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Identification of candidate cerebrospinal fluid biomarkers in parkinsonism using quantitative proteomics

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ABSTRACT

Introduction: Neurodegenerative parkinsonian syndromes have significant clinical and pathological overlap, making early diagnosis difficult. Cerebrospinal fluid (CSF) biomarkers may aid the differentiation of these disorders, but other than α-synuclein and neurofilament light chain protein, which have limited diagnostic power, specific protein biomarkers remain elusive.

Objectives: To study disease mechanisms and identify possible CSF diagnostic biomarkers through discovery proteomics, which discriminate parkinsonian syndromes from healthy controls.

Methods: CSF was collected consecutively from 134 participants; Parkinson's disease (n = 26), atypical parkinsonian syndromes (n = 78, including progressive supranuclear palsy (n = 36), multiple system atrophy (n = 28), corticobasal syndrome (n = 14)), and elderly healthy controls (n = 30). Participants were divided into a discovery and a validation set for analysis. The samples were subjected to tryptic digestion, followed by liquid chromatography-mass spectrometry analysis for identification and relative quantification by isobaric labelling. Candidate protein biomarkers were identified based on the relative abundances of the identified tryptic peptides. Their predictive performance was evaluated by analysis of the validation set.

Results: 79 tryptic peptides, derived from 26 proteins were found to differ significantly between atypical parkinsonism patients and controls. They included acute phase/inflammatory markers and neuronal/synaptic markers, which were respectively increased or decreased in atypical parkinsonism, while their levels in PD subjects were intermediate between controls and atypical parkinsonism.

Conclusion: Using an unbiased proteomic approach, proteins were identified that were able to differentiate atypical parkinsonian syndrome patients from healthy controls. Our study indicates that markers that may reflect neuronal function and/or plasticity, such as the amyloid precursor protein, and inflammatory markers may hold future promise as candidate biomarkers in parkinsonism.

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1. Introduction

Parkinson's disease (PD) is the most common neurodegenerative movement disorder and the number of patients is expected to double over the next two decades, presenting a huge social and economic challenge [1]. Atypical parkinsonian syndromes, such as progressive supranuclear palsy (PSP), multiple system atrophy (MSA) and corticobasal syndrome (CBS) represent rarer but more aggressive forms of parkinsonism. Atypical parkinsonian syndromes and PD often present in a strikingly similar manner, making an accurate early diagnosis difficult. Despite similar clinical characteristics, these diseases differ substantially in their prognosis,

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pathological features and therapeutic response [2,3]. There is an urgent need to identify biomarkers for parkinsonian disorders to enable earlier, accurate diagnosis, monitor disease progression and response to drug therapies.

Cerebrospinal fluid (CSF) has been widely investigated in parkinsonian disorders and may serve as a source of biomarkers that reflect brain-related disease processes. In PD, clinical studies of candidate proteins, such as α-synuclein and neurofilament light chain protein, show minimal changes and high assay variability and have thus far not resulted in a diagnostically useful biomarker (for a review see Ref. [4]).

To date studies employing unbiased proteomic approaches have led to the identification of various combinations of CSF proteins that differ in abundance between patient groups [5–9], but singling out promising candidate markers among the many detected proteins has proven challenging. Part of the problem is the disproportionality of the published proteomic data sets, which contain many identified proteins but only few patients, leading to a risk of over-fitting statistical models. Other complicating factors include study groups with heterogeneous disease characteristics and experimental variation.

In order to overcome these limitations, studies of larger patient groups are required. While previously limited by the long analysis times of liquid chromatography-mass spectrometry (LC-MS) used in discovery proteomics workflows, performing large studies is now possible through the development of timesaving multiplex isobaric labeling techniques, such as tandem mass tags (TMT) [10].

In this study, we used multiplex isobaric labeling to perform the largest proteomic study of parkinsonism to date, and the first study to include several atypical parkinsonian syndromes and healthy controls. Our aim was to shed light on potential disease mechanisms and explore the possibility of new diagnostic markers.

2. Methods

2.1. Study participants

This is a cross-sectional study of patients with parkinsonian disorders and healthy controls. Participants were prospectively enrolled over a two-year period from 2011 to 2013 from the movement disorders, cognitive and autonomic disorders clinics at the National Hospital for Neurology and Neurosurgery, Queen Square, London. The diagnoses of probable PD, PSP, CBS and MSA were based on consensus operational criteria [3,11–13].

Patients included in the study were 40–85 years old and under follow-up for at least two years. The clinical diagnosis was corroborated by at least two neurologists with experience in movement disorders (AJL, TTW, HRM, AJN, NM). Further information patient characterization and exclusion criteria are listed in "Supplementary Materials – Methods".

2.2. Ethics approval

The study was conducted in accordance with local clinical research regulations and an informed consent was obtained from all subjects, including access to clinical data and imaging. The study was performed in accordance with the provisions of the Helsinki declaration and the research protocol was approved by the London Queen Square research ethics committee.

2.3. CSF collection and storage

We adhered to a standardized protocol for the collection and storage of CSF as recommended by the Alzheimer's Association QC Program for AD CSF biomarkers (www.neurochem.gu.se/TheAlzAssQCProgram). Details on the procedure are given in "Supplementary Materials – Methods".

2.4. Experimental design

136 subjects were included in the analysis. Subjects were randomly divided into a discovery (13 PD, 39 atypical parkinsonian patients and 15 healthy controls) and a validation (13 PD, 43 atypical parkinsonian patients and 13 healthy controls) set. For demographic and clinical characteristics see Table 1. CSF samples from the two sets were prepared and analyzed separately. Details of CSF sample preparation are provided in "Supplementary Materials-Methods".

2.5. MS analysis

The samples were reconstituted in a solution of 2% acetonitrile, 0.1% TFA (600 μl). Aliquots of 2 μl were analyzed with a nano-LC (Ultimate 3000, Thermo Scientific) equipped with a C_{18} trap column (PepMap Acclaim 75 μm *20 mm, Thermo Scientific), and a C_{18} separation column (PepMap Acclaim 75 μm * 500 mm, Thermo Scientific), coupled to a Q-Exactive electrospray ionization mass spectrometer (Thermo Scientific), fitted with a FlexiSpray ion source. The loading buffer was 2% acetonitrile, 0.05% TFA; Buffer A was 0.1% formic acid; and Buffer B was 84% acetonitrile, 0.1% formic acid. The following gradient was used: t = 0 min, B = 3%; 140 min, B = 30%; 160 min, B = 45%; 165 min, B = 80%. The mass spectrometer was operated in the positive ion mode. Data-dependent acquisition was used, acquiring one full MS scan (R = 70 k, AGC target = 1e6, max IT = 250 ms) and up to 10 consecutive MS/MS scans (R = 17.5 k, AGC target = 5e4, max IT = 60 ms). Data processing was performed within the software ProteomeDiscoverer 1.4 (Thermo Scientific), using Mascot (MatrixScience) for protein identification (precursor Δm tolerance = 5 ppm, fragment Δm tolerance = 20 milli mass units, missed cleavages = 2, fixed modifications = carbamidomethylation, variable modifications = oxidation of methionine), searching the human subset of the UniProtKB Swiss-Prot database (release 13–10) (www.uniprot.org). Percolator (MatrixScience) was used for scoring peptide specific matches, and 1% false discovery rate (FDR) was set as threshold for identification. The following settings were used for reporter ion quantification: Integration tolerance = 150 ppm; Integration Method = Most Confident Centroid; exclusion of MS/MS spectra >50% co-isolation; normalize

Table 1
Demographic and clinical characteristics in the study groups.

Discovery set			
Study groups	HC	PD	APS
n	15	13	39
n (male/female)	5/10	8/5	21/18
avg age (range)	59 (43–76)	66 (51–83)	69 (58–81)
avg dis dur in yrs (range)	0 (0–0)	9 (2–20)	5 (1–11)
H&Y score (range)	0 (0–0)	3 (1–4)	4 (2–5)
Validation set			
Study groups	HC	PD	APS
n	13	13	43
n (male/female)	8/5	8/5	23/20
avg age (range)	61 (45–71)	71 (63–85)	66 (53–82)
avg dis dur in yrs (range)	0 (0–0)	13 (3–23)	5 (2–12)
H&Y score (range)	0 (0–0)	3 (2–5)	4 (1–5)

Abbreviations: avg: average, dis dur: disease duration, H&Y score: Hoehn and Yahr score, HC: healthy controls, PD: Parkinson's disease, APS: atypical parkinsonian syndromes.

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