Cerebrospinal Fluid Biomarkers for Parkinson Disease Diagnosis and Progression

I. Supplementary Methods

1. Participants

Participating institutions include Baylor College of Medicine, Oregon Health and Science University, the University of California at San Diego, VA Puget Sound Health Care Systems at Seattle, Portland VA Medical Center, the National Institute of Neurological Disorders and Stroke, St. Olavs Hospital (Trondheim, Norway) and the University of Washington. The inclusion and exclusion criteria for normal controls and patients with Alzheimer disease (AD) or Parkinson disease (PD) were previously described[1]. A brief description is provided below.

Controls: Control subjects were community volunteers in good health. They had no signs or symptoms suggesting cognitive impairment or neurological disease; all subjects had a MMSE score between 28 and 30; a CDR score of 0; and NYU paragraph recall scores (immediate and delayed) >6. Exclusion criteria also included moderate or heavy cigarette smoking (more than 10 packs/year), alcohol use other than socially, and any psychotherapeutic drug use. Finally, it should be emphasized that although pathological confirmation had not been obtained in most subjects, all of them had been followed for 12 months or longer (median of 3 years) without demonstrating any symptoms or signs of neurological disorders, including mild cognitive impairment.

AD: Patients were diagnosed with probable AD according to NINDS-ADRDA criteria[2], either at Oregon Health and Science University or the University of Washington, and confirmed by a clinical team consensus conference between two centers.

PD: All patients met UK PD Society Brain Bank clinical diagnostic criteria for PD[3]

except that having "more than one affected relative" was not considered an exclusion criterion. Similar to control patients, most of the PD patients were still alive at the time of CSF analysis; pathological confirmation of PD had not been obtained in most instances. Nonetheless, all patients included in this study, except for 16 *de novo* subjects, had sustained response to anti-parkinsonism drugs. 13 of the *de novo* cases responded to dopamine specific treatment after lumbar puncture, while another subject, though remained untreated at the time of the manuscript was prepared, did not show any evidence to suggest an alternative diagnosis. The remaining two cases were lost during follow up.

A validation set of PD subjects was from the DATATOP (Deprenyl and Tocopherol Antioxidative Therapy of Parkinsonism) study, in which patients with early PD who did not require symptomatic treatment were randomized to receive selegiline, tocopherol, or placebo[4, 5], but only the patients randomized to placebo were included in the current study. Misdiagnosed subjects and subjects who reached the DATATOP primary endpoint within 6 months of randomization were excluded from the current study. The primary endpoint was achieved when a subject was judged to be sufficiently impaired to warrant levodopa therapy. The annual rate of progression was calculated based on the UPDRS motor score as following: (Endpoint value – Baseline value) / Number of years between values.

MSA: Patients with multiple system atrophy (MSA) were diagnosed according to generally accepted clinical criteria[6, 7]. The parkinsonian form of MSA was identified by symptoms or signs of autonomic failure coupled with rigidity and bradykinesia, with or without clinical evidence of cerebellar failure; and the cerebellar form by autonomic and cerebellar failure without parkinsonism. All patients were tested while on their usual medications. To ensure that treatment with levodopa/carbidopa did not influence the neurochemical results, CSF data were included only for patients in whom the plasma DOPA level was less than 2,500 pg/mL (less than about 12.7 nmol/L) during supine rest. Orthostatic hypotension was defined by a decrease in

systolic blood pressure of at least 20 mm Hg and in diastolic pressure at least 10 mm Hg between supine rest for at least 15 minutes and upright posture for 5 minutes (unless symptomatic or rapid hypotension necessitated return to the supine position before 5 minutes upright). Orthostatic hypotension was determined to be neurogenic by abnormal beat-to-beat blood pressure associated with performance of the Valsalva maneuver[8]. The finding of normal 6-^{18}F-fluorodopamine-derived radioactivity in the left ventricular myocardium was also used to distinguish MSA from PD with orthostatic hypotension, because all patients with the latter have neuroimaging evidence for cardiac sympathetic denervation[9]. All MSA patients included in this study underwent formal evaluation of orthostatic hypotension by tilt table testing. All patients also underwent 6-^{18}F-fluorodopamine positron emission tomography (PET) scanning to evaluate cardiac sympathetic innervation, and all had evidence of intact innervation. Details about these MSA patients can be found in **Supplementary Table 1**.

One limitation of the current study is that patients seen by movement disorder specialists at major medical centers may not be representative of those in the community.

2. Collection of CSF and quality control

Subjects: Following written informed consent, individuals were placed in the lateral decubitus position and the L4-5 interspace was infiltrated with 1% lidocaine. Lumbar puncture (LP) was performed with a 24G spinal needle. Individuals remained at bed rest for one hour following LP. The first 2 mL of CSF were sent to a local laboratory for determination of protein, glucose and cell count. Up to 25 ml CSF was then taken from each subject, with every 5 ml pooled in one fraction. These were aliquoted into polypropylene cryotubes in 0.5 mL aliquots (labeled 1st-50th fraction, corresponding to 1st-25th ml), flash frozen, and stored at -80°C. All LP was performed in the morning to limit potential circadian fluctuation of CSF proteins and metabolites. Before analysis, all CSF samples were only thawed once when 10% protease inhibitor cocktail

(Sigma, St Louis, MO, USA) was added and samples were further aliquoted.

Reference: Reference CSF refers to the CSF samples obtained from the clinical laboratory at Harborview Medical Center (Seattle, WA), and only the samples from subjects who had been determined neurologically as well as biochemically normal were pooled and used.

Lumbar puncture is a routine neurodiagnostic procedure, with low risk of complications (< 2%) and good acceptability[10]. In addition, universal precautions were taken when handling any human specimen. Blood contamination in CSF samples can be controlled with careful and correct practice during sample collection and should be monitored in all samples collected[11, 12].

3. Luminex assays

All CSF samples were analyzed using a LiquiChip Luminex 200™ Workstation (Qiagen, Valencia, CA, USA). All incubations were performed in 96-well MultiScreen Filter plates (Millipore). Samples were analyzed in batches. Three methods were used to control for batch-to-batch variations: 1) all samples were randomly batched so that there were representations of all comparison groups in each plate; 2) every experiment was performed with an internal standard, i.e., an aliquot of a pooled reference CSF sample with known concentration of targeted proteins; and 3) only if the values of the internal standard control varied more than 10% from the average of all batches, then the results in that particular batch were normalized based on reference values.

Of note, most of the DJ-1 and α-syn data were published recently[1], but we have now expanded these analyses, and included results from several additional control and PD cases. Additionally, we have included new DJ-1 and α-syn data for a MSA cohort, an independent PD cohort and longitudinal samples from DATATOP PD subjects.

References:

1. Hong Z, Shi M, Chung KA et al. DJ-1 and alpha-synuclein in human cerebrospinal fluid as biomarkers of Parkinson's disease. Brain. 2010;133:713-726
2. McKhann G, Drachman D, Folstein M et al. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology. 1984;34:939-944
3. Gibb WR, Lees AJ. The relevance of the Lewy body to the pathogenesis of idiopathic Parkinson's disease. J. Neurol. Neurosurg. Psychiatry. 1988;51:745-752
4. Parkinson_Study_Group. DATATOP: a multicenter controlled clinical trial in early Parkinson's disease. Arch. Neurol. 1989;46:1052-1060
5. Parkinson_Study_Group. Effects of tocopherol and deprenyl on the progression of disability in early Parkinson's disease. N. Engl. J. Med. 1993;328:176-183
6. Gilman S, Low PA, Quinn N et al. Consensus statement on the diagnosis of multiple system atrophy. J. Neurol. Sci. 1999;163:94-98
7. Gilman S, Wenning GK, Low PA et al. Second consensus statement on the diagnosis of multiple system atrophy. Neurology. 2008;71:670-676
8. Goldstein DS, Tack C. Noninvasive detection of sympathetic neurocirculatory failure. Clin. Auton. Res. 2000;10:285-291
9. Goldstein DS, Holmes C, Bentho O et al. Biomarkers to detect central dopamine deficiency and distinguish Parkinson disease from multiple system atrophy. Parkinsonism Relat Disord. 2008;14:600-607
10. Peskind E, Nordberg A, Darreh-Shori T, Soininen H. Safety of lumbar puncture procedures in patients with Alzheimer's disease. Curr. Alzheimer Res. 2009;6:290-292
11. Hale JE, Gelfanova V, You JS et al. Proteomics of cerebrospinal fluid: methods for sample processing. Methods Mol. Biol. 2008;425:53-66
12. Zhang J. Proteomics of human cerebrospinal fluid - the good, the bad, and the ugly. Proteomics Clin. Appl. 2007;1:805 - 819