Background and Justification for The Parkinson's Progression Markers Initiative Lead Biomarker Candidates

Identified Lead PD Biomarker Candidates

Alpha-Synuclein

Rationale: The central role of alpha-synuclein in the pathogenesis and pathophysiology of Parkinson's disease is evident through molecular genetic studies that identified PD-causing mutations in a-synuclein gene as well as identification of alpha-synuclein as a major component in Lewy bodies, a pathological hallmark of the disease. Alpha-synuclein was first identified in the CSF of subjects in 2000 using immunoprecipitation and western blot techniques. Subsequent studies from Dr. Michael Schlossmacher and others identified the presence of alpha-synuclein both in human serum and cerebrospinal fluid by a more sensitive ELISA. Dr. Schlossmacher improved the performance of the ELISA using anti-alpha-synuclein antibodies that detects alpha-synuclein in human serum (20-40pg/uL) and CSF (2-10pg/uL).

Summary of Data: The first study by Dr. Schlossmacher compared CSF alpha-synuclein levels in 33 patients with idiopathic PD and 38 neurological control subjects. They observed reduced levels of alpha-synuclein in the cerebrospinal fluid of aged subjects and a greater reduction of alpha-synuclein in those subjects with Parkinson's disease. The figure below from Tokuda et al. (2006) shows the predicted value of log CSF in ng/mL vs. age and diagnostic group.
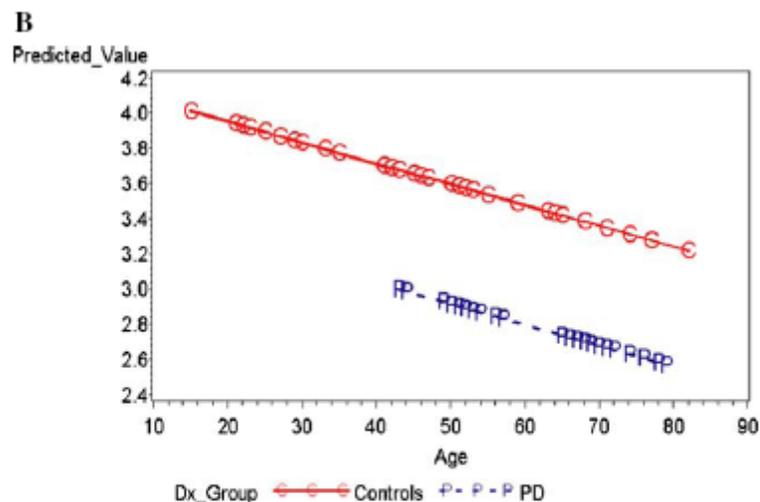

Fig. 2. (A) Scatter plot of age versus CSF α-syn in the control patient groups, as measured by sandwich ELISA. The concentration of α-syn was significantly reduced with aging. (B) Predicted values of log CSF α-syn (ng/ml) versus age, by diagnostic group (44 percent of variance accounted for; see text for details).

MJFF provided funding to Dr. Schlossmacher to improve the sensitivity of the ELISA while verifying his results in a separate cohort of individuals. In a follow up publication, Dr. Schlossmacher's group observed a similar reduction in alpha-synuclein levels in Parkinson's patients compared to neurologic controls (Mollenhauer et al., 2008).
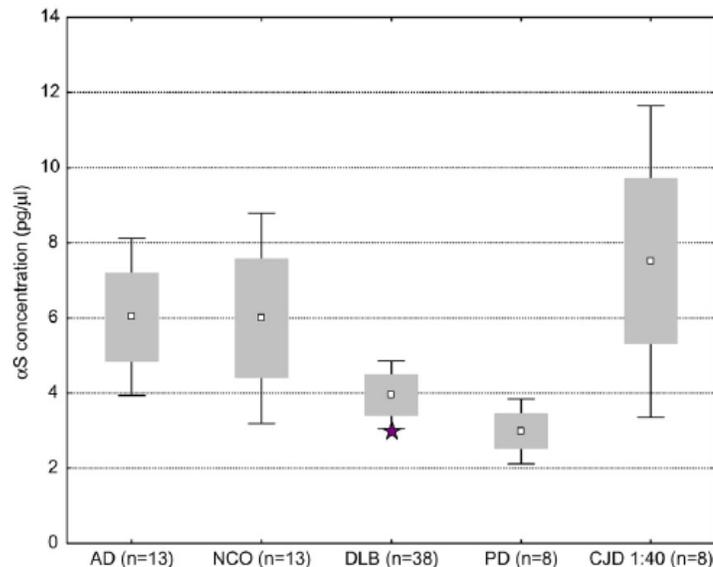

Fig. 3. Cross-sectional examination of CSF α-synuclein by ELISA. Aliquots of unconcentrated CSF from 80 living subjects were loaded in duplicate onto 384-well plates and analyzed by ELISA [mSA-1/Syn1-**BB**]. Five diagnostic groups were examined: AD denotes Alzheimer disease; PD, Parkinson disease; DLB, dementia with Lewy bodies; NCO, neurological controls; and CJD, Creutzfeldt–Jakob disease. Note, CSF specimens from CJD cases were diluted (1:40) prior to loading. The value for a single patient with autopsy confirmation is marked in the DLB cohort. □ mean; ■ mean±standard error of the mean; ⊥ bars indicate mean ±0.9 confidence interval.

Strengths and Weaknesses: While the initial cross-sectional data in the CSF of two separate cohorts of individuals suggest differences between Parkinson's subjects and neurological controls, there has not been a study examining the changes of alpha-synuclein in the *same subjects over time.* Thus it is unknown whether alpha-synuclein changes progress with the course of the disease and whether it could be used as a marker of disease progression. Additionally, detection of alpha-synuclein is possible with the current assay parameters and there are preliminary data that suggest levels are changed in the serum of Parkinson's subjects compared to controls, but verification of this observation in a larger population is critical.

Next Steps: MJFF is working with Dr. Schlossmacher to optimize the assay conditions and transfer the kit to a CRO that will run the assay on an aliquot of PPMI samples. Additionally MJFF is coordinating a clinical qualification study to determine the normal intra-subject variability of alpha-synuclein in the biofluids.

DJ-1
Rationale: The DJ-1 gene was linked to Parkinson's disease when mutations were discovered in autosomal recessive early onset PD cases. While the function of DJ-1 is not completely elucidated, there is evidence suggesting it is involved in the response to oxidative stress. Two publications from Dr. Makoto Hashimoto's group in Japan (Waragai et

al, 2006 and Waragai et al, 2007) observed elevated levels of the DJ-1 protein in CSF and plasma of PD patients compared to controls (data from publications below).
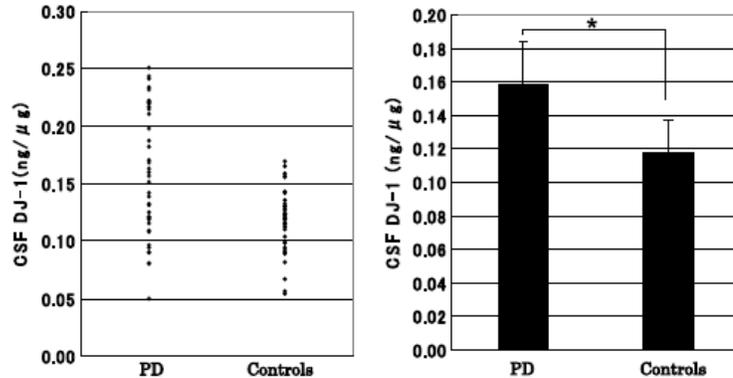

Fig. 2. Statistical analysis of CSF DJ-1 levels in PD and non-PD controls. Immunoblot analysis of CSF DJ-1 from PD and non-PD controls was performed and quantified as described in Fig. 1. The average DJ-1 levels in CSF were 0.158 ± 0.055 ng/µg (ranged from 0.050 to 0.250 ng/µg) in PD patients ($n = 40$) and 0.117 ± 0.027 ng/µg (ranged from 0.054 to 0.169 ng/µg) in non-PD controls ($n = 38$). DJ-1 levels in PD patients were significantly higher than those in controls (*$p < 0.01$ by Student's unpaired t-test).
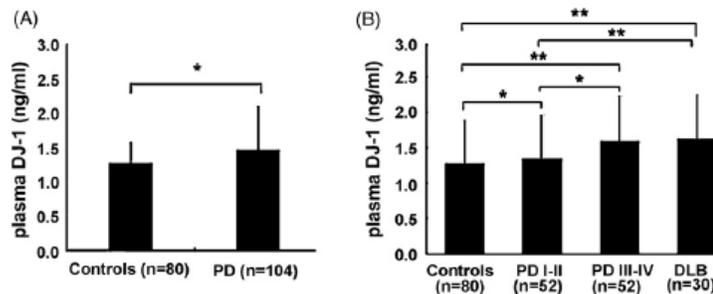

Fig. 3. Statistical analysis of plasma DJ-1 levels in PD, DLB and controls. (A) Plasma DJ-1 levels in PD are significantly higher than those in controls. *$p < 0.05$ by Student's unpaired t-test. (B) Comparison of plasma DJ-1 levels among controls, early stage PD (Yahr I–II), advanced stage (Yahr III–IV) PD and DLB. *$p < 0.05$, **$p < 0.01$ by ANOVA with post-hoc Bonferroni's test.

Strengths and Weaknesses: The causal association of DJ-1 mutations and autosomal recessive Parkinson's disease and its connection to the oxidative stress pathway increases the relevance of DJ-1 to PD. Preliminary evidence suggests DJ-1 is elevated in the CSF and plasma of Parkinson's patients compared to controls and may be utilized as an objective diagnostic marker. However, the methodology employed to detect these changes was limited by the sensitivity of immunoblotting techniques. Furthermore, only a small number of CSF samples were studied, so this has not been replicated in a larger sample set. A 2005 publication reported significant increases in the DJ-1 protein within 3 hours of the stroke onset, suggesting the marker may be a nonselective indication of neuronal damage.

Next Steps: MJFF due diligence uncovered a more sensitive commercial ELISA for detection of DJ-1 protein in biofluids. MJFF is currently supporting a study to determine the sensitivity and specificity of this assay for the detection of DJ-1. If the assay is suitable it will be integrated into the PPMI study in order to verify the initial findings of DJ-1 changes in Parkinson's disease.

Urate

Rationale: Epidemiological and clinical studies have identified urate as a biochemical marker for both risk and progression of the disease. Retrospective studies of two different clinical trial cohorts totaling around 1600 subjects revealed that patients with the highest serum urate levels had a 40% slower rate of progression to disability. While preliminary data suggest serum urate levels may predict the rate of progression of the disease, it is unknown whether serum urate levels *change* with disease progression (Schwarzschild et al 2008).

Strengths and Weaknesses: Oxidative stress has been linked to the pathophysiology of Parkinson's disease, but the molecular mechanistic connection is still unknown. The existing data from thousands of sporadic PD subjects demonstrating levels of a major antioxidant urate as a risk factor for PD further substantiates the relationship between PD and oxidative stress. However, it is still unknown whether urate levels change prior to diagnosis and with the progression of the disease. Urate is an easily measured metabolite of purine synthesis and standardized assays exist for this measurement in the blood and CSF. Therefore, it is a straightforward process to measure baseline and follow up urate levels in Parkinson's disease patients.

DAT Imaging

Rationale: Imaging tracers targeting pre-synaptic nigrostriatal function have been the most widely used biomarker to track PD progression. Most of these studies have used either F-Dopa and/or DAT
tracers to monitor dopaminergic degeneration. Numerous clinical imaging studies have shown reductions in F-Dopa, VMAT2 and DAT ligands uptake in PD patients and aging healthy subjects consistent with the expected pathology of PD and of normal aging.

In longitudinal studies of PD progression, F-Dopa, VMAT2, DAT (ß-CIT and CFT), and FDG using both PET and SPECT have demonstrated an annualized striatal rate of reduction of about 4% to 13% in PD patients compared with 0% to 2.5% change in healthy controls. Evidence from studies of hemi-PD subjects provides further insight into the rate of progression of disease. In early hemi-PD, there is a reduction in F-Dopa, VMAT2 and DAT of about 50% in the affected putamen and of 25-35% in the unaffected putamen. Since most patients will progress clinically from unilateral to bilateral in three to six years, it is therefore likely that the loss of these *in vivo* imaging markers of dopaminergic degeneration in the previously unaffected putamen will progress at about 5-10% per annum (Parkinson's Study Group, 2002; Marek et al, 2001, and Brooks et al, 2003).

Strengths and Weaknesses: DAT and F-Dopa imaging have been used to assess the effects of possible disease modifying drugs in several clinical trials. However, several caveats limit the interpretation of these imaging data. There has been concern that the drug under testing or concomitant symptomatic medications might directly regulate the imaging outcome so that it would not be a true measure of disease progression. While recent studies demonstrating that the most common symptomatic medications (levodopa and dopamine agonists) do not have a short-term regulatory effect on

DAT imaging increase confidence in DAT imaging as a measure of progression, nonetheless, imaging studies must include an assessment of the short-term effect of the test drug on the imaging outcome. A second caveat for imaging studies of disease progression has been the inconsistent correlation of changes in imaging outcomes and clinical outcomes in these clinical trials. The lack of clinical–imaging correlation may be explained since these outcomes reflect very different aspects of the disease (imaging – a physiological measure of dopamine presynaptic function; clinical – a functional measure of disability). Therefore imaging and clinical outcomes may best be considered complementary rather than correlative. Many clinical outcomes may also be confounded by symptomatic medications further complicating the correlation of clinical and imaging outcomes once symptomatic treatment has begun. In summary, the studies of dopaminergic imaging as a tool for disease progression have provided both useful and important data, but have also highlighted the difficulties in validating a progression marker and the, as yet, unmet need for additional tools to more fully and reliably assess disease progression.

Integration of Biomarkers and their Correlations to Clinical Symptoms
PPMI is an observational, multi-center study to assess progression of clinical features and imaging and biomic biomarkers in Parkinson's disease patients and healthy controls. A key deliverable coming out of this study will be the development of a comprehensive and uniformly acquired PD clinical and imaging dataset with blood, urine and CSF sampling that could be used to identify and validate clinical, imaging and biomic biomarkers of PD progression. A unique aspect of this study is that all enrolled subjects – PD patients and controls – will undergo the same assessment battery (clinical, imaging and biologics) and will provide samples on a frequent basis. The collection of this information from the entire population will enable novel analyses to be performed. Utilizing this dataset, we will be able to estimate the mean rates of change and the variability around the mean of clinical, imaging and biomic outcomes in early PD patients, and where appropriate, to compare these rates between PD and healthy subjects at study intervals from three months to 36 months. While verification of lead biomarker candidates is an important short-term objective, it is comparing and correlating the results of these lead candidates to acquired imaging and clinical data that could help identify a marker or markers that track with PD progression.

Literature Citations
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