

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

Gennaro Pagano,¹ Flavia Niccolini,¹ Heather Wilson,¹ Tayyabah Yousaf,¹ Naheed L Khan,² Davide Martino,³ Paola Piccini,⁴ Roger N, Gunn,^{5,6} Eugenii A. Rabiner,^{5,7} and Marios Politis¹

¹Neurodegeneration Imaging Group, Institute of Psychiatry, Psychology and Neuroscience (IoPPN), King's College London, UK

² Department of Neurology, Maidstone Hospital, Kent, UK

³ Department of Clinical Neurosciences, Cumming School of Medicine, University of Calgary, Canada

⁴ Neurology Imaging Unit, Centre of Neuroinflammation and Neurodegeneration, Division of Brain Sciences, Hammersmith Campus, Imperial College London

⁵ Imanova Ltd, Centre for Imaging Sciences, Hammersmith Hospital, London, UK

⁶ Division of Brain Sciences, Department of Medicine, Imperial College London, UK

⁷ Department of Neuroimaging, Institute of Psychiatry, Psychology and Neuroscience (IoPPN), Kings College London, UK

Correspondence to Professor Marios Politis, Neurodegeneration Imaging Group, Maurice Wohl Clinical Neuroscience Institute, Institute of Psychiatry, Psychology and Neuroscience (IoPPN), King's College London, 125 Coldharbour Lane, Camberwell, London SE5 9NU, UK. E-mail: marios.politis@kcl.ac.uk

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Abbreviations

PDE10A=phosphodiesterase 10A; MDS-UPDRS=Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale; cAMP=cyclic adenosine monophosphate; cGMP=cyclic guanosine monophosphate; H&Y=Hoehn & Yahr; PDQ-39=39-item Parkinson's disease Questionnaire; BP_{ND}=binding potential relative to nondisplaceable binding; BDI-II: Beck Depression Inventory-II; HDRS: Hamilton Depression Rating Scale; MMSE: Mini Mental Status Examination; MoCA: Montreal Cognitive Assessment; NMSS: Non-motor Symptoms Scale; PDSS: Parkinson's Disease Sleep Scale; ESS: Epworth Sleepiness Scale.

ABSTRACT

Recent work has demonstrated loss of phosphodiesterase 10A (PDE10A) expression in middle-stage and advanced Parkinson's patients, which was associated with motor symptom severity. In this study, we aimed to assess PDE10A expression, to compare with dopamine transporter (DAT) expression, and to explore for associations with clinical markers of disease burden in patients with early Parkinson's disease.

We enrolled 60 subjects of which 54 completed the study, and were divided into three cohorts of 17 early *de novo*, 15 early levodopa-treated patients with Parkinson's disease, and 22 age- and gender-matched healthy controls. All participants underwent [^{11}C]IMA107 PET to assess PDE10A expression, [^{11}C]PE2I PET to assess DAT expression, 3-Tesla MRI scan for volumetric measures and to aid PET analyses, and thorough clinical assessments.

Early *de novo* Parkinson's patients showed loss of [^{11}C]IMA107 non-displaceable binding potential (BP_{ND}) in caudate ($P < 0.001$) and putamen ($P < 0.001$), and loss of [^{11}C]PE2I BP_{ND} in caudate ($P < 0.05$) and putamen ($P < 0.001$). [^{11}C]PE2I BP_{ND} was reduced in the striatum contralateral to most compared to less affected side ($P < 0.001$), whereas we found no lateralisation of loss of [^{11}C]IMA107 BP_{ND} . Early levodopa-treated Parkinson's patients had three years longer disease duration and showed additional [^{11}C]IMA107 BP_{ND} loss of 10.7% in the caudate ($P < 0.001$; annual decline 3.6%) and 8.4% in the putamen ($P < 0.001$; annual decline 2.8%); whereas additional loss of [^{11}C]PE2I BP_{ND} was not significant in the caudate and was 20.3% in the putamen ($P < 0.001$; annual decline 6.8%). Loss of [^{11}C]IMA107 BP_{ND} was higher than loss of [^{11}C]PE2I BP_{ND} in the caudate ($P < 0.01$) but lower in the putamen ($P < 0.001$).

Lower [^{11}C]IMA107 BP_{ND} correlated with lower [^{11}C]PE2I BP_{ND} 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 BP_{ND} in the caudate ($\rho = -0.72$; $P < 0.001$) and putamen ($\rho = -0.48$; $P < 0.01$), and with lower [^{11}C]PE2I BP_{ND} only in the putamen ($\rho = -0.65$; $P < 0.001$). Higher burden of motor symptoms correlated with lower [^{11}C]IMA107 BP_{ND} in the caudate ($\rho = -0.42$; $P < 0.05$) and putamen ($\rho = -0.41$; $P < 0.05$), and with lower [^{11}C]PE2I BP_{ND} only in the putamen ($\rho = -0.69$; $P < 0.001$).

Our findings demonstrate that loss of PDE10A expression is an early phenomenon in the course of Parkinson's disease, and PDE10A could be a promising therapeutic target for promoting

neuronal survival and improving motor function. PDE10A and DAT expression are related and show on average a similar strength as markers of disease burden and progression. PDE10A PET is more sensitive for marking pathology in the caudate, whereas DAT PET in the putamen.

Keywords

Parkinson's disease; PET; PDE-10A; [^{11}C]IMA107; [^{11}C]PE2I; early biomarker.

INTRODUCTION

Previous imaging studies, such as with positron emission tomography (PET), have shown that changes in molecular binding profile of selected targets in brain tissue may have promise as markers of disease burden and progression, drug target identification and treatment response in therapeutic trials in patients with Parkinson's disease (Politis, 2014). Quantifying the expression of membrane-spanning proteins in the presynaptic dopaminergic system, such as the dopamine transporter (DAT), fulfil many of the requirements of a useful marker for Parkinson's disease and DAT imaging is currently used extensively in the clinical practice.

Loss of DAT signal in Parkinson's 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 (Pirker, 2003). However, molecular imaging of DAT cannot be considered an ideal biomarker for Parkinson's disease due to several limitations, including a lack of specificity for Parkinson's compared to atypical parkinsonism and of sensitivity compared to subjects with Scan Without Evidence of Dopaminergic Dysfunction (SWEDD). Moreover, although DAT mirrors the progression of the disease in the nigrostriatal pathway, dopaminergic supplementation may modulate DAT levels limiting its use as biomarker for clinical trials testing disease-modification drugs (Schapira, 2013; Fahn et al., 2004). Thus, considering these limitations, several novel targets have been evaluated as imaging markers for Parkinson's disease.

Phosphodiesterase 10A (PDE10A) is a striatal enzyme expressed in the axons of the medium spiny neurons, where it hydrolyses cAMP and cGMP (Fujishige et al., 1999a; Coskran et al., 2006). In the striatal pathways, PDE10A plays a pivotal role in the regulation of dopaminergic signalling (Nishi et al., 2008) and of several other brain functions, ranging from ion conductance to synaptic plasticity (Girault, 2012). Recent work with PET molecular imaging has demonstrated loss of PDE10A expression in moderate to advanced levodopa-treated patients with Parkinson's disease, which was associated with motor symptoms and complications (Niccolini et al., 2015a). Therefore, PDE10A is an enzyme regulating striatal output and dopaminergic signalling that has shown promise 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 and [^{11}C]PE2I in order to assess PDE10A expression, compare it to DAT expression, and explore their association with clinical markers of disease burden in patients with early Parkinson's disease including those who have never been treated.

METHODS

Participants and clinical characteristics

We enrolled 60 participants recruited from specialist Movement Disorders clinics at King's College Hospital and through public advertisement, of which 54 subjects completed the study and were included in the analyses (Table 1). Participants 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*). Patients with Parkinson's disease included 17 participants with a recent diagnosis (duration of symptoms ≤ 24 months) who were naïve to treatment for Parkinson's symptoms (*de novo*), and 15 participants 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*). None of the Parkinson's patients fulfilled the diagnostic criteria for Parkinson's disease mild cognitive impairment (Litvan I, et al. 2011) or dementia (Emre M, et al. 2007) or depression (Marsh L, et al. 2006), 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).

Motor symptom severity was assessed with the MDS-UPDRS part-III (MDS-UPDRS-III) and staged with Hoehn and Yahr (H&Y) scale. MDS-UPDRS-III subscores for rigidity, bradykinesia, tremor and axial symptoms were calculated as previously described (Niccolini et al. 2015a). Quality of life was measured with the PDQ-39. Neuropsychiatric symptoms were assessed with the Beck Depression Inventory second edition (BDI-II) and the Hamilton Depression Rating Scale (HDRS). 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 (NMSS) for Parkinson's disease was used to assess non-motor symptoms.

Sleep disturbances were assessed with the Parkinson's Disease Sleep Scale (PDSS) and the Epworth Sleepiness Scale (ESS).

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 Imanova Ltd, London, UK. All participants were scanned on Siemens Biograph Hi-Rez 6 PET-CT scanner (Erlangen, Germany). A mean dose of 278.4 MBq [^{11}C]IMA107 (SD: ± 33.5) [mean mass injected: 3.7 μg (SD: ± 1.8)] was administered intravenously as a slow bolus injection over 20s. A mean dose of 309.2 MBq [^{11}C]PE2I (SD: ± 36.5) [mean mass injected: 5.1 μg (SD: ± 2.3)] 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 caffeinate beverages for 12 hours (Fredholm et al., 1999).

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 mm³, 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]; 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) (Sudhyadhom et al., 2009) sequences for delineation of regions-of-interest and for co-registration with the PET

images. All MRI sequences used a 1 mm³ 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 (Fujishige et al., 1999a; Coskran et al., 2006), degeneration of these nuclei may affect the expression of the enzyme. Thus, we investigated volumetric changes in subcortical nuclei in our cohort of Parkinson's disease patients. We used the FreeSurfer's image analysis suite (version 5.3.0 <http://surfer.nmr.mgh.harvard.edu>) to process individual MRI scans for deriving measures of cortical and subcortical volumes, as described before (Niccolini, et al. 2015a).

[¹¹C]IMA107 and [¹¹C]PE2I PET data analysis

The Molecular Imaging and Kinetic Analysis Toolbox software package (MIAKATTM: www.miakat.org) (Searle et al., 2015), implemented in MATLAB® (The Mathworks, Natick, MA, USA) was used to carry out image processing and kinetic modelling. MIAKATTM 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. The MIAKATTM processing pipeline was followed, ensuring that all quality control steps were completed to generate parametric images and regional estimates of [¹¹C]IMA107 and [¹¹C]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 (Mansur et al., 2016). 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 MPAGE MRI.

Region of interest-based analysis

To facilitate anatomical delineation of regions of interest, PET images were anatomically co-registered and resliced to the corresponding volumetric structural T1-weighted MRI images in Statistical Parametric Mapping version 12 (SPM12) software package implemented in Matlab 8.2. Regions of interest were delineated manually on the co-registered MRIs 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 a reliable, robust and repeatable technique for manual delineation of basal ganglia structures (Tziortzi et al., 2011). Regions of interest included caudate, putamen, ventral striatum, globus pallidus (external and internal segment), substantia nigra and motor thalamic nuclei. These brain regions express PDE10A to a varying degree (Seeger et al., 2003; Coskran et al., 2006).

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 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 (Prodoehl et al., 2008). This masking drastically 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 (Friston et al., 1995). 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 8.2

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 as our PET and clinical data were normally distributed. 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 comparison test. We interrogated correlations between PET and clinical data using Spearman rho and we applied the Benjamini-Hochberg correction to reduce false discovery rate (Benjamini and Hochberg, 1995). We set the false discovery rate cut-off at 0.05. All data are presented as mean $\pm$ standard deviation, and the level α was set for all comparisons at $P < 0.05$, corrected.

RESULTS

Volumetric analysis

Freesurfer analysis showed no volumetric differences in the regions-of-interest used for the PET analyses between the groups of early *de novo* and early levodopa-treated Parkinson's patients and healthy controls (Table 2).

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

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

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

Early *de novo* patients with Parkinson's disease had lower mean [¹¹C]PE2I BP_{ND} values in total striatum ($P < 0.001$), caudate ($P < 0.05$), putamen ($P < 0.001$), ventral striatum ($P < 0.05$), globus pallidus internal ($P < 0.001$), globus pallidus external ($P < 0.001$) and substantia nigra ($P < 0.001$) compared to healthy controls (Figures 2A and 2B). There were no differences in mean [¹¹C]PE2I BP_{ND} values between early *de novo* Parkinson's patients and healthy controls in motor thalamic nuclei ($P > 0.10$).

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

In the group of early *de novo* patients with Parkinson's disease with unilateral motor symptomatology, we assessed whether the clinically affected side of the body was associated with greater decreases in contralateral brain regions-of-interest [¹¹C]IMA107 BP_{ND} and [¹¹C]PE2I BP_{ND} values. We found no differences in [¹¹C]IMA107 BP_{ND} values in any of the regions-of-interest between the most and less affected sides; whereas [¹¹C]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 side of the body compared to the less affected side (Table 3 and Figure 2C).

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

Early levodopa-treated patients with Parkinson's disease had three years longer disease duration compared to early *de novo* Parkinson's patients, and showed additional [¹¹C]IMA107 BP_{ND} loss of 10.7% in the caudate ($P < 0.001$; reflecting a 3.6% mean annual decline) and 8.4% in the putamen ($P < 0.001$; reflecting a 2.8% mean annual decline) (Figure 1A and 1B). Early levodopa-treated Parkinson's patients showed additional [¹¹C]PE2I BP_{ND} loss of 20.3% in the putamen ($P < 0.001$; reflecting a 6.8% mean annual decline), whereas changes were not significant in caudate, compared to early *de novo* Parkinson's patients (Figure 2A and 2B).

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

Voxel-by-voxel analysis of [¹¹C]IMA107 and [¹¹C]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 [¹¹C]IMA107 BP_{ND} decreases in the right and left caudate ($P < 0.001$) and right and left putamen ($P < 0.001$), and clusters of [¹¹C]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 patients compared with the group of healthy controls. SPM analysis within the striatal mask localized clusters of [¹¹C]IMA107 BP_{ND} decreases in right and left caudate ($P < 0.05$) and right and left putamen ($P < 0.05$), and clusters of [¹¹C]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 (Table 4).

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

We compared loss of [¹¹C]IMA107 BP_{ND} with loss of [¹¹C]PE2I BP_{ND} in each region-of-interest in Parkinson's patients relative to normality data from the group of healthy controls. We found that loss of [¹¹C]IMA107 BP_{ND} was greater than loss of [¹¹C]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 difference in ventral striatum ($P > 0.10$) and motor thalamic nuclei ($P > 0.10$) in early *de novo* Parkinson's patients. Similarly, we found that loss of [¹¹C]IMA107 BP_{ND} was greater than loss of [¹¹C]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 difference in ventral striatum ($P > 0.10$) and motor thalamic nuclei ($P > 0.10$) in early levodopa-treated Parkinson's patients.

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

Lower individual [¹¹C]IMA107BP_{ND} values correlated with lower individual [¹¹C]PE2IBP_{ND} values in the caudate ($r=0.51$; $P<0.01$) and putamen ($r=0.53$; $P<0.01$), but not in ventral striatum ($r=0.013$; $P>0.10$), globus pallidus internal ($r=0.21$; $P>0.10$), globus pallidus external ($r=0.46$; $P>0.05$) substantia nigra ($r=0.15$; $P>0.10$) or motor thalamic nuclei ($r=0.15$; $P>0.10$) in Parkinson's patients (Figure 2D).

Correlations between PET data and measures of Parkinson's burden

Parkinson's patients had increased MDS-UPDRS-III ($P<0.001$), NMSS ($P<0.05$), BDI-II ($P<0.05$), and HDRS ($P<0.05$) scores, and worse PDQ-39 ($P<0.05$) and PDSS ($P<0.05$) scores compared to healthy controls (Table 1). As the cohorts were carefully matched for age, we found no effect of age in [¹¹C]IMA107 and [¹¹C]PE2I BP_{ND} in all regions-of-interest across all cohorts ($P>0.10$).

Longer Parkinson's disease duration correlated with lower [¹¹C]IMA107BP_{ND} in the caudate ($r=-0.72$; $P<0.001$) and putamen ($r=-0.48$; $P<0.01$). Higher MDS-UPDRS-III total scores correlated with lower [¹¹C]IMA107BP_{ND} in the caudate ($r=-0.42$; $P<0.05$) and putamen ($r=-0.41$; $P<0.05$). Higher MDS-UPDRS-III rigidity scores correlated with lower [¹¹C]IMA107BP_{ND} in the caudate ($r=-0.52$; $P<0.01$) and putamen ($r=-0.43$; $P<0.05$). Higher MDS-UPDRS-III bradykinesia scores correlated with lower [¹¹C]IMA107 BP_{ND} in the caudate ($r=-0.45$; $P<0.01$) and putamen ($r=-0.47$; $P<0.01$) (Figure 3A).

Longer Parkinson's disease duration correlated with lower [¹¹C]PE2IBP_{ND} only in the putamen ($r=-0.65$; $P<0.001$). Higher MDS-UPDRS-III total scores correlated with lower [¹¹C]PE2IBP_{ND} only in the putamen ($r=-0.69$; $P<0.001$). Higher MDS-UPDRS-III rigidity scores correlated with lower [¹¹C]PE2IBP_{ND} only in the putamen ($r=-0.62$; $P<0.001$). Higher MDS-UPDRS-III bradykinesia scores correlated with lower [¹¹C]PE2IBP_{ND} only in the putamen ($r=-0.49$; $P<0.01$) (Figure 3B).

DISCUSSION

Our findings demonstrate that loss of striatal PDE10A expression 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 expression in the striatum in rodent models of Parkinson's disease (Giorgi et al., 2008, 2011).

We used PET molecular imaging to quantify PDE10A and DAT expression in the same cohorts of Parkinson's patients and healthy controls. In both early *de novo* and early levodopa-treated patients with Parkinson's disease both PDE10A and DAT expression were decreased in the striatum, and DAT also decreased in globus pallidus and substantia nigra. Our early *de novo* cohort of Parkinson's patients had less than 2 year of disease duration and the cohort of early levodopa-treated less than 5 years of Parkinson's symptoms and had been treated with levodopa for less than 2 years. Previously we have reported loss of striatal PDE10A expression and correlations with the burden of motor symptoms and complications in middle-stage Parkinson's patients with 7 years of disease duration and advanced Parkinson's patients with 13 years of disease duration. Collectively, these data suggest that loss of striatal PDE10A expression appears early and follows the accumulation of motor symptom burden through the course of Parkinson's disease. Loss of PDE10A expression in globus pallidus is a phenomenon appearing later in the disease (Niccolini et al., 2015a). Our findings were not affected by volumetric changes in the brain, age or gender effect and were confirmed at a voxel level.

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 PKA/DARPP-32 downstream cascade in striatal pathways (Greengard et al., 1999; Nishi et al., 2008; Girault, 2012). In striato-nigral pathway, dopamine loss leads to a reduced synthesis of D1 receptor-stimulated cAMP (Herve et al., 2001), and reduced PDE10A expression is expected to increase cAMP levels to compensate for the reduced cAMP signalling. In striato-pallidal pathway, dopamine loss decreases the inhibitory effect of D2 receptors on cAMP synthesis (Stoof and Kebabian, 1981), and reduced PDE10A expression may further increase cAMP levels by enhancing the negative consequences of dopamine loss on D2 receptor signalling and potentiate adenosine A2A receptor signalling. Therefore, early loss of PDE10A expression in the striatum may lead to an imbalance between

striato-nigral and striato-pallidal output and in turn affect thalamocortical activation, decrease motor performance and contribute to the development of Parkinson's diseasesymptoms.

We attempted to understand how informative PET molecular imaging of PDE10A and DAT could be for monitoring the progression of the disease. Our levodopa-treated patients with Parkinson's disease had three years longer disease duration compared to early *de novo* Parkinson's patients. PDE10A expression showed further decline in both caudate and putamen, whereas DAT expression was further reduced only in the putamen in early levodopa-treated compared to early *de novo* Parkinson's patients. Specifically, the loss of PDE10A in the caudate reflected a 3.6% and in putamen a 2.8% mean annual decline, whereas loss of DAT in the putamen reflected a 6.8% mean annual decline. Identification of molecular biomarkers characterising the pathological processes in Parkinson's disease could help monitor disease burden and progression, drug target identification and treatment response in therapeutic trials. So far, markers of presynaptic dopaminergic system such as quantification of striatal DAT have been extensively used for aiding diagnostics between neurodegenerative and non-neurodegenerative forms of parkinsonism and as correlates with motor symptoms. Our findings demonstrate that quantification of PDE10A has potential as a molecular imaging biomarker for Parkinson's disease motor symptom burden and progression.

We performed head-to-head comparisons between loss of PDE10A and DAT in Parkinson's 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 putamen, globus pallidus and substantia nigra in both early *de novo* and early levodopa-treated patients with Parkinson's disease. Loss of PDE10A in both caudate and putamen and loss of DAT only in 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.

Considering the underlying pathophysiology of Parkinson's 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. 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. Loss of striatal DAT signal is currently considered the gold

standard in the differential diagnosis between degenerative vs. non-degenerative parkinsonism (i.e. essential tremor, dystonic tremor or functional parkinsonism). However, this approach has also shown several shortcomings. DAT signal does not reliably distinguish Parkinson's disease from the other neurodegenerative parkinsonian syndromes, such as multiple system atrophy, progressive supranuclear palsy etc. DAT may be abnormal in asymptomatic subjects, that however are at higher risk of Parkinson's disease development, but, on the other hand, may be also normal in subjects with clinical diagnosis of Parkinson's disease, a population called SWEDD. Although there is a good correlation between DAT levels and motor symptoms, it is not the ideal biomarker of Parkinson's disease progression because its levels have a floor effect that limit its use to the initial stages of the disease. Moreover, DAT levels may be modulated by dopaminergic supplementation, with levodopa causing a decline proportional to the dose used (Fahn, et al., 2004). Clinical trials that used DAT as a marker of Parkinson's disease pathology showed controversial results, with imaging appeared to contradict the clinical results. In CALM-PD (Parkinson Study Group CALM Cohort Investigators, 2009) and REAL-PET (Whone, et al., 2003), subjects randomized to dopamine agonists showed worse clinical symptoms but lower DAT decline than subjects randomized to levodopa. In ELLDOPA trial (Fahn, et al., 2004), subjects randomized to levodopa showed better clinical symptoms but greater DAT decline than subjects on placebo. However, it is not clear whether dopaminergic treatment hastened disease progression or, rather, the results of the study simply reflected downregulation of DAT due to the use of levodopa.

PDE10A seems to be a more stable biomarker for diagnosis and for evaluating disease burden and progression. However, studies comparing PDE10A levels in Parkinson's versus other parkinsonism are needed to confirm its potential role in the differential diagnosis of Parkinson's in the clinical setting. We found a progressive decline of PDE10A in Parkinson's from the early to the advanced stages. This was independent of levodopa treatment (Niccolini, et al., 2015a) and had no floor effect, suggesting that PDE10A might be better than DAT as a potential target to monitor treatment response in clinical trials aiming at slowing the progression of Parkinson's. PDE10A and DAT were correlated in our population but PDE10A PET was better to detect pathology in the caudate whereas DAT scan was better in the putamen. On the other hand, it is important to underline that DAT levels can be measured with SPECT imaging while only PET ligands are currently available for PDE10A. Despite all these potential advantages of PDE10A, 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, in which other mechanisms, such as serotonergic deficits, seem to have a prominent role (Politis et al., 2010; Politis et al., 2011; Loane et al., 2013; Pagano et al. 2017).

In the group of early *de novo* Parkinson's patients who had unilateral motor symptomatology, we assessed whether the clinically affected side of the body was associated with greater PDE10A and DAT decreases in contralateral brain regions of interest. We found no difference in PDE10A expression in the brain between the more affected and the less affected side, whereas loss of DAT expression was higher in brain regions corresponding to the more affected side. This observation could be related to the fact that loss of striatal PDE10A expression is an earlier phenomenon compared to the loss of striatal DAT expression and an underlying mechanism associated with the reduction of dopamine release, even before motor onset. These molecular changes in affected neurons might happen when dopamine neurons are suffering and are releasing less dopamine, thus before cell death and presynaptic dopamine neuronal loss become irreversible. However, this hypothesis can be confirmed only testing PDE10A expression in prodromal Parkinson's patients, such as asymptomatic mutation carriers of Parkinson's gene (i.e. SNCA, Parkin, LRRK2 etc.). This can clarify whether the loss of PDE10A precedes clinical onset and may be considered as a pre-diagnostic biomarker, which is currently unavailable. On the other hand, normal DAT binding has been shown in asymptomatic carriers of SNCA (Krüger et al., 2001; Ahn et al., 2008) and of LRRK2 mutated genes (Khan et al., 2005a; Nandhagopal et al., 2008; Wider et al., 2008). In contrast, asymptomatic parkin mutation carriers showed reduced DAT signal in the caudate and putamen (Pellecchia et al., 2007; Khan et al., 2005b). Interestingly, loss of nigrostriatal dysfunction in parkin-linked parkinsonism occurs at a very slow rate compared to the annual loss of putamen reported for idiopathic PD (Pavese *et al.*, 2009). The gene encoding PDE10A has been identified on chromosome 6 (locus 6q26-27) (Fujishige et al., 1999b) and is nearby the gene encoding for parkin identified on the same chromosome (locus 6q25.2-q27) (Matsumine H, et al., 1997). Both PDE10A signalling and parkin mutated protein are expressed in striatum and are involved in striatal neuronal function, thus making the possibility of genetic linkage between these two intriguing. Increased parkin might be associated with higher expression of PDE10A and justify why in parkin-linked parkinsonism the disease progresses at a slower pace (Pavese et al., 2009). Further studies evaluating PDE10A by using [¹¹C]IMA107 PET molecular imaging in asymptomatic and symptomatic parkin mutation carriers are needed to shed a light on the

interaction between these two proteins involved in Parkinson's and expressed on the same chromosome.

PDE10A could be a promising therapeutic target for promoting neuronal survival and improving motor function. Recently, the modulation of PDE10A expression has shown promising results. A single oral dose of PF-02545920 increased striatal PDE10A of 14-27% at 10 mg dose and of 45-63% at 20 mg dose in healthy controls (Delnomdedieu M, et al., 2017). PF-02545920 is a highly selective PDE10A inhibitor developed for the symptomatic treatment of Huntington disease, an autosomal dominant neuropsychiatric disease that targets the cortico-striatal circuitry and is associated with loss of striatal PDE10A expression (Ahmad et al., 2014; Niccolini et al., 2015b; Wilson et al., 2016; Russell et al., 2016). However, the Amaryllis trial, a Phase 2 randomized, placebo controlled trial including 271 Huntington's disease patients in five countries testing the efficacy and the safety of PF-02545920 in subjects with Huntington's Disease, failed to show significant improvement in motor or cognitive function. PF-02545920 was indeed safe thus it might be worth testing this drug in Parkinson's disease patients. Neurodegenerative process in Parkinson's differs from Huntington's disease and boosting PDE10A expression at the earliest stage possible might reduce motor symptoms and neuronal survival in Parkinson's disease.

Two randomized controlled trials are currently investigating the effect of PF-06649751, another modulator of PDE10A, in early stages (NCT02847650) and in patients with motor fluctuations (NCT02687542).

In conclusion, our findings demonstrate that loss of PDE10A expression is an early phenomenon in the course of Parkinson's disease, and PDE10A could be a promising therapeutic target for promoting neuronal survival and improving motor function. PDE10A and DAT expression are related and show on average a similar strength as markers of disease burden and progression. PDE10A PET is more sensitive for marking pathology in the caudate, whereas DAT PET in the putamen. Similar to what was observed in our cohort of Parkinson's disease patients, loss of striatal PDE10A expression correlated with disease burden in manifest (Russell et al., 2014) and pre-manifest (Niccolini et al., 2015b) Huntington's disease gene carriers. Collectively, these observations suggest an extended role for the importance of PDE10A expression in the clinical presentation of movement disorders suggesting that PDE10A modulating drugs could potentially have a therapeutic role in slowing down the neurodegenerative processes and restore the control of movement.

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AUTHORSHIP

M.P. conceived the study, conceptualized the experimental design and acquired funding for the study. G.P. 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., 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.

REFERENCES

1. Politis M. Neuroimaging in Parkinson disease: from research setting to clinical practice. *Nat Rev Neurol*. 2014;10(12):708-22.
2. Pirker W. Correlation of dopamine transporter imaging with parkinsonian motor handicap: how close is it? *Mov Disord*. 2003;18 Suppl 7:S43-51.
3. Schapira AH. Recent developments in biomarkers in Parkinson disease. *Curr Opin Neurol*. 2013;26(4):395-400.
4. Fahn S, Oakes D, Shoulson I, Kieburtz K, Rudolph A, Lang A, Olanow CW, Tanner C, Marek K; Parkinson Study Group.. Levodopa and the progression of Parkinson's disease. *N Engl J Med*. 2004;351(24):2498-508.
5. Fujishige K, Kotera J, Omori K. Striatum- and testis-specific phosphodiesterase PDE10A isolation and characterization of a rat PDE10A. *Eur J Biochem* 1999a; 266: 1118–27.
6. Coskran TM, Morton D, Menniti FS, Adamowicz WO, Kleiman RJ, Ryan AM, et al. Immunohistochemical localization of phosphodiesterase 10A in multiple mammalian species. *J Histochem Cytochem* 2006; 54: 1205–13.
7. Nishi A, Kuroiwa M, Miller DB, O'Callaghan JP, Bateup HS, Shuto T, et al. Distinct roles of PDE4 and PDE10A in the regulation of cAMP/PKA signaling in the striatum. *J Neurosci* 2008; 28: 10460–71.
8. Girault JA. Integrating neurotransmission in striatal medium spiny neurons. *Adv Exp Med Biol* 2012; 970: 407–29.
9. Niccolini F, Foltynie T, Reis Marques T, et al. Loss of phosphodiesterase 10A expression is associated with progression and severity in Parkinson's disease. *Brain*. 2015a;138:3003-15.
10. Litvan I, Aarsland D, Adler CH, et al. MDS Task Force on mild cognitive impairment in Parkinson's disease: critical review of PD-MCI. *Mov Disord*. 2011;26:1814-24.
11. Emre M, Aarsland D, Brown R, et al. Clinical diagnostic criteria for dementia associated with Parkinson's disease. *Mov Disord*. 2007;22:1689-707.
12. Marsh L, McDonald WM, Cummings J, Ravina B; NINDS/NIMH Work Group on Depression and Parkinson's Disease.. Provisional diagnostic criteria for depression in Parkinson's disease: report of an NINDS/NIMH Work Group. *Mov Disord*. 2006;21(2):148-58.

13. Fredholm BB, Bättig K, Holmén J, et al. Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. *Pharmacol Rev* 1999; 51:83-133.
14. Sudhyadhom A, Haq IU, Foote KD, Okun MS, Bova FJ. A high resolution and high contrast MRI for differentiation of subcortical structures for DBS targeting: The Fast Gray Matter Acquisition T1 Inversion Recovery (FGATIR), *Neuroimage* 2009; 47: T44–52.
15. Searle G, Coello C, Gunn R. Realising the binding potential directly and indirectly. *Journal of Cerebral Blood Flow and Metabolism Brain PET supplement* 64 *Brain* 2015;0791:19-20.
16. Mansur A, Comley R, Boetsch C, Sanwald-Ducray P, Gunn RN, Rabiner EA, Searle G. Validation of reference tissue quantification of [11C]IMA107 for PDE10A enzyme availability in the human brain. *NRM* 2016, Abstract 40.
17. Tziortzi AC, Searle GE, Tzimopoulou S, Salinas C, Beaver JD, Jenkinson M, et al. Imaging dopamine receptors in humans with [11C]-(+)-PHNO: dissection of D3 signal and anatomy. *Neuroimage* 2011; 54: 264–77.
18. Seeger TF, Bartlett B, Coskran TM, Culp JS, James LC, Krull DL, et al. Immunohistochemical localization of PDE10A in the rat brain. *Brain Res* 2003; 985: 113–126.
19. Prodoehl J, Yu H, Little DM, Abraham I, Vaillancourt DE. Region of interest template for the human basal ganglia: comparing EPI and standardized space approaches. *Neuroimage* 2008; 39: 956–65.
20. Friston KJ, Holmes AP, Worsley KJ, Poline JB, Frith CD, Frackowiak RS. Statistical parametric maps in functional imaging: a general linear approach. *Hum Brain Mapp* 1995; 2: 189–210.
21. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc Series B Stat Methodol* 1995; 57: 289–300.
22. Giorgi M, D’Angelo V, Esposito Z, Nuccetelli V, Sorge R, Martorana A, et al. Lowered cAMP and cGMP signalling in the brain during levodopa-induced dyskinesias in hemiparkinsonian rats: new aspects in the pathogenetic mechanisms. *Eur J Neurosci* 2008; 28: 941–50.
23. Giorgi M, Melchiorri G, Nuccetelli V, D’Angelo V, Martorana A, Sorge R, et al. PDE10A and PDE10A-dependent cAMP catabolism are dysregulated oppositely in

- striatum and nucleus accumbens after lesion of midbrain dopamine neurons in rat: a key step in parkinsonism physiopathology. *Neurobiol Dis* 2011; 43: 293–303.
24. Greengard P, Allen PB, Nairn AC. Beyond the dopamine receptor: the DARPP-32/protein phosphatase-1 cascade. *Neuron*. 1999 Jul;23(3):435-47.
 25. Hervé D, Le Moine C, Corvol JC, Belluscio L, Ledent C, Fienberg AA, Jaber M, Studler JM, Girault JA. Galpha(olf) levels are regulated by receptor usage and control dopamine and adenosine action in the striatum. *J Neurosci*. 2001;21(12):4390-9.
 26. Stoof JC, Kebabian JW. Opposing roles for D-1 and D-2 dopamine receptors in efflux of cyclic AMP from rat neostriatum. *Nature*. 1981;294(5839):366-8.
 27. Pagano G, Niccolini F, Fusar-Poli P, Politis M. Serotonin transporter in Parkinson's disease: A meta-analysis of positron emission tomography studies. *Ann Neurol*. 2017;81(2):171-180.
 28. Politis M, Wu K, Loane C, Turkheimer FE, Molloy S, Brooks DJ, Piccini P. Depressive symptoms in PD correlate with higher 5-HTT binding in raphe and limbic structures. *Neurology*. 2010;75(21):1920-7.
 29. Politis M, Loane C, Wu K, Brooks DJ, Piccini P. Serotonergic mediated body mass index changes in Parkinson's disease. *Neurobiol Dis*. 2011;43(3):609-15.
 30. Loane C, Wu K, Bain P, Brooks DJ, Piccini P, Politis M. Serotonergic loss in motor circuitries correlates with severity of action-postural tremor in PD. *Neurology*. 2013;80(20):1850-5.
 31. Kruger R, Kuhn W, Leenders KL, Sprengelmeyer R, Müller T, Woitalla D, Portman AT, Maguire RP, Veenma L, Schröder U, Schöls L, Epplen JT, Riess O, Przuntek H. Familial parkinsonism with synuclein pathology: clinical and PET studies of A30P mutation carriers. *Neurology*. 2001;56(10):1355-1362.
 32. Ahn TB, Kim SY, Kim JY, et al. alpha-Synuclein gene duplication is present in sporadic Parkinson disease. *Neurology*. 2008;70(1):43-49.
 33. Khan NL, Jain S, Lynch JM, et al. Mutations in the gene LRRK2 encoding dardarin (PARK8) cause familial Parkinson's disease: clinical, pathological, olfactory and functional imaging and genetic data. *Brain*. 2005a;128(Pt 12):2786-2796.
 34. Nandhagopal R, Mak E, Schulzer M, et al. Progression of dopaminergic dysfunction in a LRRK2 kindred: a multitracer PET study. *Neurology*. 2008;71(22):1790-1795.
 35. Wider C, Skipper L, Solida A, et al. Autosomal dominant dopa-responsive parkinsonism in a multigenerational Swiss family. *Parkinsonism & related disorders*. 2008;14(6):465-470.

36. Pellecchia MT, Varrone A, Annesi G, Amboni M, Cicarelli G, Sansone V, Annesi F, Rocca FE, Vitale C, Pappatà S, Quattrone A, Barone P. Parkinsonism and essential tremor in a family with pseudo-dominant inheritance of PARK2: an FP-CITSPECT study. *Mov Disord.* 2007;22(4):559-63.
37. Khan NL, Scherfler C, Graham E, et al. Dopaminergic dysfunction in unrelated, asymptomatic carriers of a single parkin mutation. *Neurology.* Jan 11 2005b;64(1):134-136.
38. Pavese N, Khan NL, Scherfler C, et al. Nigrostriatal dysfunction in homozygous and heterozygous parkin gene carriers: an 18F-dopa PET progression study. *Movement disorders.* 2009;24(15):2260-2266.
39. Fujishige K, Kotera J, Michibata H, Yuasa K, Takebayashi S, Okumura K, Omori K. Cloning and characterization of a novel human phosphodiesterase that hydrolyzes both cAMP and cGMP (PDE10A). *J Biol Chem.* 1999b;274(26):18438-45.
40. Matsumine H, Saito M, Shimoda-Matsubayashi S, Tanaka H, Ishikawa A, et al. Localization of a gene for an autosomal recessive form of juvenile Parkinsonism to chromosome 6q25.2-27. *Am J Hum Genet.* 1997;60(3):588-96.
41. Delnomdedieu M, Forsberg A, Ogden A, Fazio P, Yu CR, Stenkrona P, et al. In vivo measurement of PDE10A enzyme occupancy by positron emission tomography (PET) following single oral dose administration of PF-02545920 in healthy male subjects. *Neuropharmacology.* 2017;117:171-181.
42. Ahmad R, Bourgeois S, Postnov A, Schmidt ME, Bormans G, Van Laere K, Vandenberghe W. PET imaging shows loss of striatal PDE10A in patients with Huntington disease. *Neurology.* 2014;82(3):279-81.
43. Niccolini F, Haider S, Reis Marques T, Muhlert N, Tziortzi AC, Searle GE, et al. Altered PDE10A expression detectable early before symptomatic onset in Huntington's disease. *Brain.* 2015b;138(Pt 10):3016-29.
44. Wilson H, Niccolini F, Haider S, Marques TR, Pagano G, Coello C, et al. Loss of extra-striatal phosphodiesterase 10A expression in early premanifest Huntington's disease gene carriers. *J Neurol Sci.* 2016;368:243-8.
45. Russell DS, Jennings DL, Barret O, Tamagnan GD, Carroll VM, Caillé F, et al. Change in PDE10 across early Huntington disease assessed by [18F]MNI-659 and PET imaging. *Neurology.* 2016;86(8):748-54.
46. Parkinson Study Group CALM Cohort Investigators. Long-term effect of initiating pramipexole vs levodopa in early Parkinson disease. *Arch Neurol.* 2009;66(5):563-70.

47. Whone AL, Watts RL, Stoessl AJ, Davis M, Reske S, Nahmias C, Lang AE, Rascol O, Ribeiro MJ, Remy P, Poewe WH, Hauser RA, Brooks DJ; REAL-PET Study Group. Slower progression of Parkinson's disease with ropinirole versus levodopa: TheREAL-PET study. *Ann Neurol*. 2003;54(1):93-101.

FIGURE LEGENDS

Figure 1 (A).Altered PDE10A expression in anatomically defined brain regions of Parkinson's disease (PD) 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 PD early *de novo* and (bottom) PD early levodopa-treated patients in stereotaxic space showing progressive loss of [^{11}C]IMA107 BP_{ND} in PD. Colour bar reflects range of PET BP_{ND} intensity. **(B) PDE10A expression in the groups of Parkinson's disease (PD) patients and healthy controls.** Column bar graphs showing mean [^{11}C]IMA107 BP_{ND} in subcortical brain regions in PD early *de novo* patients, PD 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 (PD) 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 PD early *de novo* and (bottom) PD early levodopa-treated patients in stereotaxic space showing progressive loss of [^{11}C]PE2I BP_{ND} in PD. Colour bar reflects range of PET BP_{ND} intensity. **(B) DAT expression in the groups of Parkinson's disease (PD) patients and healthy controls.** Column bar graphs showing mean [^{11}C]PE2I BP_{ND} in subcortical brain regions in PD early *de novo* patients, PD 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 (PD) 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 PD patients in stereotaxic space showing significant loss of [^{11}C]PE2I BP_{ND} in the PD 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 ($\rho = 0.527$; $P = 0.002$). PD early *de novo* in black circles and PD early levodopa-treated in grey circles.

Figure 3. Correlations between PDE10A and DAT in relation to motor symptoms in Parkinson's disease (PD) 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). PD early *de novo* in black circles and PD early levodopa-treated in grey circles.

TABLES

Table 1. Clinical characteristics of early *de novo* Parkinson's patients, levodopa-treated Parkinson's patients and healthy controls

	Healthy controls (n = 22)	PD early <i>de novo</i> (n = 17)	PD early levodopa-treated (n = 15)
Age (years $\pm$ SD)	55.68 ($\pm$ 14.9)	59.0 ($\pm$ 9.0)	61.0 ($\pm$ 5.5)
Gender	16M / 6F	11M/6F	8M/ 7F
Disease Duration (months $\pm$ SD)	-	12.1 ($\pm$ 6.9)	48.2 ($\pm$ 11.7) [^]
H&Y (mean $\pm$ SD)	-	1.1 ($\pm$ 0.3)	2.2 ($\pm$ 1.1) [^]
MDS-UPDRS-III (mean $\pm$ SD)	0 ($\pm$ 0.0)	23.3 ($\pm$ 9.7)	40.2 ($\pm$ 18.1) [^]
NMSS (mean $\pm$ SD)	6.7 ($\pm$ 9.1)	24.8 ($\pm$ 26.5)*	57.5 ($\pm$ 34.6)* [^]
PDQ-39 (mean $\pm$ SD)	0.2 ($\pm$ 0.6)	20.1 ($\pm$ 22.5)*	27.0 ($\pm$ 10.5)*
BDI-II (mean $\pm$ SD)	1.4 ($\pm$ 2.1)	6.5 ($\pm$ 8.9)	5.4 ($\pm$ 3.7)*
HDRS (mean $\pm$ SD)	1.3 ($\pm$ 1.4)	3.2 ($\pm$ 4.8)	8.0 ($\pm$ 5.2)*
MMSE (mean $\pm$ SD)	29.6 ($\pm$ 1.0)	29.7 ($\pm$ 0.7)	29.5 ($\pm$ 1.0)
MoCA (mean $\pm$ SD)	27.9 ($\pm$ 1.4)	28.4 ($\pm$ 2.0)	27.5 ($\pm$ 1.5)
PDSS(mean $\pm$ SD)	130.7 ($\pm$ 19.0)	117.8 ($\pm$ 16.8)	102.3 ($\pm$ 16.9)* [^]
ESS (mean $\pm$ SD)	4.7 ($\pm$ 3.1)	5.6 ($\pm$ 5.2)	8.2 ($\pm$ 4.8)

H&Y = Hoehn and Yahr; MDS-UPDRS III = Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale Part III - Motor; NMSS: Non-motor Symptoms Scale; PDQ-39 = 39-item Parkinson's Disease Questionnaire; BDI-II: Beck Depression Inventory-II; HDRS: Hamilton Depression Rating Scale; MMSE: Mini Mental Status Examination; MoCA: Montreal Cognitive Assessment; PDSS: Parkinson's Disease Sleep Scale; ESS: Epworth Sleepiness Scale. *P*value vs. healthy controls Bonferroni post-hoc corrected. **P* < 0.05 vs. healthy controls, [^]*P* < 0.05 vs. early *de novo* PD patients.

Table 2. FreeSurfer cortical and subcortical volumetric analyses in the groups early *de novo* Parkinson's patients, levodopa-treated Parkinson's patients and healthy controls

ROIs	Healthy Controls	PD <i>de novo</i>	PD levodopa-treated	<i>P</i> value
Caudate (mean $\pm$ SD)	2.42 ($\pm$ 0.22)	2.39 ($\pm$ 0.23)	2.32 ($\pm$ 0.31)	>0.10
Putamen (mean $\pm$ SD)	3.50 ($\pm$ 0.32)	3.34 ($\pm$ 0.28)	3.20 ($\pm$ 0.31)	>0.10
Ventral Striatum (mean $\pm$ SD)	0.34 ($\pm$ 0.07)	0.32 ($\pm$ 0.05)	0.28 ($\pm$ 0.07)	>0.10
Globus pallidus (mean $\pm$ SD)	0.94 ($\pm$ 0.13)	0.97 ($\pm$ 0.13)	0.99 ($\pm$ 0.12)	>0.10
Thalamus (mean $\pm$ SD)	4.86 ($\pm$ 0.65)	4.68 ($\pm$ 0.44)	4.22 ($\pm$ 0.39)	>0.10

¹Subcortical nuclei volumes have been corrected for total intracranial volume.

Table 3. Lateralization analysis for subcortical nuclei mean [^{11}C]IMA107BP_{ND} and [^{11}C]PE2IBP_{ND} values in the clinically most affected contralateral and homolateral side of early *de novo* patients with Parkinson's disease =

	Contralateral^a	Homolateral^a	Pvalue^b
[^{11}C]IMA107 BP_{ND}			
Caudate(mean±SD)	0.93±0.13	0.88±0.14	>0.10
Putamen (mean±SD)	1.91±0.15	1.91±0.14	>0.10
Ventral Striatum (mean±SD)	1.52±0.28	1.45±0.29	>0.10
Globus Pallidus Internal (mean±SD)	1.56±0.20	1.5±0.19	>0.10
Globus Pallidus External(mean±SD)	1.90±0.24	1.85±0.19	>0.10
Substantia Nigra (mean±SD)	0.62±0.06	0.62±0.08	>0.10
Motor Thalamic Nuclei (mean±SD)	0.56±0.09	0.56±0.10	>0.10
[^{11}C]PE2I BP_{ND}			
Caudate(mean±SD)	1.70±0.41	2.02±0.38	<0.001
Putamen (mean±SD)	1.41±0.51	2.08±0.46	<0.001
Ventral Striatum (mean±SD)	1.84±0.45	2.21±0.46	<0.001
Globus Pallidus Internal (mean±SD)	0.74±0.20	0.96±0.29	<0.001
Globus Pallidus External(mean±SD)	1.09±0.28	1.45±0.38	<0.001
Substantia Nigra (mean±SD)	0.56±0.10	0.64±0.09	<0.001
Motor Thalamic Nuclei (mean±SD)	0.49±0.06	0.47±0.05	>0.10

^aContralateral and homolateral to the most clinical affected side; ^bP values are Bonferroni corrected for multiple comparisons.

Table 4. Voxel-based analysis: bilateral striatal significant decreases in [^{11}C]IMA107 and [^{11}C]PE2IBP_{ND} in early *de novo* Parkinson's patients compared with the group of healthy controls. Voxel-based analysis: bilateral striatal significant decreases in [^{11}C]IMA107 and [^{11}C]PE2IBP_{ND} in early levodopa-treated patients compared with the group of early *de novo* Parkinson's patients.

Early <i>de novo</i> Parkinson's patients vs Healthy controls						
MNI coordinates			AreaBP _{ND}	Cluster sizes	Z-score	<i>P</i> value
x	y	z				
-12	8	14	Left Caudate[¹¹ C]IMA107 BP _{ND}	96	2.23	<0.001
16	10	18	Right Caudate[¹¹ C]IMA107 BP _{ND}	112	2.63	<0.001
-24	0	12	Left Putamen[¹¹ C]IMA107 BP _{ND}	208	2.88	<0.001
24	2	8	Right Putamen[¹¹ C]IMA107 BP _{ND}	172	3.01	<0.001
-28	-8	12	Left Putamen[¹¹ C]PE2I BP _{ND}	172	6.27	<0.001
28	-8	12	Right Putamen[¹¹ C]PE2I BP _{ND}	208	6.88	<0.001
Early levodopa-treated vs Early <i>de novo</i> Parkinson's patients						
MNI coordinates			Area	Cluster sizes	Z-score	<i>P</i> value
x	y	z				
-18	18	12	Left Caudate[¹¹ C]IMA107 BP _{ND}	112	2.56	<0.05
10	22	-2	Right Caudate[¹¹ C]IMA107 BP _{ND}	96	3.18	<0.05
-24	6	6	Left Putamen[¹¹ C]IMA107 BP _{ND}	172	2.35	<0.05

22	8	8	Right Putamen[¹¹ C]IMA107 BP _{ND}	208	2.35	<0.05
-28	-8	12	Left Putamen[¹¹ C]PE2I BP _{ND}	208	6.37	<0.05
28	-8	12	Right Putamen[¹¹ C]PE2I BP _{ND}	172	6.48	<0.05

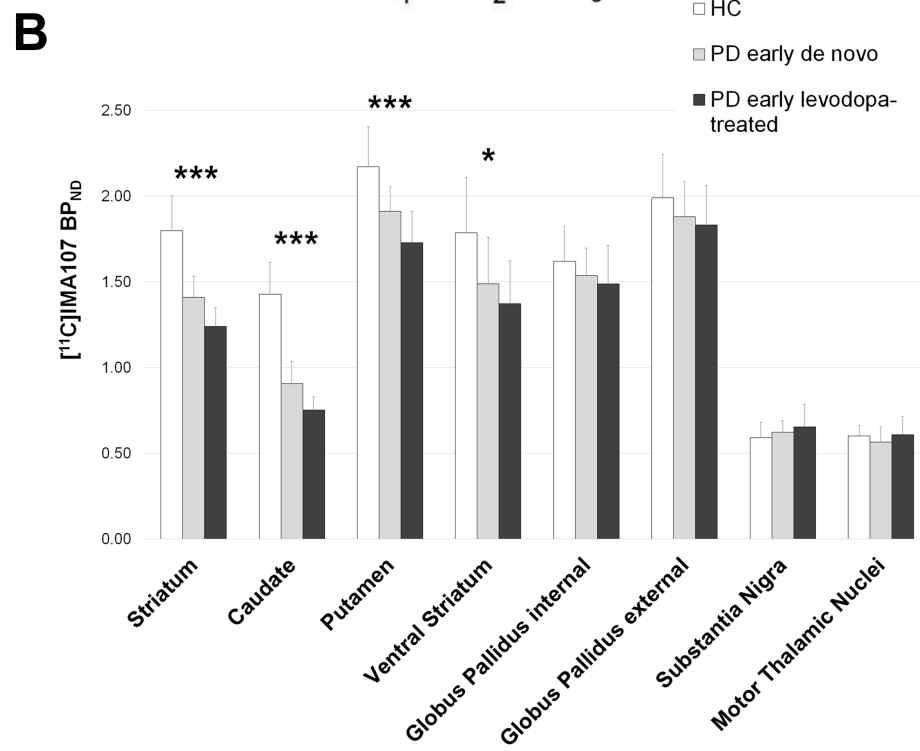
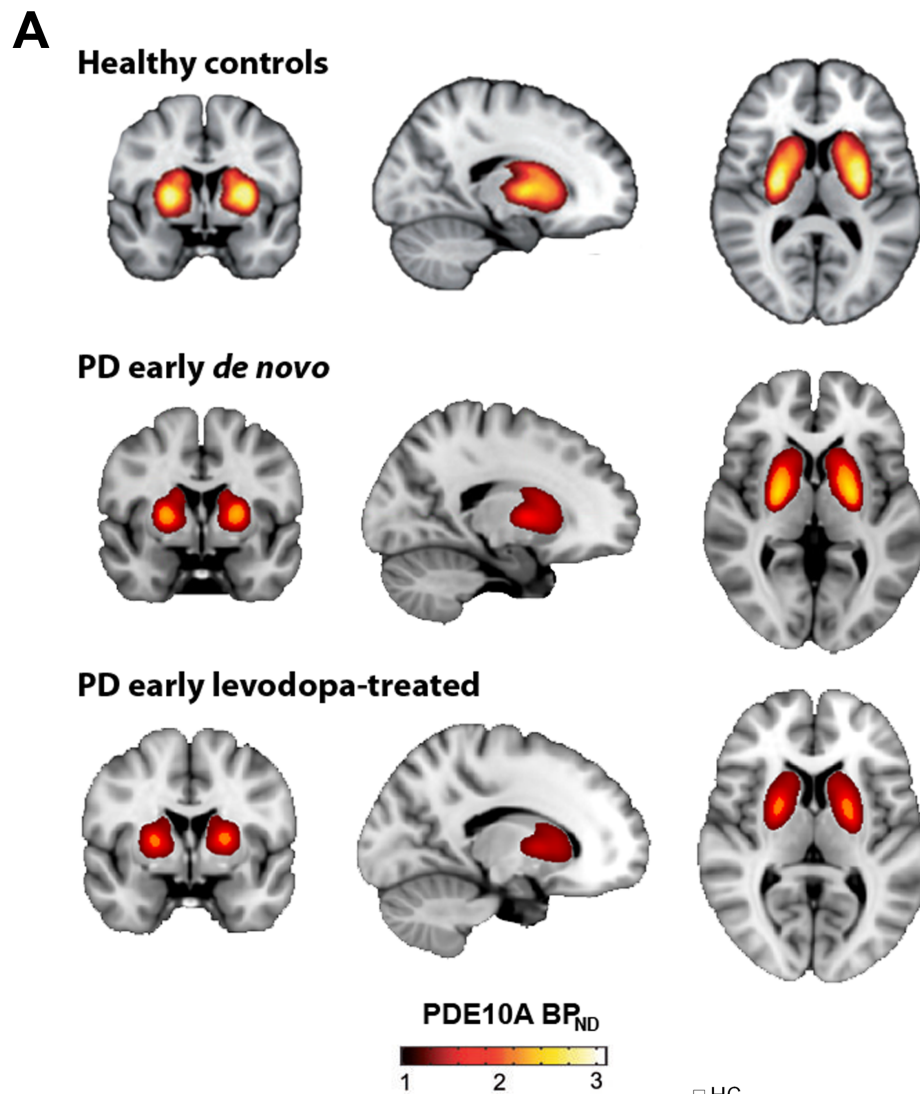


figure 1

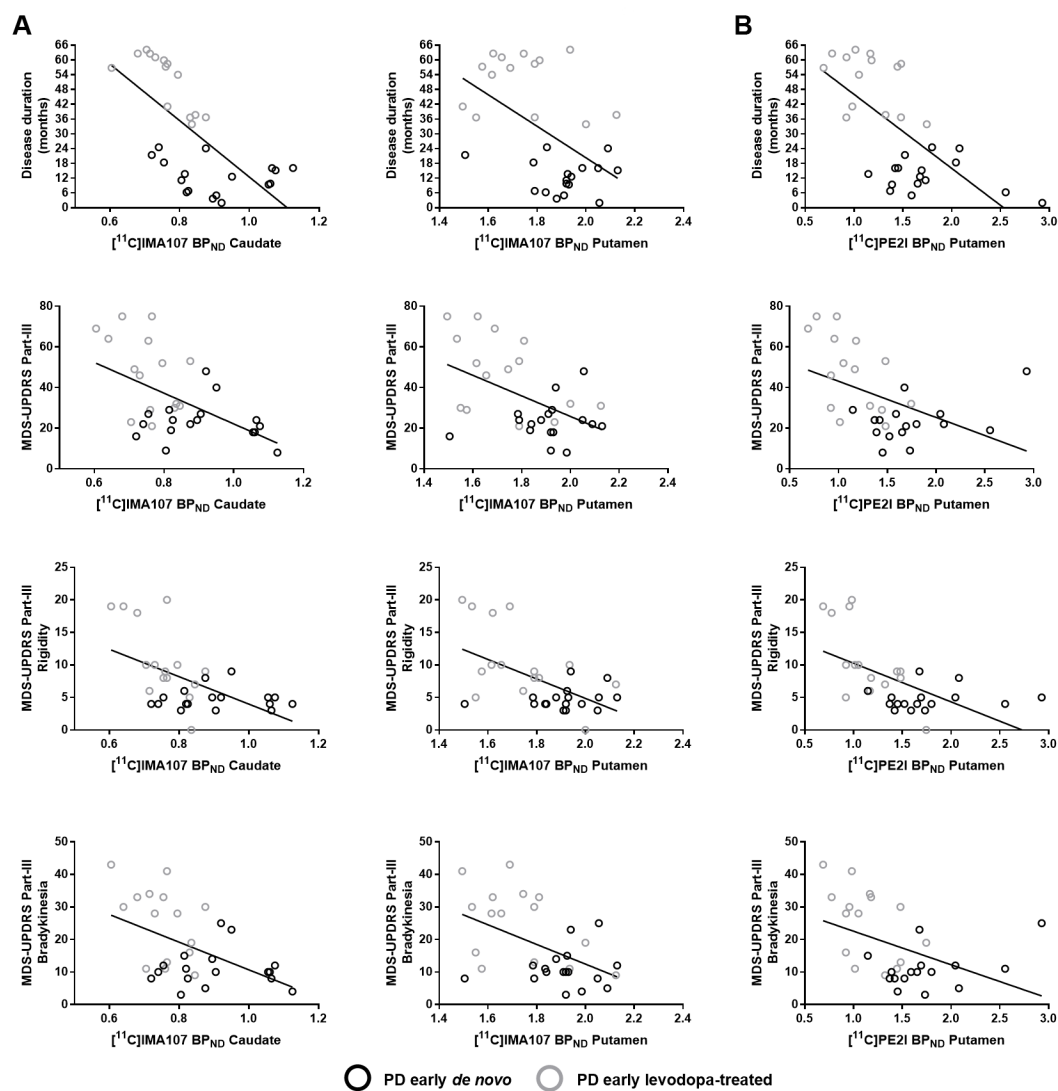


Figure 3