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Comparison of PDE10A and DAT levels as markers of disease burden in early Parkinson's disease

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Abbreviations

PDE10A=Phosphodiesterase 10A; DAT: dopamine transporter; MDS-UPDRS=Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale; cAMP=cyclic adenosine monophosphate; cGMP=cyclic guanosine monophosphate; BP_{ND}=Binding Potential relative to Non-displaceable Binding; MMSE: Mini Mental Status Examination; MoCA: Montreal Cognitive Assessment; ES: Effect Size.

ABSTRACT

BACKGROUND: Recent work has shown loss of phosphodiesterase 10A levels in middle-stage and advanced treated patients with Parkinson's disease, which was associated with motor symptom severity.

OBJECTIVE: To assess phosphodiesterase 10A levels in early Parkinson's disease, and compare with loss of dopamine transporter as markers of disease burden.

METHODS: Seventy-eight subjects were included in this study (17 early *de novo*,15 early levodopa-treated,24 moderate-advanced levodopa-treated patients with Parkinson's disease, and 22 healthy controls). All participants underwent [^{11}C]IMA107 PET, [^{11}C]PE2I PET and 3-Tesla MRI scan.

RESULTS: Early *de novo* Parkinson's disease patients showed loss of [^{11}C]IMA107 and of [^{11}C]PE2I binding in caudate and putamen (P<0.001); early levodopa-treated Parkinson's disease patients showed additional loss of [^{11}C]IMA107 in the caudate (P<0.001; annual decline 3.6%) and putamen (P<0.001; annual decline 2.8%) but loss of [^{11}C]PE2I only in the putamen (P<0.001; annual decline 6.8%). Lower [^{11}C]IMA107 correlated with lower [^{11}C]PE2I in the caudate (*rho*=0.51; P<0.01) and putamen (*rho*=0.53; P<0.01). Longer disease duration correlated with lower [^{11}C]IMA107 in the caudate (*rho*=-0.72; P<0.001) and putamen (*rho*=-0.48; P<0.01), and with lower [^{11}C]PE2I only in the putamen (*rho*=-0.65; P<0.001). Higher burden of motor symptoms correlated with lower [^{11}C]IMA107 in the caudate (*rho*=-0.42; P<0.05) and putamen (*rho*=-0.41; P<0.05), and with lower [^{11}C]PE2I only in the putamen (*rho*=-0.69; P<0.001).

CONCLUSION: Our findings demonstrate loss of phosphodiesterase 10A levels very early in the course of Parkinson's disease and is associated with the gradual and progressive increase of motor symptoms. Phosphodiesterase 10A imaging shows similar potential with dopamine transporter imaging to follow disease progression.

INTRODUCTION

Previous PET imaging studies have shown that changes in the molecular binding profile of selected brain targets may serve as promising markers of disease burden and progression, drug target identification and treatment response in therapeutic trials in patients with Parkinson's disease [1]. Loss of dopamine transporter (DAT) signal in Parkinson's disease patients reflects loss of nigrostriatal dopamine neurons, and is typically associated with dopaminergic pathology in the putamen and the motor features of bradykinesia and rigidity [2]. Phosphodiesterase 10A (PDE10A) is a striatal enzyme expressed in the axons of the medium spiny neurons, where it hydrolyses cAMP and cGMP [3, 4]. In the striatal pathways, PDE10A plays a pivotal role in the regulation of dopaminergic signalling [5] and of several other brain functions, ranging from ion conductance to synaptic plasticity [6]. Small pilot studies have shown loss of PDE10A levels in the caudate of Parkinson's disease patients, but not in the putamen [7, 8]. Our previous work with PET molecular imaging has demonstrated loss of PDE10A levels in the caudate, putamen and globus pallidus in moderate to advanced levodopa-treated patients with Parkinson's disease, which was associated with motor symptoms and complications [9]. Therefore, PDE10A, as an enzyme regulating striatal output and dopaminergic signalling, shows promise to serve as a marker of disease burden in patients with early Parkinson's disease. However, it is unknown whether PDE10A is implicated at the earlier stages of the disease and how its biomarker value compares with the gold standard DAT molecular imaging.

Here, we used PET with [^{11}C]IMA107 to assess PDE10A levels and [^{11}C]PE2I to compare DAT with PDE10A levels, and to explore for associations with clinical markers of disease burden in patients with early Parkinson's disease including those who have never been treated with dopaminergic medications. We also compared [^{11}C]IMA107 binding in our early population with data from moderate-advanced patients with Parkinson's disease, performed previously [9].

METHODS

Participants and clinical characteristics

We studied a total of 78 subjects (56 patients with Parkinson's disease and 22 healthy controls). Parkinson's patients included 17 early *de novo*, 15 early levodopa-treated, and 24 moderate-advanced levodopa-treated. While the latter group (n=24) came out of our previous study [9], we enrolled an additional 60 participants recruited from specialist Movement Disorders clinics at King's College Hospital, National Hospital of Neurology and Neurosurgery, Imperial College NHS Trust and through public advertisement, of which 54 subjects completed the study and were included in the analyses (Table 1).

We included 32 patients with idiopathic Parkinson's disease according to the Queen Square Brain Bank criteria, and 22 age- and gender-matched healthy individuals with no history of neurological or psychiatric disorders, who served as the control group *(healthy controls)*. Parkinson's disease patients included 17 subjects with a recent diagnosis (duration of symptoms ≤24 months) who were naïve to treatment for Parkinson's symptoms *(de novo)*, and 15 patients with early Parkinson's disease (duration of symptoms ≤60 months) who were recently treated with levodopa (duration of treatment ≤24 months) and had no motor complications *(early levodopa-treated)*. To get a full picture of changes in PDE10A binding at different stages of PD progression, we compared [^{11}C]IMA107 binding in our early population with data from moderate-advanced patients with Parkinson's disease. Population of moderate-advanced Parkinson's disease were recruited previously [9] and included 24 patients who had a mean disease duration of 7-13 years and were treated with levodopa for at least 24 months *(moderate-advanced levodopa-treated)*. None of the Parkinson's disease patients fulfilled the diagnostic criteria for Parkinson's disease mild cognitive impairment [10] or dementia [11] or depression [12], had any history of other neurological or psychiatric disorders, and were not under treatment with substances with known actions in PDEs (e.g. apremilast, cilomilast, luteolin, piclamilast, roflumilast and ibudilast). None of the Parkinson's disease patients included had a diagnosis of scan without evidence of dopaminergic deficit (SWEDD) after [^{11}C]PE2I PET scan.

The group of *early levodopa-treated* and *moderate-advanced levodopa-treated* PD patients were on stable levodopa treatment for at least 6 months at the time of study enrolment. Daily dopaminergic medication dose was calculated with a formula based on the theoretical

equivalence to levodopa [9]. Motor symptom severity was assessed with the MDS-UPDRS part-III and staged with Hoehn and Yahr scale in the practically defined off-medication state (after 12 hours overnight withdrawal of patient's dopaminergic medications). MDS-UPDRS-III subscores for rigidity, bradykinesia, tremor and axial symptoms were calculated as previously described[9]. Quality of life was measured with the 39-item Parkinson's Disease Questionnaire. Neuropsychiatric symptoms were assessed with the Beck Depression Inventory second edition and the Hamilton Depression Rating Scale. Mini-Mental Status Examination (MMSE) and Montreal Cognitive Assessment (MoCA) were used to assess general cognitive status. Disability was assessed by Modified Schwab and England Activities of Daily Living Scale. Non-Motor Symptoms Scale for Parkinson's disease was used to assess non-motor symptoms. Sleep disturbances were assessed with the Parkinson's Disease Sleep Scale and the Epworth Sleepiness Scale [13].

The study was approved by the institutional review boards and the research ethics committee. Written informed consent was obtained from all study participants in accordance with the Declaration of Helsinki.

Scanning procedures

All participants were screened successfully to undertake PET with [^{11}C]IMA107 and [^{11}C]PE2I, and one 3-Tesla MRI scanning under standard criteria (http://www.mrisafety.com; https://www.gov.uk/government/publications/arsac-notes-for-guidance). PET and MR imaging have been performed at Invicro London, UK. All participants were scanned on Siemens Biograph Hi-Rez 6 PET-CT scanner (Erlangen, Germany). A median dose of 297.15 (interquartile range 276.1 to 307.8) MBq [^{11}C]IMA107 [median mass injected: 3.3 ug (interquartile range 2.5 to 4.2)] was administered intravenously as a slow bolus injection over 20s. A median dose of 320.3 (interquartile range 315.2 to 340.2) MBq [^{11}C]PE2I [mean mass injected: 4.4 ug (interquartile range 3.7 to 5.8)] was administered intravenously as a bolus injection over 10s followed by a 10 ml saline bolus injection over 10s. [^{11}C]IMA107 and [^{11}C]PE2I have been performed on the same day after withholding consumption of caffeinated beverages for 12 hours [14]. The group of *early levodopa-treated* and *moderate-advanced levodopa-treated* PD patients undertake the scan in the on-medication state.

Dynamic emission data were acquired continuously for 90 minutes following the injection of [^{11}C]IMA107 and [^{11}C]PE2I. The dynamic images were reconstructed into 26 frames (8 x 15

s, 3 x 60 s, 5 x 120 s, 5 x 300 s, and 5 x 600 s), using a filtered back projection algorithm (direct inversion Fourier transform) with a 128 matrix, zoom of 2.6 producing images with isotropic voxel size of 2 x 2 x 2 mm^3, and smoothed with a transaxial Gaussian filter of 5 mm. MRI scans were acquired with a 32-channel head coil on a 3-Tesla MRI Siemens Magnetom TrioTim syngo MR B17 (Erlangen, Germany) scanner, and included a T1-weighted magnetization prepared rapid gradient echo sequence [MPRAGE; time repetition (TR) = 2300 ms, time echo (TE) = 2.98 ms, flip angle of 9°, time to inversion (TI) = 900 ms, matrix = 240 x 256] for co-registration with the PET images; fast grey matter T1 inversion recovery (FGATIR; repetition time = 3000 ms, echo time = 2.96 ms, flip angle of 8, time to inversion = 409 ms, matrix = 240 x 256)[15] sequences for delineation of regions-of-interest. All MRI sequences used a 1 mm^3 voxel size, anteroposterior phase encoding direction, and a symmetric echo.

Imaging data analysis

MRI-based volumetric analysis

Because PDE10A is an intracellular enzyme mainly expressed in the basal ganglia nuclei [3, 4], degeneration of these nuclei may affect the levels of the enzyme. Thus, we investigated volumetric changes in subcortical nuclei in our cohort of Parkinson's disease patients. We used the FreeSurfer image analysis suite (version 5.3.0 http://surfer.nmr.mgh.harvard.edu) to process individual MRI scans for deriving measures of subcortical volumes, as described before[9].

[^{11}C]IMA107 and [^{11}C]PE2I PET data analysis

The Molecular Imaging and Kinetic Analysis Toolbox software package (MIAKAT^{TM}: www.miakat.org), implemented in MATLAB® (The Mathworks, Natick, MA, USA) was used to carry out image processing and kinetic modelling. MIAKAT™ combines in-house code with wrappers for FMRIB Software Library (FSL, http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/) and Statistical Parametric Mapping (SPM, http://www.fil.ion.ucl.ac.uk/spm/) commands in order to provide state-of-the-art functionality within a coherent analysis framework. We followed the MIAKAT™ processing pipeline, ensuring that all quality control steps were completed to generate parametric images and regional estimates of [^{11}C]IMA107 and [^{11}C]PE2I non-displaceable binding potential (BP_{ND}). BP_{ND} were generated using a basis function implementation of the simplified reference tissue model, with the cerebellum as the

reference tissue for non-specific binding. Individual PET frames were corrected for head motion using frame-by-frame rigid registration using a frame with high signal-to-noise ratio as reference. PET images were co-registered to the corresponding MPRAGE MRI.

Region of interest-based analysis

To facilitate anatomical delineation of regions of interest, PET images were anatomically co-registered and re-sliced to the corresponding volumetric structural T1-weighted MRI images in Statistical Parametric Mapping version12 (SPM12) software package implemented in Matlab 2015a. Regions of interest were delineated manually on the T1 co-registered FGATIR MRI sequence using ANALYZE version 12.0 (Mayo Foundation) medical imaging software package by two assessors (G.P. and H.W.) who were blinded to groups allocation, using used a reliable, robust and repeatable technique for manual delineation of basal ganglia structures [16]. Regions of interest included caudate, putamen, whole striatum (combination of caudate and putamen), ventral striatum, globus pallidus (external and internal segments), substantia nigra and motor thalamic nuclei. These brain regions express PDE10A to a varying degree [4, 17].

Voxel-based analysis

Parametric images of [^{11}C]IMA107 and [^{11}C]PE2I BP_{ND} were spatially normalized into the T1-weighted MNI 152 template using the individual MRI with the Mutual Information Registration algorithm in SPM12. Parametric images were computed using appropriately weighted contrasts to localize significant decreases in mean voxel PET values after applying the Basal Ganglia Human Area Template [18]. This masking reduces the number of voxel-by-voxel statistical comparisons, and a cluster-corrected threshold of $P<0.05$ used to test for statistically significant effects. The contrasts were used to derive Z-scores on a voxel basis using the general linear model. The threshold for statistical significance was set to $P<0.05$ after family wise error (FWE) correction for multiple comparisons. Voxel-wise statistics for between-group comparisons were performed by using SPM12 software package implemented in Matlab 2015a.

Statistical analysis

Statistical analysis and graph illustration were performed with SPSS (version 22) and GraphPad Prism (version 6.0c) for Windows 10, respectively.

For all variables, Gaussianity was tested with Shapiro-Wilk test and we proceeded with parametric tests for normally distributed variables and non-parametric tests for non-normally distributed variables. Multivariate analysis of variance (MANOVA) was used to assess the main effects of regional [^{11}C]IMA107 and [^{11}C]PE2I BP_{ND} among the groups. If the overall multivariate test was significant, *P*-values for each variable were calculated following Bonferroni's multiple comparisons test. We interrogated correlations between PET and clinical data using Pearson r (normally distributed variables) or Spearman *rho* (non-normally distributed variables) and we applied Benjamini-Hochberg correction to reduce false discovery rate [19]. We set the false discovery rate cut-off at 0.05. All data are presented as mean ± standard deviation, and the level α was set for all comparisons at $P<0.05$, corrected.

RESULTS

Volumetric analysis

FreeSurfer analysis showed no subcortical volumetric differences in the caudate, putamen, ventral striatum, globus pallidus, and thalamus between the groups of early *de novo* and early levodopa-treated Parkinson's disease patients and healthy controls (Supplementary Table 1).

Region of interest-based [^{11}C]IMA107 BP_{ND} analysis in early de novo Parkinson's disease patients

Early *de novo* patients with Parkinson's disease had lower mean [^{11}C]IMA107 BP_{ND} values in whole striatum (ES=2.69, $P<0.001$), caudate (ES=3.75, $P<0.001$), putamen (ES=1.64, $P<0.001$) and ventral striatum (ES=1.21, $P<0.05$) compared to healthy controls (Figure 1A and 1B). There were no differences in mean [^{11}C]IMA107 BP_{ND} values between early *de novo* Parkinson's disease patients and healthy controls in globus pallidus internal (ES=0.54, $P>0.10$), globus pallidus external (ES=0.58, $P>0.10$), substantia nigra (ES=0.48, $P>0.10$) and motor thalamic nuclei (ES=0.28, $P>0.10$).

Region of interest-based [^{11}C]PE2I BP_{ND} analysis in early de novo Parkinson's disease patients

Early *de novo* patients with Parkinson's disease had lower mean [^{11}C]PE2I BP_{ND} values in whole striatum (ES=3.49, $P<0.001$), caudate (ES=1.53, $P<0.05$), putamen (ES=4.32, $P<0.001$), ventral striatum (ES=1.73, $P<0.05$), globus pallidus internal (ES=1.62, $P<0.001$),

globus pallidus external (ES=1.81, P<0.001) and substantia nigra (ES=1.67, P<0.001) compared to healthy controls (Figure 2A and 2B). There were no differences in mean [^{11}C]PE2I BP_{ND} values between early *de novo* Parkinson's disease patients and healthy controls in motor thalamic nuclei (ES=0.28, P>0.10).

Effect of lateralisation in [^{11}C]IMA107 BP_{ND} and [^{11}C]PE2I BP_{ND}

In the group of early *de novo* patients with Parkinson's disease with unilateral motor symptoms, we assessed whether the clinically affected side of the body was associated with greater decreases in contralateral brain regions of interest [^{11}C]IMA107 BP_{ND} and [^{11}C]PE2I BP_{ND} values. We found no differences in [^{11}C]IMA107 BP_{ND} values in any of the regions of interest between the most and less affected side; whereas [^{11}C]PE2I BP_{ND} values in caudate (P<0.001), putamen (P<0.001), ventral striatum (P<0.001), globus pallidus internal (P<0.001), globus pallidus external (P<0.001) and substantia nigra (P<0.001) were decreased in the contralateral to most affected compared to the less affected side of the body (Figure 2C).

[^{11}C]IMA107 BP_{ND} and [^{11}C]PE2I BP_{ND} in levodopa-treated Parkinson's disease patients

Compared to early *de novo* Parkinson's disease patients, early levodopa-treated patients with Parkinson's disease had three years longer disease duration and showed additional [^{11}C]IMA107 BP_{ND} loss of 17.2% (SD: 8.4%) in the caudate (P<0.001) and 9.5% (SD: 9.6%) in the putamen (P<0.001) (Figure 1A and 1B). Early levodopa-treated Parkinson's disease patients showed additional [^{11}C]PE2I BP_{ND} loss of was 34.7% (SD: 17%) in the putamen (P<0.001), whereas changes were not significant in the caudate, compared to early *de novo* Parkinson's disease patients (Figure 2A and 2B). Compared to early *de novo* Parkinson's disease patients, moderate-advanced patients further showed [^{11}C]IMA107 BP_{ND} loss of 13.2% (SD: 7.6%) in the caudate (P<0.001), 6.5% (SD: 5.6%) in the putamen (P<0.001) and of 10.2% (SD: 9.2%) in the globus pallidus internal (P<0.05).

Voxel-Based [^{11}C]IMA107 BP_{ND} and[^{11}C]PE2I BP_{ND} analyses

Voxel-by-voxel analysis of [^{11}C]IMA107 and [^{11}C]PE2I parametric BP_{ND} images between the groups of patients with Parkinson's disease and healthy controls confirmed results obtained with region of interest analysis. SPM analysis within the striatal mask localized clusters of [^{11}C]IMA107 BP_{ND} decreases in the right and left caudate (P<0.001) and right and

left putamen (P<0.001), and clusters of [^{11}C]PE2I BP_{ND} decreases in the right and left caudate (P<0.05) and right and left putamen (P<0.001) in the early *de novo* Parkinson's disease patients compared with the group of healthy controls. SPM analysis within the striatal mask localized clusters of [^{11}C]IMA107 BP_{ND} decreases in right and left caudate (P<0.05) and right and left putamen (P<0.05), and clusters of [^{11}C]PE2I BP_{ND} decreases in the right and left putamen (P<0.05) in the early levodopa-treated compared to early *de novo* patients with Parkinson's disease.

Head-to-head comparison between [^{11}C]IMA107 BP_{ND} and [^{11}C]PE2I BP_{ND}

We compared loss of [^{11}C]IMA107 BP_{ND} with loss of [^{11}C]PE2I BP_{ND} in each region of interest in Parkinson's disease patients relative to normality data from the group of healthy controls. We found that loss of [^{11}C]IMA107 BP_{ND} was greater than loss of [^{11}C]PE2I BP_{ND} in the caudate (P<0.01) but lower in the putamen (P<0.001), globus pallidus internal (P<0.01), globus pallidus external (P<0.001) and substantia nigra (P<0.01), and no different in ventral striatum (P>0.10) and motor thalamic nuclei (P>0.10) in early *de novo* Parkinson's disease patients. Similarly, we found that loss of [^{11}C]IMA107 BP_{ND} was greater than loss of [^{11}C]PE2I BP_{ND} in the caudate (P<0.01) but lower in the putamen (P<0.001), globus pallidus internal (P<0.05), globus pallidus external (P<0.01) and substantia nigra (P<0.05), and no different in ventral striatum (P>0.10) and motor thalamic nuclei (P>0.10) in early levodopa-treated Parkinson's disease patients.

We also evaluated the coefficient of variance (COV% = SD/mean *100) for [^{11}C]IMA107 and [^{11}C]PE2I BP_{ND}. In early *de novo* Parkinson's disease patients: (a) [^{11}C]IMA107 BP_{ND} COV% was 9% in the whole striatum, 14% in the caudate, 8% in the putamen, 18% in the ventral striatum, 10% in the globus pallidus internal, 11% in the globus pallidus external, 11% in substantia nigra, and 16% in the motor thalamic nuclei; (b) [^{11}C]PE2I BP_{ND} COV% was 19% in the whole striatum, 21% in the caudate, 26% in the putamen, 22% in the ventral striatum, 29% in the globus pallidus internal, 26% in the globus pallidus external, 16% in the substantia nigra, and 11% in the motor thalamic nuclei.

In early levodopa-treated Parkinson's disease patients: (a) [^{11}C]IMA107 BP_{ND} COV% was 9% in the whole striatum, 10% in the caudate, 11% in the putamen, 18% in the ventral striatum, 15% in the globus pallidus internal, 13% in the globus pallidus external, 20% in substantia nigra, and 17% in the motor thalamic nuclei; (b) [^{11}C]PE2I BP_{ND} COV% was 21%

in the whole striatum, 25% in the caudate, 26% in the putamen, 24% in the ventral striatum, 30% in the globus pallidus internal, 27% in the globus pallidus external, 14% in the substantia nigra, and 12% in the motor thalamic nuclei.

Correlations between [^{11}C]IMA107 BP_{ND} and[^{11}C]PE2I BP_{ND} in Parkinson's disease patients

Lower individual [^{11}C]IMA107BP_{ND} values correlated with lower individual [^{11}C]PE2I BP_{ND} values in the caudate (*rho*=0.51; *P*<0.01) and putamen (*rho*=0.53; *P*<0.01), but not in ventral striatum (*rho*=0.013; *P*>0.10), globus pallidus internal (*rho*=0.21; *P*>0.10), globus pallidus external (*rho*=0.46; *P*>0.05), substantia nigra (*rho*=0.15; *P*>0.10) or motor thalamic nuclei (*rho*=0.15; *P*>0.10) in Parkinson's disease patients (Figure 2D).

Correlations between PET data and measures of Parkinson's disease burden

Parkinson's disease patients had increased MDS-UPDRS-III (*P*<0.001), Non-motor Symptoms Scale (*P*<0.05), Beck Depression Inventory-II (*P*<0.05), and Hamilton Depression Rating Scale (*P*<0.05) scores, and worse 39-item Parkinson's disease Questionnaire (*P*<0.05) and Parkinson's Disease Sleep Scale (*P*<0.05) scores compared to healthy controls (Table 1). Only one early *de novo* Parkinson's disease patient was treated also with selective serotonin reuptake inhibitors. As the cohorts were carefully matched for age, we found no effect of age in [^{11}C]IMA107 and [^{11}C]PE2I BP_{ND} in all regions-of-interest across all cohorts (*P*>0.10). No correlations were found between cognitive status (MMSE, MoCA), depression (Beck Depression Inventory-II, Hamilton Depression Rating Scale), 39-item Parkinson's disease Questionnaire, Parkinson's Disease Sleep Scale and [^{11}C]IMA107 or [^{11}C]PE2I BP_{ND} in any area.

Longer Parkinson's disease duration correlated with lower [^{11}C]IMA107 BP_{ND} in the caudate (*rho*=-0.72; *P*<0.001) and putamen (*rho*=-0.48; *P*<0.01). Higher MDS-UPDRS-III total scores correlated with lower [^{11}C]IMA107 BP_{ND} in the caudate (*rho*=-0.42; *P*<0.05) and putamen (*rho*=-0.41; *P*<0.05). Higher MDS-UPDRS-III rigidity scores correlated with lower [^{11}C]IMA107 BP_{ND} in the caudate (*rho*=-0.52; *P*<0.01) and putamen (*rho*=-0.43; *P*<0.05). Higher MDS-UPDRS-III bradykinesia scores correlated with lower [^{11}C]IMA107 BP_{ND} in the caudate (*rho*=-0.45; *P*<0.01) and putamen (*rho*=-0.47; *P*<0.01; Figure 3A).

Longer Parkinson's disease duration correlated with lower [^{11}C]PE2I BP_{ND} only in the putamen (*rho*=-0.65; *P*<0.001). Higher MDS-UPDRS-III total scores correlated with lower [^{11}C]PE2I BP_{ND} only in the putamen (*rho*=-0.69; *P*<0.001). Higher MDS-UPDRS-III rigidity scores correlated with lower [^{11}C]PE2I BP_{ND} only in the putamen (*rho*=-0.62; *P*<0.001). Higher MDS-UPDRS-III bradykinesia scores correlated with lower [^{11}C]PE2I BP_{ND} only in the putamen (*rho*=-0.49; *P*<0.01; Figure 3B).

Correlations between PET data, levodopa equivalent daily dosage and antidepressants

In early treated PD patients, we investigated correlations between [^{11}C]IMA107 or [^{11}C]PE2I BP_{ND} and levodopa equivalent daily dosage. No correlations were found between LEDD and [^{11}C]IMA107 or [^{11}C]PE2I BP_{ND} in any area ([^{11}C]IMA107: whole striatum, rho=-0.27; P>0.10; caudate, rho=-0.28; P>0.10; putamen, rho=-0.32; P>0.10; ventral striatum, rho=-0.28; P>0.10; globus pallidus internal, rho=-0.45; P=0.095; globus pallidus external, rho=-0.39; P>0.10; substantia nigra, rho=-0.45; P=0.089; motor thalamic nuclei, rho=-0.64; P=0.091 - [^{11}C]PE2I: whole striatum, rho=-0.13; P>0.10; caudate, rho=-0.28; P>0.10; putamen, rho=-0.12; P>0.10; ventral striatum, rho=-0.01; P>0.10; globus pallidus internal, rho=-0.32; P>0.10; globus pallidus external, rho=-0.33; P>0.10; substantia nigra, rho=-0.49; P=0.063; motor thalamic nuclei, rho=-0.22; P>0.10).

We could not investigate correlations between [^{11}C]IMA107 or [^{11}C]PE2I BP_{ND} and antidepressants (e.g. selective serotonin reuptake inhibitors or tricyclic antidepressants) because we had only one patient treated with selective serotonin reuptake inhibitors.

DISCUSSION

Our findings demonstrate that loss of striatal PDE10A levels is an early phenomenon in the course of Parkinson's disease and is associated with duration and severity of motor symptoms. Our study translates into humans' previous experimental work, which has demonstrated that lesions of nigrostriatal projections with 6-hydroxydopamine, induce a downregulation of PDE10A levels in the striatum in rodent models of Parkinson's disease [20, 21].

We used PET molecular imaging to quantify PDE10A and DAT levels in the same cohorts of Parkinson's disease patients and healthy controls. In both early *de novo* and early levodopa-

treated patients with Parkinson's disease both PDE10A and DAT levels were decreased in the striatum, and DAT also decreased in globus pallidus and substantia nigra. Our early *de novo* cohort of Parkinson's disease patients had less than two year of disease duration and the cohort of early levodopa-treated less than five year of Parkinson's symptoms and were taking levodopa for less than two years. Previously, we have reported loss of striatal PDE10A levels and correlations with the burden of motor symptoms and complications in middle-stage treated Parkinson's disease patients with a mean of 7 years of disease duration and advanced treated Parkinson's disease patients with a mean of 13 years of disease duration [9]. We compared the levels of PDE10A in this population of moderate-advanced Parkinson's disease patients with our group of early *de novo*. We found in the advanced stages of Parkinson's disease further loss of PDE10A in the striatum and in the globus pallidus internal. Collectively, our data suggest that loss of striatal PDE10A levels appears early in the course of Parkinson's disease and progresses over time, and is associated with the gradual increase of motor symptom burden in different disease stages. Our findings suggest that loss of PDE10A levels in globus pallidus is a phenomenon appearing later in the disease as PDE10A levels in this region was not affected in the earlier cohorts. Our findings were not affected by volumetric changes in the brain, age or gender that were also confirmed at a voxel level for the striatum.

Loss of PDE10A correlated with loss of DAT in the striatum in early *de novo* and early levodopa-treated patients with Parkinson's disease, suggesting an association between PDE10A and dopaminergic function. PDE10A plays a key role in the regulation of dopaminergic signalling and is essential for dopamine neurotransmission through the interaction with cAMP and the activation of Protein kinase A /dopamine- and cAMP-regulated neuronal phosphoprotein 32 downstream cascade in striatal pathways [5, 6, 22].
We did not find differences in PDE10A in the substantia nigra in our populations, which could be due to the power of resolution of PET in this area and the relatively lower expression of PDE10A compared to the striatum.

We attempted to understand how informative PET molecular imaging of PDE10A and DAT could be for monitoring the disease progression, speculating on our cross-sectional data. Our levodopa-treated patients with Parkinson's disease had three years longer disease duration compared to early *de novo* Parkinson's disease patients. PDE10A levels showed further decline in both the caudate and putamen, whereas DAT levels was further reduced only in the

putamen in early levodopa-treated compared to early *de novo* Parkinson's disease patients. PDE10A showed lower variability over the time, with a SD of 8.4% in the caudate and 9.6% in the putamen, whereas DAT showed a 17% SD in the putamen. This trend of reduction was consistent in the middle and advanced stages of the disease, where the PDE10A loss extended also to the pallidus. In our study we have shown that standardized differences (Cohen's d) between early *de novo* and levodopa-treated with three-year longer disease duration are 1.48 for caudate measured with [^{11}C]IMA107, and 1.57 for putamen measured with [^{11}C]PE2I. The greater loss of [^{11}C]IMA107 in the caudate compared to the putamen might be simply due to the greater physiological levels of this protein in the caudate than putamen [4, 5]. This has also implication for future clinical trials using [^{11}C]IMA107 and [^{11}C]PE2I PET imaging as biomarkers of disease progression. Assuming in detecting a 50% reduction in these differences in a potential 18-month clinical trial testing a disease modifying drug that reduces disease progression by 50%, we will have a 0.74 Cohen's d for caudate PDE10A and a 0.78 for putamen DAT. These figures are greater than [^{18}F]FDOPA, a validated biomarker of Parkinson's progression, that showed Cohen's d of 0.79 at three-year follow-up in early *de novo* patients with Parkinson's [23], thus having a 0.39 Cohen's d in this hypothetical trial. Providing a 80% power with a two-sided test using 5% type I error rate, we may need 60 participants if we use as endpoint the changes in the caudate [^{11}C]IMA107 levels, 54 subjects if we use as endpoint the changes in the putamen [^{11}C]PE2I levels and 200 subjects if we use as endpoint the changes in the putamen [^{18}F]FDOPA levels. This suggests a similar strength for [^{11}C]IMA107-PDE10A and [^{11}C]PE2I-DAT over aromatic L-amino acid decarboxylase-[^{18}F]FDOPA as biomarkers for clinical trials. VMAT2 assessed with [^{11}C]dihydrotetrabenazine ([^{11}C]DTBZ) PET has also been suggested as a robust marker of Parkinson's progression and would be interesting to see how this is compared to PDE10A PET [24].

We performed head-to-head comparisons between loss of PDE10A and DAT in Parkinson's disease patients relative to normality data from the group of healthy controls. We found that loss of PDE10A was greater than loss of DAT in the caudate, but lower in the putamen, globus pallidus and substantia nigra in both early *de novo* and early levodopa-treated patients with Parkinson's disease. In early *de novo* and levodopa-treated patients with Parkinson's disease, PDE10A tracer showed a 10% lower COV variability in the striatum, caudate and putamen compared to DAT. This could suggest that PDE10A is a more stable biomarker but also that PDE10A may have a lower sensitivity to dopaminergic disease process or that the

greater COV for DAT may simply reflect that it is a better marker of primary disease pathology in Parkinson's disease; imperfect correlations between DAT and PDE10A would be compatible with related but different pathology. Loss of PDE10A in both the caudate and putamen, and loss of DAT only in the putamen correlated with longer Parkinson's duration, total burden of motor symptoms, and with increased rigidity and bradykinesia. The level of correlations between PDE10A and DAT and clinical scales were similar. However, correlations with disease duration and with motor symptoms for PDE10A have lower regression (*rho*) values for both caudate and putamen than corresponding values for DAT in the putamen. We also found higher z-scores for DAT imaging at voxel-level compared to PDE10A, which further suggests that [^{11}C]PE2I-DAT might have higher sensitivity compared to [^{11}C]IMA107-PDE10A.

Considering the underlying pathophysiology of Parkinson's disease includes progressive deposition of α-synuclein, the ideal neuroimaging biomarker to monitor disease progression should be able to quantify regional deposition of abnormal α-synuclein accumulation. Development of such a molecular imaging radiotracer has been the focus of much research but is not yet forthcoming and assessment of DAT represents the most direct approach able to quantify presynaptic nigrostriatal dopaminergic neurons. However, within the postsynaptic striatal neurons, cAMP signalling is finely regulated by the PDE10A. Lesions in nigrostriatal dopaminergic projections in animal models of Parkinson's disease leads to an increase in cAMP levels, which could be due to a reduction in PDE10A levels/activity, and treatment with levodopa reduces the high cAMP levels observed in the denervated striatum [21, 25]. Altered striatal second messenger signalling may be due to the lost ability of striatal neurons to induce both depotentiation and long-term depression [25]. Hence, stimulation of postsynaptic striatal neurons from damaged dopaminergic terminals would fail, and dysregulation of PDE10A could be a pathogenic mechanism underlying the dysfunction of second messenger signalling. This enzyme might be the final regulator of striatal output and modulating its level crucial for the control of fine-tuned movements.

We demonstrated a bilateral loss of PDE10A in the striatum (with no lateralization) at the early stages of Parkinson's disease, when loss of DAT was only unilateral. This suggests that dysregulation of postsynaptic PDE10A might happen earlier than dopaminergic terminals loss and quite far from the motor onset of Parkinson's disease. As confirmation of this theory, an early involvement of PDE10A has been previously demonstrated in pre-symptomatic

Huntington's disease gene carriers [26, 27]. However, one concern with PDE10-targeting tracers is that they may be modulated by drugs that do not directly target the PDE enzyme, either through direct or allosteric competition *via* altered cyclic nucleotide levels, or by drug-induced changes in PDE10 levels, independently of the disease state [28]. Thus, the use of [^{11}C]IMA107 PET imaging as an outcome in clinical trials should be considered with caution in treated subjects.

To minimize the variability over time, we decided to perform [^{11}C]IMA107 and [^{11}C]PE2I PET scans on the same day. However, in the levodopa-treated group, we had to perform the scan in the on-medication state, because the patients were not able to tolerate a whole day off-medication. Although this could induce a potential bias, because we are not able to exclude the possibility of an acute effect of dopaminergic medication on PDE10A levels, all our patients were on the same medication regimen for at least 6 months and we included the levodopa equivalent daily dose as covariate. We found no correlations between levodopa equivalent daily dose and PDE10A or DAT levels in any brain areas. Moreover, PDE10A levels were reduced in a population of PD patients who were never treated with any dopaminergic drugs, suggesting that reduction of PDE10A is probably not influenced by the dopaminergic treatment. We had no power to investigate the influence of other drugs on PDE10A levels. Based on our findings, we do not expect PDE10A levels to be vulnerable to dopamine medications. However, only acute challenge studies with [^{11}C]IMA107 PET imaging performed before and after the drug administration could clarify this issue entirely.

PDE10A imaging could be a robust alternative to DAT imaging regarding diagnosis and for evaluating disease burden and progression. We found a gradual progressive decline of PDE10A in Parkinson's from very early to advanced stages. This makes PDE10A imaging potentially attractive to explore its clinical use further. It is important to note that both [^{11}C]IMA107 and [^{11}C]PE2I are kinetically well-behaved tracers with good-to-very good reproducibility [29], and the differences in their binding in the striatum are probably related to the pathological processes underlying Parkinson's disease, more than characteristics of the tracers. A combined approach using both radiotracers may be useful because of their sensitivity to different underlying pathological processes. Despite these potential advantages of PDE10A imaging, only PET ligands are currently available for PDE10A whereas DAT levels can be measured with SPECT imaging. This limitation reduces the cost-effective use of

this novel molecular imaging technique until a SPECT PDE10A tracer becomes available. Neither DAT or PDE10A are good biomarkers for other Parkinson's symptoms, like tremor or depression, where other mechanisms, such as serotonergic transporter deficit, seems to have a prominent role [30-33].

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AUTHORSHIP

M.P. conceived the study, conceptualized the experimental design and acquired funding for the study. G.P. and F.N. performed the imaging and clinical assessments and acquired the data. G.P. and M.P. organised the study, wrote the first draft and prepared the manuscript. G.P., H.W., T.Y. generated the figures. G.P. and H.W. analysed the data. M.P., G.P., E.A.R. and R.N.G interpreted the data. G.P., F.N., T.F., N.K., D.M. and P.P. recruited the subjects. All authors revised and gave input to the manuscript.

POTENTIAL CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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FIGURE LEGENDS

Figure 1. (A) Altered PDE10A expression in anatomically defined brain regions of Parkinson's disease patients. Axial, sagittal and coronal (MNI co-ordinates: x = 19, y = −8, z = 4) mean summed PET images derived from (top) 22 healthy controls, (middle) 17 Parkinson's disease early de *novo* and (bottom) Parkinson's disease early levodopa-treated patients in stereotaxic space showing progressive loss of [^{11}C]IMA107 BP_{ND} in PD. Colour bar reflects range of [^{11}C]IMA107 BP_{ND} intensity. **(B) PDE10A expression in the groups of Parkinson's disease patients and healthy controls.** Column bar graphs showing mean [^{11}C]IMA107 BP_{ND} in subcortical brain regions in Parkinson's disease early de *novo* patients, Parkinson's disease early levodopa-treated patients and healthy controls. **P* <0.05, ***P* <0.01, ****P* <0.001.

Figure 2. (A) Altered DAT expression in anatomically defined brain regions of Parkinson's disease patients. Axial, sagittal and coronal (MNI co-ordinates: x = 19, y = −8, z = 4) mean summed PET images derived from (top) 22 healthy controls, (middle) 17 Parkinson's disease early de *novo* and (bottom) Parkinson's disease early levodopa-treated patients in stereotaxic space showing progressive loss of [^{11}C]PE2I BP_{ND} in Parkinson's disease. Colour bar reflects range of PET BP_{ND} intensity. **(B) DAT expression in the groups of Parkinson's disease patients and healthy controls.** Column bar graphs showing mean [^{11}C]PE2I BP_{ND} in subcortical brain regions in Parkinson's disease early de *novo* patients, Parkinson's disease early levodopa-treated patients and healthy controls. **P* <0.05, ***P* <0.01, ****P* <0.001. **(C) Laterality of mean loss of DAT expression in Parkinson's disease patients.** Axial, coronal and sagittal (MNI co-ordinates: x = 19, y = −8, z = 4) mean summed PET images derived from (top) most affected right and (bottom) left of Parkinson's disease patients in stereotaxic space showing significant loss of [^{11}C]PE2I BP_{ND} in the Parkinson's disease patients. Colour bar reflects range of [^{11}C]PE2I BP_{ND} intensity. **(D) Correlations between PDE10A and DAT in Parkinson's disease (PD) patients.** Correlation between [^{11}C]IMA107 and [^{11}C]PE2I BP_{ND} (top) in the caudate (rho=0.514; *P*=0.003) and (bottom) in the putamen (rho=0.527; *P*=0.002). Parkinson's disease early *de novo* in black circles and Parkinson's disease early levodopa-treated in grey circles.

Figure 3. Correlations between PDE10A and DAT in relation to motor symptoms in Parkinson's disease patients. Correlation between disease duration (first line) and [^{11}C]IMA107 BP_{ND} in the caudate (rho=-0.721; *P*<0.0001) and [^{11}C]IMA107 and [^{11}C]PE2I

in the putamen (rho=-0.481; *P*=0.005 for [^{11}C]IMA107 and rho=-0.645; *P*<0.0001 for [^{11}C]PE2I). Correlation between MDS-UPDRS-III motor scores (second line) and [^{11}C]IMA107 BP_{ND} in the caudate (rho=-0.423; *P*=0.016) and [^{11}C]IMA107 and [^{11}C]PE2I in the putamen (rho=-0.410; *P*=0.020 for [^{11}C]IMA107 and rho=-0.692; *P*<0.0001 for [^{11}C]PE2I). Correlation between MDS-UPDRS-III rigidity subscores (third line) and [^{11}C]IMA107 BP_{ND} in the caudate (rho=-0.515; *P*=0.003) and [^{11}C]IMA107 and [^{11}C]PE2I in the putamen (rho=-0.432; *P*=0.014 for [^{11}C]IMA107 and rho=-0.620; *P*<0.0001 for [^{11}C]PE2I). Correlation between MDS-UPDRS-III bradykinesia subscores (forth line) and [^{11}C]IMA107 BP_{ND} in caudate (rho=-0.448; *P*=0.010) and putamen (rho=-0.467 ; *P*=0.007 for [^{11}C]IMA107 and rho=-0.493; *P*=0.005 for [^{11}C]PE2I). Parkinson's disease early *de novo* in black circles and Parkinson's disease early levodopa-treated in grey circles.