


RESEARCH ARTICLE

Impaired synaptic function is linked to cognition in Parkinson's disease

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Abstract

Objective: Cognitive impairment is frequent in Parkinson's disease, but the underlying mechanisms are insufficiently understood. Because cortical metabolism is reduced in Parkinson's disease and closely associated with cognitive impairment, and CSF amyloid- β species are reduced and correlate with neuropsychological performance in Parkinson's disease, and amyloid- β release to interstitial fluid may be related to synaptic activity; we hypothesize that synapse dysfunction links cortical hypometabolism, reduced CSF amyloid- β , and presynaptic deposits of α -synuclein. We expect a correlation between hypometabolism, CSF amyloid- β , and the synapse related-markers CSF neurogranin and α -synuclein. **Methods:** Thirty patients with mild-to-moderate Parkinson's disease and 26 healthy controls underwent a clinical assessment, lumbar puncture, MRI, ¹⁸F-fludeoxyglucose-PET, and a neuropsychological test battery (repeated for the patients after 2 years). **Results:** All subjects had CSF amyloid- β 1-42 within normal range. In Parkinson's disease, we found strong significant correlations between cortical glucose metabolism, CSF A β , α -synuclein, and neurogranin. All PET CSF biomarker-based cortical clusters correlated strongly with cognitive parameters. CSF neurogranin levels were significantly lower in mild-to-moderate Parkinson's disease compared to controls, correlated with amyloid- β and α -synuclein, and with motor stage. There was little change in cognition after 2 years, but the cognitive tests that were significantly different, were also significantly associated with cortical metabolism. No such correlations were found in the control group. **Interpretation:** CSF A β , α -synuclein, and neurogranin concentrations are related to cortical metabolism and cognitive decline. Synaptic dysfunction due to A β and α -synuclein dysmetabolism may be central in the evolution of cognitive impairment in Parkinson's disease.

Introduction

Cognitive impairment is an important nonmotor symptom of Parkinson's disease (PD).¹ Mean duration from clinical disease onset to dementia is 10 years, but cognitive impairment has been demonstrated already at the time of diagnosis.²

Several factors argue for early involvement of synaptic pathology in PD. Many of the proteins relevant in PD pathophysiology are involved in synaptic physiology (reviewed in³). α -synuclein is considered the core pathological substrate of PD, and in addition to accumulation in Lewy bodies, a major deposition site of CNS oligomeric α -synuclein is the presynapse, accompanied by transsynaptic dendritic spine loss.^{4,5} The presynaptic terminal is the main site of function for both amyloid precursor protein (APP) and α -synuclein. α -synuclein is linked to synaptic-vesicle complex formation and APP to synaptic-vesicle docking and priming. Neurogranin, a substrate of protein kinase C, is abundantly expressed, and located preferentially at the postsynaptic dendritic spine plasma membrane.⁶

Intracellular metabolism of these proteins is performed by overlapping sets of enzymes and subcellular compartments.^{7–10} Presynaptic activity and endocytosis influence release of amyloid- β ($A\beta$) species to the interstitial fluid.¹¹ APP is initially cleaved at the presynaptic terminal to several biologically active metabolites, including $A\beta$ 1–38 ($A\beta$ 38), $A\beta$ 1–40 ($A\beta$ 40), and $A\beta$ 1–42 ($A\beta$ 42), the latter particularly prone to brain parenchymal deposition.^{12,13}

Most studies of CSF $A\beta$ species in PD have reported slight reductions.^{14,15} We and others have found a correlation between CSF $A\beta$ 42 levels and memory performance in PD,^{15,16} also in subjects with normal CSF $A\beta$ 42 levels (thus without evidence for amyloid plaques).^{15,17} These changes relate to hippocampus subfield volumes¹⁸; experimental studies show detrimental preplaque $A\beta$ effects on synaptic function,¹⁹ and human $A\beta$ turnover decreases from approximately 40 years of age,²⁰ suggesting preplaque effects of $A\beta$ on cognition and synaptic physiology.

MRI studies show that slight gray matter atrophy is involved in PD cognitive decline.²¹ Studies combining neuroimaging and CSF in PD are scarce, but CSF $A\beta$ correlate with reduced gray matter volume and associated neuropsychological function.^{22,23} FDG-PET studies have identified a widespread, specific pattern of reduced glucose metabolism in PD,²⁴ most prominent in the frontoparietal and occipital cortices, even in the early stages of the disease.²⁵ Cerebral glucose metabolism reflects cortical activation and synaptic density.²⁶ Several studies associate this hypometabolism with cognitive impairment, suggesting that hypometabolism in PD primarily reflects cognitive deterioration.^{27,28} Though a very recent

postmortem study suggests that loss of synaptic markers predicts cognitive decline in Lewy body diseases,²⁹ to the best of our knowledge, no direct links have been established between reduced glucose metabolism in PD and independent synaptic markers.

Because (1) cortical glucose metabolism is reduced and correlates with cognitive impairment in PD, and (2) CSF $A\beta$ species are decreased and correlate with neuropsychological performance, and amyloid- β release to interstitial fluid may be related to synaptic activity; we hypothesize that synapse dysfunction is a common link between cortical hypometabolism, CSF amyloid- β concentrations, and presynaptic deposits of α -synuclein. We thus expect to find a correlation between levels of CSF $A\beta$ species and relative glucose metabolism, and also to possible markers of synaptic dysfunction or loss, such as CSF α -synuclein and neurogranin.

Materials and Methods

Subjects

This study is based on an ongoing longitudinal follow-up of patients in a university hospital-based neurological clinic. Thirty nondemented patients with mild-to-moderate stage PD were recruited between 2011 and 2014 and examined by a neurologist with extensive experience in movement disorders, as previously described.¹⁵

The patient group and inclusion criteria in this study is the same (aside from one subject excluded due to missing FDG-PET) as the one described in ref 15. In short, inclusion criteria were diagnosis of PD according to the UK Parkinson's Disease Society Brain Bank clinical diagnostic criteria,³⁰ Hoehn and Yahr stage <3, and disease duration <6 years. All PD subjects had a pathological dopamine transporter single photon emission computed tomography (DaT-SPECT). Exclusion criteria were dementia (see below for procedure) and other somatic or psychiatric diseases that might contribute to cognitive impairment. The Unified Parkinson's Disease Rating Scale (UPDRS), part III motor examination and Hoehn & Yahr staging were performed. Patients had their medication as usual when assessed. Total daily levodopa equivalent dose was calculated. All PD subjects underwent cognitive assessment employing a well-validated neuropsychological test battery thoroughly described previously.^{15,31} The battery covers memory: *Delayed verbal recall* (Rey Auditory Verbal Learning Test (RAVLT)),³² and *Delayed visual reproduction* (Rey Complex Figure Test (RCFT)), (Meyers & Meyers, 1995) and aspects of executive functions: *Psychomotor speed* (Trail Making Test-A (TMT-A)), *Divided attention* (Trail Making Test-B (TMT-B)) (Reitan & Wolfson 1985), *Word fluency* (Controlled Oral Word

Association Test (COWAT)) (Benton & Hamsher 1989), and *Response inhibition* (Stroop Color-Word, D-KEFS) (Delis *et al.*, 2001). All examinations were conducted within a 3-month period, and repeated after 2 years for the PD subjects.

In addition to PD patients, a healthy control group was included. Healthy controls were recruited from spouses of included patients (three subjects), and the remaining controls were recruited before lumbar puncture in conjunction with orthopedic surgery. Twenty-six subjects assented and successfully underwent MRI and FDG-PET. Exclusion criteria were as for PD, in addition to relevant subjective cognitive memory symptoms.

All subjects gave their written consent, and the Regional Committee for Medical and Health Research Ethics South-East evaluated (based on the Norwegian Health and Research Act and the Helsinki Declaration of 1964; revised 2013) and approved the study. All further study conduct was based on and in line with these guidelines.

CSF analysis

CSF samples were collected from all participants by lumbar puncture, performed between 9 and 12 AM, and consecutively after inclusion. Following lumbar puncture and standardized handling, CSF A β 42 > 700 ng/L were considered normal.

CSF A β 38, A β 40, and total α -synuclein were analyzed with electrochemiluminescence immunoassays (Meso Scale Discovery, US) in all PD patients. Due to possible effects of hemolysis, total α -synuclein were only included if CSF hemoglobin was <350 ng/mL. α -synuclein values for five patients were thus excluded.

CSF neurogranin concentration was measured using a previously described in-house ELISA.⁶ Briefly, an assay based on the monoclonal antibody Ng7 (epitope including amino acids 52–65 on neurogranin) was used for capture, a polyclonal neurogranin anti-rabbit antibody (ab23570; Upstate Biotechnology, Lake Placid, NY, USA) for detection, and full-length neurogranin protein as calibrator. We were able to measure neurogranin concentrations in all included PD patients. For 19 of the 26 included controls there was sufficient CSF available to assess neurogranin concentrations, and we added an additional 11 controls for which there was no available PET data. (The neurogranin healthy control material, $N = 30$, mean age 61.5 [range: 40–78; SD: 9.34], 18 females.)

FDG-PET and MRI imaging

FDG-PET/CT imaging was performed with a Siemens Biograph 64 PET/CT scanner for the PD group, and a Siemens Biograph 16 PET/CT scanner for the healthy

control group. All subjects fasted at least 4 h before image acquisition, and had an intravenous bolus of 218 MBq (± 23 MBq) injected, 45 min prior to scanning. A low-dose CT scan for attenuation correction was acquired. Patients were scanned for 15 min in three-dimensional mode using one bed position (axial range = 16 cm). Acquired data were corrected for random events, dead time, attenuation (CT-derived μ -map), scatter (model based), and decay. PET volumes were reconstructed using an iterative algorithm (OSEM 2D, four iterations, eight subsets), and a postreconstruction three-dimensional Gaussian filter of width 3.5 mm full-width at half maximum was applied. The axial image format was 256 \times 256 (pixel size: 2.67 mm \times 2.67 mm), with a slice thickness of 2.00 mm. Blood glucose at FDG injection time was measured routinely, and the patient was excluded if blood glucose exceeded 8.0 mmol/L.

For both PD and control subjects, MRI scans were acquired on a Philips Achieva 3 Tesla system. A single three-dimensional turbo field echo (TFE) sequence was acquired for morphometrics. Cortical reconstruction and volumetric segmentation was performed with FreeSurfer.^{33,34} The exact sequence parameters and reconstruction methods are described elsewhere.³⁵

Whole head PET volumes were coregistered with the anatomical volume using a rigid body spatial registration as implemented in the Spatial Parametrical Mapping (SPM 8, Wellcome Trust Centre for Neuroimaging, UCL, UK) co-registration tool. PET volumes were resliced into 1 mm MRI. For each subject, a voxel-based intensity normalization to the mean uptake in the cerebellar white matter was performed. (Prior to intensity normalization, the cerebellar white matter mask was eroded to avoid influence due to partial volume effects, inaccurate segmentation, or coregistration). Intensity normalized volumes were used for all further analyses. Because there is both global cerebral and cerebellar cortical hypometabolism in PD, neither global cortical mean nor cerebellar cortical normalization were suitable. We therefore chose to normalize the FDG-PET uptakes against mean cerebellar white matter.²⁵

Statistical analysis

Statistical maps were generated using the general linear model as implemented in FreeSurfer. Auxiliary statistical analyses were performed using IBM SPSS version 22 (Chicago, IL). Statistical maps of correlations (while correcting for effects of age and gender) between normalized FDG-PET uptake (or cortical thickness) and each CSF biomarker concentration were created. This operation was performed separately in the PD and control groups. Also, similar statistical maps of correlations between cortical

thickness and CSF biomarker concentrations were created. These statistical operations on global data and surface maps were generated by computing a general linear model of the effects of CSF biomarker concentrations on FDG uptake (or cortical thickness) at each vertex, while controlling for effects of age and gender. A cluster-wise correction for multiple comparisons was employed, based on ref 36. A cluster-forming threshold of $P < 0.01$ was chosen, and only clusters with a cluster-wise $P < 0.01$ (Bonferroni corrected for examining both hemispheres) were kept.

The coefficient of determination, R^2 was determined for the relation between CSF biomarkers (separately) and FDG uptake or cortical thickness (in pertinent areas as determined above). The effects of age and sex were regressed out of mean FDG uptake or cortical thickness, and the resulting residual was entered in a simple regression model as dependent variables, with the CSF biomarker concentration as explanatory variable.

To assess the relationship with cognitive function, mean FDG uptake (corrected for age and sex) in the pertinent areas as determined above for each CSF biomarker was also correlated (Spearman) with T-scores of neuropsychologic tests.

According to examination of histograms and as assessed by the Kolmogorov–Smirnov test of normality, CSF neurogranin concentrations were not normally distributed in neither the PD nor the control group. We therefore assessed group differences using Mann–Whitney U . There were no significant correlations (Spearman's rho) between CSF neurogranin concentrations and age or sex (neither within the PD group nor within the control group). We did therefore not correct for age or sex when comparing the groups. One outlier in the PD group with values 10–20 times the mean in the group, was removed, but included in additional analyses for reference.

Within the PD group, the distribution of CSF neurogranin, A β 38, 40, and 42 concentrations as well as UPDRS scores were borderline normally distributed according to examination of histograms and as assessed by the Kolmogorov–Smirnov test of normality. We therefore chose to assess the correlation between these variables with Spearman correlations. (The estimations of these correlations were repeated with parametric Pearson correlations with very similar results, data not shown.)

Two-year follow-up cognitive data were available for the PD subjects. To assess the association between cognition, time, and FDG-PET clusters as determined above, a mixed models approach was used: each of the examined neuropsychological variables were entered as dependent variables accounting for repeated measures (in separate models) with age, the FDG-PET cluster corresponding to CSF A β 42, and also an interaction between time and the

FDG-PET cluster (to model the association between FDG-PET and change over time) as independent variables. A priori, the FDG-PET cluster corresponding to CSF A β 42 was chosen for this analysis because it is one of the larger, representative clusters and the well-documented role of A β 42 in synaptic pathology.

Results

Demographical, CSF, and neuropsychological characteristics of the groups

There were no significant differences in age, gender composition, education, or MMSE between the PD and the healthy control group. Neuropsychological function was in the normal range for both groups, except slightly below normal on a measure of divided attention in the PD group. Group composition and demographics are outlined in Table 1 and neuropsychological characteristics in Table 2.

Relationship between the CSF biomarkers

CSF neurogranin concentration was significantly lower in the PD group as compared to the control group (Mann–Whitney $U = 211$, $P = 0.001$). (Not excluding the outlier, the difference was still significant (Mann–Whitney $U = 150$, $P = 0.006$.) (Fig. 1).

There were no significant correlations between CSF neurogranin concentration and age or sex in either of the diagnostic groups (in the PD group, respectively, $\rho = 0.192$; $P = 0.319$, $\rho = -0.260$; $P = 0.173$, in the control group, respectively, $\rho = -0.224$; $P = 0.235$, $\rho = 0.283$, $P = 0.130$). There was a significant correlation between α -synuclein and age ($\rho = 0.533$, $P = 0.006$) in the PD group.

CSF neurogranin concentrations were significantly correlated with the concentrations of CSF A β 38 ($\rho = 0.694$; $P < 0.001$), A β 40 ($\rho = 0.653$; $P < .001$), A β 42 ($\rho = 0.434$; $P = 0.019$), and α -synuclein ($\rho = 0.759$; $P \leq 0.001$). CSF neurogranin concentrations were significantly inversely correlated with UPDRS score ($\rho = -0.369$; $P = 0.039$), as was α -synuclein ($\rho = -0.483$, $P = 0.020$). There were no significant correlations between UPDRS scores and concentrations of CSF A β 38, 40, or 42.

FDG-PET and A β in patients with PD

There was a significant correlation between CSF A β 42 concentrations and FDG-PET uptake in PD; lower CSF A β 42 concentrations were found with more severe reductions on FDG-PET. The statistical map (Fig. 2) shows decreased cortical metabolism in mainly parietal, frontal, and occipital areas. For both hemispheres, there was one frontal and one

Table 1. Overview of demographics and group characteristics.

	Parkinson's disease	Healthy controls
N subjects	30	26
Age	65.0 (47–74) years	65.0 (47–81) ¹
Females/males	11/19	13/13 ²
Education	13.0 (8–19) years	12.6 (8–20) ³
MMSE	28.6 (22–30)	28.7 (26–30) ⁴
Geriatric depression scale score	1.3 (0–6)	1.4 (0–6) ⁵
Duration from motor symptom onset	2.6 (1–6) years	n.a.
UPDRS part III motor score	15.4 (6–31)	n.a.
Hoehn and Yahr stage	1.55 (1–2.5)	n.a.
Total daily levodopa equivalent dose	390.0 (0–924)	n.a.
CSF A β 38; ng/L	1877 (819–4551)	n.a.
CSF A β 40; ng/L	5125 (2212–10512)	n.a.
CSF A β 42; ng/L	943 (707–1280)	987 (634–1290) ⁶
CSF total α -synuclein; ng/L	278 (160–476) ⁷	n.a.
CSF neurogranin; ng/L	251 (125–556) ⁸	397 (119–988) ^{9,10}

Entries are mean (range) unless otherwise stated. Bold entries are significantly different from Parkinson's disease.

A β , amyloid- β ; A β 38, amyloid- β 1-38; A β 40, amyloid- β 1-40; A β 42, amyloid- β 1-42; MMSE, Mini-Mental State Examination; n.a., not available; UPDRS, Unified Parkinson's Disease Rating Scale.

¹Age: Parkinson's disease versus Controls: *T*-test $P = 0.989$.

²Gender: Between-group gender composition differences; Pearson chi-square, $P = 0.315$.

³Education: Parkinson's disease versus Controls: Mann-Whitney $U = 349$, $P = 0.498$.

⁴MMSE: Parkinson's disease versus Controls: Mann-Whitney $U = 380$, $P = 0.864$.

⁵Geriatric depression scale: Parkinson's disease versus Controls: Mann-Whitney $U = 357.5$, $P = 0.757$.

⁶A β 42: Parkinson's disease versus Controls: Mann-Whitney $U = 329$, $P = 0.312$.

⁷ $N = 25$. Missing for five subjects with Parkinson's disease.

⁸Neurogranin: One outlier in the Parkinson's disease group excluded, see methods section.

⁹ $N = 30$ for Controls, see methods section.

¹⁰Parkinson's disease versus Controls: Mann-Whitney $U = 211$, $P = 0.001$.

posterior (comprising parietal, occipital, and some temporal vertices) cluster. There were no correlations between CSF A β 42 concentrations and cortical thickness in PD.

In addition, there was a positive correlation between CSF A β 38 concentrations and FDG-PET uptake in PD. The statistical map (Fig. 3) shows the widespread clusters, most notably posterior lateral areas in which cortical metabolism correlated with CSF A β 38 concentrations.

There were also slight correlations between CSF A β 40 concentrations and FDG-PET uptake in PD. The

Table 2. Neuropsychological characteristics of the groups.

Neuropsychological test battery	Parkinson's disease		Healthy controls
	Baseline	Follow-up	
N subjects (missing neuropsychological data)	28 (2)	29 (1)	26 (0)
RAVLT delayed verbal recall of 15 words after 30 min, T-score	50.4 (31–75)	53.0 (25–71)	47.3 (32–67) ¹
RCFT delayed visual reproduction after 30 min, T-score	46.5 (20–75)	60.1 (22–80)	n.a.
TMT-A psychomotor speed, T-score	41.0 (26–62)	47.5 (32–62)	n.a.
TMT-B divided attention, T-score	39.9 (10–59)	47.0 (27–66)	50.2 (28–65) ²
SCW response inhibition, T-score	50.7 (27–67)	50.7 (20–70)	49.0 (20–63) ³
COWAT verbal fluency, T-score	48.1 (33–69)	52.0 (32–73)	48.7 (25–69) ⁴

Entries are mean (range) unless otherwise stated. Bold entries are significantly different from Parkinson's disease at baseline.

A β , amyloid- β ; COWAT, Controlled Oral Word Association Test; RAVLT, Rey Auditory Verbal Learning Test; RCFT, Rey Complex Figure Test; SCW, Stroop Color-Word; TMT-A, Trail Making Test-A; TMT-B, Trail Making Test-B.

¹RAVLT delayed verbal recall: Parkinson's disease versus Controls: Mann-Whitney $U = 297$, $P = 0.24$.

²TMT-B divided attention (available for 25 controls): Parkinson's disease versus Controls: Mann-Whitney $U = 207.5$, $P = 0.011$.

³SCW response inhibition: Parkinson's disease versus Controls: Mann-Whitney $U = 345.5$, $P = 0.936$.

⁴COWAT verbal fluency (available for 25 controls): Parkinson's disease versus Controls: Mann-Whitney $U = 331.5$, $P = 0.728$.

statistical map (Fig. 4) shows small posterior lateral clusters in both hemispheres in which cortical metabolism correlated with CSF A β 40 concentrations.

FDG-PET and A β in healthy controls

There were no correlations between CSF A β 42 concentrations and cortical FDG-PET uptake in the control group. (Not corrected for multiple comparisons, still no voxels reached the $P < 0.01$ threshold). Further, there were no correlations between CSF A β 42 concentrations and cortical thickness in the control group. (Not corrected for multiple comparisons, with the threshold set at $P < 0.01$ some small, distributed areas were detected.)

FDG-PET and α -synuclein in patients with PD

There was a significant correlation between CSF total α -synuclein concentrations and FDG-PET uptake in PD;

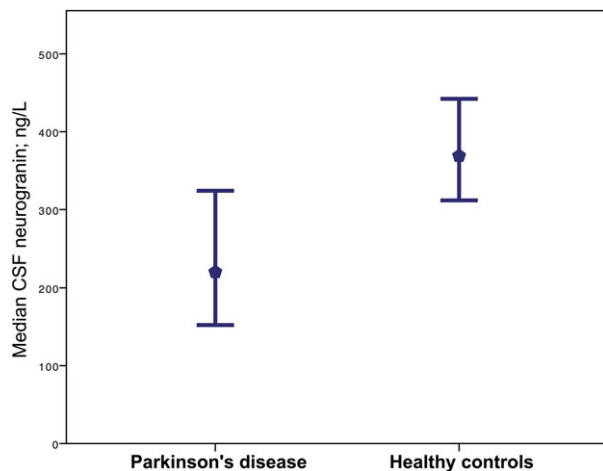


Figure 1. Median CSF neurogranin concentrations. The median concentration of CSF neurogranin in patients with mild-to-moderate Parkinson's disease and healthy controls, error bars represent 95% confidence intervals. (Mean CSF neurogranin in the Parkinson's disease subjects was 251 (range: 125–556), and in healthy controls, 397 (range: 119–988).)

lower CSF total α -synuclein concentrations were found with more severe reductions on FDG-PET. The statistical map (Fig. 5) shows decreased cortical metabolism in several cortical clusters. Cluster-wise $P = 0.0002$ for the more frontal left clusters and the lateral right clusters, and $P = 0.021$ for the remaining left, and $P = 0.028$ for the remaining right cluster.

There were no correlations between CSF total α -synuclein concentrations and cortical thickness in PD.

FDG-PET and neurogranin in patients with PD

There was a significant correlation between CSF neurogranin concentrations and FDG-PET uptake in PD; lower CSF neurogranin concentrations were found with more severe reductions on FDG-PET. The statistical map (Fig. 6) shows decreased cortical metabolism in several cortical clusters. Cluster-wise $P = 0.008$ for the lateral left and $P = 0.002$ for the medial left cluster, whereas $P = 0.0002$ for the single, larger right cluster.

There was a significant correlation between CSF neurogranin concentrations and cortical thickness in PD. (Not shown). There were five small clusters, cluster-wise $P = 0.001$ for a frontal left and $P = 0.002$ for a parietal left cluster, whereas $P = 0.042$ for a parietal right, and $P = 0.021$ for a frontal, medial right cluster. Also, there was a right inferior temporal cluster, $P = 0.002$. The clusters comprised 3% of the left and 3% of the right hemisphere. R^2 was 0.655 in the left and 0.630 in the right combined hemisphere clusters.

Relationship between FDG-PET clusters and cognition in patients with Parkinson's disease

For the cortical areas in which glucose metabolism was correlated with either CSF $A\beta_{38}$, $A\beta_{40}$, $A\beta_{42}$, total α -synuclein, or neurogranin, there were strong correlations between mean FDG uptake and most tests in the neuropsychological test battery, including measures of executive function (word fluency, response inhibition) and verbal and visual recall (Table 3). P -values for COWAT verbal fluency, SCW response inhibition, RAVLT delayed verbal recall, and RCFT delayed visual reproduction correlations with FDG-PET CSF-based clusters were all <0.01 . Correlations to TMT-B divided attention were not significant.

Relationship between FDG-PET clusters, cognition, and cognitive decline in patients with Parkinson's disease

For the cortical areas in which glucose metabolism was correlated with CSF $A\beta_{42}$, there were strong correlations ($P \leq 0.002$) between mean FDG uptake and all tests in the neuropsychological test battery at baseline (as assessed by the mixed model approach described above). Only TMT-A ($F = 5.684$, $P = 0.27$) and COWAT verbal fluency ($F = 4.431$, $P = 0.48$) were significantly changed from baseline to follow-up, and for these tests, FDG-PET cluster uptake was associated with change. (The interaction between repeated neuropsychological testing and cluster FDG-PET uptake is a measure of this. $F = 5.035$, $P = 0.36$ for TMT-A and $F = 5.041$, $P = 0.36$ for COWAT verbal fluency). TMT-A performance and verbal fluency improved, and for both higher FDG-PET uptake was associated with better performance at follow-up.

Discussion

Combining clinical, imaging, and CSF-proteomic techniques, this study links cortical glucose metabolism to $A\beta$ metabolism, CSF α -synuclein and neurogranin and cognitive impairment in PD. To the best of our knowledge, this is the first study to explore regional correlations between FDG-PET, $A\beta$ species, and cognition, and also relate these measures to an independent synaptic marker. The study results support our initial hypothesis, that is, that synaptic dysfunction is related to both reduced glucose metabolism and reduced levels of $A\beta$ species, putatively related to presynaptic deposition of α -synuclein. The highly significant associations to a wide range of cognitive functions, including memory and aspects of executive functions, demonstrate the clinical relevance of our

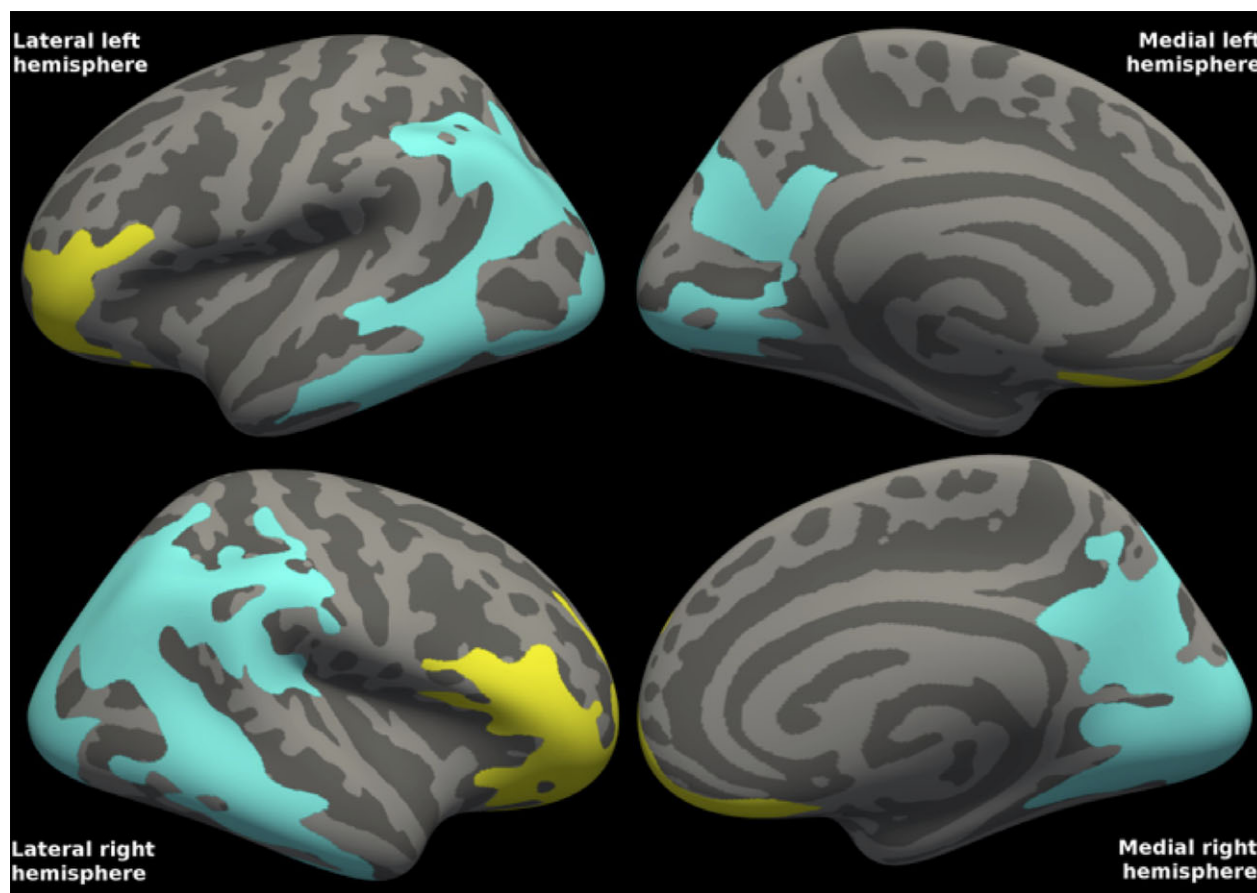


Figure 2. Correlations between FDG-PET and CSF A β 42 in Parkinson's disease. The statistical map shows two clusters in each hemisphere, one frontal (yellow) and one posterior (turquoise) cluster, in which cortical metabolism in mild-to-moderate Parkinson's disease is correlated with CSF A β 42 concentrations. Mean CSF A β 42 was 943 ng/L (range 707–1280). Cluster-wise $P = 0.0001$ for all clusters. The clusters comprised 36% of the total surface area (27% of the left and 45% of the right hemisphere surface area). R^2 was 0.389 in the left and 0.433 in the right combined hemisphere clusters (excluding the effects of age and gender).

findings. Although there was little change in cognitive performance from baseline to follow-up in the PD on group level, performance changes were significantly associated with FDG-PET.

We describe correlations between CSF A β 38 and A β 42 concentrations and FDG-PET in parietal association, occipital and lateral frontal areas in PD even though levels of A β 42 were within normal range for all included PD subjects. Similar associations were not found in healthy controls. Furthermore, within these same areas there were strong FDG-PET correlations to CSF neurogranin concentrations. CSF neurogranin was also strongly correlated with concentrations of the A β species, and were significantly reduced compared to healthy controls at a group level. The broad cortical areas involved are mirrored by broad and highly significant cognitive associations.

As described in the introduction, the reduced FDG-PET signal, the lowered CSF A β species and the reduced

levels of α -synuclein and neurogranin may all be linked to reduced synaptic activity. In AD, increased CSF neurogranin concentrations probably reflects synaptic degeneration with loss of integrity of postsynaptic spines, that results in increased CSF levels, and this is hypothesized specifically for Alzheimer's disease.^{37,38} In PD, cortical presynaptic pathology has been demonstrated in the form of accumulation of α -synuclein oligomers, accompanied by loss of dendritic spines but other postsynaptic injury was not described.⁵ This is consistent with our present findings of a reduced CSF neurogranin in the present PD population as compared to healthy controls. Thus, though synapses are a focus of both the Alzheimer and PD processes, the different mechanisms of attack may explain the marked differences in neurogranin levels observed in these diseases.³⁸ The strong association observed here between CSF α -synuclein and neurogranin is in line with such a mechanism, but other factors such as altered degradation or transport mechanisms may also be implicated. Late in

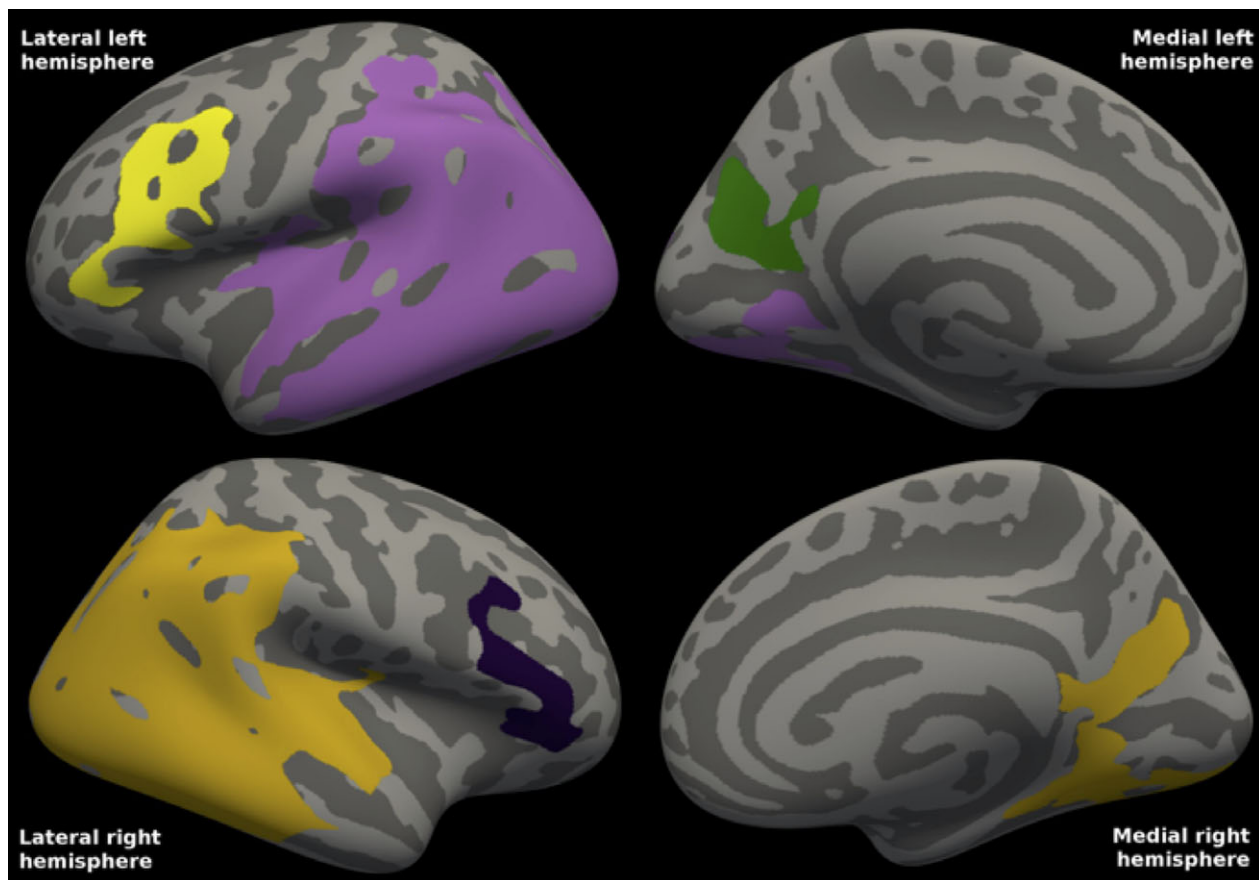


Figure 3. Correlations between FDG-PET and CSF A β 38 in Parkinson's disease. The statistical map shows the three clusters in the left hemisphere and the two clusters in the right hemisphere in which cortical metabolism in mild-to-moderate Parkinson's disease is positively correlated with CSF A β 38 concentrations. For the left hemisphere, cluster-wise $P = 0.0001$ for the two mainly lateral clusters, whereas for the mainly medial (precuneal; green) cluster, cluster-wise $P = 0.0074$. For the right hemisphere, cluster-wise $P = 0.0001$ for the larger, posterior cluster, whereas cluster-wise $P = 0.00170$ for the frontal. The clusters comprised 36% of the total surface area (38% of the left hemisphere surface area, and 33% of the right). R^2 was 0.319 in the left and 0.304 in the right combined hemisphere clusters (excluding the effects of age and gender). Mean CSF A β 38 was 1877 ng/L (range: 819–4551).

the disease-course PD cases suffer from more extensive multifactorial neurodegeneration, including amyloid pathology. The present cohort consisted of early cases (Table 1, mean disease duration 2.6 years), whereas mean disease duration in the PD cohort described in ref. 38 was 8 years. If active amyloid-associated neurodegeneration causes the rise of CSF neurogranin in Alzheimer's disease, this may possibly explain why the latter cohort also had higher values than the present cohort. Thus, the present findings suggest that several disease markers and clinical parameters like UPDRS and cognitive function are linked to synaptic dysfunction in PD, and putatively demonstrate a clinical relevance of CSF neurogranin in PD via its correlation with the UPDRS motor scale.

Though reduced FDG-PET uptake points to cortical involvement and the described cortical presynaptic accumulation of α -synuclein points to a cortical focus, we

cannot exclude subcortical pathology as contributing factors. The nucleus basalis of Meynert (in the basal forebrain) has widespread projecting cholinergic neurons to the entire neocortex, and is known to degenerate in PD (as well as Alzheimer's disease), leading to cortical dysfunction and predicting cognitive decline.^{39–41} Corticopetal dopaminergic pathways mainly project to the frontal cortex. In PD, reduced dopaminergic and cholinergic transmission has been demonstrated in a pattern coinciding well with the frontal and posterior clusters demonstrated in the present work.⁴² As such, the present findings in the PD group could, hypothetically, be construed as an effect of reduced dopaminergic (the frontal cluster) and reduced cholinergic transmission (the posterior cluster) in accordance with the dual syndrome hypothesis. However, here we describe very strong links between cognition, cortical glucose metabolism, and CSF

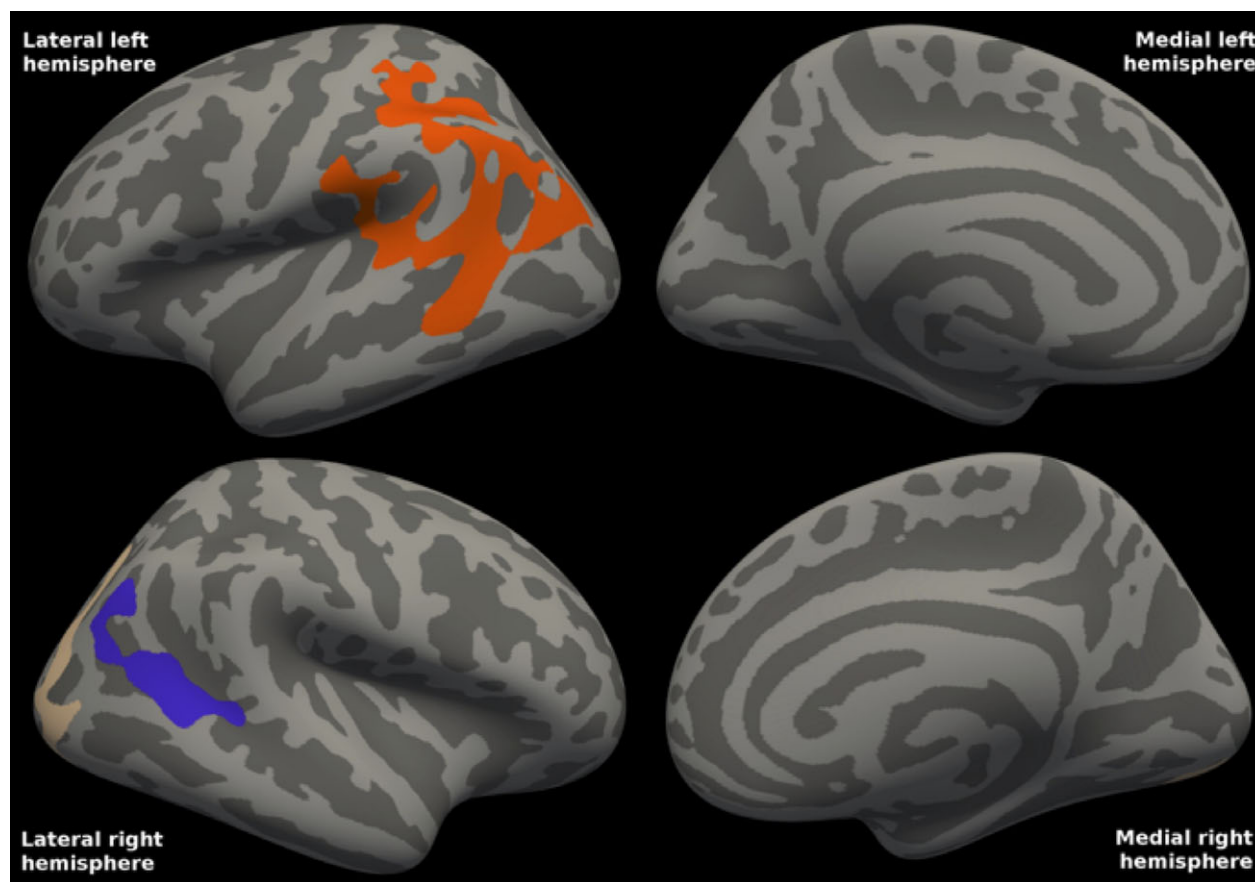


Figure 4. Correlations between FDG-PET and CSF A β 40 in Parkinson's disease. The statistical map shows one cluster in the left hemisphere and two clusters in the right hemisphere in which cortical metabolism in mild-to-moderate Parkinson's disease is positively correlated with CSF A β 40 concentrations. For the left hemisphere cluster and the right hemisphere occipital cluster, cluster-wise $P = 0.0001$. For the right hemisphere parietal cluster (shown in blue), cluster-wise $P = 0.0061$. The clusters comprised 6% of the total surface area (8% of the left hemisphere surface area, and 5% of the right). R^2 was 0.277 in the left and 0.250 in the right combined hemisphere clusters (excluding the effects of age and gender). Mean CSF A β 40 was 5125 ng/L (range: 2212–10512).

A β species, both potentially linked to a cortical etiology. Also, neurobiological underpinnings linking A β and α -synuclein physiology in the presynaptic terminal, evidence for cortical presynaptic buildup of α -synuclein aggregates and postsynaptic spine retraction is in line with our demonstration of links to CSF neurogranin and α -synuclein, and primary cortical etiology. Although the present findings do not primarily address mechanistic links, decades of preplaque A β pathology including oligomerization may well influence presynaptic α -synuclein metabolism, and there is experimental evidence that A β 42 may escalate production of toxic α -synuclein oligomers – directly disrupting SNARE-dependent vesicle function.^{43,44}

Although we find strong links to A β , a reverse causality cannot be excluded, in that synaptic dysfunction could be due to presynaptic α -synuclein aggregates slightly reducing A β in proportion to the degree of α -synuclein-dependent neurodegeneration.^{5,45} Alternatively, defective

presynaptic proteolysis and lysosomal degradation could affect both A β and α -synuclein metabolism.^{7,8,46} In either case, postsynaptic spines would be affected secondarily, likely leading to the observed reduced levels of neurogranin. Combined, pre- and postsynaptic dysfunction would result in loss of clinical and cognitive function as measured by the UPDRS and the cognitive measures. Putatively, these hypotheses could be tested or differentiated by also measuring presynaptic markers, like CSF synaptosomal-associated protein 25 (SNAP25, a component of the trans-SNARE complex)⁴⁷ or synaptotagmin, expected to be changed if vesicle fusion is truly hampered.

There are limitations to the current work that needs to be addressed. The PET scans of the PD and control groups were acquired on two similar, but not identical scanners. However, the scan and image reconstruction parameters, and also the postprocessing procedure were

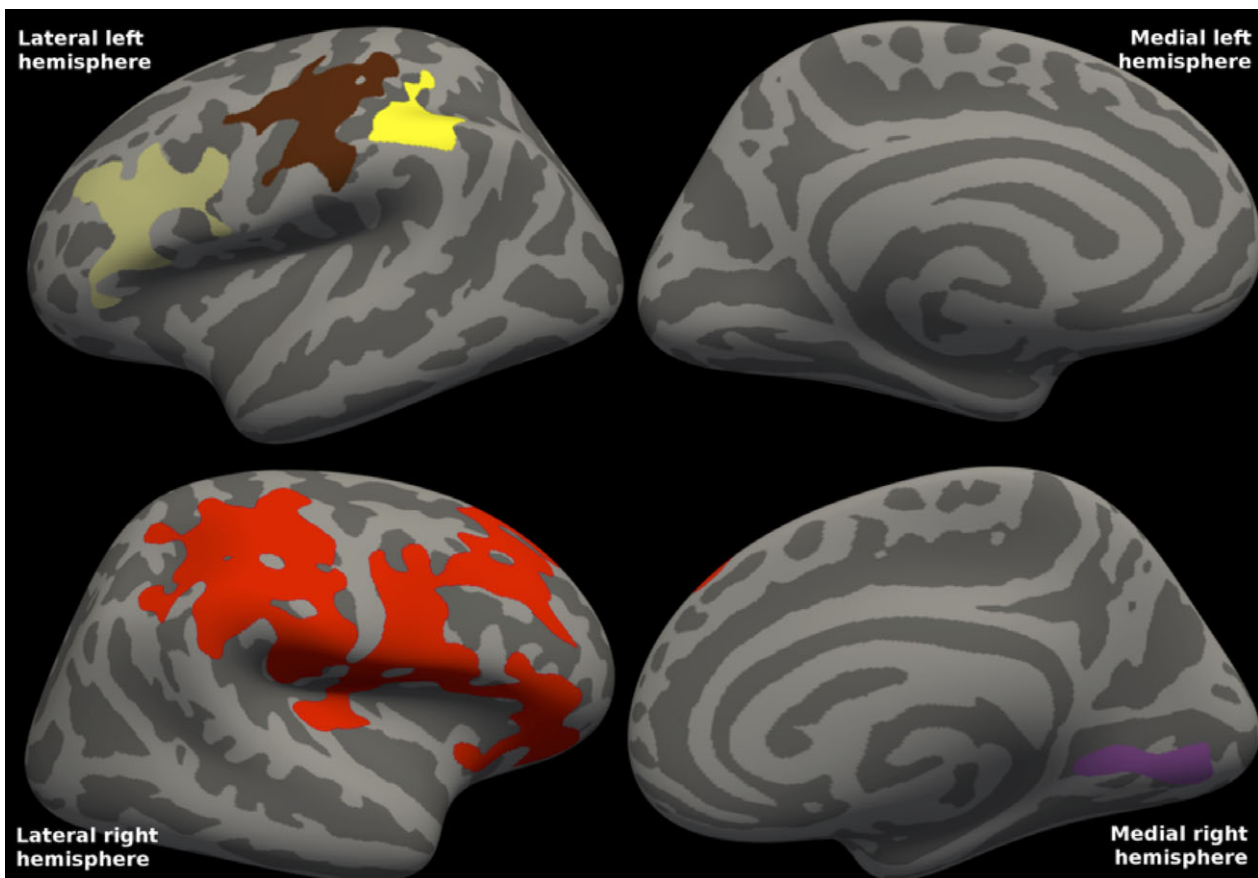


Figure 5. Correlations between FDG-PET and CSF total α -synuclein in Parkinson's disease. The statistical map shows the clusters in each hemisphere in which cortical metabolism in mild-to-moderate Parkinson's disease is correlated with CSF t - α -syn concentrations. Mean CSF t - α -syn was 278 (range: 160–476). Cluster-wise $P = 0.0002$ for the more frontal left clusters and the lateral right clusters, and $P = 0.021$ for the remaining left, and $P = 0.028$ for the remaining right cluster. The clusters comprised 13% of the total surface area (8% of the left and 19% of the right surface area). R^2 was 0.337 in the left and 0.339 in the right combined hemisphere clusters (excluding the effects of age and gender).

identical between the scanners and all analyses were conducted within, and not between, groups. Care should nevertheless be taken when comparing the results between groups. Also, the sample size in the present work is limited, and our findings need to be reproduced in other populations (including drug naive), and although fluctuations are relatively uncommon at the present disease stage (Hoehn and Yahr stage <3 , disease duration <6 years) the subjects should preferably have been tested at a standardized time in relation to medication. The sample size for the FDG-PET analyzes with α -synuclein and neurogranin were even smaller than for $A\beta$, and one might expect more widespread, significant clusters with a larger sample. Further, although FDG uptake reflects synaptic function, there exists no direct or absolute measure of synaptic function, and this should be taken into account when interpreting the present results.

All PD subjects had normal levels of CSF $A\beta_{42}$, and were thus unlikely to harbor amyloid plaques. However, a

presence of soluble, synaptotoxic $A\beta$ oligomers cannot be excluded and could be an alternative explanation for the present findings related to $A\beta$. Measuring $A\beta$ oligomers in the CSF of patients with PD may be a way to test this alternative explanation.

We were able to investigate the correlations between cortical metabolism and the alternative amyloid species $A\beta_{38}$ and $A\beta_{40}$ (considered less prone to aggregate) in the PD group, and interestingly the correlations between FDG-PET and $A\beta_{38}$ were comparable to those with $A\beta_{42}$ (whereas the correlations with $A\beta_{40}$ were more sparse). As described earlier,¹⁸ CSF $A\beta$ species and α -synuclein concentrations correlate strongly, strengthening the supposition that presynaptic α -synuclein and $A\beta$ metabolism may interact to produce the current findings.

On group level, the PD patients performed as well as presumptively healthy controls on neuropsychological tests except on a measure of divided attention (functions that may be affected both by impaired dopaminergic and

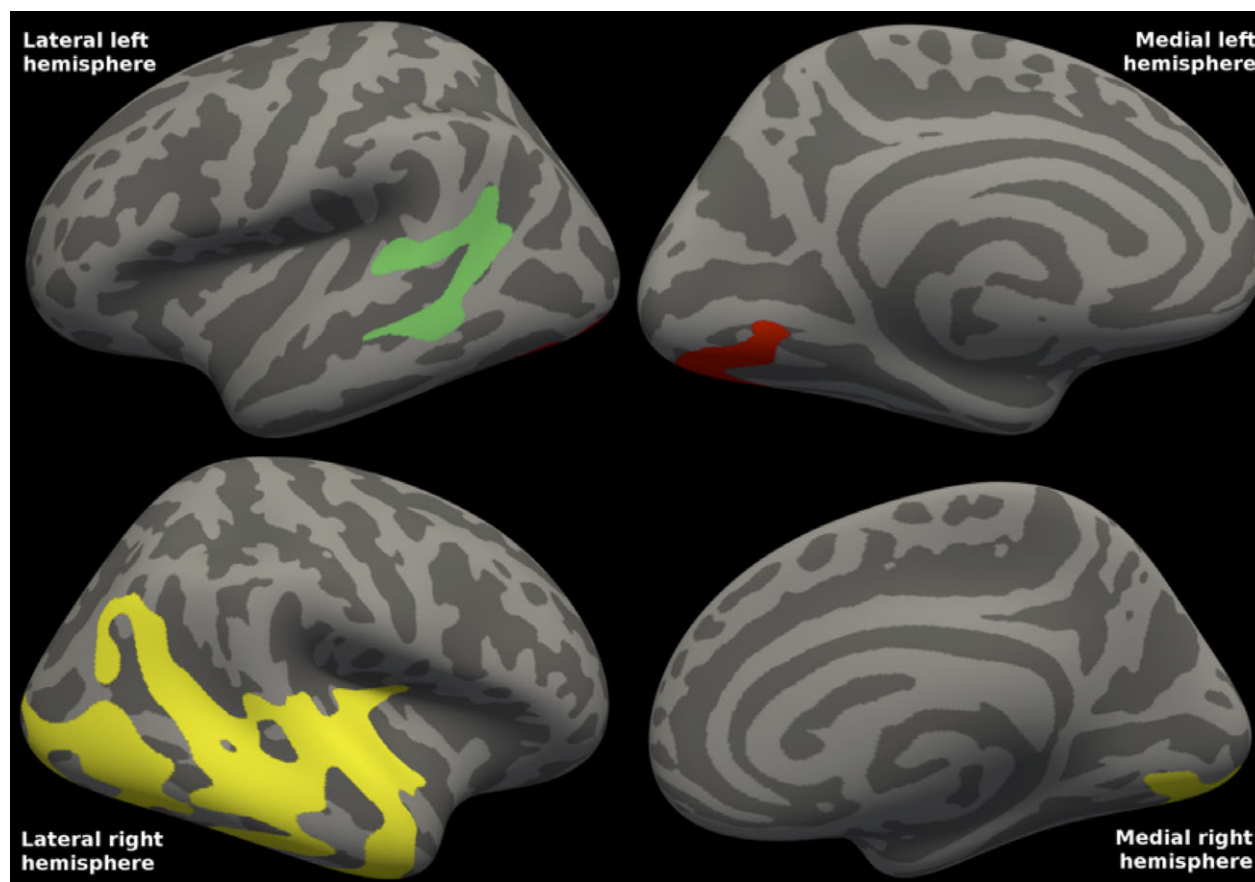


Figure 6. Correlations between FDG-PET and CSF neurogranin in Parkinson's disease. The statistical map shows the clusters in each hemisphere in which cortical metabolism in mild-to-moderate Parkinson's disease is correlated with CSF neurogranin concentrations. Mean CSF neurogranin was 251 (range: 125–556). Cluster-wise $P = 0.008$ for the lateral left and $P = 0.002$ for medial left cluster, whereas $P = 0.0002$ for the single, large right cluster. The clusters comprised 10.5% of the total surface area (4% of the left and 16% of the right surface area). R^2 was 0.330 in the left and 0.346 in the right combined hemisphere clusters (excluding the effects of age and gender).

Table 3. Correlations between cognition and mean FDG-PET in cortical clusters related to CSF biomarkers in Parkinson's disease.

Neuropsychological test battery	FDG-PET CSF biomarker-based cortical clusters				
	$A\beta_{42}^1$	$A\beta_{38}^1$	$A\beta_{40}^1$	α -synuclein ²	Neurogranin ³
RAVLT delayed verbal recall of 15 words after 30 min, T-score	0.566 (0.001)	0.615 (<0.001)	0.651 (<0.001)	0.561 (0.004)	0.616 (<0.001)
RCFT delayed visual reproduction after 30 min, T-score	0.608 (<0.001)	0.655 (<0.001)	0.667 (<0.001)	0.551 (0.005)	0.605 (0.001)
TMT-A divided attention, T-score	0.650 (<0.001)	0.602 (<0.001)	0.607 (<0.001)	0.727 (<0.001)	0.504 (0.005)
TMT-B divided attention, T-score	0.277 (0.145)	0.248 (0.195)	0.283 (0.136)	0.249 (0.252)	0.184 (0.350)
SCW response inhibition, T-score	0.617 (<0.001)	0.613 (<0.001)	0.621 (<0.001)	0.549 (0.005)	0.585 (0.001)
COWAT verbal fluency, T-score	0.499 (0.006)	0.499 (0.006)	0.539 (0.003)	0.653 (0.001)	0.488 (0.008)

Entries are Spearman's rho Correlation Coefficient (P -value). Bold entries are significant.

$A\beta$, amyloid- β ; COWAT, Controlled Oral Word Association Test; RAVLT, Rey Auditory Verbal Learning Test; RCFT, Rey Complex Figure Test; SCW, Stroop Color-Word; TMT-B, Trail Making Test-B. ¹⁻³Names refer to the clusters significantly correlated with that CSF biomarker.

¹ $N = 30$ for RAVLT, RCFT, and SCW. $N = 29$ for TMT-B, COWAT, WAIS-III.

² $N = 24$ for RAVLT, RCFT, SCW, and COWAT. $N = 23$ for TMT-B and WAIS-III.

³ $N = 29$ for RAVLT, RCFT, and SCW. $N = 28$ for TMT-B, COWAT, and WAIS-III.

cholinergic networks and frontoparietal hypometabolism⁴⁸). The present results may have been more informative were the included PD subjects more cognitively impaired, or if the time from baseline to follow-up was longer. However, there were strong correlations between cortical glucose metabolism and cognitive function in the PD group. Although there was sparse change in cognition at 2-year follow-up, cortical metabolism was significantly associated with this change. We hypothesize that baseline cortical glucose metabolism will predict further cognitive change and predict time from disease onset to dementia.

Herein, we describe a correlation between cortical glucose metabolism, cognitive functions, cognitive change, CSF A β species, neurogranin, and α -synuclein in PD. The findings were robust even though CSF A β 42 was within normal levels for all included subjects with PD, suggesting that cortical synaptic function is related to cognitive functioning, and is affected in PD. If confirmed, the present results will have important implications for understanding the role of synaptic dysfunction, A β and α -synuclein in the evolution of PD. We further find lower CSF concentrations of neurogranin in these patients with mild-to-moderate PD, but further studies are needed to assess whether this may be of clinical relevance. Additionally, the findings question the previously postulated association of reduced CSF α -synuclein concentration in PD with Lewy body pathology, in that reduced CSF α -synuclein concentration may reflect synaptic degeneration and reduced release.

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Author Contributions

PS drafted the manuscript, contributed to the conception and design of the study, acquisition and analysis of data. ALS contributed in revising a substantial portion of the manuscript and acquisition of data. KKJ contributed in revising a substantial portion of the manuscript and acquisition of data. AB contributed to the conception and design of the study, and acquisition of data. CC contributed to the acquisition and analysis of data. EA, LK, and ISA revised the manuscript and contributed to the acquisition of data. EH contributed to the conception and design of the study, and acquisition of data. HZ, KB,

DA, and TF contributed in the conception and design of the study as well as acquisition of data and revision of the manuscript. All authors approved the final draft.

Conflicts of Interest

Dr. Selnes, Dr. Stav, Dr. Johansen, Dr. Bjørnerud, Dr. Kalheim, Dr. Almdahl, Dr. Hessen, and Dr. Auning have nothing to disclose. Dr. Coello reports personal fees from Imanova Ltd, outside the submitted work. Dr. Zetterberg reports personal fees from Roche Diagnostics, non-financial support from Eli Lilly, personal fees from Pharmasum Therapeutics, outside the submitted work; and HZ is co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Venture-based platform company at the University of Gothenburg. Dr. Blennow reports personal fees from Fujirebio Europe, personal fees from Roche Diagnostics, personal fees from IBL International, outside the submitted work; and Co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Venture-based platform company at the University of Gothenburg. Dr. Aarsland reports personal fees from Novartis, personal fees from H Lundbeck, grants from Astra-Zeneca, outside the submitted work. Dr. Fladby reports grants from Norwegian Research Council, grants from EU-JPND, during the conduct of the study; in addition, Dr. Fladby has a patent PCT/EP2009/001210 issued, and a patent PCT/US2011/062233 pending.

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