD,38 our results suggest that \( \alpha \)-synuclein may serve as a plasma biomarker for cognitive decline in PD. However, as plaques of amyloid-\( \beta \) and tau neurofibrillary tangles, the hallmark pathologies of Alzheimer’s dementia, are also observed, and correlate with cognitive status in patients with PDD,39 future longitudinal follow-up studies concomitantly incorporating assessing \( \alpha \)-synuclein, amyloid-\( \beta \) protein, total and phospho-tau in plasma levels of PDD are needed to better understand the pathophysiology of cognitive deterioration in PD.

In our study, a negative, but important observation, is that although plasma \( \alpha \)-synuclein levels increase as motor symptoms progress (figure 2), their values did not correlate with UPDRS part III scores after considering confounders (age, gender and disease duration (table 2)). This observation suggests that, in contrast to PD-related cognitive decline, there is not a straightforward relationship between plasma \( \alpha \)-synuclein and motor symptoms. Similar findings have been observed in previous cross-sectional investigations that examined patients’ levels of \( \alpha \)-synuclein in CSF.39 40 One possible explanation is that the UPDRS part III scores primarily reflect deficits arising from nigrostriatal degeneration, whereas plasma or CSF \( \alpha \)-synuclein levels are influenced by whole brain \( \alpha \)-synuclein loading. However, the reason why plasma \( \alpha \)-synuclein level correlates cognitive decline but not motor symptoms severity remains unclear. Further studies are warranted to decipher the pathophysiology behind motor and cognitive symptoms of PD.

The major advantage of this study is the use of the IMR-based method to detect plasma levels of \( \alpha \)-synuclein, as well as enrolling control subjects, and PD patients with varying levels of disease severity. In plasma samples, heterophilic antibodies commonly interfere with immunoassays and are major confounders when assaying low-level target proteins using the traditional ELISA method.41 Previous studies have shown that IMR manifests a low-interference and high-specificity for detecting target proteins comparing with ELISA.13 15 When IMR signals are measured, the magnetic particles oscillate, which establishes centrifugal forces. Should these forces exceed the binding forces that couple an antibody to a non-specific target, selective removal of weakly bound antibody/antigen targets will occur. This selection diminishes the interference effects of both heterophile antibody action, and naturally occurring plasma biomolecules derived from frequently used drugs that can bind to magnetic nanoparticles.13 These characteristics feature IMR a novel and sensitive assay to detect plasma level of \( \alpha \)-synuclein. However, our study has some limitations. First, we only assessed cognitive function using MMSE, a simple measurement for global cognitive function. Detailed neuropsychological tests evaluating individual cognitive domains are warranted for further assessing the correlation...
between plasma α-synuclein level and individual cognitive domain decline in patients with PD. Second, the relatively small number of enrolled subjects and the cross-sectional design of this study may limit the extent to which our data can be extrapolated to all patients with PD. Future, large cohort studies with a long follow-up period are needed to validate our results. Third, the mean age of control subjects was younger than the mean age of patients with PD in the current study. Although we did not find any significant correlation between age and plasma α-synuclein levels in control subjects of varying age (online supplementary figure 1), further studies with age-matched control subjects are needed. Finally, we did not check plasma levels of amyloid-β and tau in this study. As plaques of amyloid-β and tau neurofibrillary tangles are also observed in the postmortem findings of patients with PDD,11 future longitudinal follow-up studies concomitantly incorporating assessing α-synuclein, amyloid-β protein, total and phospho-tau in plasma levels of patients with cognitive decline are warranted to have a better understanding of the pathophysiology of PDD.

In summary, our findings suggest that plasma levels of α-synuclein could serve as a non-invasive biomarker for PD risk and severity. Furthermore, plasma α-synuclein levels, which correlated with cognitive decline in patients with PD, may help clinicians to identify patients at risk of cognitive impairment in the future.

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Correction notice Since this notice was first published online figure 1 has been updated.

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REFERENCES


