Altered PDE10A expression detectable early before symptomatic onset in Huntington’s disease

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There is an urgent need for early biomarkers and novel disease-modifying therapies in Huntington’s disease. Huntington’s disease pathology involves the toxic effect of mutant huntingtin primarily in striatal medium spiny neurons, which highly express phosphodiesterase 10A (PDE10A). PDE10A hydrolyses cAMP/cGMP signalling cascades, thus having a key role in the regulation of striatal output, and in promoting neuronal survival. PDE10A could be a key therapeutic target in Huntington’s disease. Here, we used combined positron emission tomography (PET) and multimodal magnetic resonance imaging to assess PDE10A expression in vivo in a unique cohort of 12 early premanifest Huntington’s disease gene carriers with a mean estimated 90% probability of 25 years before the predicted onset of clinical symptoms. We show bidirectional changes in PDE10A expression in premanifest Huntington’s disease gene carriers, which are associated with the probability of symptomatic onset. PDE10A expression in early premanifest Huntington’s disease was decreased in striatum and pallidum and increased in motor thalamic nuclei, compared to a group of matched healthy controls. Connectivity-based analysis revealed prominent PDE10A decreases confined in the sensorimotor-striatum and in striatonigral and striatopallidal projecting segments. The ratio between higher PDE10A expression in motor thalamic nuclei and lower PDE10A expression in striatopallidal projecting striatum was the strongest correlate with higher probability of symptomatic conversion in early premanifest Huntington’s disease gene carriers. Our findings demonstrate in vivo, a novel and earliest pathophysiological mechanism underlying Huntington’s disease with direct implications for the development of new pharmacological treatments, which can promote neuronal survival and improve outcome in Huntington’s disease gene carriers.

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Introduction

Huntington’s disease is a monogenic, progressive and fatal neurodegenerative disorder affecting motor, cognitive and neuropsychiatric functions (Walker, 2007). Currently, there is no cure or disease-modifying therapy for Huntington’s disease, while symptomatic treatment is limited. There is an urgent need for new therapies and robust pharmacodynamics measures for the development of novel therapeutic interventions and for allowing the monitoring of disease progression at the time that matters the most: before the development of overt symptoms (premanifest stage).

Phosphodiesterase 10A (PDE10A) is a dual substrate enzyme highly expressed in the striatal medium spiny neurons (Fujishige et al., 1999; Coskran et al., 2006). Preclinical research in transgenic Huntington’s disease animal models suggests a direct effect of mutant huntingtin on PDE10A expression via the alteration of transcription, synthesis and trafficking (Hu et al., 2004; Leuti et al., 2013). At a molecular striatal level, PDE10A regulates cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) downstream signalling cascades (e.g. cAMP/PKA/DARPP-32) that control the phosphorylation state and activity of several physiological effectors including gene transcription factors such as CREB, and various neurotransmitter receptors and voltage-gated ion channels (Nishi et al., 2008; Girault, 2012). Thus, toxic huntingtin effects on PDE10A could be detrimental for neuronal survival and for the regulation of basal ganglia functions through the dopamine-D1 direct and dopamine-D2 indirect pathways (Hebb et al., 2004; Giampa et al., 2009, 2010).

PDE10A could be a molecular target of critical therapeutic interest in Huntington’s disease with possible application in other basal ganglia disorders (Siuciak et al., 2006; Giampa et al., 2009, 2010; Leuti et al., 2013; Piccart et al., 2013). Previous work explored PDE10A expression in transgenic Huntington’s disease mice, showing decreased PDE10A protein and mRNA levels in the striatum (Hebb et al., 2004; Leuti et al., 2013), and increased PDE10A levels in the perikarya of striatal medium spiny neurons (Leuti et al., 2013). In humans, decreased PDE10A levels were found in post-mortem striatal tissue (Hebb et al., 2004). Recent PET studies found reductions in the striatal PDE10A binding in manifest Huntington’s disease patients with significant striatal atrophy (Ahmad et al., 2014; Russell et al., 2014) and premanifest Huntington’s disease gene carriers who were a mean of 12 years from disease onset (Russell et al., 2014). Experimental studies have suggested that PDE10A inhibition could be beneficial in Huntington’s disease (Giampà et al., 2009, 2010; Leuti et al., 2013).

Although previous data suggest an important role of PDE10A enzyme in the pathophysiology of Huntington’s disease, it is unclear how the alteration of PDE10A expression is related to the neuropathological salient networks, and whether the changes in PDE10A are functionally significant.

Here, we hypothesized that altered PDE10A expression could be one of the earliest changes in Huntington’s disease due to its immediate link with primary Huntington’s disease pathology. If this is true PDE10A could be of crucial importance in mechanisms modulating motor, cognitive and neuropsychiatric functions. We combined state-of-the-art PET molecular imaging and multimodal magnetic resonance-based structural imaging in vivo to study a unique cohort of early premanifest Huntington’s disease gene carriers. Our investigations led to a discovery of the earliest reported biochemical abnormality in Huntington’s disease, linking altered PDE10A signalling with predicted risk of imminent symptomatic conversion and with potential implications for the development of new treatments.

Materials and methods

Participants

We identified and studied 12 early premanifest Huntington’s disease gene carriers, who were the furthest from the predicted disease onset (90% probability to symptoms onset = 25 ± 6.9 years, mean ± SD; range: 17–43 years; Table 1), from the Huntington’s disease gene carrier registry database of National Hospital of Neurology and Neurosurgery, Queen Square, London. To estimate time to symptom onset we used a validated variant of the survival analysis formula described by Langbehn et al. (2004). This formula can be transformed into a probability distribution for age of diagnosis and subsequently years from symptomatic onset that depends on both the subject’s CAG expansion length and current age (Paulsen et al., 2008). To estimate time from symptomatic onset, the probability distribution was truncated to account for the fact that a subject has reached their current age without yet receiving a clinical diagnosis. Then, the mean of this revised distribution was calculated (Paulsen et al., 2008).

Details of this revised formula and original conditional probability of symptomatic onset derived from the survival analysis formula of Langbehn et al. (2004) are provided in Supplementary Table 1. All early premanifest Huntington’s disease gene carriers were asymptomatic based on the standardized total motor score (TMS) subscale (TMS = 0) of the Unified Huntington Disease Rating Scale (UHDRS) with a
diagnostic confidence level of 0 (The Huntington Study Group, 1996). Twelve healthy individuals, matched for age and gender, who served as the control group, were recruited by public advertisement. All participants screened successfully to undertake PET and MRI scanning under standard criteria, had no history of other neurological or psychiatric disorders, and were not under treatment with substances with known actions in phosphodiesterases (Supplementary Tables 2 and 3). The study was approved by the institutional review boards and the research ethics committee. Written informed consent was obtained from all study participants.

### Clinical assessments

Motor function was assessed with the UHDRS TMS (The Huntington Study Group, 1996). Functional capacity was assessed with clinician-based [Total Functional Capacity Scale (TFC), Independence Scale (IS), UHDRS functional assessment] (The Huntington Study Group, 1996) and participant self-reported [36-Item Short Form Health Survey (SF-36); Ware et al., 1993] functional and quality-of-life measures and assessments. Neuropsychiatric symptoms were assessed with the shortened form of the problem behaviour assessment (PBA; Craufurd et al., 2001), the Beck Depression Inventory-II (BDI-II; Beck et al., 1996), and the Hamilton Depression Rating Scale (HDRS; Hamilton, 1960). Cognitive assessments were carried out using the Cambridge Neuropsychological Test Automated Battery (CANTAB1) and included assessments related to episodic memory (Paired Associate Learning), visual memory (Pattern Recognition Memory), attention (Reaction Time), executive function (One Touch Stockings of Cambridge) and language processing (Graded Naming Test).

### Imaging assessments

PET and MRI were performed at Imanova Ltd, London, UK. All participants were scanned on Siemens Biograph Hi-Rez 6 PET-CT scanner (Erlangen, Germany) following the injection of an intravenous bolus mean dose of 258 MBq $^{11}$C-IMA107 [standard deviation (SD): ±56.5; mean mass injected: 3.8 µg (SD: ±2.2)]. All participants were scanned after withholding consumption of caffeinated beverages for 12 h (Fredholm et al., 1999). Dynamic emission data were acquired continuously for 90 min following the injection of $^{11}$C-IMA107. The dynamic images were reconstructed with in-house software, into 26 frames (8 × 15 s, 5 × 30 s, 5 × 300 s, and 5 × 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 × 2 × 2 mm³, and a transaxial Gaussian filter of 5 mm.

MRI scans were acquired with a 32-channel head coil on a Siemens Magnetom Verio, 3 T MRI scanner and included a $T_1$-weighted magnetization prepared rapid gradient echo sequence [MPRAGE; time repetition = 2300 ms, time echo = 2.98 ms, flip angle of 9°, time to inversion = 900 ms, matrix = 240 × 256] for co-registration with the PET images and for voxel-based morphometry analysis; fast grey matter $T_1$ inversion recovery (FGATIR; repetition time = 3000 ms, echo time = 2.96 ms, flip angle of 8°, time to inversion = 409 ms, matrix = 240 × 256) (Sudhyadhom et al., 2009) and fluid and white matter suppression (FLAWS; repetition time = 5000 ms, echo time = 2.94 ms, flip angle of 5°, inversion time = 409/1100 ms, matrix = 240 × 256) (Tanner et al., 2013) sequences for improving delineation of subcortical brain regions. All sequences used a 1 mm³ voxel size, anteroposterior phase encoding direction, and a symmetric echo.

Diffusion-weighted data were acquired for performing a two-layered probabilistic tractography and connectivity-based functional parcellation of the striatum using echo planar imaging (EPI; repetition time = 8000 ms, echo time = 96 ms, flip angle of 90° and voxel size of 2 × 2 × 2 mm³). The diffusion weighting was isotropically distributed along the 30 directions (b-value = 1000 s/mm²), and a non-diffusion-weighted imaging (B0) was acquired at the beginning of each scan. EPI acquisitions are prone to geometric distortions that can lead to errors in tractography. To minimize this, two image sets were acquired with the phase-encoded direction reversed—‘blip-up’ and ‘blip-down’—resulting in images with geometric distortions of equal magnitude but in the opposite direction allowing for the calculation of a corrected image (Andersson et al., 2003). Before correcting geometric distortions, each image set—blip-up and blip-down—was corrected for motion and eddy current-related distortions. Diffusion data analysis was
performed with the FSL tools (FMRIB Centre Software Library, Oxford University; http://www.fmrib.ox.ac.uk/fsl/).

Data processing

Voxel-based morphometry

Images were segmented into grey matter, white matter and CSF tissue classes using the statistical parametric mapping (SPM) version 8 software package (Wellcome Department of Imaging Neuroscience, London, UK, http://www.fil.ion.ucl.ac.uk/spm/). Grey and white matter images were then normalized to a grey and white matter population template, generated from the complete image set using the diffeomorphic anatomical registration using exponentiated lie-algebra (DARTEL) registration method (Ashburner, 2007). This non-linear warping technique minimizes between-subject structural variations. All images were checked following spatial normalization to ensure registration accuracy. The final voxel resolution was 1 x 1 x 1 mm. Spatially normalized images were modulated by the Jacobian determinants so that intensities represent the amount of deformation needed to normalize the images, and then smoothed with an 8-mm full-width at half-maximum Gaussian kernel.

Voxel-based multiple regression analysis (based on the general linear model) was carried out using SPM8 with voxel-wise grey and white matter volumes as the dependent variables. Age and gender were added as nuisance covariates and total intracranial volume, calculated by summing the values of the native space tissue segmentations using the 'get_totals' function in SPM8, were added as a global measure. Multiple regression analysis was then performed to assess for changes in grey matter and white matter volumes between premanifest Huntington’s disease gene carriers and healthy controls. The threshold for statistical significance was set at $P < 0.05$ after family wise error (FWE) correction for multiple comparisons.

Freesurfer MRI volumetric analysis

The FreeSurfer image analysis suite (version 5.3.0 http://surfer.nmr.mgh.harvard.edu) processing pipeline was used to derive measures of subcortical volumes. The automated procedures for volumetric measures of these different brain structures have been previously described (Fischl et al., 2002). This procedure automatically assigns a neuroanatomical label to each voxel in an MRI volume based on probabilistic information automatically estimated from a manually labelled training set. In brief, the segmentation is carried out as follows: first, an optimal linear transform is computed that maximizes the likelihood of the input image, given an atlas constructed from manually labelled images. Next, a non-linear transform is initialized with the linear one, and the image is allowed to further deform to better match the atlas. Finally, a Bayesian segmentation procedure is carried out, and the maximum a posteriori estimate of the labelling is computed. The segmentation uses three pieces of information to disambiguate labels: (i) the prior probability of a given tissue class occurring at a specific atlas location; (ii) the likelihood of the image intensity given that tissue class; and (iii) the probability of the local spatial configuration of labels given the tissue class. This technique has previously been shown to be comparable in accuracy to manual labelling (Fischl et al., 2002). Adjustments for intracranial volume were calculated for each region of interest using validated methods within the FreeSurfer toolkit (Buckner et al., 2004).

$^{11}$C-IMA107 PET data

Movement correction

Subjects were positioned supine with their transaxial planes parallel to the line intersecting the anterior-posterior commissure line. Head position was maintained with the help of individualized foam holders, monitored by video and was repositioned if movement was detected. Subjects were in a resting state with low light. Intrascan notes for participant’s movement were acquired during scanning. Minimal head movements were noted only in 3 of 24 subjects (12.5%).

After reconstruction of the dynamic $^{11}$C-IMA107 image volumes, we applied regions of interest to the dynamic data set. We tested correction for movement using a frame-by-frame realignment procedure as previously described (Montgomery et al., 2006) with in-house software (c-wave) implemented in Matlab 8.2 (The MathWorks Inc.). Non-attenuated corrected images were used for realignment, to provide additional information by reducing the influence of redistribution of radiotracer producing erroneous realignments (Dagher et al., 1998). The non-attenuated corrected images were de-noised using a level 2, order 64 Battle Lemarie wavelet filter (Turkheimer et al., 1999). The de-noised frames were then realigned using a mutual information algorithm (Studholme et al., 1997). Frames of the original time series were then resliced and reassembled into a movement-corrected dynamic scan.

The decay-corrected time-activity curves were computed and compared to those without movement correction for all subjects including the three subjects with minimal head movement. Amount and timing of any movement were assessed graphically and compared with intrascan records. Visual inspection of time-activity curves pre- and post-correction determined that no correction for movement needed to be applied in these datasets.

Parametric images

Parametric images of $^{11}$C-IMA107 non-displaceable binding potential (BP$_{ND}$) were generated from the dynamic $^{11}$C-IMA107 scans using a basis function implementation of the simplified reference tissue model, with the cerebellum as the reference tissue for non-specific binding using an in-house software (c-wave) implemented in Matlab 8.2 (Gunn et al., 1997). Previous PET studies have shown lower PDE10A uptake in the cerebellum (Plisson et al., 2011, 2014; Barret et al., 2014) and $^{11}$C-IMA107 binding in the cerebellum not changed after the administration of PDE10A selective blocker (Imanova internal data), confirming the suitability of the cerebellum as a reference region for the determination of the regional estimation of BP$_{ND}$.

Anatomically defined regions of interest

To facilitate anatomical delineation of regions of interest, PET images were anatomically co-registered and resliced to the corresponding volumetric FLAWS and FGATIR magnetic resonance images and spatially normalized into the T1-weighted MNI 152 template using the Mutual
Information Registration algorithm in SPM8 software package implemented in Matlab 8.2. Regions of interest were delineated manually on the co-registered MRIs using ANALYZE version 11 (Mayo Foundation) medical imaging software package by the assessor who was blinded to groups allocation. We manually delineated basal ganglia structures because of the poor performance of automated parcellation techniques on structures such as the substantia nigra, largely due to poor contrast in these regions on structural T₁-weighted magnetic resonance images. To compensate for this, we acquired state-of-the-art FGATIR and FLAWS MRI scans for each individual, which use T₁-nulling to minimize white matter signal and, by improving contrast, increase the definition of basal ganglia structures. We have then used 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, substantia nigra and motor thalamic nuclei. These brain regions express higher PDE10A levels (Seeger et al., 2003; Coskran et al., 2003; Coskran et al., 2006).

Connectivity-based parcellations of regions of interest according to cortico-striatal projections

Probabilistic tractography was performed on each subjects’ diffusion data to functionally parcellate striatum into limbic, cognitive and sensorimotor areas. The methods applied have been described previously (FSL library: http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Atlases/striatumconn) (Tziortzi et al., 2014). In brief, tractography was performed in the subjects’ continuous space, and the results were output in the subjects’ structural space. To register the diffusion data to the T₁-weighted images the epi_reg script was used (Jenkinson et al., 2002). FMRIB’s diffusion toolbox (FTD, http://www.fmrib.ox.ac.uk/fsl/fdt) was used to perform probabilistic tractography with a partial volume model allowing for up to two fibre directions in each voxel (Behrens et al., 2007). From each striatal voxel 10 000 sample tracts were generated to enable estimates of the striatal connectivity profile with each of the cortical target.

Five cortical regions, which correspond to the limbic, cognitive, rostral motor, caudal motor and parietal lobes defined on the MNI template were used (Tziortzi et al., 2014). The rostral motor, caudal motor and parietal regions were merged and constituted the sensorimotor target. Thereafter the FMRIB’s linear image registration tool (FLIRT) and FMRIB’s nonlinear image registration tool (FNIRT) functions (Jenkinson et al., 2002) were sequentially applied to normalize the MNI template to each subjects’ T₁-weighted image. To tailor the cortical regions of interest to the subjects’ individual anatomy, the subjects’ segmented grey matter was used to mask the regions of interest. The lower threshold for the grey matter mask was set at 0.25. The striatum was manually defined on the subjects’ T₁-weighted image following published guidelines (Tziortzi et al., 2011).

The connectivity maps of each cortical target were processed in two different ways: (i) for each subject, exclusive connectivity-based functional regions of interest were created following the procedures described by Johansen-Berg et al. (2005) where each voxel is assigned to the cortical target with the highest connection probability; and (ii) each subject’s connectivity maps were thresholded at 5% of the maximum connectivity value to minimize noise and voxels with low connectivity values (Fig. 2A). This allowed functional subdivisions to have a certain degree of overlap. Subsequently, cortical-striatal connectivity maps were applied to ¹³C-IMA107 binding potential (BPND) images to calculate the regional estimates of BPND.

Connectivity-based parcellations according to striatonigral and striatopallidal projections

Striatal parcellations according to striatonigral, striatopallidal internal and striatopallidal external projections were carried out using the following steps:

(i) The substantia nigra, external and internal segments of the globus pallidus were defined on each participant’s FLAWS/FGATIR image. Their FLAWS/FGATIR image was then linearly registered to their diffusion images using FLIRT, and the transform was then applied to the substantia nigra, globus pallidus internus and externus mask to move it into diffusion space.

(ii) FDT was used to determine the connectivity of each putamen voxel (from the striatal mask, as defined for the striatal parcellations according to cortical-connectivity) with the substantia nigra/globus pallidus internus and globus pallidus externus targets in diffusion space. Ten thousand sample tracts were used. Distance correction was applied since connectivity distribution drops with distance from the seed mask and substantia nigra is located further from the putamen than the globus pallidus.

(iii) The resulting connectivity maps of each subcortical target (substantia nigra/globus pallidus internus and globus pallidus externus) were then processed by creating exclusive connectivity-based functional regions of interest and 5% thresholded connectivity maps (as described for the striatal parcellations according to cortical-connectivity).

(iv) The striatal-sensorimotor maps were then masked using the exclusive connectivity-based functional regions of interest and 5% thresholded connectivity maps from ‘step iii’. This generated maps of striatal voxels specifically involved in sensorimotor networks, which projected to the substantia nigra/globus pallidus internus or globus pallidus externus, and so reflect the major parts of the direct or indirect pathways, respectively (Fig. 2B). Our striatonigral/striatopallidal internal and striatopallidal external maps are consistent with findings obtained with tract-tracing methods in primates indicating that dorsolateral striatal neurons project mainly to the substantia nigra and dorsomedial striatal neurons form bundles that run caudally toward the pallidal external complex (Parent and Hazzard, 1995). Moreover, overlapping fibres with those located in the more medial bundles reflecting the nigrostriatal tract were carefully excluded (Hedreen et al., 1991).

Statistical analysis

Statistical analysis and graphical representations of data were performed with GraphPad Prism (version 6.0c) and SPSS (version 22) for MAC OS X. For all variables, variance homogeneity and Gaussianity were tested with Bartlett and Kolmogorov-Smirnov tests, 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 clinical and imaging variables between the group of early premanifest Huntington’s disease (HTT) gene carriers and healthy controls. 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’s r and applied the
Hochberg multiple-comparison correction using PPLot (version 1.0) in Matlab 8.2 (Turkheimer et al., 2001). All data are presented as mean ± SD, and the level α was set for all comparisons at P < 0.05, corrected.

Results
All subjects underwent a battery of clinical assessments, which showed no deficits in motor and functional (P > 0.10), neuropsychiatric (P > 0.10), and cognitive (P > 0.10) performance in early premanifest Huntington’s disease gene carriers compared to healthy controls (Supplementary Tables 4–6). Neuropsychiatric assessments showed small non-significant differences between healthy controls and premanifest Huntington’s disease gene carriers; however, the neuropsychiatric burden in our cohort of early premanifest Huntington’s disease gene carriers was minimal.

As grey and white matter volumetric brain changes have been reported as one of the earliest changes in the course of Huntington’s disease (Tabrizi et al., 2009), we wanted to explore whether this was also true for the earlier cohort of premanifest Huntington’s disease gene carriers studied here. We applied both voxel-based morphometry and volumetric analysis with Freesurfer. We found no volume changes at a voxel level in any brain regions, and averaged volumes of Freesurfer subcortical and ventricle volumetric measures did not show significant differences between healthy controls and early premanifest Huntington’s disease gene carriers (P > 0.05; Supplementary Table 7).

We then assessed the expression of PDE10A enzyme in vivo with 11C-IMA107 PET, which is a specific and highly potent PDE10A-selective PET radioligand for human use (Plisson et al., 2014). We performed a three-layered analysis of the 11C-IMA107 PET BPND in: (i) magnetic resonance-based anatomically defined regions of interest (caudate, putamen, ventral striatum, globus pallidus, substantia nigra and motor thalamic nuclei); (ii) connectivity-based parcellations of regions of interest (limbic, cognitive and sensorimotor striatum) according to corticostriatal connectivity profiles; and (iii) connectivity-based parcellations of regions of interest (striatonigral/striatopallidal internal and striatopallidal external projecting segments of striatum) based on striatal connections with globus pallidus externus and substantia nigra/globus pallidus internus, and so reflect the major parts of the ‘indirect’ and ‘direct’ pathways, respectively.

PDE10A expression in anatomically defined brain regions
We found significant group differences in anatomical 11C-IMA107 BPND between the early premanifest Huntington’s disease gene carriers and healthy controls (P < 0.001). 11C-IMA107 BPND was decreased in caudate (P < 0.001; −33%), putamen (P < 0.001; −30.5%) and globus pallidus (P = 0.01; −25.6%), and increased in motor thalamic nuclei (P = 0.01; +34.5%), with no significant changes observed in substantia nigra (P > 0.10; +9%) and ventral striatum (P > 0.10; −16.9%) in early premanifest Huntington’s disease gene carriers compared to healthy controls (Fig. 1A, B and Table 2).

Individual 11C-IMA107 BPND analysis was also performed in the early premanifest Huntington’s disease gene carriers. The normal range was defined as including values ±2 SD of the healthy controls mean 11C-IMA107 BPND. Ten of 12 early premanifest Huntington’s disease gene carriers (83.3%) had abnormally reduced striatal and abnormally increased motor thalamic nuclei 11C-IMA107 BPND values (Fig. 1C–F).

PDE10A expression in the functionally defined striatum
We calculated 11C-IMA107 BPND in functional striatal subdivisions following probabilistic tractography and connectivity-based functional striatal parcellation (Fig. 2A and B). Cortico-striatal connectivity-based functional analysis showed 28–30% decreases in sensorimotor (P = 0.003) and cognitive (P = 0.02) striatum 11C-IMA107 BPND in early premanifest Huntington’s disease gene carriers compared to healthy controls (Table 2). Connectivity maps of each cortical target were then thresholded at 5% of the maximum connectivity value to minimize noise and voxels with low connectivity values, therefore allowing functional subdivisions to have a certain degree of overlap (Fig. 2A). Following thresholding, group differences remained significant (P = 0.002); however, post hoc analysis showed 11C-IMA107 BPND decreases only in the sensorimotor (P < 0.001; −34%) but not in limbic (P > 0.10) and cognitive (P > 0.10) striatum in early premanifest Huntington’s disease gene carriers compared to healthy controls (Fig. 2C and Table 2).

Hence, we used the sensorimotor striatum as a seed mask as it was the only subdivision with significant PDE10A decreases, to perform striatonigral and striatopallidal connectivity-based functional analysis (Fig. 2B). We found 23–28% 11C-IMA107 BPND decreases in striatonigral/striatopallidal internal and striatopallidal external projecting sensorimotor striatal segments (P = 0.008) (Table 2), which further decreased to a mean of 33–34% after thresholding at 5% of the maximum connectivity value in early premanifest Huntington’s disease gene carriers compared to healthy controls (P = 0.002) (Fig. 2D and Table 2).

Correlations between PDE10A expression and clinical characteristics
We found correlations between: (i) higher motor-thalamic-nuclei/striatal 11C-IMA107 BPND ratio and
higher 2- and 5-year probability for symptomatic conversion ($r = 0.59$, $P = 0.044$; and $r = 0.59$, $P = 0.043$, respectively) (Fig. 3A); (ii) higher motor-thalamic-nuclei/sensorimotor $^{11}$C-IMA107 $B_{\text{ND}}$ ratio and higher 2-, 5-, 10- and 15-year probability for symptomatic conversion ($r = 0.66$, $P = 0.020$; $r = 0.66$, $P = 0.019$; and $r = 0.63$, $P = 0.027$, respectively) (Fig. 3B); and (iii) higher motor-thalamic-nuclei/ striatopallidal $^{11}$C-IMA107 $B_{\text{ND}}$ ratio and higher 2-, 5-, 10- and 15-year probability for symptomatic conversion ($r = 0.85$, $P = 0.001$; $r = 0.82$, $P = 0.001$; $r = 0.76$, $P = 0.004$; $r = 0.65$, $P = 0.023$, respectively) (Fig. 3C). All other interactions between clinical and imaging data were not significant following the Hochberg multiple-comparison correction.
**Table 2** ¹¹C-IMA107 BPND in the groups of early premanifest Huntington’s disease gene carriers and healthy controls

<table>
<thead>
<tr>
<th>Anatomical regions of interest</th>
<th>Healthy controls</th>
<th>Premanifest Huntington’s disease</th>
<th>P-value*</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caudate (mean ± SD)</td>
<td>1.37 (±0.2)</td>
<td>0.92 (±0.2)</td>
<td>&lt;0.001</td>
<td>-33.0%</td>
</tr>
<tr>
<td>Putamen (mean ± SD)</td>
<td>2.21 (±0.3)</td>
<td>1.55 (±0.3)</td>
<td>&lt;0.001</td>
<td>-30.3%</td>
</tr>
<tr>
<td>Ventral striatum (mean ± SD)</td>
<td>1.01 (±0.1)</td>
<td>0.84 (±0.2)</td>
<td>&gt;0.10</td>
<td>-16.9%</td>
</tr>
<tr>
<td>Substantia nigra (mean ± SD)</td>
<td>0.49 (±0.1)</td>
<td>0.54 (±0.1)</td>
<td>&gt;0.10</td>
<td>+9.0%</td>
</tr>
<tr>
<td>Globus pallidus (mean ± SD)</td>
<td>1.56 (±0.2)</td>
<td>1.18 (±0.3)</td>
<td>0.01</td>
<td>-25.6%</td>
</tr>
<tr>
<td>Motor thalamic nuclei (mean ± SD)</td>
<td>0.44 (±0.07)</td>
<td>0.60 (±0.1)</td>
<td>0.01</td>
<td>+34.5%</td>
</tr>
</tbody>
</table>

**Exclusive cortico-striatal connectivity-based regions of interest: striatum**

| Cognitive (mean ± SD) | 1.48 (±0.3) | 1.07 (±0.3) | 0.02 | -27.6% |
| Sensorimotor (mean ± SD) | 1.77 (±0.3) | 1.24 (±0.3) | 0.003 | -29.6% |

**Cortico-striatal connectivity-based regions of interest: striatum with overlaps**

| Limbic (mean ± SD) | 0.83 (±0.3) | 0.65 (±0.2) | >0.10 | -11.3% |
| Cognitive (mean ± SD) | 1.30 (±0.3) | 1.06 (±0.3) | >0.10 | -18.5% |
| Sensorimotor (mean ± SD) | 1.95 (±0.3) | 1.29 (±0.4) | <0.001 | -34.0% |

**Exclusive striatonigral and striatopallidal connectivity-based regions of interest: striatum**

| Striatonigral and Striatopallidal internal [direct pathway] (mean ± SD) | 1.49 (±0.3) | 1.08 (±0.3) | 0.004 | -27.9% |
| Striatopallidal external [indirect pathway] (mean ± SD) | 1.47 (±0.4) | 1.13 (±0.3) | 0.036 | -23.2% |

**Striatonigral and striatopallidal connectivity-based regions of interest: striatum with overlaps**

| Striatonigral and Striatopallidal internal [direct pathway] (mean ± SD) | 1.61 (±0.3) | 1.09 (±0.3) | 0.008 | -32.6% |
| Striatopallidal external [indirect pathway] (mean ± SD) | 1.73 (±0.4) | 1.15 (±0.3) | <0.001 | -33.9% |

*P-values are Bonferroni corrected.

**Discussion**

Here we report altered PDE10A expression detectable with non-invasive imaging decades before the predicted development of symptoms. We have studied a unique cohort of early premanifest Huntington’s disease gene carriers, with normal clinical functions, who were on average 25 years before the predicted symptomatic onset (90% probability), and had no brain atrophy. We have used combined PDE10A PET molecular and magnetic resonance-based structural imaging, and demonstrated bidirectional changes in PDE10A expression within the brain networks, which are linked to symptomatic conversion in premanifest Huntington’s disease gene carriers.

Previous studies have reported significant changes in premanifest Huntington’s disease gene carriers that appear years before onset of clinical symptoms and include neurovascular and metabolite brain changes (8 years) (Unschuld et al., 2012; Hua et al., 2014), brain volume loss (up to 10 years; Tabrizi et al., 2009), increased interleukin 6 levels in substantia nigra and ventral striatum. Then, we used probabilistic tractography to parcellate each subject’s striatum into functional segments according to cortical connections. Our findings showed that striatal PDE10A signal loss was mostly confined in the sensorimotor striatum, whereas PDE10A signal was not significantly altered in limbic and cognitive striatum at this stage of the disease and with the sample examined. Subsequently we examined the sensorimotor striatum, which was parcellated on each subject according to segments projecting to substantia nigra and globus pallidus internus and globus pallidus externus. We found PDE10A signal loss in both striatonigral/striatopallidal internal and striatopallidal external projecting segments of striatum, though decreased PDE10A expression in the striatopallidal external segment was one level more significant. The latter observation is in line with previous studies indicating preferential degeneration of striatopallidal external projection neurons in Huntington’s disease (Albin et al., 1992; Richfield et al., 1995). We found substantial overlap between striatonigral/striatopallidal internal and striatopallidal external projecting regions of the striatum, in line with the known densities of D1 and D2 receptors throughout the striatum (Sun et al., 2012), albeit with marginally greater prevalence of striatonigral projecting regions in lateral and dorsal regions of the striatum, as predicted by animal studies (Villalba and Smith, 2013).
We looked for correlations between PET and clinical data. The balance between PDE10A signal loss in the striatum and increased PDE10A signal in the motor thalamic nuclei correlated with higher probability for symptomatic conversion. Within the striatal denominator of this ratio, PDE10A signal loss in the striatopallidal projecting segments of the sensorimotor striatum was the most critical for this correlation. Previous studies using similar samples have reported correlations at a 0.05 \( \alpha \)-level between decreased D2-receptors (van Oostrom et al., 2009) and increased activated microglia (Politis et al., 2011) in the striatum, and predicted 5-year probability of Huntington’s disease onset. In our study, the balance between decreased PDE10A in striatopallidal projections and increased PDE10A in motor thalamic nuclei correlated with the risk for symptomatic conversion up to a 0.001 \( \alpha \)-level with power to associate with predicted onset up to 15 years. To our knowledge, this is the strongest reported correlation with the risk of symptomatic Huntington’s disease conversion. Nonetheless, this is a cross-sectional study and further longitudinal studies are needed to elucidate the predictive value of \( ^{11} \text{C-IMA107} \) PET for determining symptomatic onset and its utility for determining disease progression rates.

Decreased levels of PDE10A have been reported in transgenic Huntington’s disease mice and in post-mortem tissue of three patients with Huntington’s disease (Hebb et al., 2004). A pilot PDE10A PET study reported 60–70% decreases in striatal PDE10A expression in five patients with manifest Huntington’s disease with significant striatal atrophy (Ahmad et al., 2014). Using PET with \( ^{18} \text{F-MNI-659} \), Russell et al. (2014) have found 47.6%
decreases in striatal and pallidal PDE10A expression in eight patients with early manifest Huntington’s disease and lower striatal 18F-MNI-659 binding was associated with disease severity and disease burden of pathology. Three premanifest Huntington’s disease gene carriers, who were a mean of 12 years from predicted onset, displayed decreases in striatal PDE10A expression to a lesser degree compared to the group of manifest Huntington’s disease patients (Russell et al., 2014). Our anatomical analysis demonstrated 31–33% loss of striatal PDE10A expression at early premanifest stages indicating that striatal PDE10A loss is an early phenomenon, which may progresses over the course of the disease. Altered PDE10A expression may be due to altered transport of this protein (Leuti et al., 2012). Given the significant body of data suggesting that mutant huntingtin affects intracellular transport (Ross and Tabrizi, 2011), it would then be likely that altered PDE10A expression is a secondary event in the pathogenesis of Huntington’s disease. Also, our group of early premanifest Huntington’s disease gene carriers had no brain atrophy indicating that decreases in PDE10A do not reflect loss of medium spiny neurons. However, it is possible that the small number of subjects studied here is underpowered to detect volumetric differences. A larger study in the future would be able to elucidate whether at this early stage, premanifest Huntington’s disease gene carriers show significant subcortical atrophy.

**Figure 3** Correlations between PDE10A expression and probability for symptomatic conversion in early premanifest Huntington’s disease gene carriers. (A) Higher thalamic motor nuclei (MN)/striatal 11C-IMA107 BPND ratio correlated with higher 2- ($r = 0.59; P = 0.044$) and 5- ($r = 0.59; P = 0.043$) year probability for symptomatic conversion; (B) higher motor-thalamic-nuclei/sensorimotor 11C-IMA107 BPND ratio correlated with higher 2- ($r = 0.66; P = 0.020$), 5- ($r = 0.66; P = 0.019$) and 10- ($r = 0.66; P = 0.027$) year probability for symptomatic conversion, and (C) higher motor-thalamic nuclei/striatopallidal 11C-IMA107 BPND ratio correlated with higher 2- ($r = 0.85; P = 0.001$), 5- ($r = 0.82; P = 0.001$), 10- ($r = 0.76; P = 0.004$) and 15- ($r = 0.65; P = 0.023$) year probability for symptomatic conversion. Probabilities for symptomatic conversion were calculated according with the survival analysis formula described by Langbehn et al. (2004).
We did not find significant decreases in PDE10A expression in the substantia nigra of early premanifest Huntington’s disease gene carriers. Previous data from animal model of Huntington’s disease have shown significant decreases in PDE10A immunoreactivity in the substantia nigra of R6/2 mice (Leuti et al., 2012). This discrepancy could be explained by the fact that PDE10A decreases were observed in 9–13-weeks-old R6/2 mice, which were fully symptomatic with severe motor impairment. In our study, we have assessed a cohort of early premanifest Huntington’s disease gene carriers who were clinically normal, thus we cannot exclude the possibility that significant decreases in substantia nigra PDE10A expression may be present only in symptomatic Huntington’s disease patients, or at least not in the early premanifest stages studied here.

Our functional analysis revealed that the striatal PDE10A signal loss was mostly confined to the dorsal striatum, which receives sensorimotor information from the cortex (Choi et al., 2012). Previous MRI studies have shown that striatal atrophy in Huntington’s disease follows a topographical dorso-ventral and caudo-rostral gradient (Kassubek et al., 2004; Douaud et al., 2006). As PDE10A expression is important for neuronal survival our findings are consistent with the pattern of volume loss that can be detected at later stages of Huntington’s disease with MRI.

Mutant huntingtin, by gaining a toxic effect on PDE10A (Hu et al., 2004; Leuti et al., 2013), would impair its function for regulating the striatonigral and striatopallidal downstream signalling cascades, which work in a coordinated manner for the fine-tuning of movement (Nishi et al., 2008; Girault, 2012; Cui et al., 2013). Imbalance of striatal output would lead to abnormal thalamo-cortical input, which contributes to the development of Huntington’s disease symptoms (André et al., 2010). Our findings show 33–34% PDE10A signal loss in striatonigral/striatopallidal internal and striatopallidal external projecting striatal segments and 35% increases of PDE10A signal in the motor thalamic nuclei. Previous 18F-FDG PET studies have reported increases in motor thalamic nuclei metabolism associated with decreases in striatal metabolism in premanifest Huntington’s disease gene carriers (Feigin et al., 2001, 2007). Premanifest Huntington’s disease gene carriers, who converted to symptomatic Huntington’s disease, showed a progressive loss of thalamic metabolism activity at 4- (Feigin et al., 2007) and 7-year follow-up PET scan (Tang et al., 2013). The ventral anterior and lateral motor thalamic nuclei receive GABAergic signals from the striatopallidal and striatonigral neurons and convey their input to the cortex (Parent and Hazrati, 1995). Therefore, our findings might reflect a compensatory thalamic mechanism to counterbalance the decreased inhibitory incoming signals and consequently, physiologically stimulate the cortex (Fig. 4A). As the disease progresses this compensatory mechanism could gradually fail leading to an overflow of excitatory glutamatergic activity in the cortex, and consequently manifestation of chorea (Fig. 4B) (Albin et al., 1991). However, it is also possible that the observed thalamic increases in PDE10A might reflect an upregulatory reaction to an ongoing loss of inhibitory striatal output. The importance of thalamic signalling in motor execution is further justified as motor thalamic nuclei provide feedback to the sensorimotor striatum through direct glutamatergic projections (McFarland and Haber, 2000), which if impaired contribute to the development of Huntington’s disease symptoms (Deng et al., 2013). Thus, the interplay of signals between striatopallidal and motor thalamic nuclei could explain the critical importance of PDE10A expression in these brain areas for the development of symptoms as indicated by our results.

Preclinical studies have postulated arguments in order to determine the use of PDE10A inhibitors in the clinical management of Huntington’s disease (Giampa et al., 2009, 2010; Leuti et al., 2013). PDE10A inhibition with TP-10 was able to reduce the loss of striatal and cortical neurons, to increase striatal and cortical CREB phosphorylation, and to delay the development of neurological deficits in Huntington’s disease animal models (Giampa et al., 2009, 2010). Thus, PDE10A may be a target to ameliorate both pathogenesis by promoting neuronal survival and pathophysiology by delaying the onset of symptoms of Huntington’s disease. In premanifest Huntington’s disease stages, PDE10A inhibition would activate the cAMP/PKA/DARPP-32 signalling cascade by simultaneously potentiating adenosine A2A receptor signalling and inhibiting dopamine D2-receptor signalling in striatopallidal external neurons (Nishi et al., 2008). In the striatonigral/striatopallidal internal pathway, PDE10A inhibitors would potentiate the dopamine D1-receptor-induced increase in DARPP-32 phosphorylation (Nishi et al., 2008). PDE10A inhibitors do not decrease PDE10A levels but the enzyme activity (Leuti et al., 2013). Thus, they could restore cellular mechanisms and the expression of several transcriptional factors and genes by increasing cAMP/PKA/DARPP-32 signalling cascade (Schmidt et al., 2008; Giampa et al., 2009, 2010; Threlfell et al., 2009). However, it is unclear whether this increased signal would be beneficial in Huntington’s disease. If decreased striatal PDE10A expression is a pathological effect of mutant huntingtin, PDE10A inhibitor therapy by further reducing the residual low-activity of PDE10A may not be beneficial.

In conclusion, our findings highlight very early neurochemical changes in premanifest Huntington’s disease gene carriers. PDE10A expression could be a biomarker of striatal medium spiny neurons integrity and 11C-IMA107 PET may be a useful tool for future trials of disease-modifying therapeutics aiming to delay the onset and slow the progression of Huntington’s disease. However, these data need to be extended in a larger cohort over different stages of the disease to confirm the relationship between PDE10A and clinical symptomatology.
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Supplementary material

Supplementary material is available at Brain online.

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