Microglial activation in presymptomatic Huntington’s disease gene carriers

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Microglial activation may play a role in the pathogenesis of Huntington’s disease (HD). Using 11C-(R)-PK11195 (PK) positron emission tomography (PET), we investigated microglial activation in HD presymptomatic gene carriers (PGCs), its relationship with striatal neuronal dysfunction measured with 11C-raclopride (RAC) PET, and the role of PK PET as a possible marker of subclinical disease progression in PGCs. Eleven HD PGCs underwent PK and RAC PET. Their results were compared with those of healthy controls. PK and RAC binding was measured using region-of-interest analysis. Regional increases in PK binding were also localized with voxel-based statistical parametric mapping. HD PGCs had lower striatal RAC binding than the controls but significantly higher striatal and cortical PK binding. Individual levels of higher striatal PK binding in PGCs correlated with lower striatal RAC binding and, after excluding one outlier, with a higher probability of developing HD in 5 years. The inverse association between striatal PK and RAC binding in PGCs continues into early to moderate stages of HD. This study demonstrated for the first time in vivo widespread microglial activation in preclinical HD which correlated with striatal neuronal dysfunction. These findings indicate that microglial activation is an early event in the pathogenic processes of HD and is associated with subclinical progression of disease. PK PET may be a useful marker of active subclinical disease and a means of investigating the efficacy of neuroprotection strategies in PGCs.

Keywords: Huntington’s disease; presymptomatic; microglial activation; positron emission tomography; PK11195

Abbreviations: BP = binding potential; HD = Huntington’s disease; PET = positron emission tomography; PGCs = presymptomatic gene carriers; SPM = statistical parametric mapping.


Introduction

Huntington’s disease (HD) is an autosomal dominant neurodegenerative disease caused by a CAG repeat expansion in the IT15 gene on chromosome 4 (The Huntington’s Disease Collaborative Research Group, 1993). The exact mechanisms of cell death are uncertain but microglial activation has been suggested to play a role (Sapp et al., 2001). Microglia are the major intrinsic immunocompetent cells in the central nervous system and are normally present in a quiescent state. Upon exposure to neuronal insults such as infection, ischaemia or the presence of abnormal protein aggregations (including mutant huntingtin), microglia become activated and release pro-inflammatory cytokines (e.g. TNF-α and IL-1β). These in turn lead to free-radical production (Kim and de Vellis, 2005), NMDA-mediated excitotoxicity (Tikka and Koistinaho, 2001) and caspase activation (Wang et al., 2005), all of which may contribute to neuronal death. A post-mortem study of HD brains found a significant accumulation of activated microglia in regions affected by HD, such as the basal ganglia and the frontal cortex, and their density correlated with the severity of HD pathology, suggesting a close association between microglial activation and neuronal death (Sapp et al., 2001).

When microglia become activated, their mitochondria express peripheral benzodiazepine binding sites (PBBS). 11C-(R)-PK11195 (PK), a positron emission tomography...
(PET) tracer that binds selectively to the PBBS, has detected microglial activation in vivo in neurodegenerative diseases such as Alzheimer’s disease (Cagnin et al., 2001) and Parkinson’s disease (Gerhard et al., 1999). Using PK PET, we have also found significant microglial activation in the striatum and cortical regions of symptomatic HD patients, and reported that striatal PK binding correlates with loss of striatum and cortical regions of symptomatic HD patients (Sapp et al., 2001), validating the use of PK PET as a marker of microglial activation in HD.

The aims of the current study were to investigate in vivo with PK PET the extent of microglial activation in HD presymptomatic gene carriers (PGCs), and the relationship between microglial activation and subclinical striatal neuronal dysfunction, reflected by loss of D2 binding measured with 11C-raclopride (RAC) PET (Pavese et al., 2006). The results are in agreement with the post-mortem findings (Sapp et al., 2001), validating the use of PK PET as a marker of microglial activation in HD.

The material and methods section describes the study design, including the recruitment of participants, the use of PET scanning, and the analysis of the data. The study involved 11 presymptomatic HD PGCs and 10 healthy controls. The PET scans were performed using an ECAT EXACT HR++ (CTI/Siemens 966, Knoxville, TN) tomograph with a total axial field of view of 23.4 cm. The camera has a transaxial spatial resolution of 4.8 ± 0.2 mm and axial resolution of 5.6 ± 0.5 mm after image reconstruction (Spinks et al., 2000). A 5-min transmission scan was performed prior to injection of tracer to correct for tissue attenuation of 511 keV gamma radiation. A mean dose of 286 MBq PK was administered intravenously over 30 s. Dynamic data were collected over 60 min as 18 time frames. The mean injected dose for RAC was 190 MBq. Scanning began at the start of tracer infusion generating 20 time frames over 65 min. RAC and PK were supplied by Hammersmith Imanet.

### Table 1 Demographic and clinical details of the 11 presymptomatic Huntington’s disease (HD) carriers

<table>
<thead>
<tr>
<th>Subject no</th>
<th>Age (years)</th>
<th>Sex</th>
<th>CAG repeat length</th>
<th>HD diagnostic confidence</th>
<th>5-year probability of developing HD</th>
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<tbody>
<tr>
<td>1</td>
<td>41</td>
<td>M</td>
<td>43/38</td>
<td>0</td>
<td>0.43</td>
</tr>
<tr>
<td>2</td>
<td>44</td>
<td>M</td>
<td>40/17</td>
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<td>0.26</td>
</tr>
<tr>
<td>3</td>
<td>33</td>
<td>F</td>
<td>47/11</td>
<td>1</td>
<td>0.15</td>
</tr>
<tr>
<td>4</td>
<td>43</td>
<td>F</td>
<td>39/17</td>
<td>0</td>
<td>0.34</td>
</tr>
<tr>
<td>5</td>
<td>41</td>
<td>M</td>
<td>44/20</td>
<td>1</td>
<td>0.60</td>
</tr>
<tr>
<td>6</td>
<td>37</td>
<td>M</td>
<td>48/25</td>
<td>2</td>
<td>0.29</td>
</tr>
<tr>
<td>7</td>
<td>61</td>
<td>F</td>
<td>40/18</td>
<td>1</td>
<td>0.04</td>
</tr>
<tr>
<td>8</td>
<td>40</td>
<td>F</td>
<td>46/17</td>
<td>1</td>
<td>0.34</td>
</tr>
<tr>
<td>9</td>
<td>32</td>
<td>F</td>
<td>46/19</td>
<td>0</td>
<td>0.04</td>
</tr>
<tr>
<td>10</td>
<td>46</td>
<td>F</td>
<td>41/10</td>
<td>0</td>
<td>0.04</td>
</tr>
<tr>
<td>11</td>
<td>46</td>
<td>M</td>
<td>41/10</td>
<td>0</td>
<td>0.04</td>
</tr>
</tbody>
</table>

*0 = no abnormalities; 1 = non-specific motor abnormalities or soft signs (<50% confidence); 2 = motor abnormalities that may be early signs of HD (50 to 89% confidence); 3 = motor abnormalities that are likely signs of HD (90 to 98% confidence); 4 = motor abnormalities that are unequivocal signs of HD (≥99% confidence). Derived from (Langbehn et al., 2004) based on subject’s age and CAG repeat length. Homozygous carrier.
Each subject also received a volumetric T1 MRI using a 1.5 tesla MRI scanner (Eclipse systems Picker International; pulse sequence: RF-fast; acquisition time: TR = 30 ms, TE = 3 ms; flip angle = 30°) for coregistration with the PET images.

**Image analysis**

The investigator (Y.F.T.) analysing the scans was blinded to the clinical and demographic data of the participants. Parametric images of PK binding potential (BP) were generated using a simplified reference tissue model. Due to the potentially widespread and patchy distribution of activated microglia in neurodegenerative conditions like HD, it is often difficult to define a reference region free of microglial activation. Therefore, cluster analysis was used to identify a cluster of voxels representing non-specific PK uptake (equivalent to normal cortical grey matter) in order to provide a tissue input function, as previously described (Banati, 2002; Pavese et al., 2006). In short, the cluster analysis divides the brain voxels into 10 clusters based on the shape of their time-activity curves. The cluster with the time–activity curve which most resembles that of the mean for a normal population of cortical voxels, tested for similarity using a χ² test (P < 0.05), is selected as the reference cluster. Parametric images of RAC BP were generated using a basis function implementation of the simplified reference tissue model with a cerebellar reference tissue (Gunn et al., 1997). PK and RAC parametric images of each subject were co-registered to their respective volumetric MRI scans using a mutual information registration algorithm implemented in SPM99 software (Wellcome Functional Imaging Laboratory, London). We chose SPM99 in order to compare the PK findings of HD PGCs with the published data of 11 symptomatic HD patients (Pavese et al., 2006). Striatal regions-of-interest were manually traced on the MRI scans using Analyze 6.0 software (Mayo Clinic, MN), and then applied to the corresponding coregistered parametric images to sample the BP. The BP values reported are the averaged values of left and right sides.

We measured the cortical PK BPs of the images after spatially transforming the PK parametric BP images into Montreal Neurological Institute (MNI) stereotaxic space using SPM99 software. To perform this, the individual subject’s MRI was first spatially normalized to the T1 MRI template in SPM99, and the transformation parameters were then applied to the co-registered PK parametric image. We then applied a standardized cortical object map which was defined on the single-subject T1 MRI template contained in SPM99 software as described previously (Pavese et al., 2006).

In addition, we also performed a voxel-based analysis of PK BP images using SPM99, an approach which compares the PK binding over the entire brain in HD PGCs with controls applying no a priori hypothesis. The normalized PK BP images were smoothed using an isotropic kernel of 6 mm. Between-group comparisons were made with a voxel threshold of P < 0.005. All clusters with a P-value of < 0.05 after correcting for multiple comparisons across the whole brain were deemed significant.

**Statistical analysis**

Non-parametric statistical tests (using SPSS12 statistical software, SPSS Inc., Chicago, IL) were used for between-group comparisons (Mann–Whitney U test) and to interrogate correlations between BP values and variables of interest (Spearman’s correlation). All significance levels reported are two-tailed.

The study was approved by the Research Ethics Committee of Hammersmith, Queen Charlotte’s and Chelsea and Acton Hospitals Trust. Permission to administer radioactive substance was granted by the Administration of Radioactive Substances Advisory Committee of the UK. All participants gave written informed consent to take part in this study in accordance with the Declaration of Helsinki.

**Results**

**Striatal binding**

The mean striatal PK BP of HD PGCs was significantly higher than that of healthy controls (0.27 ± 0.04 versus 0.13 ± 0.01, mean ± SE; P = 0.001), while their mean striatal RAC BP was significantly lower (2.37 ± 0.18 versus 2.99 ± 0.06; P = 0.014) (Table 2 and Fig. 1). Defining normality as values within two standard deviations of the controls’ mean, 9 out of 11 HD PGCs had abnormally reduced striatal RAC BP and 8 had abnormally raised striatal PK BP (Figs 1 and 2). Subject 1, who was a homozygous HD gene carrier, had normal striatal RAC BP but abnormally raised striatal PK BP. Subjects 2 and 8 showed low striatal RAC binding but normal striatal PK BP. Subject 4, who had the lowest 5-year probability of developing HD amongst all the subjects, exhibited normal PK and RAC binding.

**Cortical binding**

The cortical PK BP values of the HD PGCs were also significantly higher than the controls (1.3–1.5 times greater than controls—Table 2) though the increases were less than that observed for the striatum (2.2 times).

**SPM**

SPM of PK parametric images localized increased PK binding in the striatum and cortical areas in HD PGCs confirming the region-of-interest findings. In addition, SPM also detected significantly increased PK binding in

<table>
<thead>
<tr>
<th>Table 2</th>
<th>¹¹C-(R)-PK11195 binding potential</th>
<th>P value</th>
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<tbody>
<tr>
<td></td>
<td>Healthy controls, n = 10</td>
<td>HD gene carriers, n = 11</td>
</tr>
<tr>
<td>Striatum</td>
<td>0.13 ± 0.01</td>
<td>0.27 ± 0.04</td>
</tr>
<tr>
<td>Frontal lobe</td>
<td>0.15 ± 0.01</td>
<td>0.20 ± 0.01</td>
</tr>
<tr>
<td>Parietal lobe</td>
<td>0.13 ± 0.01</td>
<td>0.19 ± 0.01</td>
</tr>
<tr>
<td>Temporal lobe</td>
<td>0.15 ± 0.01</td>
<td>0.19 ± 0.01</td>
</tr>
<tr>
<td>Occipital lobe</td>
<td>0.17 ± 0.01</td>
<td>0.23 ± 0.01</td>
</tr>
</tbody>
</table>

Region-of-interest measurements of ¹¹C-(R)-PK11195 binding potential in healthy controls and Huntington’s disease (HD) gene carriers. Mean ± standard error.
globus pallidus, thalamus and brainstem nuclei (Fig. 3 and Table 3).

**Correlations**

Individual HD PGC levels of striatal PK BP correlated inversely with levels of striatal RAC BP (Spearman’s $\rho = -0.65$, $P = 0.03$, Fig. 4). The 5-year probability of developing clinical HD did not correlate with striatal PK ($\rho = 0.37$, $P = 0.26$) or striatal RAC ($\rho = -0.24$, $P = 0.47$) binding. Subject 8 had been taking high dose creatine, which is being investigated as a putative neuroprotective agent in HD, for 4 years and this may have influenced both PET findings and the probability of disease onset. As can be seen in Fig. 5, subject 8 was a clear outlier in the correlation between striatal PK BP and the probability of disease onset. If we excluded this subject from the analysis, there was a strong correlation between the 5-year probability of disease onset and striatal PK BP ($\rho = 0.71$, $P = 0.02$, Fig. 5), but not RAC BP ($\rho = -0.38$, $P = 0.28$). The overall significance of the other statistical comparisons reported earlier remained unchanged with the exclusion of this subject.

**Comparisons with symptomatic HD patients**

The mean striatal PK binding of HD PGCs was lower than that of symptomatic HD patients (0.31 ± 0.03) (Pavese et al., 2006) though this was not statistically
significant \((P = 0.39)\). Combining the data from PGCs and symptomatic patients, the correlation between striatal PK and RAC binding remained significant \((r = 0.538, \ P = 0.018)\).

### Discussion

We report for the first time the presence in vivo of microglial activation in presymptomatic HD gene carriers and its association with striatal neuronal dysfunction. This association continues from presymptomatic to early/moderate symptomatic stages. The widespread presence of microglial activation in HD PGCs suggests that this process is an early event in HD pathogenesis. Whilst it is unlikely to be the initiating event, the close association of microglial activation with striatal neuronal dysfunction indicates that it may contribute to neuronal death and propagate disease progression. Nevertheless, the cross-sectional nature of this study means that we have demonstrated an association, rather than causal relationship, between microglial activation and striatal neuronal dysfunction in HD PGCs.

Minocycline, which inhibits caspase and microglial activation, was reported to slow disease progression in a R6/2 transgenic mouse model of HD (Chen et al., 2000) and protect against microglia-mediated excitotoxicity (Tikka and Koistinaho, 2001). However, these results have not been replicated by other groups (Smith et al., 2003). While activation of microglia may lead to neuronal dysfunction, it may also have a neuroprotective role by releasing growth factors (Streit, 2002). Pilot studies using
minocycline in human symptomatic HD patients are already underway (Huntington Study Group, 2004). Our findings of early and widespread microglial activation lend support to extending these trials to PGCs, particularly if the results are positive in the symptomatic subjects.

Putative neuroprotective agents are more likely to be effective if applied at the presymptomatic stage since the pathogenic process in HD commences years before clinical manifestation (Paulsen et al., 2006). However, there are inherent difficulties when designing such studies involving PGCs. The ‘gold standard’ primary outcome in these studies is likely to be the time to disease onset. Currently available data (Langbehn et al., 2004) do not accurately predict an individual HD PGC’s age of onset, particularly for those with smaller CAG repeat expansions. Many PGCs in these trials will not develop symptomatic HD for years, resulting in the need to recruit a large number of subjects and to follow them up for prolonged periods before a statistically meaningful outcome can be measured.

A biomarker which is ‘objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes or pharmacologic responses to intervention’ (Downing, 2000) could circumvent these potential issues by providing a surrogate primary outcome measure (Biglan and Halmagyi, 2006).

There is currently no proven biomarker available for HD. RAC PET, while it reflects underlying striatal dysfunction in HD patients and PGCs (Antonini et al., 1996), does not predict the likelihood of disease onset, nor does it correlate longitudinally with disease progression (Pavese et al., 2003). Striatal MRI volumetry predicts disease onset in PGCs (Aylward et al., 2004), but it is less sensitive than RAC and FDG PET in detecting subclinical abnormalities (van Oostrom et al., 2005). PK PET detects subclinical disease activity and reflects an underlying pathogenic process. In addition, higher baseline striatal PK binding in PGCs correlated with a shorter predicted time to symptomatic onset of HD, albeit only after excluding a creatine exposed

![Figure showing significant correlation between striatal $^{11}$C-(R)-PK11195 binding of Huntington’s disease gene carriers and their predicted 5-year probability of developing Huntington’s disease ($r = 0.71$, $P = 0.02$) after excluding a subject (open circle) who had been taking high dose creatine, and was an outlier in this correlation.](image)
outlier. Therefore, PK PET potentially provides a useful biomarker in HD PGCs if therapies targeting this pathway are considered. However, further longitudinal clinical series with PK PET characterization of HD PGCs are needed before such an approach can be validated. Such a longitudinal study will also ascertain whether PK PET improves predictability of symptomatic onset in PGCs, particularly for those with shorter CAG repeat expansions. If it does, PK PET will provide important prognostic information for individual HD PGCs, and may also help select subjects who are nearer disease onset to increase statistical power in neuroprotective studies.

The prevalence of reduced striatal RAC binding was similar to that of raised striatal PK binding in PGCs. The cross-sectional design of our study did not allow us to determine whether striatal neuronal dysfunction precedes microglial activation, or vice versa. Subject 4, who had the lowest 5-year probability of developing HD among the PGCs, had normal PK and RAC PET. Subject 2, who had the second-lowest probability of disease onset, and subject 8 had abnormally low striatal RAC BP but normal PK BP. Subject 8 had been taking creatine for several years. While creatine is not known to interfere with PK binding or influence microglial activation, it has been reported to have potential for neuroprotection and for delaying disease onset (Tabrizi et al., 2005). This, along with the data shown in Fig. 5 where she appears to be a clear outlier, justified a re-analysis of the data without this subject. Her striatal PK binding was much lower than expected for the group given her probability of disease onset, raising an interesting question regarding creatine’s potential to suppress microglial activation. Removing this subject strengthened the correlation between levels of striatal PK uptake and predicted symptomatic onset but did not alter the significance of the other PET findings. Subject 1, the homozygous HD gene carrier, showed significant PK binding but normal RAC binding. We hypothesize that this may be due to the loss of the putative neuroprotective function of the wild-type huntingtin in homozygous carriers (Squitieri et al., 2003), resulting in activation of caspase-8 and apoptosis (Gervais et al., 2002), which then activates microglia to phagocytose the apoptotic cells.

The finding of widespread increases in brainstem, basal ganglia and cortex (particularly frontal) PK binding in the HD PGCs reflects early involvement of these structures by HD pathology, as has been shown by other imaging studies (Rosas et al., 2003). Other regions such as the occipital cortex, which are known to be affected in HD (Halliday et al., 1998), have not been shown to be affected at this early presymptomatic stage. Thus the increased PK binding here may simply reflect remote trans-synaptic activation of microglia away from the site of pathology (Yeterian and Van Hoesen, 1978), a known characteristic of activated microglia (Banati, 2002; Kreutzberg, 1996). The pattern of activation seen in HD bears some similarity to the patterns reported in movement disorders such as Parkinson’s disease (Gerhard et al., 2006a) and corticobasal degeneration (Gerhard et al., 2006b). Microglial activation is a non-specific response to both local and distant neuronal insults in the central nervous system and, while PK PET provides a marker of underlying disease activity, it is not a specific diagnostic marker.

The difference in age between the HD PGCs and PK controls is unlikely to account for the results shown here. Cagnin et al. (2001) reported that the only brain region which showed significant age-related changes in PK binding is the thalamus, where microglial activation increases with age. In our study, the HD PGCs had higher PK binding in the thalamus than the controls despite being younger. Using a group of age-matched controls could have made the difference in the thalamus binding larger, but would have been unlikely to alter the conclusions of this study.

In summary, our study has demonstrated early and widespread microglial activation in presymptomatic HD gene carriers which was associated with subclinical striatal neuronal loss of dopamine D2 receptor binding, indicating a potential role of activated microglia in HD pathogenesis. This finding has potential therapeutic implication supporting the trialing of agents that inhibit microglial activation in PGCs to try and delay their onset of clinical symptoms. PK PET may also provide prognostic information in HD PGCs and have value as a biomarker in neuroprotective studies though further studies are required to confirm this.

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