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TITLE PAGE

Increased microglial activation in patients with Parkinson disease using [¹⁸F]-DPA714 TSPO PET imaging

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ABSTRACT

Introduction

Increasing evidence suggests that neuroinflammation is active in Parkinson disease (PD) and contribute to neurodegeneration. This process can be studied *in vivo* with PET and radioligands targeting TSPO, upregulated in activated microglia. Initial PET studies investigating microglial activation in PD with the [¹¹C]-PK11195 have provided inconclusive results. Here we assess the presence and distribution of neuroinflammatory response in PD patients using [¹⁸F]-DPA714 and to correlate imaging biomarkers to dopamine transporter imaging and clinical status.

Methods

PD patients (n=24, Hoehn and Yahr I-III) and 28 healthy controls were scanned with [¹⁸F]-DPA714 and [¹¹C]-PE2I and analyzed. They were all genotyped for TSPO polymorphism. Regional binding parameters were estimated (reference Logan graphical approach with supervised cluster analysis). Impact of TSPO genotype was analyzed using Wilcoxon signed-rank test. Differences between groups were investigated using a two-way ANOVA and Tukey *post hoc* tests.

Results

PD patients showed significantly higher [¹⁸F]-DPA714 binding compared to healthy controls bilaterally in the midbrain ($p < 0.001$), the frontal cortex ($p = 0.001$), and the putamen contralateral to the more clinically affected hemibody ($p = 0.038$). Microglial activation in these regions did not correlate with the severity of motor symptoms, disease duration nor putaminal [¹¹C]-PE2I uptake. However, there was a trend toward a correlation between cortical TSPO binding and disease duration ($p = 0.015$ uncorrected, $p = 0.07$ after Bonferroni correction).

Conclusion

[¹⁸F]-DPA714 binding confirmed that there is a specific topographic pattern of microglial activation in the nigro-striatal pathway and the frontal cortex of PD patients.

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Key words : Microglia; TSPO; [¹⁸F]-DPA714; PET; Parkinson disease, neuroinflammation

1 **BACKGROUND**

2 In Parkinson disease (PD), neuroinflammation is thought to play an important role in the
3 progression of the neurodegeneration process [1-3]. Studies have described the presence of
4 reactive microglia in *postmortem* brain samples of PD patients [1]. Specifically, major
5 histocompatibility complex class II immunoreactive microglia was identified in the Substantia
6 nigra (SN) and the striatum [4]. Located in the vicinity of the remaining nigral dopaminergic
7 (DA) neurons, these microglial cells displayed morphologies characteristic of activated and
8 phagocytic cells, similar to those seen in aging [1, 5]. Studies of *postmortem* human brains
9 and animal models of parkinsonism further strongly suggest an involvement of
10 neuroinflammation in the pathological process. However, several questions remain
11 unresolved, such as the topography of neuroinflammation *in vivo* compared to *postmortem*,
12 the relationship between the severity of the disease and the intensity of neuroinflammatory
13 response in the brain and the time sequence of this process as primary or secondary marker of
14 the disease [6].

15 To investigate these questions in PD patients, positron emission tomography (PET)
16 imaging studies have widely relied on a radioligand, the [¹¹C]-PK11195, that binds to the
17 translocator protein (TSPO). This protein is expressed at low levels in healthy brains but is
18 markedly upregulated on activated microglia and astrocytes in the presence of inflammation
19 in several acute and degenerative disorders [7]. Studies with [¹¹C]-PK11195 have led to
20 considerable advancement in understanding the pathophysiological implication of microglial
21 activation in several neurodegenerative diseases. In PD patients, early PET studies conducted
22 by Gerard *et al.* and Ouchi *et al.* revealed an increase in [¹¹C]-PK11195 binding in
23 nigrostriatal structures (basal ganglia and midbrain) [8, 9]. However, the use of this
24 radioligand is limited by low brain permeability, a high nonspecific binding (to brain and to
25 plasma proteins, monocytes and platelets) and a relatively short half-life of carbon-11 (see

26 reviews [10, 11]). These restrictions have prompted over the last twenty years the
27 development of second-generation TSPO radioligands with higher signal to noise ratio in an
28 attempt to improve quality of TSPO imaging. Owen *et al.* reported that these new TSPO
29 ligands however are sensitive to the single-nucleotide polymorphism, rs6971, located in the
30 TSPO gene in humans that accounts for variable affinity patterns [15]. Therefore, PET scans
31 using second-generation TSPO ligands should be analyzed only after considering the rs6971
32 polymorphism of each subject. Some of these TSPO ligands such as [¹⁸F]-FEPPA, [¹¹C]-
33 PBR28, [¹¹C]-DPA713 have been used to investigate microglial activation in PD [10, 12, 14,
34 16].

35 Among second-generation TSPO radiotracers, [¹⁸F]-DPA714 has shown high affinity
36 for TSPO, a high brain penetration and favorable pharmacokinetics [17] with the Logan
37 graphical analysis. Its quantification has been validated in healthy volunteers using first the
38 two-tissue compartment model with arterial input function and second, and the supervised
39 cluster analysis (SVCA) [18]. In the present study, we investigated whether TSPO imaging
40 with [¹⁸F]-DPA714 could be used as a potential and sensitive biomarker of microglial
41 activation in PD and how it compares with previous studies using other second-generation
42 radioligands.

43 By accounting for the TSPO polymorphism, we studied microglial activation in healthy
44 controls (HC) and in PD patients at different disease stages and investigated the microglial
45 activation along with presynaptic dopamine transporter (DAT) density imaging using the
46 [¹¹C]-PE2I radioligand. We sought to measure the regional brain distribution of activated
47 microglia especially in the midbrain and various dopaminergic pathways of PD patients and
48 to determine in these regions whether neuroinflammation correlates with disease severity
49 measured through clinical motor scores and presynaptic DAT abnormalities.

50

51 **METHODS**

52 **Subjects**

53 Twenty-four patients meeting UK Brain Bank criteria for the diagnosis of idiopathic PD
54 (63.5 ± 9.8 years; 8 women, 16 men; NCT02319382) and 28 age-matched healthy controls
55 (HC) (57.4 ± 13.2 years; 15 women, 13 men, NCT02319382, NCT01775696) were enrolled in
56 this study. All participants provided written informed consent. The protocols were approved
57 by the French Medical Bioethics Committee and were in accordance with French legislation
58 and the Declaration of Helsinki 1975 (revised in 1983). Patients were recruited at the Henri
59 Mondor hospital and controls were recruited by in-house advertisements.

60 None of the subjects had any evidence of severe cognitive decline or history of hallucinations.
61 Exclusion criteria for all participants included: (1) history of head injury, psychiatric or other
62 neurological disease, except PD for the patients; (2) alcohol or drug abuse; (3) contra-
63 indications for MRI scanning; (4) use of any drug interacting with DAT or TSPO binding
64 (e.g., benzodiazepines or derivatives that are not specific to central benzodiazepine binding
65 site) or TSPO expression (nonsteroidal anti-inflammatory drugs more than 50 days within the
66 last year or more than 7 days within the last month); and (5) clinically significant lesion on
67 brain MRI.

68 The selection of PD patients purposely included patients at different stages: early drug-naïve
69 patients (n=9), patients with longer disease duration (>36 mo, < 5 years) without motor
70 fluctuations (n=9) and more advanced patients having motor fluctuations and disease for at
71 least 5 years (n=6). Disease duration was calculated from the first clinical diagnosis of PD.

72 All participants underwent PET and anatomical MRI scans on the same day. Medications of
73 PD patients were withdrawn about 15 hours before scanning (last dose was taken the night
74 before; two nights before for extended release dopamine agonists). Clinical evaluations were
75 performed using the MDS-UPDRS motor score in on-state during the hospital visit a few days

76 or up to 6 weeks before the PET sessions. MDS-UPDRS in off-state and quality of life with
77 the PDQ-39 were evaluated by the same neurologist on the day of PET imaging.

78 Genomic DNA from blood samples was used to genotype the rs6971 polymorphism of the
79 TSPO gene to stratify all subjects into high (HAB), mixed (MAB) and low (LAB) affinity
80 binders.

81 **Imaging protocol**

82 MR images were acquired to ensure the absence of brain lesion and for co-registration with
83 the PET images to anatomically delineate the Volumes-of-Interest (VOIs). T1-weighted
84 imaging was performed using a turbo spin echo sequence on a 1.5 Tesla Philips Achieva
85 system (Best, The Netherlands) or a MPRAGE sequence on a 3.0 Tesla TRIO MRI scanner
86 (Siemens Healthcare, Germany).

87 [¹⁸F]-DPA714 was prepared according to standard conditions [19] and [¹¹C]-PE2I synthesis
88 was performed as previously reported [20]. The short half-life of 11-Carbene (20 minute)
89 allowed both PET scans to be performed in a single day for each participant. All exams were
90 acquired on a High Resolution Research Tomograph (HRRT; CTI/Siemens Molecular
91 Imaging) allowing imaging of small volume structures. A custom-fitted mask was applied for
92 each subject to minimize head movements. PET dynamic acquisitions lasted up to 60 minutes
93 after injection of [¹¹C]-PE2I (mean 293.4±78 MBq) and [¹⁸F]-DPA714 (mean 207.9±42.7
94 MBq) was injected 3 hours later (90-min acquisitions).

95 **Image processing**

96 The partial volume effect was reduced by directly incorporating resolution modelling inside
97 the iterative algorithm during reconstruction of PET images [21].

98 The TSPO protein has a widespread distribution and consequently, no single region free of
99 this target can be identified as a reference for non-specific ligand binding. Supervised cluster
100 analysis (SVCA), previously described and validated with [¹⁸F]-DPA714 images [18], was

101 therefore used to extract a distributed cluster of voxels with a time activity curve that
102 represents the reference curve of a normal population.

103 A VOI-based approach was performed using an automatic segmentation of grey matter on
104 individual MRI using the VBM package implemented in SPM8 (Statistical Parametric
105 Mapping, Wellcome Trust Centre for Neuroimaging) and the Volbrain pipeline for sub-
106 cortical regions [22]. The automated anatomical labelling atlas (AAL), previously applied in
107 PD patients [23], was deformed to each MRI and applied after co-registration to the PET
108 dynamic data in order to measure [^{11}C]-PE2I and [^{18}F]-DPA-714 uptakes in 90 anatomical
109 regions. These VOIs were defined in both hemispheres and were then regionally pooled into
110 larger anatomical VOIs. We defined the following VOIs: caudate (7.13 ± 0.96 mL), putamen
111 (7.80 ± 0.86 mL), globus pallidus (2.39 ± 0.33 mL), cerebellum (49.81 ± 5.94 mL), thalamus
112 (10.47 ± 0.97), amygdala (1.59 ± 0.22 mL) and gray matter of frontal, temporal, parietal and
113 occipital cortices (93.72 ± 12.89 , 106.46 ± 13.60 , 35.36 ± 5.02 and 58.32 ± 8.43 mL, respectively).

114 ‘Whole cortical’ [^{18}F]-DPA714 binding was considered as the average of values in the
115 cortical regions. Midbrain region was manually delineated by an experienced neurologist (PR)
116 on individual MRI to include the whole substantia nigra (2.88 ± 0.60 mL) (Figure 1A). In
117 patients, more (+) and less (-) sides for sub-cortical regions were defined as contralateral to
118 the clinically more and less affected sides based on MDS-UPDRS measurements. In HC, we
119 found no significant difference in microglial activation between the right and left sides for all
120 the regions explored. Therefore, measurements of both hemispheres were averaged.

121 The Logan's Reference Tissue Model (Pmod[®] software) used for [^{18}F]-DPA714 analysis,
122 yielded regional non-displaceable binding potential (BP_{ND}) estimates in each VOI [24]. The
123 Simplified Reference Tissue Model with cerebellum as reference input function was used for
124 [^{11}C]-PE2I to calculate the DAT binding in striatal structures (BP_{ND}) [25].

125

126 **Statistics**

127 All data are presented as mean \pm SD. Statistical analysis was performed using the R project
128 software. Demographic and clinical measures were compared using a two-way ANOVA with
129 disease (PD and controls) and TSPO genotype as factors. The impact of TSPO genotype in
130 participants was analyzed using Wilcoxon signed-rank test. Differences in [¹⁸F]-DPA714
131 BP_{ND} values between HC and patients and between patients at the three different disease
132 stages were analyzed in all regions, using a two-way ANOVA and Tukey *post hoc* tests, with
133 genotype and disease stage as factors. To remove the effect of affinity on clinical-PET
134 correlations, individual BP_{ND} estimates in each cortical region were normalized by the mean
135 BP_{ND} of their corresponding affinity group (HAB or MAB) to correlate normalized BP_{ND} with
136 disease duration. All correlations were analyzed using the Pearson correlation test (Pearson
137 correlation coefficient *r*). The Hampel method and the Grubbs test were used to reveal any
138 clinical data outlier and a Bonferroni correction was applied for multiple comparisons.
139 Statistical significance was set to $p < 0.05$.

140

141 **RESULTS**

142 ***Subjects***

143 One patient was excluded from the analyses because he could not complete the [¹⁸F]-DPA714
144 scan. Two HC were excluded from the study due to frontal lesion on the brain MRI and
145 essential tremor. Therefore, 23 PD patients and 26 HC were analyzed. Demographic and
146 clinical data of patients are shown in Table 1. The diagnosis of PD was supported by
147 quantitative evaluation of DAT binding using [¹¹C]-PE2I PET. BP_{ND} values in the putamen
148 were significantly lower in patients (BP_{ND}= 2.65 \pm 1.27) than in controls (BP_{ND}=8.7 \pm 3.33,
149 $p < 0.001$). In all PD patients, the lowest putaminal DAT binding was contralateral to the
150 clinically more affected side.

151 Genomic analysis in HC and patients revealed 13 and 7 HABs, 12 and 13 MABs and 1 and 3
152 LABs, respectively. Analyses performed on LABs could not accurately quantify the [¹⁸F]-
153 DPA714 binding nor provide accurate BP_{ND} values unlike in MAB and HAB subjects.
154 Therefore, LABs were excluded from further statistical analyses and interpretation. No
155 difference in disease duration, disease severity or DAT binding was found between the HAB
156 and the MAB groups and no statistical outliers in the clinical data were observed.

157

158 *TSPO binding in PD patients*

159 Factorial ANOVAs revealed that the [¹⁸F]-DPA714 binding was significantly higher in the
160 midbrain of PD patients compared to HC (Figure 2). This increase was found both in the
161 predominantly affected side ($p < 0.001$ in midbrain+; +68.8% in HABs and +58.1% in
162 MABs) and the less affected side ($p < 0.001$ in midbrain-, +12.6% in HABs and +44.1% in
163 MABs). In PD patients, [¹⁸F]-DPA714 binding was significantly higher in midbrain+ than in
164 the midbrain- region ($p = 0.045$; +49.9% and +9.7% in HABs and MABs, respectively, Figure
165 1B).

166 In addition, microglial activation was significantly higher in patients than in HC in the
167 Putamen+ ($p = 0.038$; 27.3% and +68.9% in HABs and MABs, respectively). There was an
168 increase of microglial activity in Putamen- region which did not reach statistical significance
169 ($p = 0.08$; +21.6% and +66.0% in HABs and MABs).

170 In the frontal cortex, TSPO binding was significantly higher in patients than in HC with an
171 average increase of +38.2 % ($p = 0.001$, HAB +34.7%, MAB +41.6%, Table 2). In the other
172 cortical regions, there was no significant increase of TSPO expression in the PD patients
173 (Table 2).

174 Differences in regional [¹⁸F]-DPA714 binding between patients and healthy controls were
175 also investigated with a voxel-based analysis using SPM. Voxel clusters extracted with this

176 method were found in similar regions than those displaying significant [¹⁸F]-DPA714 increase
177 in the ROI-based approach (Supplementary Figure1).

178

179 *Correlations*

180 There was no correlation between [¹⁸F]-DPA714 binding and age in controls and in patients
181 across regions. In patients, we found no significant correlation between putaminal [¹¹C]-PE2I
182 DAT binding and [¹⁸F]-DPA714 binding in the midbrain and the striatum. In addition, there
183 was no correlation between disease duration or MDS-UPDRS motor scores and [¹⁸F]-
184 DPA714 BP_{ND} in the midbrain, putaminal and frontal regions. Nevertheless, [¹⁸F]-DPA714
185 binding in the whole cortex was correlated with disease duration (Pearson, $r = 0.55$, $p = 0.015$
186 which did not stand up to Bonferroni correction: $p = 0.071$, supplementary Figure 2).

187

188 *TSPO polymorphism effect*

189 Overall, there was a significant main effect of TSPO genotype in HC ($p < 0.001$) and in
190 patients ($p = 0.007$) with significantly higher [¹⁸F]-DPA714 BP_{ND} estimates in HABs
191 compared to MABs (+21.8 ±10.0% in HC and +9.2 ±16.7% in patients). Regions that had the
192 greatest percent differences included the thalamus, putamen and globus pallidum (Table 2).

193

194 **DISCUSSION**

195 There is a consensus on the probable role of neuroinflammation in the degenerative process of
196 PD, which might therefore be considered as a potential therapeutic target for neuroprotective
197 studies. This study is the first use of [¹⁸F]-DPA714 to compare microglial activation in
198 Parkinsonian patients and HC, all genotyped for TSPO polymorphism. Our analyses
199 demonstrated significantly higher [¹⁸F]-DPA714 BP_{ND} in the midbrain, putaminal and frontal
200 cortical regions in patients compared to HC. The ROI-based analysis was further confirmed

201 by the voxel-based approach. This binding was not correlated with markers of disease
202 progression, such as disease duration, MDS-UPDRS motor scores or DAT binding. We found
203 a trend for a positive correlation between [¹⁸F]-DPA714 binding in the whole cortex and
204 disease duration, suggesting an accumulation of inflammation in cortical areas over years in
205 PD.

206 ***Microglial activation in the midbrain***

207 We found increased TSPO binding bilaterally in the midbrain/SN of PD patients. This is in
208 line with five previous studies using either [¹¹C]-PK11195 [9, 13, 26, 27] or the more recent
209 [¹¹C]-DPA713 [16]. Midbrain/SN was not investigated in two studies [12, 28]. Conversely, no
210 significant increase of TSPO was found in the midbrain/SN in three studies using either [¹¹C]-
211 PK11195 [8, 29] or the second-generation ligand [¹¹C]-PBR28 [14]. Altogether, midbrain/SN
212 activation can be found in most studies considering that region and using either [¹¹C]-
213 PK11195 or second-generation – more specific – TSPO radioligands. These results obtained
214 *in vivo* confirm the role of microglial activation in the pathophysiology of PD, in agreement
215 with postmortem studies showing the presence of microglia activation nearby the
216 dopaminergic neurons degeneration in the SN pars compacta [3, 4].

217 In our PD patients, the presence of activated microglia was bilateral in the midbrain but was
218 significantly more pronounced in the side more affected by the disease process. This suggests
219 that inflammation accumulates with disease progression, independently of the number of
220 remaining dopaminergic neurons. In line with most previous studies, we found no correlation
221 between disease duration or severity and TSPO binding in the midbrain regions. Conversely,
222 Ouchi *et al.* [30] found a significant relationship between midbrain TSPO binding and two
223 markers of disease severity: UPDRS motor score and DAT binding in the putamen,
224 suggesting that TSPO binding increases with disease severity. However, this analysis was
225 performed in a small group of patients at an early stage of the disease and was not reproduced

226 thereafter, even in the present study with a larger number of patients at different stages of the
227 disease and using identical disease severity markers. This lack of correlation could be
228 explained on one hand by the fact that microglial activation may be present long before
229 symptoms onset of PD and that there might be a threshold of microglial activity necessary for
230 cell death to occur. On the other hand, microglial activity and phenotype might fluctuate over
231 time independently of clinical progression of the disease, which would be more related to the
232 progressive dysfunction of the dopaminergic system and counterbalancing compensatory
233 mechanisms [31].

234 ***Microglial activation in the striatum***

235 Interestingly, microglial activation was present in the more affected putamen and did not
236 reach statistical significance ($p = 0.08$) in the less affected putamen. This asymmetry
237 reproduces the asymmetry of microglial activation observed at the midbrain/SN level
238 described above to a lesser extent. Therefore, it might be related to the degenerative process
239 involving the SN neurons projecting mainly to the putamen. Across the literature, striatal
240 increase of TSPO binding using PET imaging has been inconsistently reported. Gerhard *et al.*
241 [8] and Terada *et al.* [16] found an increase in TSPO binding in the whole striatum of their
242 patients who were at different stages of the disease. Iannacone *et al.* [13] and Kang *et al.* [26]
243 found an increase of TSPO binding in the putamen only, whereas Edison *et al.* [28] found a
244 striatal increase only in demented PD patients. In addition, increased TSPO binding has also
245 been reported in the striatum of patients with multisystem atrophy and progressive
246 supranuclear palsy [8, 32]. These latter examples suggest that direct neuropathological lesions
247 of the putamen definitively contribute to inflammatory response in the striatum. However, the
248 amplitude of [^{18}F]-DPA714 increase being more important in the midbrain than in the
249 putamen of our non-demented patients, our hypothesis is that the latter is secondary to the
250 former and might therefore be considered as a spreading of the disease process along the

251 nigro-striatal dopaminergic system. This is in line with a report from Stockholm et al., [33]
252 showing increased microglial activation in the midbrain but not the putamen of subjects with
253 REM-sleep behavior disorders exhibiting a premanifest decrease of dopamine functions.

254

255 *Microglial activation in the cortex*

256 We also found microglial activation in the frontal region in PD relative to HC. In previous
257 PET studies, Iannacone *et al.* [13] and Edison *et al.* [28] found a marked [¹¹C]-PK11195
258 increase (respectively +50% and +30%) of frontal TSPO binding which however did not
259 reach statistical significance, in early PD patients. Terada and colleagues found a significant
260 increase of [¹¹C]-DPA713 binding in the frontal cortex of more advanced patients [16].
261 Again, none of these studies, including the present one, could report a relationship between
262 disease severity or [¹¹C]-PE2I striatal binding and the amount of frontal inflammation
263 markers. However, this does not discard a trend to a progressive cortical inflammation
264 process occurring in PD. Indeed, Terada *et al.*, found an increase of TSPO binding in the
265 frontal cortex of PD patients that were rescanned one year after the first examination [28].
266 Moreover, in our patients, there is a trend for a correlation between disease duration and the
267 binding of [¹⁸F]-DPA714 in the whole cortical mantle ($r = 0.55$, $p = 0.071$ after Bonferroni
268 correction), suggesting a subtle inflammatory spreading process to the whole cortex over
269 years, as reported by Gerhard and colleagues using [¹¹C]-PK11195 [8]. Moreover, other
270 authors have reported significantly increased cortical TSPO binding in patients with PD-
271 dementia [28] and diffuse Lewy body disease [13]. Our interpretation of these data is that
272 cortical markers of inflammation might be present early in the frontal cortex of PD patients
273 and spread to the whole cortical mantle over time. It is unclear whether such progression
274 might be related to Lewy bodies spreading and to the risk of developing dementia, as it has
275 been shown in Alzheimer disease [34], but this hypothesis might be explored in future studies.

276
277
278

Methodological issues and sensitivity to TSPO polymorphism

279 There have been eleven previous PET studies investigating inflammation in PD and results
280 have been heterogeneous. These discrepancies can be attributed to several factors such as the
281 various stages of the disease in the patients, the small cohorts of patients, the resolution of the
282 scanners employed or the use of different radioligands across studies. And future studies
283 using [¹⁸F]-DPA714 in a larger and different cohort of PD patients will be necessary to
284 confirm our results. Moreover, unlike with [¹¹C]-PK11195, the rs6971 polymorphism has
285 been observed with several second-generation TSPO tracers both in controls and in PD
286 patients [12, 14] and the lack of TSPO genotyping had likely an impact on the results of
287 former PET studies in PD [16]. Our data confirm the marked impact of this polymorphism on
288 the binding of the [¹⁸F]-DPA714 radioligand to its target, both in HC and in PD patients, that
289 consequently affects PET images and interpretation. This has already been observed with
290 other second-generation TSPO tracers in HC and PD patients [12, 14]. It is therefore
291 mandatory to stratify participants into matched affinity groups to compare patients to controls
292 but this implies the genotyping of all participants and probably the exclusion of LAB subjects
293 whose TSPO PET binding is not quantifiable. In our study, 13% of PD patients were LABs
294 and therefore excluded. In a representative caucasian population, LABs represent 5-10% of
295 the subjects [15] which induces a minimal loss of representativity of the population if LABs
296 are excluded.

297

CONCLUSION

299 Using the [¹⁸F]-DPA714 second-generation TSPO radioligand, we found a significant
300 neuroinflammatory response in the midbrain, the putamen and the frontal cortex of PD
301 patients. It seems that inflammation progressively involves the whole cortical mantle over

302 years in PD, and might reveal spreading of pathological process. [¹⁸F]-DPA714 might be used
303 to explore the impact of specific anti-inflammatory drugs in this disease.

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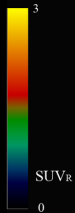
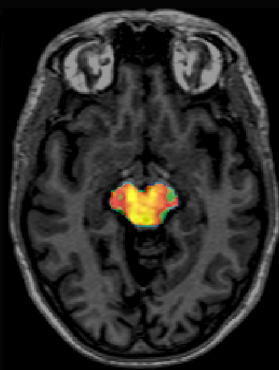
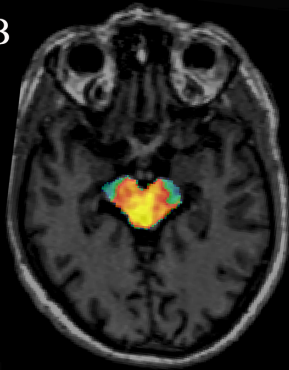
FIGURE LEGENDS

Figure 1 : **A**-Midbrain regions of interest superimposed on an individual representative MRI; **B**-Representative merged [¹⁸F]-DPA714 PET and MRI images at the midbrain level.

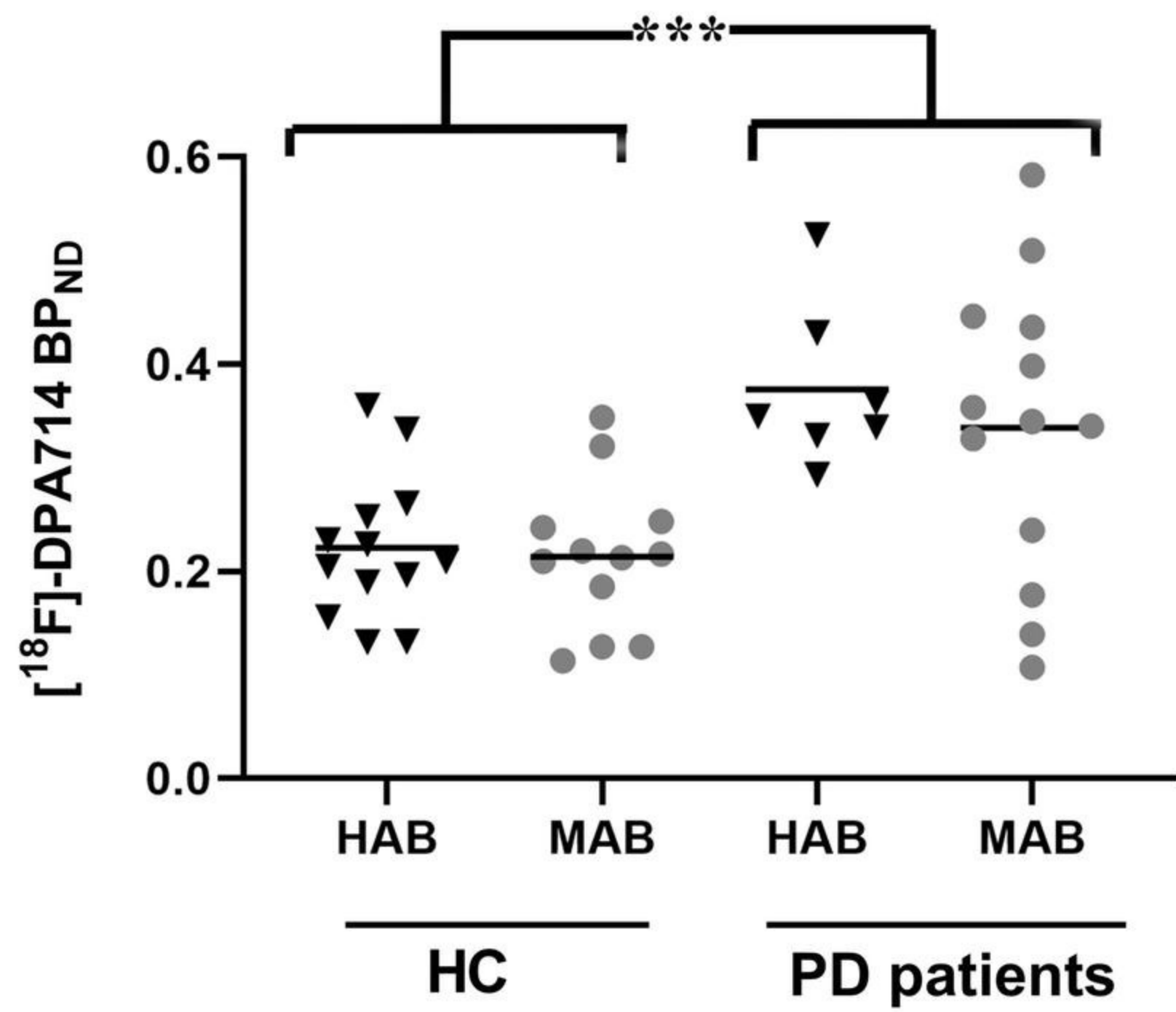
Legend: Scaling of the images is fixed for visual comparison. PET images are corrected for weight and injected dose and are normalized by the radioactivity value in the SVCA reference region (SUV_R). A mask of the midbrain has been applied on the PET image to show [¹⁸F]-DPA714 activity in this region only. Normalized images are summed from 60 to 90 minutes. Middle: HAB HC and right: HAB PD patient. PET images are coregistered to the individual MRI.

Figure 2 : [¹⁸F]-DPA714 BP_{ND} individual values in the putaminal and midbrain regions

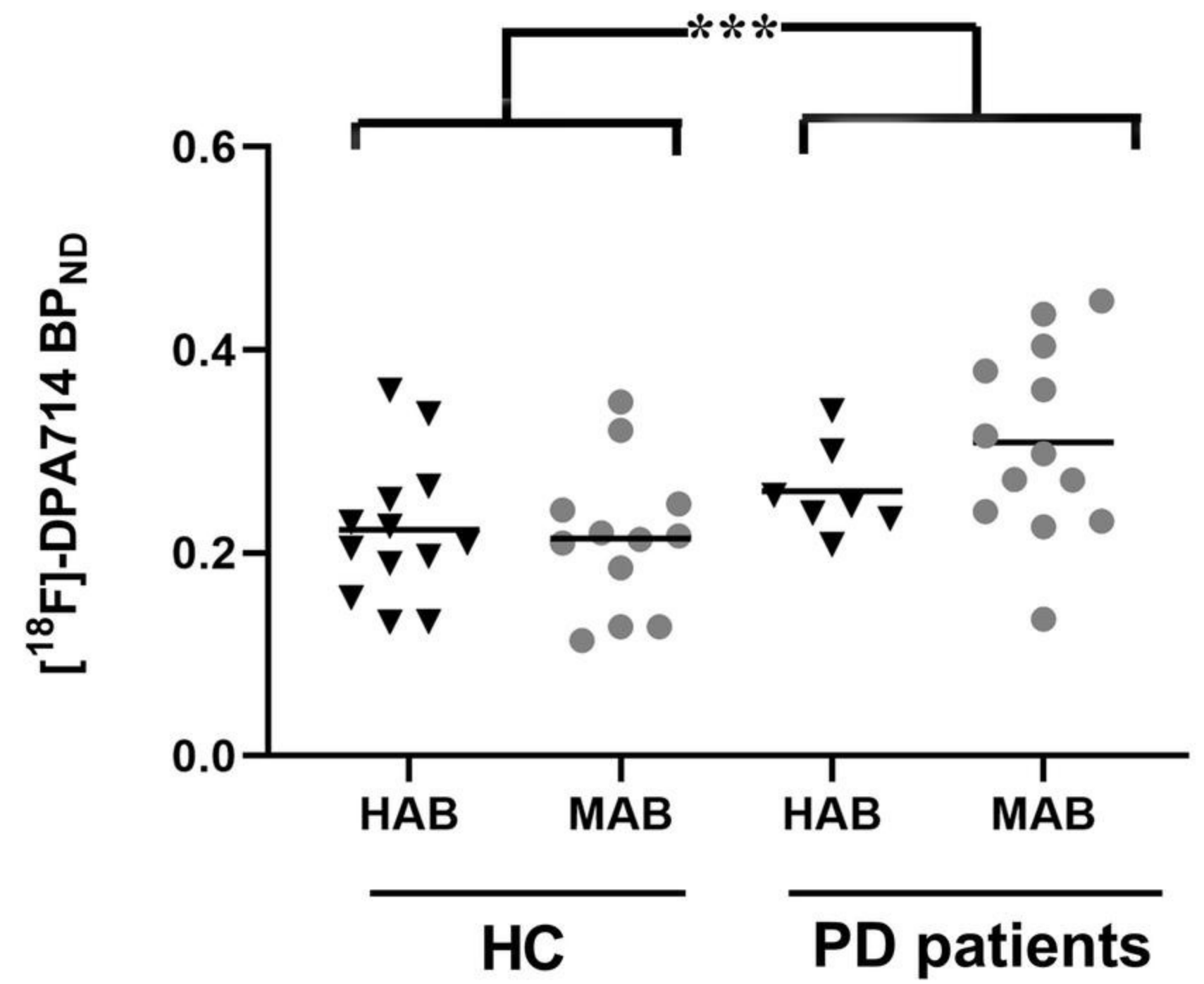
*Legend: PD patients compared to HC bilaterally in the midbrain and in the putamen. + and – refer respectively to more and less affected sides in patients. BP_{ND} from left and right hemispheres are averaged in HC. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$, 2-way ANOVA followed by Tukey post hoc.*

A**B**

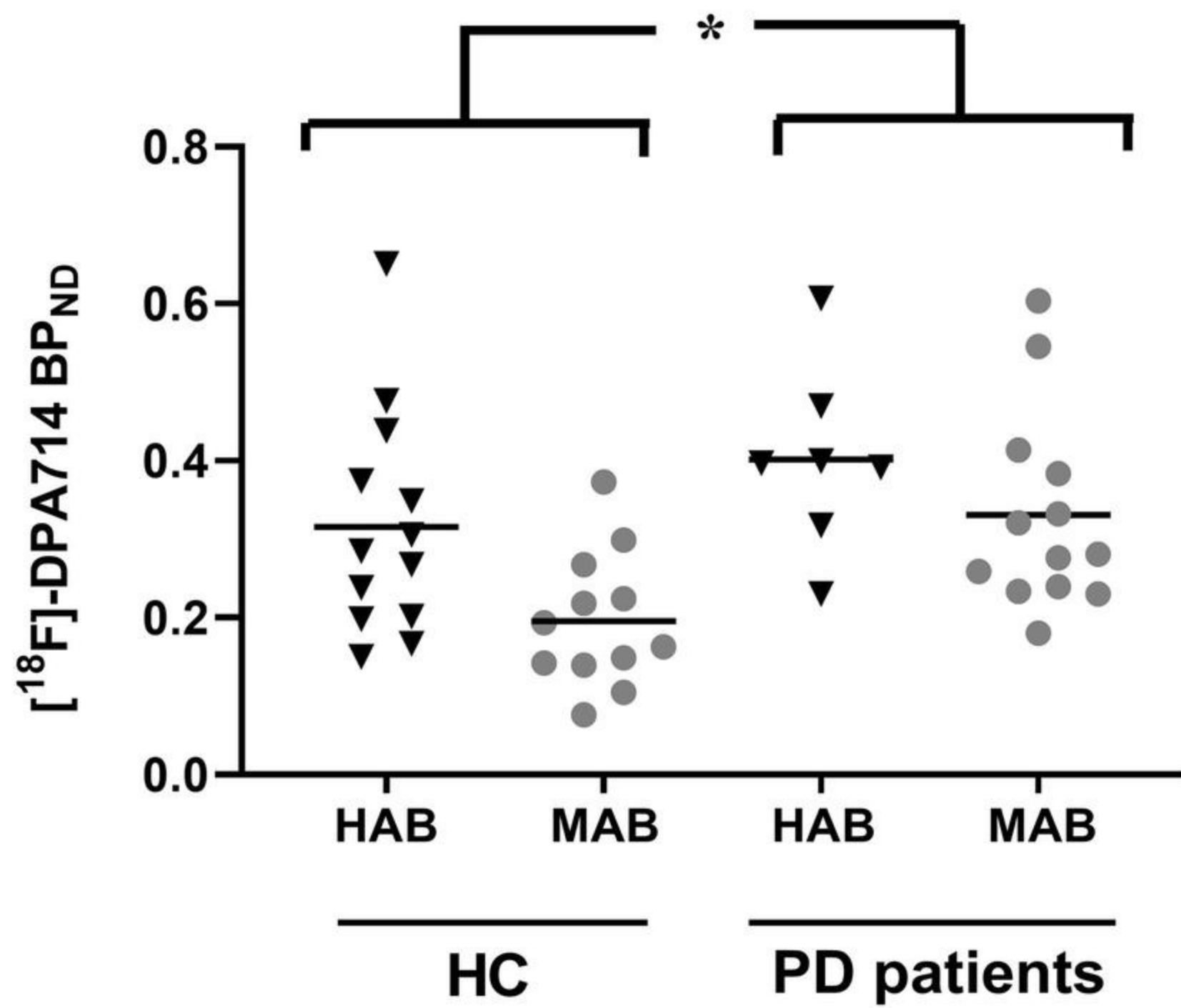
Midbrain+ REGION



Midbrain- REGION



Putamen+ REGION



Putamen- REGION

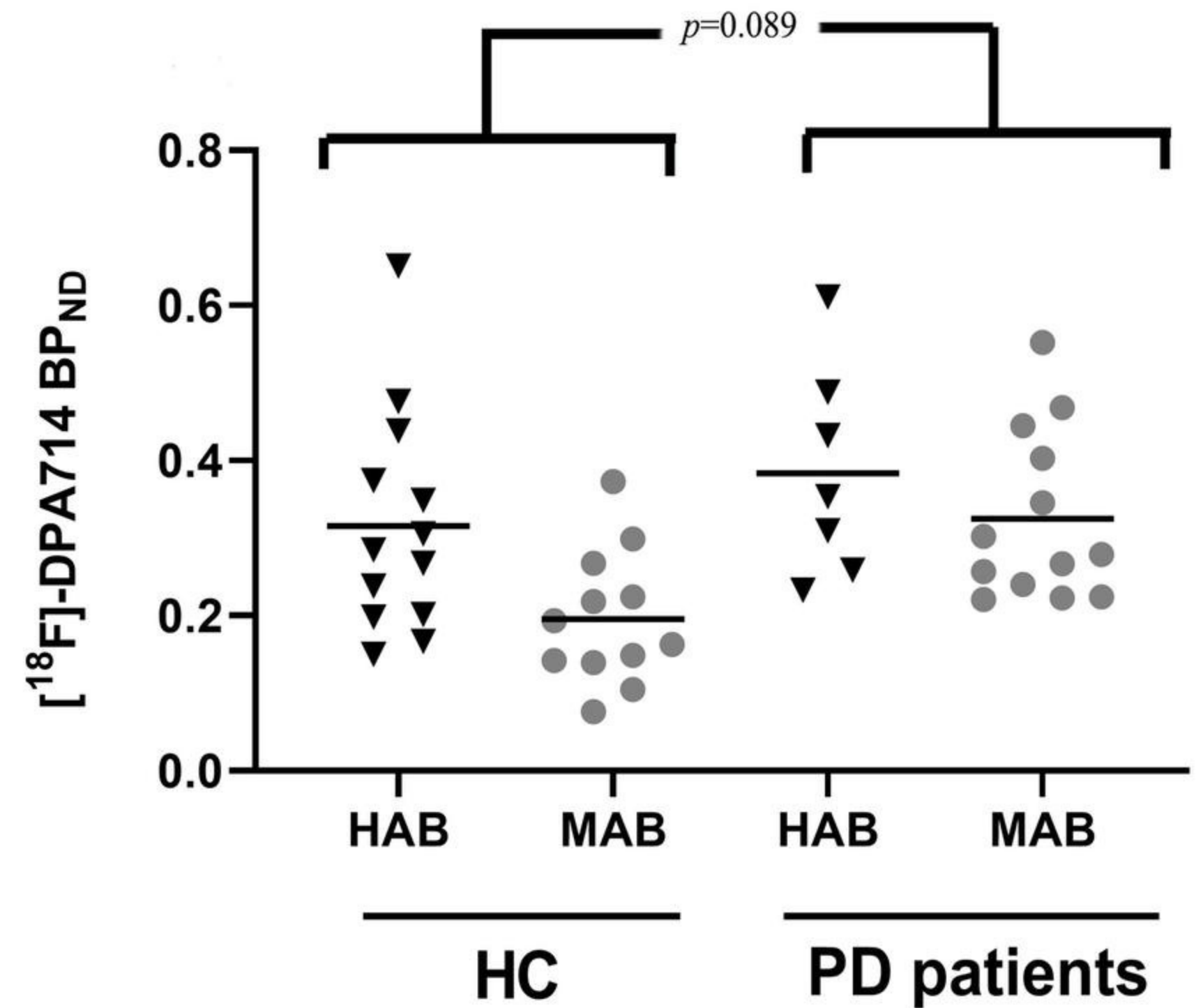


Table 1 : Clinical characteristics of the Parkinson's disease patients.

Patients	Gender	Age	Genotype	Disease duration (mo)	UPDRS I	UPDRS II	UPDRS-III (off)	UPDRS IV	Hoehn et Jahr
1	F	63	MAB	30	0	7	6	0	2
2	M	58	HAB	36	0	0	12	0	2.5
3	M	57	MAB	30	1	5	13	0	2
4	F	52	MAB	1	1	2	13	0	1.5
5	M	69	MAB	1	0	1	14	0	1.5
6	M	55	HAB	18	1	4	14	0	1.5
7	F	72	LAB	30	1	4	17	0	2.5
8	F	63	MAB	60	1	4	19	0	1
9	M	46	HAB	18	2	6	19	0	2.5
10	M	66	MAB	120	1	8	19	5	2.5
11	F	78	MAB	6	4	8	19	0	2.5
12	M	80	na	2	0	4	13	0	1
13	M	59	LAB	5	1	5	20	0	3
14	M	60	HAB	36	0	4	21	0	2.5
15	M	76	LAB	156	2	8	21	6	3
16	F	66	MAB	13	4	22	23	1	1.5
17	M	78	MAB	1	2	14	23	0	2.5
18	M	71	HAB	108	2	7	24	6	3
19	M	66	HAB	72	4	16	25	4	2.5
20	M	72	MAB	6	3	5	26	0	2.5
21	M	66	HAB	132	13	16	27	8	2.5
22	M	63	MAB	30	1	6	28	0	2
23	F	67	MAB	60	0	7	29	1	2.5
24	F	58	MAB	108	1	2	34	3	3

Legend: HAB, MAB, LAB are respectively high affinity, mixed affinity and low affinity binders of [¹⁸F]-DPA714. Disease duration is in months. MDS-UPDRS: Movement Disorder Society – Unified Parkinson Disease Rating Scale.

Table 2 : Regional microglial activation in HC and PD subjects : mean [¹⁸F]-DPA-714 BP_{ND} and standard deviation values in different VOIs

	HC (BP _{ND} ± SD)		PD patients (BP _{ND} ± SD)		P-values - Diff PD/HC	P-values - TSPO polymorphism
	MAB	HAB	MAB	HAB		
Midbrain-			0.31± 0.09	0.25± 0.09	<0.001	NS
Midbrain+	0.21±0.07	0.22±0.07	0.34± 0.14	0.37± 0.07	<0.001	NS
Caudate -			0.02± 0.146	0.12± 0.161	NS	NS
Caudate +	-0.07±0.13	0.010±0.11	0.06± 0.191	0.05± 0.283	NS	NS
Putamen -			0.33± 0.109	0.38± 0.135	NS	0.045
Putamen +	0.20±0.086	0.31±0.14	0.33± 0.126	0.40± 0.118	0.038	0.016
Thal -			0.50± 0.144	0.60± 0.090	NS	0.006
Thal +	0.45±0.09	0.58±0.08	0.43± 0.176	0.57± 0.131	NS	<0.001
GP -			0.24± 0.086	0.32± 0.089	NS	<0.001
GP +	0.20±0.07	0.32±0.09	0.26± 0.096	0.40± 0.167	NS	<0.001
Occipital	0.30± 0.11	0.35± 0.15	0.35± 0.091	0.34± 0.10	NS	NS
Frontal	0.42 ± 0.10	0.53 ± 0.42	0.60 ± 0.17	0.71 ± 0.20	0.001	NS
Parietal	0.32 ± 0.18	0.41 ± 0.15	0.40 ± 0.13	0.46± 0.17	NS	NS
Temporal	0.20 ± 0.038	0.25 ± 0.071	0.24± 0.05	0.26± 0.12	NS	NS

Legend : Regional mean binding potential values (BP_{ND}) for all subjects. + and - refer respectively to the most and less affected sides in patients. Left and right hemispheres are averaged for cortical regions. SD = standard deviation. Statistical differences between HC (healthy controls) and patients (HABs+MABs) are provided as p-values in the third column when significant (two-ways ANOVA). Last column: statistical impact of the TSPO polymorphism on the HC and PD patients difference. NS = non significant