Aggregated α-synuclein in erythrocytes as a protentional biomarker for idiopathic Parkinson's Disease

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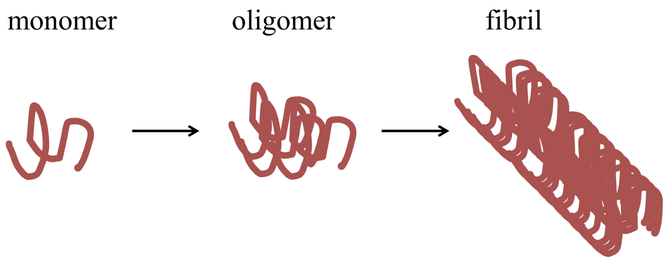
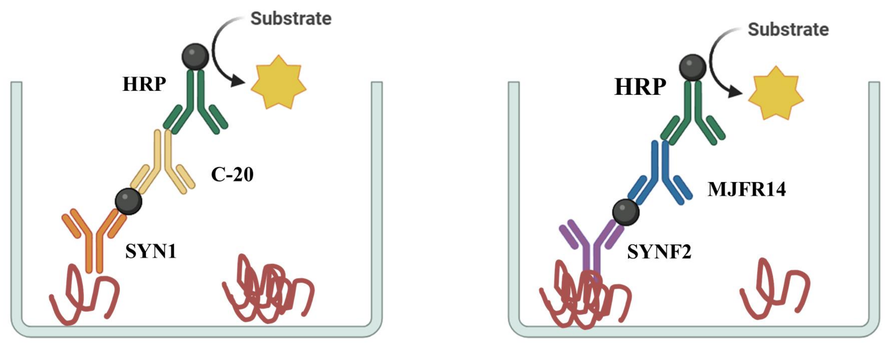
Background Mostly known for its implication in synucleinopathies, including Parkinson's disease (PD), α-synuclein is predominantly expressed in the nervous system. Most of the peripheral α-synuclein is found in erythrocytes, and several studies have examined a possible association between erythrocytic α-synuclein and PD.

Methods We have used a recently developed ELISA that selectively detects fibrillar and oligomeric α-synuclein to measure aggregated α-synuclein in red blood cells (RBCs) collected from PD patients and age/sex-matched control individuals (n=35). Optimization studies have been previously performed to develop the aggregated α-synuclein ELISA. The mouse monoclonal Syn-F2 antibody, which preferentially binds to mature amyloid fibrils and high molecular weight oligomers, was used to capture fibrillar and oligomeric α-synuclein assemblies. To ensure selectivity, the aggregate-specific rabbit monoclonal antibody MJFR-14-6-4-2 was used as the detection antibody. Sonicated PFFs were used as calibrators. The PD group included patients without any common mutation (genetically undetermined group, GU-PD, n=56) as well as mutation carriers in the α-synuclein gene (A53T-PD, n=28) and glucocerebrosidase gene (GBA-PD, n=24).

Basic conformations of α-synuclein's



Conformation-specific ELISA



Results

A. Specificity of the measurements using the conformation-specific ELISA in lysed RBCs (Figure 1).
(A) Assessment of serial dilutions of a control RBC sample. Dilutions were made in TBS-T/BSA buffer and measured in triplicate.
(B) Assessment of a PD RBC sample before and after the addition of 1.2 or 3.6 ng/ml PFFs. Aggregated α-synuclein after the PFF addition was estimated using the standard addition method. Data in A and B are presented as mean ± SEM.
(C) Measurement of total protein concentration in lysed RBCs from the control (n=34) and GU-PD (n=56) groups using the Bradford method. Statistics were performed by Student's t-test (p=0.4024).

B. Aggregated, but not total, α-synuclein is elevated in erythrocytes from PD patients compared to controls (Figure 2).
(A) Aggregated α-synuclein was measured in RBCs from controls (n=35) and GU-PD patients (n=56). Statistics were performed by Mann Whitney test (***p=0.0067*).
(B) The levels of aggregated α-synuclein found in GU-PD patients were compared to PD patients carrying GBA and A53T mutations. Statistics by Kruskal-Wallis test followed by Dunn's multiple comparisons test (*p=0.0102*, *0.9616*, and *>0.9999* for GU-PD, GBA-PD and A53T-PD vs controls, respectively).
(C) The concentration of aggregated α-synuclein is compared to total α-synuclein in RBCs from 9 controls and 9 GU-PD patients. In each case, control vs GU-PD groups were compared by Student's t-test (**p=0.02319* for aggregated α-synuclein, *p=0.9327* for total α-synuclein).
(D) The concentration of aggregated α-synuclein is compared to the aggregated:total ratio in RBCs from 9 controls and 9 GUPD patients. Control vs GU-PD groups were compared by Mann-Whitney test (**p=0.0326* for aggregated α-synuclein, ***p=0.0078* for aggregated:total ratio).

C. The accumulation of α-synuclein aggregates in PD erythrocytes is not due to aging (Figure 3). (A, B) Correlation analysis of aggregated α-synuclein concentration with the age of the control (A, R2=0.1845) or the GU-PD (B, R2=0.02628) groups. (C, D) Correlations between aggregated α- synuclein and age of onset (C, R2=0.05688) or disease duration (D, R2=0.03989).

Figure 1

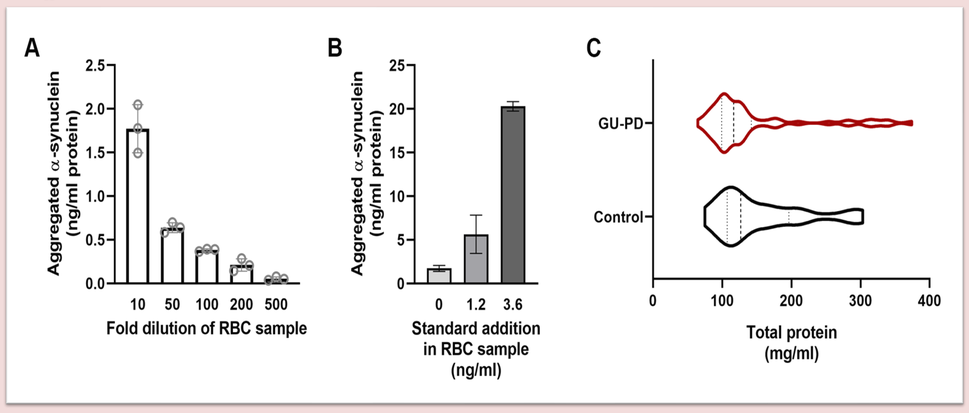


Figure 2

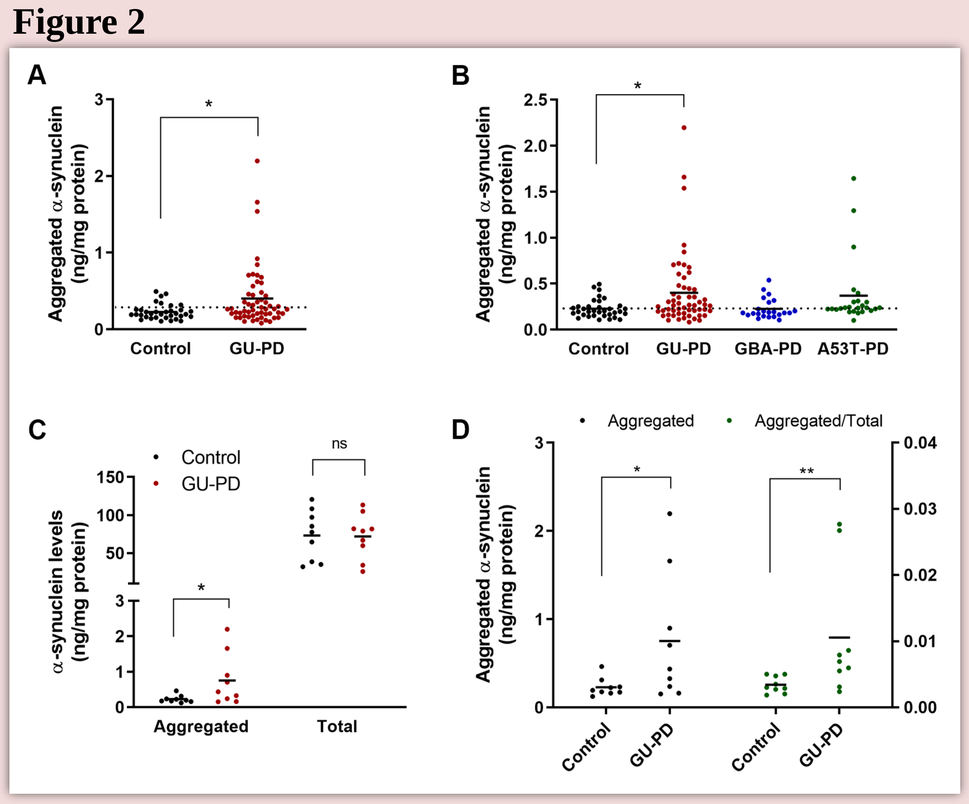
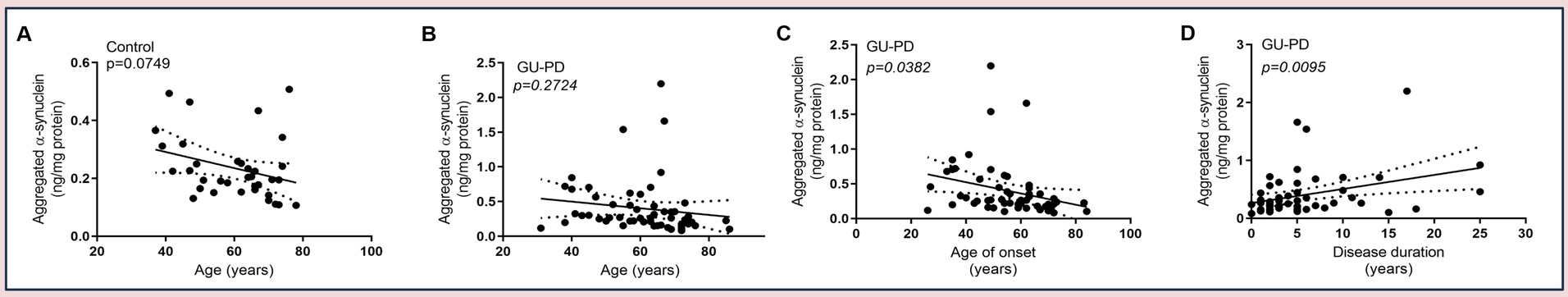


Figure 3



Conclusions

- A recently developed conformation-specific ELISA can detect aggregated forms of α-synuclein in erythrocytes from both PD patients and control individuals.
- Aggregated, but not total, α-synuclein is elevated in erythrocytes from PD patients compared to controls.
- The accumulation of α-synuclein aggregates in erythrocytes is not due to aging and does not depend on the severity of disease.
- Erythrocytic aggregated α-synuclein can discriminate PD from control subjects and could be a potential biomarker for PD.

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