Neuroinflammation In Parkinson's Disease: A Study With [11C]PBR28 PET And Cerebrospinal Fluid Markers

Haidar Al-Abdulrasul (Haidar.al-abdulrasul@helsinki.fi)
Turku PET Centre: Turun PET keskus https://orcid.org/0000-0001-9788-134X
Riikka Ajalin
Turku PET Centre: Turun PET keskus
Jouni Tuisku
Turku PET Centre: Turun PET keskus
Henrik Zetterberg
University of Gothenburg Institute of Neuroscience and Physiology: Goteborgs universitet Institutionen for neurovetenskap och fysiologi
Kaj Blennow
University of Gothenburg Institute of Neuroscience and Physiology: Goteborgs universitet Institutionen for neurovetenskap och fysiologi
Tero Vahlberg
University of Turku Faculty of Medicine: Turun yliopisto Laaketieteellinen tiedekunta
Laura Ekblad
Turku PET Centre: Turun PET keskus
Semi Helin
Turku PET Centre: Turun PET keskus
Sarita Forsback
Turku PET Centre: Turun PET keskus
Juha Rinne
Turku PET Centre: Turun PET keskus
Anna Brück
Turku PET Centre: Turun PET keskus

Research Article

Keywords: Parkinson's disease,18 kDa translocator protein, Neuroinflammation, [11C]PBR28, alpha-synuclein

Posted Date: June 27th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1772425/v1

Abstract

Purpose

To investigate neuroinflammation in Parkinson's disease (PD) with [^{11}C]PBR28 positron emission tomography (PET) and cerebrospinal fluid (CSF) biomarkers, and the relationship to dopaminergic functioning measured with 6-[^{18}F]-fluoro-L-dopa ([^{18}F]FDOPA) PET.

Methods

The clinical cohort consisted of 20 patients with PD and 51 healthy controls (HC). All HC and 15 PD participants underwent [^{11}C]PBR28 High Resolution Research Tomograph (HRRT) PET examination for the quantitative assessment of cerebral binding to the translocator protein (*TSPO*), a neuroinflammation marker. CSF samples were available from 17 patients with PD and 21 HC and were examined for soluble triggering receptor expressed on myeloid cells 2 (sTREM2), chitinase 3-like 1 protein (YKL-40), neurogranin (NG), alpha-synuclein (aSyn) and oligo-alpha-synuclein. All patients with PD underwent [^{18}F]FDOPA HRRT PET examination.

Results

Subjects with PD and HC did not differ in the total volume of distribution (V_T) of [^{11}C]PBR28 in any studied brain regions. In the CSF analyses, higher levels of sTREM2 and NG were associated with more severe motor symptoms evaluated by The Unified Parkinson's Disease Rating Scale motor part (UPDRS-III) (p = 0.041 and p = 0.016 respectively). Additionally, in the PD group increased V_T in the basal ganglia and substantia nigra (SN) were related to higher levels of YKL-40 (p < 0.01). No significant correlations were found between [^{11}C]PBR28 VT and [^{18}F]FDOPA uptake or between [^{11}C]PBR28 VT and UPDRS-III in any studied region. No significant correlations were observed between the CSF markers and [^{18}F]FDOPA uptake in the SN or the striatum. No significant correlations were found between [^{11}C]PBR28 VT and aSyn, oligo-aSyn or their ratio.

Conclusion

Associations between CSF biomarkers, motor disability and [^{11}C]PBR28 VT in the striatum and SN may support a role for neuroinflammation in PD.

Introduction

The aetiology of Parkinson's disease (PD) is still unclear but growing evidence suggests that neuroinflammation has a crucial role in its pathology. It has been associated with PD in studies of

activated microglia [1, 2] and inflammatory cytokines [3] in postmortem brain tissue and cerebrospinal fluid (CSF) [4]. Higher levels of inflammatory transmitters and activated immune defence cells have been detected in CSF and blood in patients with PD, compared to healthy controls [5–7]. However, the relationship between neuroinflammation and neurodegeneration and clinical features of PD remains poorly understood.

Microglial mitochondria expresses an 18kDa translocator protein (*TSPO*) which has a low expression in resident microglia but is significantly increased in activated microglia[8], which represents a hallmark in neuroinflammation. It is also overexpressed in the reactive astrocytes [9]. [^{11}C]PK11195 is a PET radioligand which binds to *TSPO* and has been widely used to image neuroinflammation *in vivo*. Patients with PD showed significantly increased [^{11}C]PK11195 binding [10–13] and the increased binding seems to correlate with the severity of PD motor symptoms [11]. The findings have not, however, been consistently confirmed [14]. [^{11}C]PK11195 has a poor signal to noise ratio which has led to the development of so-called second-generation *TSPO* tracers, such as [^{11}C]PBR28. The binding of [^{11}C]PBR28 is affected by a single nucleotide polymorphism (rs6971) [15] and recently, it was demonstrated that also other biological factors may contribute to the binding, including body mass index (BMI), age and sex [16].

In this study, we examined the distribution and severity of neuroinflammation in PD with [^{11}C]PBR28 PET and CSF biomarkers for neuroinflammation, neurodegeneration and alfa-synuclein levels in relation to dopaminergic functioning measured with 6-[^{18}F]-fluoro-L-dopa ([^{18}F]FDOPA) PET.

Materials And Methods

Subjects and study design

The participant sample consisted of 20 patients with PD and 10 healthy controls (HC). All participants were interviewed thoroughly for their medical history and a clinical neurological examination was performed by a clinical neurologist. Participants with neurodegenerative disease or a significant neurological illness other than PD were excluded. Other significant exclusion criteria included regular use of anti-inflammatory medication, history of stroke, contraindication for MRI and major focal lesions in brain MRI. The healthy control group included also 41 participants from another study (CIRI Study) from the Turku PET Centre focusing on risk factors for Alzheimer's disease, especially *APOE* ε4 genotype and midlife insulin resistance (IR) [17]. The study population consisted of elderly individuals without cognitive impairment, half of whom had IR in midlife. In addition, 50% were *APOE* ε4 carriers. The exclusion criteria were similar to the present study. The IR-status did not, however, affect the [^{11}C]PBR28 V_T in any brain region [18]. Thus, we were able to include this sample in our study with the permission of the corresponding researchers.

All participants were genotyped for the rs6971 polymorphism of the *TSPO* gene prior to imaging. Only high-affinity (HAB) and mixed-affinity (MAB) binders were included in the study. Recruited PD participants fulfilled the UK Brain Bank Research criteria for the diagnosis of idiopathic PD. Demographics are presented in Table 1.

All PD participants were on their individual standard PD medication including dopamine agonists, levodopa and MAO-B inhibitors. The Unified Parkinson's Disease Rating Scale motor part (UPDRS-III) was evaluated during the OFF stage (levodopa was discontinued for a minimum of 12 hours in advance, dopamine agonists and MAO-B inhibitors 24h in advance). To confirm the clinical diagnosis of PD, the patients with PD underwent [^{18}F]FDOPA PET examination showing typical findings for PD.

Cerebrospinal fluid

Thirty-eight subjects (17 PD: age years ± SD: 66.7 ± 6.1, F/M: 3/14 and 21 HC: 67.4 ± 6.0, F/M: 10/11) (age $p = 0.7$, sex $p = 0.02$) gave their consent to CSF sampling. The procedure was carried out by an experienced MD in an aseptic manner with the locally accepted protocols. The CSF samples were centrifuged at 2200 x g for 10 minutes with the temperature set at + 20°C. After that 0.5-1.0 mL aliquots of the samples were pipetted into labelled cryotubes to be frozen and stored at -80°C before the transfer. The CSF samples were transferred on dry ice to the Clinical Neurochemistry Laboratory of the University of Gothenburg for the biochemical measurements. CSF soluble triggering receptor expressed on myeloid cells 2 (sTREM2) concentration was measured using an in-house electrochemiluminescence assay on a Meso Scale Discovery (MSD) SECTOR imager 6000 (MSD, Rockville, MD), as previously described [19]. CSF neurogranin (NG) concentration was measured using a combination of NG2 and NG36 anti-NG antibodies in an in-house ELISA as previously described [20]. CSF chitinase 3-like 1 protein (YKL-40) concentration was measured using a commercially available YKL-40 ELISA kit (R&D Systems, Minneapolis, MN, USA). An enzyme-linked immunosorbent assay (ELISA) kit (catalog number: 844101, Legend Max, BioLegend, USA) was employed to measure total α-syn levels in CSF samples. Oligo-alpha-synuclein (oligo-aSyn) concentration was measured using an in-house Single molecule array (Simoa) assay employing an oligo-aSyn-selective capture antibody, as previously described [21].

Radiochemistry

The [^{11}C]PBR28 was prepared as described previously from its corresponding desmethyl-PBR28 precursor (PharmaSynth AS, Tartu, Estonia) [16]. [^{18}F]FDOPA was prepared as described previously [22].

Imaging procedures

Before PET imaging, structural MR imaging was conducted using a 3T scanner (Philips Ingenuity TF PET/MR, Philips Medical Systems, Cleveland, OH, USA) and evaluated by an experienced clinical neuroradiologist to exclude contributing pathologies. T1-weighted MR images were used for delineation of anatomical regions after co-registration with PET data.

All PET examinations were performed using a HRRT scanner (Siemens/CTI, Knoxville, TN, USA). All healthy controls (age years ± SD: 70.0 ± 4.9, F/M: 28/23) and 17 patients with PD (age years ± SD: 66.5 ± 6.4, F/M: 4/13, age $p = 0.018$, sex $p = 0.025$) underwent a [^{11}C]PBR28 PET but due to failure in obtaining arterial blood samples in two patients a valid data was available only for 15 patients with PD (age years ± SD: 66.0 ± 6.6, F/M: 4/11, age $p = 0.011$, sex $p = 0.054$). A thermoplastic mask was used with a head fixation system to minimize participant movement artefacts. After the transmission scan (using ^{137}Cs point

source) [^{11}C]PBR28 (injected activity avg ± SD: 493 ± 18,5MBq) was injected intravenously as a rapid bolus followed by a 70 min dynamic PET acquisition. During the first 3 minutes, continuous blood sampling was performed by an automated blood sampling system (ABSS, Allogg AB, Mariefred, Sweden) after which blood samples were drawn manually at fixed time points (4, 6, 8,10, 15, 20, 25, 30, 40, 50 and 70 min). Blood plasma radioactivity was measured at these time points and the [^{11}C]PBR28 metabolites were determined to generate a metabolite corrected arterial plasma input curve. [^{11}C]PBR28 image reconstruction and blood data processing were carried out as described previously [16].

Patients with PD underwent [^{18}F]FDOPA imaging to examine the presynaptic dopaminergic function. The patients were given 150 mg of carbidopa 60 minutes before the scan to block the peripheral decarboxylation of [^{18}F]FDOPA. After the transmission scan, a rapid bolus of [^{18}F]FDOPA (injected activity avg ± SD: 188.3 ± 9.6MBq) was given intravenously and a 90-minute dynamic scan was performed. No blood samples were drawn during this scan. [^{18}F]FDOPA images were reconstructed as described previously [23]. [^{11}C]PBR28 and [^{18}F]FDOPA scans were performed on different days with an average of 107.7 ± 91.3 days in between.

PET imaging analyses

The PET data were realigned and co-registered with the anatomical MR T1-weighted images using SPM12 software (Wellcome Trust Centre for Neuroimaging, London, UK) running in MATLAB (The Mathworks, Natick, MA). Global grey matter and six bilateral regions of interest (ROIs) at the anterior and posterior cingulate cortices, thalamus, caudate nucleus, putamen and cerebellum were delineated automatically with FreeSurfer software (vs 6) [24]. Additionally, a bilateral substantia nigra ROI was generated by using the Hammers atlas [25].

For quantitative assessment of [^{11}C]PBR28 binding, regional distribution volumes (V_T) of different ROIs were estimated using Logan's method within 30–70 min by using the metabolite corrected arterial plasma time-activity curve (TAC) as an input function. The reference tissue input Patlak method [26] with the occipital region as the reference region was used to calculate the influx constant K_i^{ref} between 15 min and 90 min following the [^{18}F]FDOPA injection.

Statistical Analyses

Statistical analyses were performed with the IBM SPSS Statistics program (version 25) and JMP Pro 14. The distributions were explored with Shapiro-Wilk's test and histograms. The difference in the regional [^{11}C]PBR28 V_T in different ROIs between groups was analyzed with General Linear Model (GLM). Analyses were adjusted for sex and age. Since the residuals of the V_T values in some ROIs were not considered normally distributed, we conducted a sensitivity analysis using log-transformed values to confirm the results. Subgroup analyses were also made based on the *TSPO* genotype with the same method in the HAB subgroup. Whereas the differences in the MAB subgroup were explored with the two-samples t-test and for the V_T values in some ROIs that were not considered normally distributed the results were also explored

with Mann-Whitney U-test. The differences in [11C]PBR28 V_T between males and females were explored with the two-samples t-test.

The correlation between K_i^{ref} and [11C]PBR28 V_T values in contralateral and ipsilateral (to the predominant side of motor symptoms) putamen and caudate nucleus were tested with Pearson partial correlation coefficients while the effect of the *TSPO* genotype was adjusted. The K_i^{ref} of contralateral and ipsilateral putamen and caudate nucleus were compared with paired samples t-test. Contralateral and ipsilateral putamen and caudate nucleus [^{11}C]PBR28 V_T were tested with paired samples t-test and the [^{11}C]PBR28 V_T in contralateral and ipsilateral substantia nigra was tested with Wilcoxon signed-rank test due to the distribution.

The correlation between the UPDRS-III, duration of the disease, NG, YKL-40, aSyn, oligo-aSyn and their ratio and [^{18}F]FDOPA K_i^{ref} in the PD group were examined with Pearson correlation coefficient. When exploring the correlation between the UPDRS-III, duration of the disease, NG, aSyn, oligo-aSyn and their ratio and the regional V_T of [^{11}C]PBR28 in the PD group we used the Pearson partial correlation coefficient where the effect of the *TSPO* genotype was adjusted. The same method was applied to explore the correlation between YKL and the regional V_T of [^{11}C]PBR28 in the PD group with *TSPO* genotype and age set as covariates. sTREM2 was not considered normally distributed, thus the correlations with UPDRS-III and the regional V_T of [11C]PBR28 in the PD group were estimated with Spearman's correlation coefficient. Due to the small sample size, the association between [^{11}C]PBR28 and CSF biomarkers were also explored with Spearman's correlation coefficient in which the two genotypes were analyzed separately. The differences between CSF biomarkers were explored with the two-samples t-test, except for YKL-40, where GLM was used for age adjustment. The level of significance was set at $p < 0.05$ in all tests.

Results

The [^{11}C]PBR28 V_T in PD and healthy controls

The effect of the *TSPO* genotype on [^{11}C]PBR28 V_T was seen in all studied regions both in subjects with PD and healthy controls ($p < 0.001$). There were no statistically significant differences in the V_T between patients with PD and healthy controls in any of the studied regions. The same observations were also made when the HAB and MAB genotypes were tested separately (Table 2). A significant correlation between BMI and [^{11}C]PBR28 V_T was observed as [^{11}C]PBR28 V_T decreased with higher BMI in all studied regions ($p < 0.01$). There was no significant difference in [11C]PBR28 VT between males and females or correlation between VT of [11C]PBR28 and age ($p > 0.05$).

Correlation between [^{11}C]PBR28 V_T and [^{18}F]FDOPA K_i^{ref} and UPDRS-III

No significant relationship was found between [^{18}F]FDOPA K_i^{ref} and [^{11}C]PBR28 V_T in any studied region ($p > 0.1$). There was no significant differences between the [^{11}C]PBR28 V_T in the contralateral and ipsilateral caudate nucleus (mean 2.79 vs 2.78, $p = 0.84$), putamen (mean 3.88 vs 3.92, $p = 0.54$) and substantia nigra (median 4.49 vs 4.03, $p = 0.39$). The contralateral [^{18}F]FDOPA K_i^{ref} values were lower than those in the ipsilateral side both in caudate nucleus (median 0.0068 vs 0.0075, $p = 0.002$) and in putamen (median 0.0047 vs 0.0054, $p = 0.047$) as expected.

There was no correlation between UPDRS-III motor part total score and [^{11}C]PBR28 V_T in any studied region ($p > 0.1$). No statistically significant correlation was found between UPDRS-III and the duration of the disease ($p > 0.1$) nor between the duration of the disease and [^{11}C]PBR28 V_T ($p > 0.1$). The correlation between UPDRS-III and [^{18}F] FDOPA K_i^{ref} did not reach statistical significance in the contralateral (r=-0.412, $p = 0.080$) or ipsilateral putamen (r=-0.32, $p = 0.18$) or the contralateral (r=-0.33, $p = 0.16$) or ipsilateral caudate nucleus (r=-0.36, $p = 0.13$).

CSF markers in PD subjects and healthy controls

YKL-40 levels increased with age (r = 0.59, $p < 0.001$) but similar observations were not made with sTREM2 (r = 0.22, $p = 0.30$) or NG (r = 0.23, $p = 0.11$). No significant differences were observed between sex and any of the CSF markers and thus only age was included in the analyses as a covariate. Patients with PD tended to have higher sTREM2 compared to controls, but this did not reach statistical significance ($p = 0.07$). Surprisingly, the control group had higher NG values than the PD group (median 162 vs 180, $p = 0.044$) while YKL-40 did not differ between groups ($p > 0.1$) (Fig. 1). There were no significant differences in aSyn, oligo-aSyn or their ratio between groups ($p > 0.1$). Also, no significant correlations were observed between aSyn, oligo-aSyn or their ratio and [^{11}C]PBR28 V_T in PD or HC groups.

UPDRS-III total score increased in correlation with sTREM2 and NG (Fig. 2, r = 0.52, $p = 0.041$ and r = 0.59, $p = 0.016$ respectively). A similar association was not observed between YKL-40 and UPDRS-III total score ($p = 0.15$, Fig. 2). Disease duration was not associated with any of the CSF marker levels. No significant correlations were observed between CSF markers and [^{18}F]FDOPA K_i^{ref} in substantia nigra, caudate nucleus or putamen ($p > 0.1$). aSyn, oligo-aSyn or their ratio did not correlate with UPDRS-III nor with [^{18}F]FDOPA K_i^{ref} in substantia nigra, caudate nucleus or putamen ($p > 0.1$).

Interestingly, in a subgroup analysis including patients with PD who had undergone both [^{11}C]PBR28 PET and CSF sampling (n = 12) YKL-40 levels were found to increase with higher [^{11}C]PBR28 V_T in the ipsilateral putamen (r = 0.65, $p = 0.043$) and contralateral thalamus (r = 0.66, $p = 0.039$) when the genotype and age were included as covariates. Due to the small sample size, this association was also explored with Spearman's correlation coefficient in which the two genotypes were analyzed separately. In the HAB group (n = 8) YKL-40 increased with [^{11}C]PBR28 V_T in the substantia nigra (r = 0.88), caudate (r = 0.86) and the putamen (r = 0.86) ($p < 0.01$, in all regions) but not in cortical regions. Similar associations were not observed between the other CSF markers and [^{11}C]PBR28 V_T or in the healthy controls ($p > 0.1$).

aSyn was found to increase in association with higher sTREM2 (r = 0.80, $p < 0.01$) and NG levels (r = 0.80, $p < 0.01$) in both groups and with YKL-40 only in PD group (r = 0.58, $p = 0.02$). Oligo-aSyn did not correlate significantly with any of the studied CSF neuroinflammation markers ($p > 0.1$). The ratio of Oligo-aSyn and aSyn showed to decrease in association with higher NG levels only in PD group (r = -0.52, $p = 0.03$). This ratio showed a significant correlation with sTREM2 in HC (r = -0.44, $p = 0.048$) but not in PD (r = -0.43, $p = 0.09$).

Discussion

In the present study, patients with PD and healthy controls underwent PET examination with a second-generation *TSPO* radioligand [^{11}C]PBR28. The results showed no statistically significant differences in [^{11}C]PBR28 binding between patients and healthy controls. The results are in accordance with previous findings obtained using second-generation radioligands [^{18}F]FEPPA [27, 28] and [^{11}C]PBR28 [29]. As an extension to previous studies, we also investigated neuroinflammation markers in CSF in a subset of subjects and found that an increased level of neuroinflammation markers was associated with more severe motor symptoms in PD patients.

PET studies using the first generation *TSPO* tracer [^{11}C]PK11195 have reported significantly increased binding in patients with PD compared to controls in substantia nigra, pons, basal ganglia and cortical regions [10, 11, 13]. Although significant differences in participant sample demographics such as age and disease stage might explain some of the confounding results, the most likely explanation lies in the tracers used.

Several factors have been reported to contribute to the [^{11}C]PBR28 binding, single nucleotide polymorphism (*rs6971*) being the most significant one affecting the binding of all second-generation *TSPO* tracers developed to date [30]. A recent study investigated the effect of the polymorphism on [^{11}C]PK11195 binding and did not observe sensitivity to the genotype [31]. However, the authors noted that a minor sensitivity might not have been detected due to the study's small sample size and the poor signal-to-noise ratio of [11C]PK11195. Currently, new tracers are being developed which would not include the *rs6971* sensitivity [30]. Other factors contributing significantly to the high variation of the [^{11}C]PBR28 binding include age, BMI and sex as pointed out in our earlier study [16]. BMI correlated inversely to *TSPO* binding also in our study. Possible explanations for this finding include the dysregulation of *TSPO* in obese people and endogenous ligands capable of binding to *TSPO* such as cholesterol which are typically higher in obese individuals [16, 32].

Additionally, based on the evidence from animal studies it has been suggested that microglial cells react to their surroundings and change not only their phenotype but also their functioning resulting in a sum effect which can either be anti- or pro-inflammatory depending on the stimulus. Thus, it is possible that microglia can have a beneficial or a harmful role depending on the disease stage. There is currently no evidence that the *TSPO* PET tracers in use today can differentiate between these two functional states [33].

In the current study, we did not observe any significant correlation between [^{11}C]PBR28 binding and the dopaminergic functioning measured with [^{18}F]FDOPA. Neither was the [^{11}C]PBR28 binding related to the UPDRS-III motor part total score. Regardless of the statistically significant decrease of the dopaminergic function on the contralateral side to the side with dominant motor symptoms, no significant differences between contralateral and ipsilateral sides on the [^{11}C]PBR28 V_T binding were seen in the striatum or any other regions studied. These results are in accordance with the previous study reporting no association between [^{11}C]PBR28 binding and [^{18}F]FE-PE2I, a dopamine transporter ligand [29]. [^{11}C]PK11195 binding in the mid-brain has been reported to correlate inversely to dopaminergic activity in the putamen in the mild initial stage of the disease, but not later in the disease course [10, 11]. Also, the [^{11}C]PK11195 binding did not change in patients with PD during the follow-up period of two years suggesting that microglial activation occurs early in the disease process [10]. Most of our patients were at a moderate stage which could partially explain the lack of association between [^{11}C]PBR28 V_T and dopaminergic activity. It is possible that *TSPO* binding correlates with the dopaminergic function in the early stages of the disease, or perhaps the correlation is nonlinear which would require larger sample sizes to be detected. In the current study, we did not detect any association between the duration of the disease and [^{11}C]PBR28 binding supporting this notion.

In addition to [^{11}C]PBR28 PET imaging, the CSF neuroinflammation biomarkers sTREM2 and YKL-40 were investigated. sTREM2 and YKL-40 are associated with microglia and astrocyte activity respectively and increased levels of these glial markers have been reported in early Alzheimer's disease (AD) [34, 35] and in dementia with Lewy bodies in co-existence with AD type pathology [35]. No differences have been reported between patients with PD and healthy controls in sTREM2 levels in an earlier study [36] whereas in a more recent one there has been a significant difference [37].

In the current study subjects with PD showed slightly increased CSF sTREM2 levels compared to controls although this did not reach statistical significance. Interestingly, higher YKL-40 levels were associated with increased [^{11}C]PBR28 V_T in SN and the striatum in patients with PD but not in healthy controls, but given that the sample size was very small in this analysis, these findings should be interpreted with caution. However, the findings could reflect the upregulation of *TSPO* in astrocytes being a sign of astrocyte activation as previous observations have suggested [38]. This finding is following the neuropathological evidence of activated astrocytes in the regions of α-synuclein accumulation [39]. A similar relation between YKL-40 and [^{11}C]PBR28 binding (measured as standardized uptake volume ratio with respect to cerebellum reference region) was found in the cerebral cortex in regions where amyloid accumulation is typically first seen in early AD in a study investigating the association of neuroinflammation in the early stages of cerebral amyloid accumulation [18].

Another finding in the current study was the association between higher levels of sTREM2 and NG with the increased motor disability in PD. A recent multi-centre study with a larger sample reported similar findings of higher levels of neuroinflammation markers sTREM2 and YKL-40 in more advanced Hoehn & Yahr stages [40]. Thus it seems that although [^{11}C]PBR28 binding does not correlate with the motor symptoms

in PD the association between CSF glial markers and motor disability does indicate a role for neuroinflammation in PD symptoms. Further studies investigating the relationship between different motor subtypes and neuroinflammation in PD are required. For example, it will be of interest to study whether the postural instability and gait disorder, PIGD, a subtype of PD, which is associated with a more rapid disease progression and increased risk for dementia, differs from other subtypes in the neuroinflammatory markers.

In this study, we observed a correlation between aSyn and sTREM2 and NG in both groups. The CSF biomarkers, especially aSyn, are known to be sensitive to the blood contamination [41]. Therefore, blood contamination could lead to false correlations. On the other hand, the ratio of oligo-aSyn and aSyn has been found to be more reliable regardless of possible blood contamination [41]. The correlation between the ratio and NG only in the PD group makes the possibility of blood contamination less likely. Both NG and aSyn, represent a marker for synaptic loss and therefore the correlation could be expected.

In conclusion, our study suggests that neuroinflammation has a role in PD as indicated by the CSF results and their association with motor symptom severity. It also seems that [^{11}C]PBR28 is not an optimal PET tracer to study neuroinflammation in PD. Further studies are required with larger sample sizes with patients in different stages of the disease using multidisciplinary approaches including more advanced PET tracers, which could identify the functional status of the activated microglia rather than the phenotype, and CSF biomarkers reflecting different contributors in neuroinflammation and neurodegeneration.

Table 1
Demographics and characteristics of all subjects with PD and healthy controls.

	PD	Healthy Controls	*p*-value
Number (Male/female)	20 (4/16) 15 (4/11) #	51 (28/23) (41 (18/23)) *	0.008 0.054§
Age (years), mean ± SD	66.2 ± 5.9 66.0 ± 6.6#	70.0 ± 4.9 (71.12 ± 3.2) *	0.003 0.011§
BMI, mean ± SD	27.0 ± 6.6#	26.3 ± 3.6 (26.5 ± 3.5) *	0.88
TSPO genotype (HAB/MAB)	12/5 (10/5)#	28/23 (22/19) *	0.26 (0.56)§
Motor UPDRS score, mean ± SD	27.1 ± 8.0	-	-
Duration of disease (years), mean ± SD	8.6 ± 5.2	-	-
# Subjects with valid [11C] PBR28 data in PD group (n = 15)			
§ PD patients with valid [11C] PBR28 data (n = 15) compared to healthy controls (n = 51)			

Table 2

[^{11}C]PBR28 V_T in PD and healthy controls in HAB and MAB *TSPO* subtype.

ROI	PD (n = 15) Adjusted mean (SE)* (ml·cm^{-3})	HC (n = 51) Adjusted mean (SE)* (ml·cm^{-3})		HAB			MAB		
			p-value	PD (n = 10) Adjusted mean (SE)* (ml·cm^{-3})	HC (n = 28) Adjusted mean (SE)* (ml·cm^{-3})	*p*-value	PD (n = 5) Mean (SD) (ml·cm^{-3})	HC (n = 23) Mean (SD) (ml·cm^{-3})	*p*-value
ACC	4.20 (0.43)	3.87 (0.22)	0.45	4.99 (0.45)	4.94 (0.26)	0.92	2.68 (0.49)	2.46 (0.52)	0.38
PCC	4.14 (0.43)	3.98 (0.22)	0.67	4.87 (0.45)	5.10 (0.27)	0.69	2.72 (0.56)	2.53 (0.46)	0.43
CAU	2.83 (0.30)	2.78 (0.16)	0.96	3.23 (0.35)	3.54 (0.21)	0.47	1.90 (0.46)	1.85 (0.38)	0.79
PUT	3.93 (0.39)	3.65 (0.20)	0.49	4.68 (0.42)	4.64 (0.25)	0.94	2.51 (0.50)	2.38 (0.44)	0.55
THA	4.38 (0.48)	4.14 (0.25)	0.70	5.14 (0.52)	5.36 (0.31)	0.74	2.75 (0.46)	2.62 (0.50)	0.58
CER	3.56 (0.39)	3.50 (0.20)	0.75	4.30 (0.40)	4.49 (0.24)	0.70	2.25 (0.46)	2.18 (0.37)	0.71
SN	4.70 (0.99)	5.20 (0.51)	0.55	5.13 (1.36)	6.77 (0.80)	0.33	2.99 (0.72)	3.45 (2.81)	0.72
GM ctx	3.99 (0.43)	3.78 (0.22)	0.61	4.70 (0.46)	4.89 (0.27)	0.73	2.59 (0.46)	2.34 (0.43)	0.25
WM	3.57 (0.37)	3.42 (0.19)	0.64	4.21 (0.41)	4.34 (0.24)	0.80	2.37 (0.44)	2.20 (0.41)	0.42

* Sex and age adjusted means from general linear model

Sex and age were included as covariates when both genotypes were explored together and in the HAB genotype subgroup. MAB subgroup was explored with the two-samples t-test. ACC: anterior cingulate

cortex, PCC: posterior cingulate cortex, CAU: caudate nucleus, PUT: putamen, THA: thalamus, CER: cerebellum, SN: substantia nigra, GM ctx: global cortical grey matter cortex, WM: white matter

Declarations

Competing Interests:

HA reports no disclosures. AB reports no disclosures. HZ has served at scientific advisory boards and/or as a consultant for Abbvie, Alector, Annexon, Artery Therapeutics, AZTherapies, CogRx, Denali, Eisai, Nervgen, Pinteon Therapeutics, Red Abbey Labs, Passage Bio, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave, has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure, Biogen, and Roche, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work). KB has served as a consultant, at advisory boards, or at data monitoring committees for Abcam, Axon, BioArctic, Biogen, Julius Clinical, Lilly, MagQu, Novartis, Roche Diagnostics, and Siemens Healthineers, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work). LE reports no conflicts of interest. JOR serves as a consultant neurologist for Clincal Research Services Turku (CRST) and as a member of data monitoring board (Lundbeck) and global expert panel (Novo Nordisk). SH reports no conflicts of interest.

Acknowledgements

HZ is a Wallenberg Scholar supported by grants from the Swedish Research Council (#2018-02532), the European Research Council (#681712), Swedish State Support for Clinical Research (#ALFGBG-720931), the Alzheimer Drug Discovery Foundation (ADDF), USA (#201809-2016862), the AD Strategic Fund and the Alzheimer's Association (#ADSF-21-831376-C, #ADSF-21-831381-C and #ADSF-21-831377-C), the Olav Thon Foundation, the Erling-Persson Family Foundation, Stiftelsen för Gamla Tjänarinnor, Hjärnfonden, Sweden (#FO2019-0228), the European Union’s Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 860197 (MIRIADE), European Union Joint Program for Neurodegenerative Disorders (JPND2021-00694), and the UK Dementia Research Institute at UCL. KB is supported by the Swedish Research Council (#2017-00915), the Alzheimer Drug Discovery Foundation (ADDF), USA (#RDAPB-201809-2016615), the Swedish Alzheimer Foundation (#AF-742881), Hjärnfonden, Sweden (#FO2017-0243), the Swedish state under the agreement between the Swedish government and the County Councils, the ALF-agreement (#ALFGBG-715986), European Union Joint Program for Neurodegenerative Disorders (JPND2019-466-236), and the Alzheimer’s Association 2021 Zenith Award (ZEN-21-848495). LE was supported by Emil Aaltonen Foundation. JOR was supported by grants from the Academy of Finland research grants (#310962), the Sigrid Juselius Foundation and Finnish State Research Funding (VTR). AB was supported by grants from the Finnish Parkinson Foundation and Finnish State Research Funding (VTR). HA was supported by grants from the Finnish Parkinson Foundation and Helsinki University research funds.

Author Contributions

Brück Anna and Rinne Juha Designed and conceptualized the study. The data was acquired by Al-abdulrasul Haidar, Ajalin Riikka, Brück Anna, Tuisku Jouni, Zetterberg Henrik, Blennow Kaj, Ekblad Laura, Helin Semi and Forsback Sarita. Statistical analyses were conducted by Al-Abdulrasul Haidar, Ajalin Riikka and Brück Anna with the guidance of Vahlberg Tero. The first draft of the manuscript was written by Al-Abdulrasul Haidar, Ajalin Riikka and Brück Anna and all authors critically revised the first draft of the manuscript. All authors read and approved the final manuscript. All authors and contributors agree to the conditions outlined in the Authorship and Contributorship section of the Information for Authors. Authors take full responsibility for the data, the analyses and interpretation, and the conduct of the research; full access to all of the data; and the right to publish any and all data.

Data Availability

The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval

The study was approved by the Ethics Committee of the Hospital District of Southwest Finland on the 7th of February, 2014 and was conducted according to the World Medical Association Declaration of Helsinki (Ethical Principles for Medical Research Involving Human Subjects) and following Good Clinical Practice guidelines.

Consent to participate

Written informed consent was obtained from all participants according to the declaration of Helsinki.

References

1. McGeer PL, Itagaki S, Boyes BE, McGeer EG. Reactive microglia are positive for HLA-DR in the: Substantia nigra of Parkinson's and Alzheimer's disease brains. Neurology. 1988;38:1285–91.
2. Imamura K, Hishikawa N, Sawada M, Nagatsu T, Yoshida M, Hashizume Y. Distribution of major histocompatibility complex class II-positive microglia and cytokine profile of Parkinson's disease brains. Acta Neuropathol. 2003;106:518–26.
3. Hirsch EC, Hunot S. Neuroinflammation in Parkinson's disease: a target for neuroprotection? Lancet Neurol. 2009;8:382–97.
4. Mogi M, Harada M, Narabayashi H, Inagaki H, Minami M, Nagatsu T. Interleukin (IL)-1β, IL-2, IL-4, IL-6 and transforming growth factor-α levels are elevated in ventricular cerebrospinal fluid in juvenile parkinsonism and Parkinson's disease. Neurosci Lett. 1996;211:13–6.
5. Blum-Degena D, Müller T, Kuhn W, Gerlach M, Przuntek H, Riederer P. Interleukin-1β and interleukin-6 are elevated in the cerebrospinal fluid of Alzheimer's and de novo Parkinson's disease patients. Neurosci Lett. 1995;202:17–20.

6. Brodacki B, Staszewski J, Toczyłowska B, Kozłowska E, Drela N, Chalimoniuk M, et al. Serum interleukin (IL-2, IL-10, IL-6, IL-4), TNFα, and INFγ concentrations are elevated in patients with atypical and idiopathic parkinsonism. Neurosci Lett. 2008;441:158–62.
7. Nicoletti A, Fagone P, Donzuso G, Mangano K, Dibilio V, Caponnetto S, et al. Parkinson's disease is associated with increased serum levels of macrophage migration inhibitory factor. Cytokine. 2011;55:165–7.
8. Cumming P, Burgher B, Patkar O, Breakspear M, Vasdev N, Thomas P, et al. Sifting through the surfeit of neuroinflammation tracers. J Cereb Blood Flow Metab. 2018;38:204–24.
9. Lavisse S, Guillermier M, Hérard A-SS, Petit F, Delahaye M, Van Camp NV, et al. Reactive astrocytes overexpress TSPO and are detected by TSPO positron emission tomography imaging. J Neurosci [Internet]. 2012;32:10809–18. Available from: http://www.jneurosci.org/cgi/doi/10.1523/JNEUROSCI.1487-12.2012.
10. Gerhard A, Pavese N, Hotton G, Turkheimer F, Es M, Hammers A, et al. In vivo imaging of microglial activation with [11C](R)-PK11195 PET in idiopathic Parkinson's disease. Neurobiol Dis. 2006;21:404–12.
11. Ouchi Y, Yoshikawa E, Sekine Y, Futatsubashi M, Kanno T, Ogusu T, et al. Microglial activation and dopamine terminal loss in early Parkinson's disease. Ann Neurol. 2005;57:168–75.
12. Iannaccone S, Cerami C, Alessio M, Garibotto V, Panzacchi A, Olivieri S, et al. In vivo microglia activation in very early dementia with Lewy bodies, comparison with Parkinson's disease. Park Relat Disord. 2013;19:47–52.
13. Edison P, Ahmed I, Fan Z, Hinz R, Gelosa G, Ray Chaudhuri K, et al. Microglia, amyloid, and glucose metabolism in parkinson's disease with and without dementia. Neuropsychopharmacology. 2013;38:938–49.
14. Bartels AL, Willemsen ATM, Doorduin J, de Vries EFJ, Dierckx RA, Leenders KL. [11C]-PK11195 PET: Quantification of neuroinflammation and a monitor of anti-inflammatory treatment in Parkinson's disease? Park Relat Disord. 2010;16:57–9.
15. Paul S, Gallagher E, Liow JS, Mabins S, Henry K, Zoghbi SS, et al. Building a database for brain 18 kDa translocator protein imaged using [11C]PBR28 in healthy subjects. J Cereb Blood Flow Metab. 39: SAGE Publications Ltd; 2019. pp. 1138–47.
16. Tuisku J, Plavén-Sigray P, Gaiser EC, Airas L, Al-Abdulrasul H, Brück A, et al. Effects of age, BMI and sex on the glial cell marker TSPO — a multicentre [11C]PBR28 HRRT PET study. Eur J Nucl Med Mol Imaging. 2019;46:2329–38.
17. Ekblad LL, Johansson J, Helin S, Viitanen M, Laine H, Puukka P, et al. Midlife insulin resistance, APOE genotype, and late-life brain amyloid accumulation. Neurology [Internet]. Lippincott Williams and Wilkins; 2018;90:e1150–7. Available from: http://www.neurology.org/lookup/doi/10.1212/WNL.0000000000005214.
18. Toppala S, Ekblad LL, Tuisku J, Helin S, Johansson JJ, Laine H, et al. Association of Early Beta-amyloid Accumulation and Neuroinflammation Measured with [11C]PBR28 in Elderly Individuals Without Dementia. Neurology. 2021.

19. Gisslén M, Heslegrave A, Veleva E, Yilmaz A, Andersson L-MM, Hagberg L, et al. CSF concentrations of soluble TREM2 as a marker of microglial activation in HIV-1 infection. Neurol Neuroimmunol NeuroInflammation [Internet]. Lippincott Williams and Wilkins; 2019;6:e512. Available from: http://nn.neurology.org/lookup/doi/10.1212/NXI.0000000000000512.
20. Clarke MTMM, Brinkmalm A, Foiani MS, Woollacott IOCC, Heller C, Heslegrave A, et al. CSF synaptic protein concentrations are raised in those with atypical Alzheimer's disease but not frontotemporal dementia. Alzheimers Res Ther [Internet]. BioMed Central Ltd.; 2019;11:105. Available from: https://alzres.biomedcentral.com/articles/10.1186/s13195-019-0564-2.
21. von Euler Chelpin M, Söderberg L, Fälting J, Möller C, Giorgetti M, Constantinescu R, et al. Alpha-Synuclein Protofibrils in Cerebrospinal Fluid: A Potential Biomarker for Parkinson's Disease. J Parkinsons Dis Netherlands. 2020;10:1429–42.
22. Forsback S, Eskola O, Haaparanta M, Bergman J, Solin O. Electrophilic synthesis of 6-[18F]fluoro-L-DOPA using post-target produced [18F]F2. Radiochim Acta. 2008;96:845–8.
23. Majuri J, Joutsa J, Johansson J, Voon V, Alakurtti K, Parkkola R, et al. Dopamine and Opioid Neurotransmission in Behavioral Addictions: A Comparative PET Study in Pathological Gambling and Binge Eating. Neuropsychopharmacol Nat Publishing Group. 2017;42:1169–77.
24. Fischl B, Salat DH, Busa E, Albert M, Dieterich M, Haselgrove C, et al. Whole brain segmentation: Automated labeling of neuroanatomical structures in the human brain. Neuron. 2002;33:341–55.
25. Hammers A, Allom R, Koepp MJ, Free SL, Myers R, Lemieux L, et al. Three-dimensional maximum probability atlas of the human brain, with particular reference to the temporal lobe. Hum Brain Mapp [Internet]. 2003;19:224–47. Available from: http://doi.wiley.com/10.1002/hbm.10123.
26. Patlak CS, Blasberg RG. Graphical evaluation of blood-to-brain transfer constants from multiple-time uptake data. Generalizations J Cereb Blood Flow Metab. 1985;5:584–90.
27. Koshimori Y, Ko JH, Mizrahi R, Rusjan P, Mabrouk R, Jacobs MF, et al. Imaging striatal microglial activation in patients with Parkinson's disease. PLoS One. Public Library of Science; 2015;10.
28. Ghadery C, Koshimori Y, Coakeley S, Harris M, Rusjan P, Kim J, et al. Microglial activation in Parkinson's disease using [18F]-FEPPA. J Neuroinflammation [Internet]. BioMed Central Ltd.; 2017;14:8. Available from: http://jneuroinflammation.biomedcentral.com/articles/10.1186/s12974-016-0778-1.
29. Varnäs K, Cselényi Z, Jucaite A, Halldin C, Svenningsson P, Farde L, et al. PET imaging of [11 C]PBR28 in Parkinson's disease patients does not indicate increased binding to TSPO despite reduced dopamine transporter binding. Eur J Nucl Med Mol Imaging. 2019;46:367–75.
30. Best L, Ghadery C, Pavese N, Tai YF, Strafella AP. New and Old TSPO PET Radioligands for Imaging Brain Microglial Activation in Neurodegenerative Disease. Curr Neurol Neurosci Rep. 2019;19:24.
31. Kobayashi M, Jiang T, Telu S, Zoghbi SS, Gunn RN, Rabiner EA, et al. 11 C-DPA-713 has much greater specific binding to translocator protein 18 kDa (TSPO) in human brain than 11 C-(R)-PK11195. J Cereb Blood Flow Metab [Internet]. SAGE Publications Ltd; 2018 [cited 2020 Mar 1];38:393–403. Available from: http://journals.sagepub.com/doi/10.1177/0271678X17699223.

32. Papadopoulos V, Baraldi M, Guilarte TR, Knudsen TB, Lacapère JJ, Lindemann P, et al. Translocator protein (18 kDa): new nomenclature for the peripheral-type benzodiazepine receptor based on its structure and molecular function. Trends Pharmacol Sci. 2006;27:402–9.
33. Joers V, Tansey MG, Mulas G, Carta AR. Microglial phenotypes in Parkinson's disease and animal models of the disease. Prog Neurobiol [Internet]. Elsevier Ltd; 2017;155:57–75. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0301008215300538.
34. Wennström M, Surova Y, Hall S, Nilsson C, Minthon L, Hansson O, et al. The inflammatory marker YKL-40 is elevated in cerebrospinal fluid from patients with Alzheimer's but not Parkinson's disease or dementia with Lewy bodies. PLoS One Public Library of Science. 2015;10:1–13.
35. Morenas-Rodríguez E, Alcolea D, Suárez-Calvet M, Muñoz-Llahuna L, Vilaplana E, Sala I, et al. Different pattern of CSF glial markers between dementia with Lewy bodies and Alzheimer's disease. Sci Rep. 2019;9:7803.
36. Bekris LM, Khrestian M, Dyne E, Shao Y, Pillai J, Rao S, et al. Soluble TREM2 and biomarkers of central and peripheral inflammation in neurodegenerative disease. J Neuroimmunol. 2018;319:19–27.
37. Schulz I, Kruse N, Gera RG, Kremer T, Cedarbaum J, Barbour R, et al. Systematic Assessment of 10 Biomarker Candidates Focusing on α-Synuclein-Related Disorders. Mov Disord. John Wiley and Sons Inc; 2021.
38. Kuhlmann AC, Guilarte TR. Cellular and subcellular localization of peripheral benzodiazepine receptors after trimethyltin neurotoxicity. J Neurochem. 2000;74:1694–704.
39. Braak H, Sastre M, Del Tredici K. Development of α-synuclein immunoreactive astrocytes in the forebrain parallels stages of intraneuronal pathology in sporadic Parkinson's disease. Acta Neuropathol. 2007;114:231–41.
40. Brosseron F, Kolbe CC, Santarelli F, Carvalho S, Antonell A, Castro-Gomez S, et al. Multicenter Alzheimer's and Parkinson's disease immune biomarker verification study. Alzheimer's Dement [Internet]. 2020;16:292–304. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1016/j.jalz.2019.07.018.
41. Barkovits K, Kruse N, Linden A, Tönges L, Pfeiffer K, Mollenhauer B, et al. Blood Contamination in CSF and Its Impact on Quantitative Analysis of Alpha-Synuclein. Cells. 2020;9.

Figures

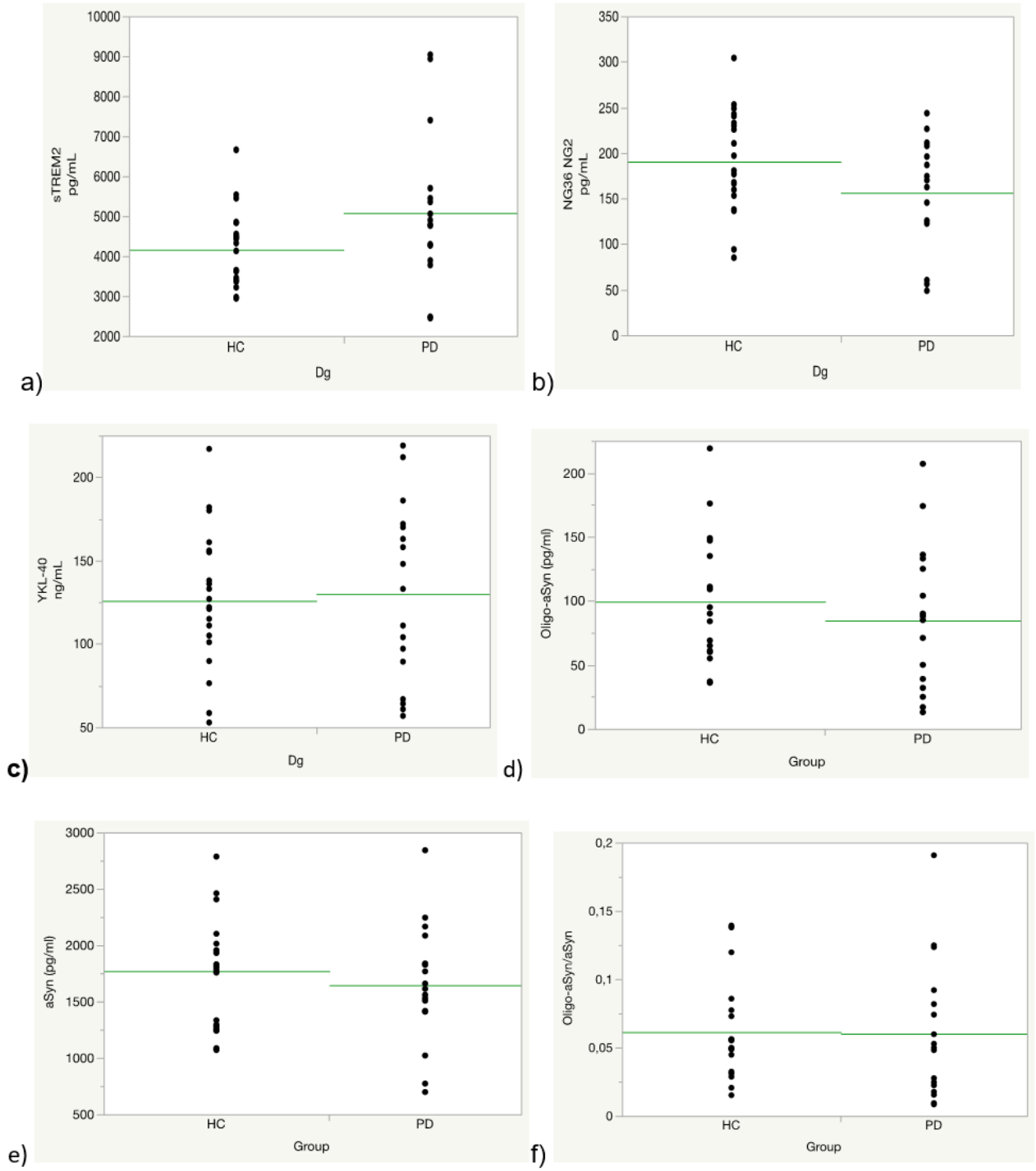

Figure 1

CSF markers in PD and HC mean ± SD. a) sTRTEM2 PD: 5030±353 pg/mL, HC: 4160±318 pg/mL, *p*=0.07, b) NG PD: 152.47±56.7 pg/mL, HC: 190.5±55.3 pg/mL, *p*=0.044 and c) YKL-40 PD: 126±8.90 ng/mL, HC: 126±8.00 ng/mL, *p*=0.77 when age set as covariate, d) Oligo-aSyn PD: 84.7±56.3 pg/mL, HC: 98.6±46.0 pg/mL, *p*=0.41 e) aSyn PD:1644±532 pg/mL, HC: 1779±472 pg/mL, *p*=0.41 f) Oligo-aSyn/aSyn PD: 0.060±0.050, HC: 0.060±0.035, *p*=0.99. The green line showing the mean in each group.

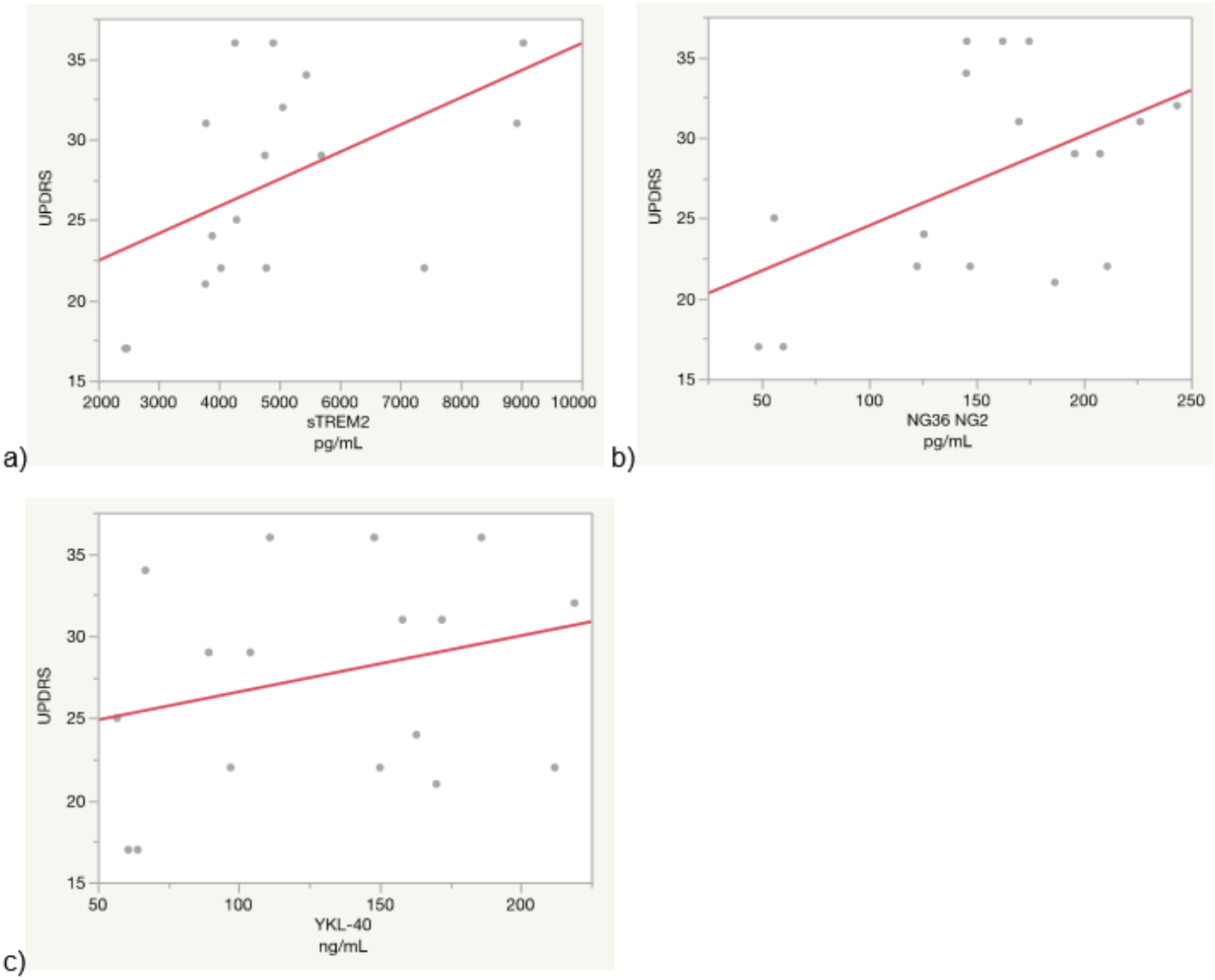

Figure 2

UPDRS III total score in relation to CSF markers. A) sTREM2 r_s=0.52, *p*=0.041, b) NG r=0.59, *p*=0.016 and c) YKL-40 r=0.38, *p*=0.15.

r_s = Spearman correlation coefficient

r = Pearson correlation coefficient

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- submissionletterEJNMMI.docx