

Development of α -synuclein as a potential molecular marker for Parkinson's Disease

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INTRODUCTION

Parkinson's disease (PD) is characterized pathologically by the selective loss of dopaminergic neurones in the substantia nigra of the brain and the presence of Lewy bodies (LBs) in surviving cells and Lewy neurites in brain parenchyma. The major protein component of these lesions is α -synuclein (α -syn), which accumulates in a phosphorylated and aggregated form. Rare cases of familial PD are caused by missense mutations in the *SNCA* gene and duplication and triplication of this gene gives rise to late-onset and young-onset familial PD, respectively. These and other findings suggest that α -syn plays an important role in the pathogenesis of PD and other related ' α -synucleinopathies'. It is now clear that α -syn is released from cells and is present in human body fluids including blood plasma (El-Agnaf *et al.*, 2003). We aim to determine if α -syn in blood plasma can be used as a molecular biomarker for the diagnosis and/or progression of PD.

We have developed ELISA methods for the measurement of 'total' and 'soluble oligomeric' forms of both normal and phosphorylated (at Ser-129) α -syn, and have employed them to assay blood plasma samples from a longitudinal cohort of patients with PD, as well as single blood plasma samples from a group of normal, healthy controls.

PATIENT POPULATION AND DEMOGRAPHICS

The target of our study is to follow 200 patients with PD over a period of 2-3 years, reviewing them at 4-6 monthly intervals. This study is ongoing, but we also followed the first 32 patients more intensively over the initial phases of the study and this group were seen at monthly intervals for the first 3 months. It is the initial results from these first 32 patients that are presented here. Their demographic details are as follows:-

Gender (male/female)	23/9
Hoehn & Yahr 1.0	5
Hoehn & Yahr 1.5	3
Hoehn & Yahr 2.0	24
Median PD onset age (years (interquartile range))	61.93 (55.53 – 69.75)
Age at study recruitment (years (interquartile range))	68.39 (62.27 – 73.80)
Disease duration at study recruitment (interquartile range)	4.95 (3.09 – 9.27)

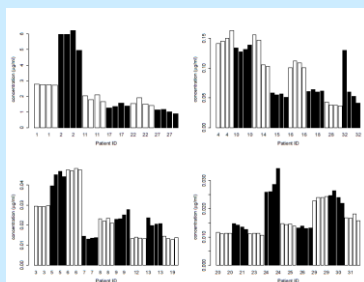
We also obtained single plasma samples from a group of 30 healthy controls, consisting of 13 males and 17 females, with an average age of 61.45 years (youngest 42, oldest 75).

ELISA METHODS

We have set up and validated 4 different sandwich ELISAs:-

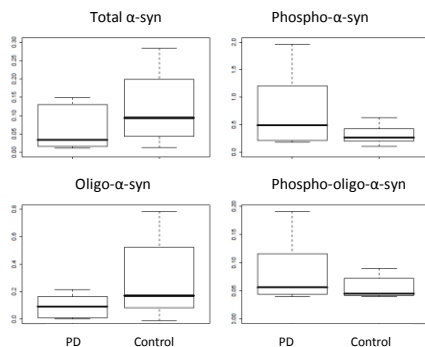
1. **Total α -syn:** Mab 211 capture/ FL-140 detect
2. **Oligomeric α -syn:** Mab 211 capture and detect
3. **Phospho- α -syn:** N19 capture/ Phospho (pS129) detect
4. **Phospho-oligo- α -syn:** Phospho (pS129) capture and detect

THE LEVELS OF ALL FORMS OF α -SYN VARIED GREATLY BETWEEN INDIVIDUALS, BUT WERE HIGHLY CONSISTENT WITHIN EACH INDIVIDUAL OVER TIME



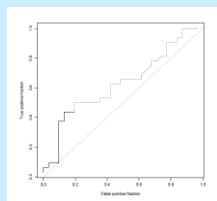
The bar charts show longitudinal data for total α -syn in blood plasma from the 32 PD patients. Consecutive bars for each patient show the mean level (n=3) of total α -syn in blood plasma samples taken at 0, 1, 2 and 3 months.

ONLY THE LEVELS OF PHOSPHO- α -SYN DIFFERED BETWEEN PD CASES AND HEALTHY CONTROLS



Under a two-sample t-test, the average level of phospho- α -syn (on a log scale) was found to be marginally significantly higher ($P=0.053$) in the PD samples than the healthy controls, whereas there was no significant difference across the average levels of PD patients and controls with regard to total α -syn ($P=0.244$), oligo- α -syn ($P=0.221$) or phospho-oligo- α -syn ($P=0.181$).

PHOSPHO- α -SYN HAS LIMITED DIAGNOSTIC VALUE



The area under this ROC curve (AUC) of 0.68 suggests that phospho- α -syn has limited value as a discriminant between PD patients and controls, in this small sample set.

Similar curves constructed for the other assays gave much lower AUC values of 0.28 for total α -syn, 0.22 for oligo- α -syn and 0.62 for oligo-pS- α -syn, suggesting that, on the other hand, they have no practical value as a diagnostic tool.

THERE WAS A WEAK CORRELATION BETWEEN NON-PHOSPHORYLATED α -SYN AND AGE

The 32 PD patients in this cohort were evaluated at each assessment (time point) using the following rating scales:-

1. Mini mental state (MMSE); 2. UPDRS; 3. Schwab and England Scale; 4. PDQ39; 5. EuroQol Visual Analogue Scale; 6. EuroQol EQ5D

The following baseline covariates were also recorded:-

1. Age at first visit; 2. Disease duration at recruitment into study; 3. Gender; 4. Current drug treatment for PD; 5. Hoehn & Yahr score; 6. Family history of PD; 7. Familial history of other neurodegenerative disorders

To investigate whether there was any association between these covariates and the measured α -syn levels, under all four assays, we used a linear mixed model in which we regressed the levels upon the covariates while accounting for the natural heterogeneity across the profiles of the PD patients. Although a few instances of potentially significant effects were found, the estimated effect sizes in these cases were essentially zero, rendering the findings of no scientific or clinical significance.

For the total and oligomeric α -syn assays, age was a marginally significant ($P=0.052$) and significant ($P=0.045$) predictor, respectively, of α -syn levels. On the other hand, the p-values corresponding to the effect of age upon α -syn levels, under the phospho- α -syn and phospho-oligo- α -syn assays, were 0.412 and 0.274, respectively.



DISCUSSION AND MAIN CONCLUSIONS

The levels of α -syn in blood plasma vary greatly between individuals, within the range 0.01 – 6 μ g/ml for total α -syn, but are remarkably consistent over time within the same individual.

Red blood cells (rbc) are the main source of α -syn in blood (Barbour *et al.*, 2008), but rbc contamination cannot explain the consistently high levels of α -syn found in the repeat plasma samples from some individuals.

There was no change with time in any of the measured levels of plasma α -syn over the 3-month longitudinal sampling period, but a more extended period will be required to determine if α -syn can act as a marker of disease progression.

Previous studies of α -syn as a potential diagnostic marker in plasma have suggested increased levels in patients with PD compared to healthy controls (Lee *et al.*, 2006), decreased levels (Li *et al.*, 2007) or no significant change (Shi *et al.*, 2010). Also, oligomeric α -syn has been reported to be elevated in PD plasma (El-Agnaf *et al.*, 2006). We found no change in levels of either total α -syn or oligo- α -syn in PD plasma compared to healthy controls.

Our data suggest that phosphorylated α -syn shows more promise as a diagnostic marker than the non-phosphorylated protein. Only the average level of phospho- α -syn was found to be higher in plasma from patients with PD compared to healthy controls. This was reflected in the ROC analysis (AUC=0.68 for phospho- α -syn).

The α -syn deposited in LBs is predominantly phosphorylated (Fujiwara *et al.*, 2002) and this form of α -syn is more likely to reflect the fundamental neuropathology of PD and than the non-phosphorylated protein (Foulds *et al.*, 2010).

Furthermore, age was a marginally significant predictor of the levels of non-phosphorylated α -syn, but age was not a confounding variable for the phosphorylated forms.

More extensive studies of phosphorylated α -syn as a potential molecular biomarker for PD and related disorders are warranted, and are ongoing in our laboratories.

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ACKNOWLEDGEMENTS

This work was supported by a Project Grant from The Medical Research Council and we are grateful for the support of DeNDRoN North West, who have adopted this study.