Published in final edited form as:

Neurobiol Dis. 2018 August ; 116: 53–59. doi:10.1016/j.nbd.2018.04.015.

Plasma α -synuclein and cognitive impairment in the Parkinson's Associated Risk Syndrome: A pilot study

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Abstract

Plasma total and nervous system derived exosomal (NDE) α -synuclein have been determined as potential biomarkers of Parkinson's disease (PD). To explore the utility of plasma α -synuclein in the prodromal phase of PD, plasma total and NDE α -synuclein were evaluated in baseline and 2-year follow-up samples from 256 individuals recruited as part of the Parkinson's Associated Risk Syndrome (PARS) study. The results demonstrated that baseline and longitudinal increases in total α -synuclein predicted progression of cognitive decline in hyposmic individuals with dopamine transporter (DAT) binding reduction. On the other hand, a longitudinal decrease in NDE α -synuclein predicted worsening cognitive scores in hyposmic individuals with DAT binding reduction. Finally, in individuals with faster DAT progression, decreasing NDE/total α -synuclein ratio was associated with a larger reduction in DAT from baseline to follow-up. These results suggest that, though underlying mechanisms remain to be defined, alterations in plasma total and NDE α -synuclein concentrations are likely associated with PD progression, especially in the aspect of cognitive impairment, at early stages of the disease.

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Financial disclosure/conflict of interest

All authors report no disclosure relevant to the manuscript.

Keywords

Plasma; α -Synuclein; Parkinson's; Prodromal; Risk

1. Introduction

Parkinson's disease (PD), a progressive neurodegenerative disease, has a prodromal phase, which precedes the onset of classic parkinsonism (de Lau et al., 2006; Gonera et al., 1997). Development of novel tools to evaluate disease progression at the prodromal stage, which can include motor or nonmotor symptoms, could benefit patients, particularly in the use of potential disease-modifying interventions, which would likely be most efficacious when started in the earliest stages of the disease (Athauda et al., 2015). Clinically, a sense of smell that is impaired (hyposmia) or absent (anosmia) could be a useful marker of premotor PD, as significant loss of olfactory function has been reported in PD patients and the extent of smell loss is independent of disease duration, disease stage or medication (Doty et al., 1988). However, the loss of olfactory function is neither sensitive nor specific enough to be used as a sole biomarker for PD, as a subset of PD patients do not suffer from loss of olfactory function, and it can also occur in other settings such as brain trauma and Alzheimer's disease (AD). Thus, the utility of hyposmia/anosmia as a biomarker might be improved when used in combination with additional specific imaging or biochemical biomarkers.

Although dopamine transporter (DAT) imaging reveals nigrostriatal dopaminergic dysfunction, like many imaging based screening tests, it is associated with a number of limitations, making the development of a more convenient biochemical or molecular marker in body fluids attractive. Among the candidate biomarkers that have been tested, α -synuclein (α -syn) is considered the most promising, based on its status as the primary component of Lewy bodies and its implication in the pathogenesis of PD, including early-onset familial PD, caused by duplication, triplication (Miller et al., 2004) or point mutations in the gene that encodes it (SNCA) (Ahn et al., 2008; Polymeropoulos et al., 1997). In addition, several large studies have shown that CSF total α -syn is lower in patients with PD compared to controls, when controlling for hemoglobin contamination (Atik et al., 2016; Hall et al., 2012; Hong et al., 2010; Kang et al., 2013; Mollenhauer et al., 2011; Mollenhauer et al., 2013; Parnetti et al., 2011; Parnetti et al., 2014; Tokuda et al., 2006). Although blood (plasma/serum) is more readily accessible, thus preferable for screening, changes in α -syn concentration in blood have been less consistent (Atik et al., 2016; Duran et al., 2010; El-Agnaf et al., 2006; Foulds et al., 2013, Foulds et al., 2011; Lee et al., 2006; Li et al., 2007; Shi et al., 2010) in part due to the abundance of α -syn in red blood cells and platelets that can influence plasma or serum α -syn level substantially. More recently, it has been demonstrated that α -syn in a population of relatively central nervous system-specific exosomes isolated from plasma is higher in PD patients compared to controls. Moreover, the diagnostic sensitivity and specificity of plasma nervous system derived exosomal (NDE) α -syn were superior to that of whole plasma, comparable to those of CSF α -syn, and NDE α -syn was associated with PD severity in the cross-sectional study (Shi et al., 2014). However, the previous study considered individuals already diagnosed with PD, and it remains unclear

if plasma total or NDE α -syn may be useful as a biomarker during the prodromal phase of PD.

The purpose of this study was to determine the use of plasma total and NDE α -syn as biomarkers in individuals identified as at risk of PD based on olfactory status and DAT imaging as part of the Parkinson's Associated Risk Syndrome (PARS) study. With controlling for several key variables, total and NDE plasma α -syn were measured, and their use as potential biomarkers in monitoring the severity of motor and cognitive symptoms was examined both at baseline and longitudinally.

2. Materials and Methods

2.1. Study design and subjects

PARS, a multicenter study conducted in the United States, was prompted by the need for better diagnostic tools for the prodromal stage of PD. Its purpose is to better understand individuals who may be at risk of PD, by evaluating specific tests in order to determine whether these tests can detect early signs of PD. PARS subjects were recruited using a two-stage screening strategy, with the objective of identifying those with olfactory and imaging signs of being at elevated risk of PD, suggesting they may be in the prodromal stage at the time of recruitment.

Procedures were approved by the Western Institutional Review Board, the Human Research Protection Office at the US Army Medical Research Material and Command, and the local institutional review boards at participating centers. Written consent was obtained from all subjects under the supervision of institutional review boards of the study sites.

Using recruitment and assessment methods described previously (Jennings et al., 2014; Siderowf et al., 2012), individuals were recruited by 16 movement disorder clinics. Briefly, PD patients from participating movement disorder clinics were asked to recruit first-degree relatives who were required to complete a demographic form and brief questionnaire to determine eligibility. Additionally, mailings to clinics and posting on the PARS website or other media were used to target individuals with or without a family history of PD. The inclusion criteria were (1) no diagnosis of PD or other neurodegenerative disorder, (2) age older than 50 years (or within 10 years of the age of onset of an affected PD relative) and (3) no known reason for abnormal olfaction such as nasal trauma, sinus infection or sinus surgery.

263 participants (87 normosmic and 176 hyposmic) who completed the baseline clinical and imaging evaluations for the PARS study were included in the current study, 7 normosmic subjects with DAT deficits were excluded from the analyses (see Table 1).

2.2. Self-assessment: olfactory screening and self-report questionnaires

Self-administration assessments as well as informed consent forms were mailed to eligible subjects, which included assessment of non-motor and subtle motor symptoms and a 40-item University of Pennsylvania Smell Identification Test (UPSIT) (Jennings et al., 2014). Subjects who scored 15th percentile based on age, sex and cohort specific norms

(developed based on the first 2,200 subjects enrolled) were classified as hyposmic and those in the >15th percentile were classified as normosmic.

2.3. Imaging and Clinical Evaluation

Subjects identified as hyposmic were invited to participate in the imaging and clinical assessments. Hyposmic subjects were matched with normosmic subjects by age and sex at a 2:1 ratio. Consenting subjects underwent DAT imaging using [¹²³I]-fluoro-propyl-beta-carbomethoxy-3beta-(4-idophenyl) tropane ([¹²³I]β-CIT) single-photon emission computed tomography (SPECT), performed at a single imaging center (Institute for Neurodegenerative Disorders), as described previously (Seibyl et al., 1995), at baseline and two follow-up time points, 2 years and 4 years. SPECT image analysis included evaluation of the striatal binding ratio using a standardized region-of-interest analysis method (Seibyl et al., 1998). As DAT striatal binding declines with age (van Dyck et al., 2002), imaging outcome was adjusted by determining the percent of age-expected [¹²³I] β-CIT binding in the lowest putamen compared with a previously acquired database of healthy subjects (van Dyck et al., 2002; Jennings et al., 2004). Scans were then categorized as DAT deficit (< 65% age-expected lowest putamen [¹²³I] β-CIT binding), indeterminate (65–80% age-expected lowest putamen [¹²³I] β-CIT binding), or no DAT deficit (>80% age-expected lowest putamen [¹²³I] β-CIT binding) (van Dyck et al., 2002; Jennings et al., 2004).

Cognitive performance and other clinical measurements including the Unified Parkinson's Disease Rating scale (UPDRS; total and motor), Mini-Mental Status Examination (MMSE), the Montreal Cognitive Assessment (MoCA) and Hoehn and Yahr (H&Y) scales were also performed at baseline and 2 years and then at 4 years (UPDRS, MMSE and H&Y only) by investigators unaware of olfactory status and DAT imaging data.

2.4. Sample collection

Whole blood was collected (Institute for Neurodegenerative Disorders, New Haven, CT, USA) in EDTA-coated tubes and centrifuged at 1500 x g for 15 minutes (4°C). Plasma was then transferred to sterile polypropylene tubes on ice and stored at –70°C and sent to the Movement Disorders Centre at the University of Pennsylvania, PA, USA, for further processing. Once received, samples were immediately moved to –80°C until aliquoted. Prior to aliquoting, samples were thawed at room temperature and aliquoted into volumes of 0.5mL and stored at –80°C prior to shipment to University of Washington for analysis. Before analysis, all samples were thawed, treated with protease inhibitor cocktail (Sigma, St Louis, MO, USA) and further aliquoted.

2.5. Biofluid assays

α-Syn was measured in plasma exosomes derived from the CNS using methods published previously (Shi et al., 2014). Briefly, exosomes were isolated from plasma using anti-L1CAM (a putative CNS specific marker) antibody-coated superparamagnetic microbeads. Anti-L1CAM antibodies (clone UJ127, Abcam, Cambridge, MA, USA) or normal mouse IgGs (Santa Cruz Biotechnology, Dallas, TX, USA) as negative controls, were coated onto M-270 Epoxy beads using a Dynabeads® Antibody Coupling Kit (Life Technologies, Grand Island, NY, US) according to the manufacturer's instructions. Plasma samples diluted with

phosphate buffered saline (PBS; pH 7.4) were incubated with antibody-coated beads for ~24 h at 4°C with gentle rotation. Beads were washed with 0.1% bovine serum albumin (BSA)/PBS (pH 7.4), transferred to a new tube and exosomes lysed by incubating the beads in 1% Triton X-100 plus 10% of a protease inhibitor cocktail (prepared in 10 mL of H₂O) in 0.1% BSA/PBS (pH 7.4) for 1 h at room temperature with gentle shaking. Exosomes were extracted in batches with two reference plasma samples added to each batch in order to control batch variations.

Exosomal preparations were frozen at -80°C until immediately before measurement of α -syn levels by Luminex assays (Luminex, Austin, TX), according to our previously published protocol (Hong et al., 2010). Total α -syn concentrations in plasma were also measured by Luminex assays as previously described (Shi et al., 2010). All samples were evaluated using a Luminex 200 Workstation (Qiagen, Hilden, Germany). Hemoglobin (HGB) was measured in all samples by ELISA (Bethyl Lab, Inc., Montgomery, TX), according to the manufacturer's instructions, to establish an index of the degree of red blood cell contamination or hemolysis. Soluble P-Selectin (sP-Selectin) levels were also measured using a Human sP-Selectin/CD62P ELISA Quantitation Kit from R&D Systems (Minneapolis, MN, USA) according to the manufacturer's instructions, as an index of the degree of platelet contamination, on the basis of our previous findings that total α -syn correlates strongly with HGB and sP-Selectin in plasma (Shi et al., 2010). Reference plasma samples were included in each exosome isolation batch and each immunoassay run/plate to control for assay variations. The average inter-batch coefficient of variation (CV) for plasma exosomal preparations was ~20%, and the average inter-run CV for α -syn Luminex immunoassay was <10% in this study. In all assays, assay operators were blind to the status or time point of samples and samples randomized to ensure all groups were equally represented on each assay plate.

2.6. Statistical analysis

All statistical analyses were performed using IBM SPSS version 18 (Armonk, NY). Plasma α -syn, HGB and sP-Selectin levels were log(10) transformed to compensate for non-normal distribution of the raw measurements. Univariate analysis of variance (UNIANOVA) was used to compare groups with respect to α -syn, HGB, sP-Selectin, UPDRS and cognitive scores, with controlling for potential confounding factors such as age, sex, sP-Selectin and HGB (α -syn results only) at baseline and then at follow-up (year 2). Longitudinal changes in α -syn, UPDRS and cognitive scores (baseline to 2 years) were assessed by general linear model (GLM) repeated measures with adjusting for age and sex for cognitive scores and age, sex, sP-Selectin and HGB for α -syn levels. To investigate whether baseline plasma NDE, total α -syn, or a ratio of the two measures could predict PD progression, with the outcome being assessment at 2 and 4 years in hyposmic individuals, as measured by UPDRS or cognitive scores, a linear regression model (Pepe et al., 2013) was implemented controlling for age, sex, sP-Selectin, HGB and baseline cognitive scores. Similarly, whether the change in α -syn (exosomal, total and ratio) from baseline to 2 years could predict PD progression, with the outcome being assessment at 4 years in hyposmic individuals, as measured by UPDRS or cognitive scores, a linear regression model was implemented controlling for age,

sex, sP-Selectin, HGB and change in cognitive score from baseline to 2 years. Results with P values <0.05 were regarded as significant.

3. Results

3.1. Demographic and cross-sectional analysis at baseline and 2 years

Among the 263 subjects who completed the baseline clinical and imaging evaluations for the PARS study, 7 normosmic subjects with DAT deficits were excluded, 80 were in the normosmia/–DAT reduction group (DAT $>80\%$), 133 were in the hyposmia/–DAT reduction group (DAT $>80\%$) and 43 were in the hyposmia/+DAT reduction group (DAT $<65\%$ or $65\text{--}80\%$). All of these 256 subjects had follow-up data (DAT, UPDRS, MMSE, MoCA and H&Y) up to 2 years, 172 of which had follow-up data (DAT, UPDRS, MMSE and H&Y) up to 4 years. Of the 256 subjects recruited for the study, 17 subjects from the hyposmia/+DAT group and 6 subjects from the hyposmia/–DAT group converted to PD by 6 years. Clinical and demographic data, as well as plasma α -syn (exosomal, total and ratio) levels, at baseline and 2 years are presented in Table 1.

Cross-sectionally, UPDRS (total and motor) was significantly higher in subjects with hyposmia and DAT reduction at both baseline and 2 years (Table 1), while much lower than in cohorts consisting of subjects with established PD (e.g., in our typical PD cohort, UPDRS motor scores averaged 28.4 ± 12.6 , H&Y averaged 2.4 ± 0.7 , and MMSE averaged 28.0 ± 2.6) (Shi et al., 2014). Longitudinally, UPDRS (total and motor) increased from baseline to 2 years in all groups (GLM repeated measures interaction of diagnosis with follow-up time; $p < 0.05$), but more quickly in the hyposmia/+DAT reduction group. There were no significant differences in other cognitive scores at baseline, whereas H&Y score was obviously increased in the hyposmia/+DAT reduction group at 2 years. Baseline total α -syn (log-transformed for normal distribution) was significantly higher in hyposmic groups, with DAT category having no significant effect, when controlling for HGB (an index of hemolysis or contamination of red blood cells) and sP-Selectin (an index of platelet contamination) (Table 1). Longitudinally, total α -syn was significantly different between all groups (GLM repeated measures, diagnosis effect, $p = 0.008$); however, the difference between groups became less significant with follow-up time ($p = 0.05$). There were no major cross-sectional differences between groups in NDE α -syn and the NDE to total α -syn ratio at both time points.

3.2. Predictive value of baseline plasma α -syn with longitudinal clinical scores in hyposmic individuals

To account for the important influence of DAT status in PD, the hyposmic subjects were grouped by DAT status (–DAT, $>80\%$; +DAT, $<80\%$). Analyzing the predictive values of plasma α -syn, with controlling for age, sex, HGB, sP-Selectin and baseline scores, we found that higher total α -syn was predictive of a lower MoCA score at 2 years in the hyposmia/+DAT reduction group (interaction coefficient, -8.50 ± 2.98 , $p = 0.008$; Figure 1A). Additionally, higher baseline NDE α -syn was associated with better MoCA score at 2 years in the hyposmia/–DAT reduction group (interaction coefficient, 1.71 ± 0.76 , $p = 0.025$; Figure 1B). No significant associations were observed between baseline plasma α -syn and other motor or cognitive scores at 2 years (e.g., baseline total α -syn vs MMSE at 2 years,

$p=0.35, 0.97$, -DAT and +DAT, respectively; baseline NDE α -syn vs MMSE at 2 years, $p=0.31, 0.92$, -DAT and +DAT, respectively) or any score at 4 years in either of the two groups.

3.3. Predictive value of longitudinal change in plasma α -syn with 4 year UPDRS and cognitive scores in hyposmic individuals

To determine whether these variables in individuals further affect the relationship between α -syn and clinical outcomes, the longitudinal change of plasma α -syn levels was included in the analytical model, with controlling for age, sex, HGB, sP-Selectin and longitudinal change in scores. In the hyposmia/+DAT reduction group the longitudinal change in total α -syn from baseline to 2 years predicted total UPDRS score at 4 years, with a larger increase in total α -syn associated with worse UPDRS score (interaction coefficient, 29.2 ± 12.3 , $p = 0.024$; Figure 2A). Moreover, in this group the longitudinal change in NDE α -syn, specifically a larger decrease (or a smaller increase) in plasma NDE α -syn is associated with worse MMSE score (4.73 ± 2.03 , $p = 0.026$; Figure 2B). Note that MoCA scores are not available at 4 years and the relationships cannot be assessed. In the hyposmia/-DAT group, however, the longitudinal change in plasma total or NDE α -syn in 2 years did not predict UPDRS or cognitive scores at 4 years.

3.4. Association of plasma α -syn with change in DAT from baseline to 2 years

Considering rapid progression in DAT score indicates the severity of the disease, we also assessed the predictive value of plasma α -syn on changes in DAT score. The whole hyposmic group was separated by whether their DAT was reduced from baseline to 2 years (progressors) or not reduced from baseline to 2 years (non-progressors). The progressors were further divided in quartiles ($n=27-29$ per group) based on the severity of their DAT change. Quartile 1 had a mean DAT change of -0.16 ± 0.01 , quartile 2, -0.37 ± 0.01 , quartile 3, -0.64 ± 0.02 and quartile 4, -1.15 ± 0.04 . In quartile 4, a larger decrease in the change in the NDE to total α -syn ratio was associated with a larger reduction in DAT from baseline to 2 years (interaction coefficient, 0.61 ± 0.18 , $p = 0.003$; Figure 3A-C). In other quartiles, no correlations were found between DAT progression and plasma α -syn.

4. Discussion

There is still an unmet need for better understanding of early, pre-motor changes in PD and the use of these changes as better predictive tools for the prodromal stage of PD. Among the PARS subjects, it appears that use of both smell and DAT imaging deficits could identify a cohort enriched with individuals presumed to be at risk of PD. Indeed, in this study we found that at both baseline and 2 years, total and motor UPDRS worsened in hyposmic subjects, particularly those with reduced DAT. This is consistent with previous findings that cognitive performance was worse in the group of individuals at risk of PD due to the presence of hyposmia and DAT reduction (Chahine et al., 2016). In addition to these early motor and cognitive changes, in this study we further demonstrated that changes in plasma α -syn levels might also be an early sign and plasma total and NDE α -syn could potentially predict progression of cognitive decline and motor dysfunction in these presumed pre-motor PD subjects.

When cognitive differences were considered, deficits in the PARS subjects were most pronounced on the global cognitive measure, specifically executive abilities, suggesting that these cognitive abnormalities are part of the prodromal stage of PD, particularly when none of the participants in any group met the criteria for PD diagnosis (Chahine et al., 2016). Notably, clinical scores for both cognitive and movement tests were mostly in typical control ranges at both baseline and year 2, similar to other cohorts of preclinical PD (Stewart T et al., 2015). Thus, while the groups differed from each other, and demonstrated signs of progression, even the later stages likely reflect preclinical disease stages in the majority of cases (i.e., non-converting subjects), and underlying PD pathology needs to be further confirmed.

Another major observation is that both baseline and longitudinal increases in total plasma α -syn predicted progression of cognitive decline, as indicated by both MoCA and total UPDRS score respectively, in individuals presenting with hyposmia and DAT reduction, a group likely to develop PD. In contrast, a longitudinal decrease in plasma NDE α -syn in individuals identified as hyposmic was predictive of worsening MMSE score in the hyposmia/+DAT reduction group. MMSE is commonly used to assess global cognitive impairment, but MoCA may be more sensitive to discern some subtle cognitive deficits in patients with PD. While the cause of cognitive dysfunction in the PARS cohort is unclear (Chahine et al., 2016), and whether α -syn plays a direct role in the development of this cognitive impairment is unknown, in this study we see an association of α -syn and clinical scores (MoCA or MMSE) in individuals diagnosed as hyposmic with DAT reduction. This suggests that both the levels of α -syn and DAT may be associated with PD within this group. Indeed an association between DAT binding and cognitive function has been demonstrated previously (Erixon-Lindroth et al., 2005; Ravina et al., 2012), where lower DAT binding has been shown to be associated with global cognitive decline.

To further investigate the possible association of variable plasma α -syn and DAT score with PD, in this study, we identified hyposmic individuals whose DAT scores decreased longitudinally, and further subdivided this group into quartiles based on their DAT change. Interestingly, we found that in individuals with faster DAT progression, specifically those in the fourth quartile, progressively decreasing NDE to total α -syn ratio, was associated with larger reductions in DAT from baseline to 2 years. This altered ratio seems to be driven largely by a change in NDE α -syn. Taking into account that plasma α -syn was also associated with cognitive scores, in this study it appears that more plasma NDE α -syn is associated with better clinical outcome, while an increase in total α -syn indicates a worse clinical outcome. It is possible that increased NDE α -syn occurs as a result of a protective mechanism. That is, as more α -syn accumulates in the brain, excess α -syn, possibly those toxic species, might be cleared into blood via exosomes, as a mechanism to decrease the load of potentially toxic α -syn in the brain, as suggested previously (Shi M et al., 2014; Matsumoto et al., 2017). One explanation for the current findings might be that this proposed mechanism of α -syn clearance is dependent on PD severity. That is, as PD progresses α -syn clearance rate increases, as reflected by faster DAT progression and decreasing NDE to total α -syn ratio. However, the significant association was only observed a subset (quartile) of hyposmic participants, and thus more caution should be taken to

interpret these results. Clearly, the mechanisms and implications of this hypothesis require further investigation.

We also observed that plasma total α -syn was significantly higher at baseline in hyposmic subjects without DAT reduction. Previous studies assessing plasma total α -syn concentrations in PD patients are contradictory (Duran et al., 2010; Foulds et al., 2011; Lee et al., 2006; Shi et al., 2010; Mata et al., 2010; Park et al., 2011), higher plasma α -syn in early PD compared to healthy controls has been found in some studies (Duran et al., 2010; Lee et al., 2006), however, the plasma α -syn concentrations reported in these studies differ from those reported in the present study, likely due to differences in methods of measurement and cohort as well as its pre-clinical/early stage. Nonetheless, the concentrations reported here are comparable to a larger study using the same assay to measure plasma total α -syn (Shi et al., 2010). Although the cause for this increase in plasma total α -syn is unknown, its appearance in these subjects at an increased risk of PD suggest that alterations in α -syn may begin early during the prodromal stage of PD, and are likely important in the disease process. However, the group difference (increase in the hyposmic group) at 2 years became less significant. Therefore, whether this is indeed an early change in prodromal PD and whether this increase would disappear at later disease stages, need to be further investigated and validated (e.g., in larger early PD PPMI [Parkinson Progression Marker Initiative, 2011] or DeNoPa [Mollenhauer et al., 2013] cohorts). Additionally, the predictive value of baseline α -syn, particularly when used with other surrogate markers (e.g., smell test and DAT imaging), also needs further assessment and validation. While plasma total α -syn concentrations may be contradictory, the plasma NDE α -syn concentrations reported here in the two hyposmic groups (without and with DAT deficit) at baseline are higher than those in the normosmic group, and closely reflect previously published PD NDE α -syn concentrations (Shi et al., 2014). In this previous study using matching CSF and plasma from patients with PD and healthy controls, there was a weak correlation between plasma total α -syn and CSF α -syn, but plasma NDE α -syn did not correlate with CSF α -syn (Shi et al., 2014). This has led us to speculate that although α -syn (in its free form and contained within exosomes) may be transported bi-directionally between the brain (CSF) and blood (Shi et al., 2014; Matsumoto et al., 2017), the mechanisms involved in its transportation to blood vs CSF might be quite different. In any case, these findings highlight the complex nature of plasma α -syn and its interaction with α -syn in the brain and CSF during PD pathogenesis and progression.

A major caveat of this study centers on the fact that only a small number of PARS subjects have progressed to the point of a PD diagnosis at this time. Since no definitive markers for early PD exist yet (indeed, that is a primary motivation of this study), the current work must rely on a combination of proxy markers (olfactory function and DAT) and progression. While the PARS study is ideally suited to answer these questions as more subjects convert to PD, the lack of significant findings in the small group that has already converted emphasizes that these findings should be revisited as progression among this cohort continues, in order to assess the relationships between α -syn and clinical outcomes. However, the finding of multiple associations between NDE α -syn specifically with cognitive, rather than motor, outcomes mitigates the multiple comparisons problem by providing an internally consistent

corroboration. Ultimately, our findings should be replicated in future cohorts such as PPMI and DeNoPa.

In this study, we employed an immunocapture technology based on a putative CNS- or brain cell-specific NDE marker, L1CAM, to examine CNS-derived NDEs in peripheral blood. The isolation technology, including its characterization and validation, has been reported by us (Shi et al., 2014; Shi et al., 2016) and others (Mustapic et al., 2017) and adopted by multiple groups to study brain-derived biomarkers in blood for CNS disorders (Fiandaca et al., 2015; Goetzl et al., 2015; Kapogiannis et al., 2015; Winston et al., 2016; Hamlett et al., 2017). L1CAM is the founding member of a subfamily of cell adhesion molecules primarily expressed in the nervous system that is thought to be a surface marker of CNS-derived exosomes (Simpson, et al., 2012; Kenwrick et al., 2000; Shi et al., 2014). Recent proteomic studies demonstrated that L1CAM-carrying exosomes in plasma have higher concentrations of several markers (e.g., phosphorylated tau, neuron-specific enolase, microtubule associated protein 2, neurofilament light chain, and L1CAM) that are reasonably specific to neurons compared to total plasma exosomes (Mustapic et al., 2017), highly suggesting the neuronal (and, to some degree, CNS) origin of these exosomes. However, as discussed in our previous studies (Shi et al., 2014; Shi et al., 2016), L1CAM is also known to be expressed in certain cancer and specialized cells, including some kidney cells (Kenwrick et al., 2000). Although there is no evidence indicating that L1CAM-containing exosomes are secreted from kidney and other peripheral cell types, the CNS- or neuron-specificity of L1CAM-positive NDEs in blood and the CNS origin of α -syn contained in these NDEs need to be further investigated.

In conclusion, we observed that plasma α -syn in individuals at risk of PD correlates with measures of cognitive function as well as with DAT imaging. Importantly, we found that, in contrast to total α -syn, an increase in NDE α -syn was associated with better cognitive and DAT outcomes, which could be indicative of a possible protective mechanism. Regardless of the mechanisms involved, however, our results suggest combining plasma α -syn concentrations with olfactory status and clinical profile likely to be useful in assessing the risk of future degeneration in prodromal PD.

Acknowledgments

We deeply appreciate those who have donated blood for our studies. This study was supported by grants from the National Institutes of Health (NIH) (U01 NS082137 and U01 NS091272 to JZ) and from the Department of Defense (W81XWH-06-067 to KM).

Abbreviation:

PD	Parkinson's disease
DAT	dopamine transporter
PARS	Parkinson's Associated Risk Syndrome
AD	Alzheimer's disease
α-syn	alpha-synuclein

CSF	cerebrospinal fluid
UPSIT	University of Pennsylvania Smell Identification Test
[¹²³I]β-CIT	[¹²³ I]-fluoro-propyl-beta-carbomethoxy-3beta-(4-idophenyl) tropane
SPECT	single-photon emission computed tomography
UPDRS	the Unified Parkinson's Disease Rating Scale
MMSE	Mini-Mental Status Examination
MoCA	the Montreal Cognitive Assessment
H&Y	Hoehn and Yahr
BSA	bovine serum albumin
HGB	hemoglobin
sP-Selectin	soluble P-Selectin
CV	coefficient of variation
GLM	general linear model

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Highlight:

- Increased total α -syn in individuals predicted progression of cognitive decline.
- Longitudinal decreased exosomal α -syn predicted worsening cognitive scores.
- Decreased exosomal to total α -syn ratio was associated with faster DAT progression.
- Altered plasma total and exosomal α -syn likely indicate progression in prodromal PD.

Figure 1. Association of baseline α-syn with 2 year MoCA score.

Higher total α-syn was predictive of a lower MoCA score at 2 years in the hypsmia/+DAT reduction group (unadjusted scatter plot; interaction coefficient, -8.50 ± 2.98 , $p = 0.008$; A), whereas a higher NDE α-syn in the hypsmia/-DAT reduction group associated with a better MoCA score (unadjusted scatter plot; interaction coefficient, 1.71 ± 0.76 , $p = 0.025$; B).

Figure 2. Association of longitudinal change in α-syn with total UPDRS and MMSE score.

In the hyposmia/+DAT reduction group the longitudinal change in total and NDE α-syn significantly predicted total UPDRS (A) and MMSE score (B) respectively, with a larger increase in total α-syn predictive of a worse UPDRS score (unadjusted scatter plot; interaction coefficient, 29.2 ± 12.3 , $p = 0.024$) and a larger decrease or smaller increase in NDE α-syn associated with a significant reduction or worse MMSE score (unadjusted scatter plot; 4.73 ± 2.03 , $p = 0.026$).

Figure 3. Association of longitudinal change in α -syn with change in DAT (quartile 4) from baseline to 2 years.

In quartile 4 (individuals with a faster progression in DAT score) a smaller increase or larger decrease in the change in NDE to total α -syn ratio was associated with a larger reduction in DAT from baseline to 2 years (unadjusted scatter plot; interaction coefficient, 0.61 ± 0.18 , $p = 0.003$; A). There was no statistically significant interaction of NDE (unadjusted scatter plot; B) or total (unadjusted scatter plot; C) α -syn with DAT from baseline to 2 years.

Demographics of study participants, cognitive scores and plasma marker levels at baseline and at 2 years

Table 1

	Normosmia/-DAT reduction	Hyposmia/-DAT reduction	Hyposmia/+DAT reduction	P value
Total number of cases	80	133	43	
Age (baseline)	62.8± 9.08	65.2± 7.98	64.6± 7.63	
Sex (F/M)	48/32	67/66	14/29	
*Baseline UPDRS (18–31)	1.20± 1.76	1.35± 2.02	2.63± 3.92	0.001
*Baseline UPDRS (1–31)	2.33± 2.87	2.57± 3.1	5.02± 5.97	<0.001
*Baseline MMSE	29.4± 0.89	29.1± 1.07	29.2± 0.87	0.149
*Baseline MoCA	26.3± 2.09	25.8± 2.51	25.2± 2.98	0.102
*Baseline H&Y total	0.05± 0.22	0.05± 0.21	0.02± 0.15	0.879
**Baseline Log total α -syn (ng/mL)	1.50± 0.27	1.63± 0.25	1.59± 0.23	0.001
**Baseline Log NDE α -syn (pg/mL)	0.97± 0.43	1.11± 0.4	1.10± 0.46	0.358
**Baseline Log NDE to total α -syn ratio	-0.53± 0.28	-0.52± 0.25	-0.49± 0.28	0.601
Total number of cases	80	133	43	
Age (year 2)	65± 9.06	67.3± 7.94	66.7± 7.56	
Sex (F/M)	48/32	67/66	14/29	
*Year 2 UPDRS (18–31)	1.49± 2.44	2.05± 2.92	5.30± 7.10	<0.001
*Year 2 UPDRS (1–31)	3.26± 3.71	3.76± 4.5	9.12± 9.80	<0.001
*Year 2 MMSE	29.2± 1.19	29.0± 1.15	28.6± 1.86	0.096
*Year 2 MoCA	26.2± 2.66	25.5± 2.83	25.0± 2.74	0.188
*Year 2 H&Y total	0± 0	0.02± 0.15	0.33± 0.71	<0.001
**Year 2 Log total α -syn (ng/mL)	1.72± 0.23	1.68± 0.24	1.63± 0.29	0.100
**Year 2 Log NDE α -syn (pg/mL)	1.31± 0.36	1.24± 0.41	1.24± 0.48	0.892
**Year 2 Log NDE to total α -syn ratio	-0.41± 0.26	-0.45± 0.29	-0.39± 0.32	0.605

Data shown are mean±SD.

Analyte data were log transformed to compensate for non-normal distribution.

* Comparisons were made using a UNIANOVA where age and sex were used as co-variables

** Comparisons were made using a UNIANOVA where age, sex, sP-Selectin and HGB were used as co-variables.

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