The distribution of structural neuropathology in pre-clinical Huntington’s disease

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Summary
Putative neuroprotective agents in Huntington’s disease may have particular application before brain pathology becomes manifest clinically. If these agents were to be tested in clinical trials, a reliable marker of the burden and rate of progression of pathological change in the pre-clinical group would be needed. The present study investigates whether the Huntington’s disease genotype is associated with regional differences in brain structure, particularly differences that could not be predicted from clinical or neuropsychological assessment. A secondary aim is to seek indirect evidence of pathological progression in the form of changes in local tissue volume with age, specific to the Huntington’s disease genotype. Formal motor examination, neuropsychological assessment, and T1-weighted cerebral MRI were performed in 34 subjects who had undergone predictive genetic testing for Huntington’s disease. Clinical and cognitive testing were performed blinded to gene status. A linear discriminant analysis revealed the combination of test scores (the ‘optimal clinical score’) which best differentiated 18 subjects carrying the Huntington’s disease gene mutation (the ‘gene-positive’ group). Voxel-based morphometry (VBM) was used to identify regions of significant main effect of Huntington’s disease gene status on grey and white matter volume and regions of significant interaction of gene status with age. In the gene-positive group, there was significant reduction in grey matter volume in the left striatum, bilateral insula, dorsal midbrain and bilateral intra-parietal sulcus relative to ‘gene-negative’ controls. There was a significant reduction of periventricular white matter volume with age bilaterally in the gene-positive relative to the gene-negative group. Changes remained significant when controlled for differences in optimal clinical score between subjects. This study provides evidence of distributed grey matter pathology and progressive white matter atrophy with age before clinical onset of Huntington’s disease. This suggests that VBM may be useful in monitoring cross-sectional and longitudinal changes in brain structure in pre-clinical Huntington’s disease and for determining the efficacy of neuroprotective agents.

Keywords: Huntington’s disease; VBM; MRI; striatum; asymmetry

Abbreviations: MNI = Montreal Neurological Institute; UHDRS = United Huntington’s Disease Rating Scale; VBM = voxel-based morphometry

Introduction
Twenty-five years ago, Lange and colleagues (Lange et al., 1976) lamented that ‘descriptive neuropathology based on classical methods has made only minor contributions’ to the understanding of Huntington’s disease. Most descriptions, it was felt, were ‘limited too exclusively to damage to the striatum’. While valuable for consistent classification, the introduction of a grading system of post-mortem neuropathology in Huntington’s disease based almost entirely on two standard coronal sections through the head of caudate and putamen (Vonsattel et al., 1985) has tended to perpetuate the belief that regional pathology outside the striatum is insignificant.

Since the discovery that the Huntington’s disease phenotype results from an expanded CAG trinucleotide repeat within the IT15 gene located on chromosome 4 (Huntington’s Disease Collaborative Research Group, 1993), progress towards an understanding of the pathological process has been much more rapid. The mutant gene product huntingtin is
expressed throughout the brain in Huntington’s disease (Aronin et al., 1995), although there is some evidence that polyglutamine load differs between tissues due to differential instability of the CAG mutation (Kennedy and Shelbourne, 2000). Huntingtin fragments aggregate into neuronal intranuclear inclusions (Davies et al., 1997; DiFiglia et al., 1997). Neuronal intranuclear inclusions are widely distributed in the cortex (Becher et al., 1998). However, there are relatively few neuronal intranuclear inclusions in the striatum (Gutekunst et al., 1999), where the classical histopathological changes of Huntington’s disease (neuronal loss and reactive astrocytosis) are emphasized (Vonsattel et al., 1985; Vonsattel and DiFiglia, 1998).

Perhaps then the striatum is more vulnerable to the effects of accumulating mutant huntingtin than other tissues. The abnormal gene product may unmask regional developmental susceptibility to normally non-lethal stressors (Mehler and Gokhan, 2000) such as excitotoxins. Impairment of mitochondrial function in the striatum, evident as decreased cerebral glucose metabolism on PET (Kuhl et al., 1982; Mazziotta et al., 1987) and as elevated lactate on magnetic resonance spectroscopy (Jenkins et al., 1998), might allow glutamatergic cortical afferents to trigger oxidative stress and ultimately neuronal death (Petersen et al., 1999; Calabresi et al., 2000). A recent serial PET study of the caudate nucleus in Huntington’s disease implied a ‘one-hit model’ for cell death on a background of unchanging genetic vulnerability (Clarke et al., 2000) rather than cumulative damage to striatal cells over time.

There have been several attempts to correlate the pattern of neuronal loss within the striatum with clinical stage of Huntington’s disease. Involuntary movement disorder and ‘frontal’ dementia (Watkins et al., 2000) are generally attributed to interruption of motor and cognitive basal ganglia loops (Alexander et al., 1986) in the putamen and caudate, respectively, particularly the loss of enkephalin-containing medium spiny projection neurones in the matrix compartment (Reiner et al., 1988; Albin et al., 1991) that are part of the ‘indirect’ pathway (Albin et al., 1989). Atrophy of other relays in this pathway, including globus pallidus externa (Vonsattel et al., 1985; Halliday et al., 1998) and subthalamic nucleus (Lange et al., 1976) is thought to be secondary to the loss of striatal neurones. Loss of projection neurones of the indirect pathway (measured with PET as decreased striatal dopamine D2 receptor binding) correlates with performance in cognitive tasks requiring serial ordering of responses (Lawrence et al., 1998).

Vonsattel and DiFiglia (1998) suggested that ‘with the progression of the disease, the striatal degeneration appears to move in a caudo-rostral direction and in a dorso-ventral/meio-lateral direction’. However, these observations were based on post-mortem cases with long-established Huntington’s disease and the stage of ’progression’ could only be assessed by a ‘disability rating’ shortly before death (Vonsattel et al., 1985). Loss of basal ganglia volume has been reported in pre-clinical cases (Aylward et al., 1994; Harris et al., 1999), suggesting that striatal neuronal loss occurs early in the clinical course. However, progressive motor and cognitive impairment after clinical onset, as measured by the Quantified Neurologic Examination and neuropsychological test batteries, are perhaps better explained by loss of white matter volume (Aylward et al., 1997, 1998), reflected in enlargement of the frontal horns of the lateral ventricles (de la Monte et al., 1988; Harris et al., 1999). Although striatal volume declines over time at a greater rate in younger affected subjects (Aylward et al., 1997; de la Monte et al., 1988), it seems that it is loss of white matter volume—particularly at coronal levels including the lateral ventricles—that best predicts duration of chorea (Halliday et al., 1998).

Several focal cognitive deficits and focal neurological signs in early Huntington’s disease are difficult to explain purely on the basis of striatal dysfunction. First, there is a specific deficit in the recognition of the facial expression of disgust in established Huntington’s disease (Sprengelmeyer et al., 1996) and pre-clinical carriers of the Huntington’s disease gene mutation (Gray et al., 1997). This might reflect a more general limitation of the experience of disgust, and may also relate to the specific loss of olfactory experience in Huntington’s disease (Nordin et al., 1995; Moberg and Doty, 1997; Hamilton et al., 1999). Attempts have been made to explain these deficits by striatal interruption of a ventral basal ganglia loop with inputs from orbitofrontal cortex and outputs via the medio-dorsal thalamus (Alexander et al., 1986; Gray et al., 1997; Lawrence et al., 2000). However, recent imaging studies suggest that focal damage to insular cortex might be a more parsimonious explanation. Bilateral insular activation upon presentation of ‘disgust’ faces can be measured in normals with functional MRI (Phillips et al., 1997). Moreover, a focal lesion of the left insula in the otherwise normal brain results in an identical deficit in disgust recognition to Huntington’s disease (Calder et al., 2000).

Secondly, there are multiple lines of evidence for midbrain involvement in Huntington’s disease. There is pathological evidence of loss of amineergic neurones in the locus coeruleus (Zweig et al., 1992), and a decrease in striatal monoaminergic terminals identified with PET (Bohnen et al., 2000). Studies of oculomotor physiology reveal an increased latency of the pupillary light reflex attributed to involvement of the pretectal nucleus, Edinger–Westphal nucleus and intercalated neurones in the rostral midbrain (Den Heijer et al., 1988). There is slowing of oculomotor saccades in the vertical plane (Leigh et al., 1983)—even early in disease (Kirkwood et al., 2000)—possibly related to loss of burst neurones in the rostral interstitial nucleus of the medial longitudinal fasciculus (Leigh et al., 1985) or perhaps in the nucleus pontis centralis caudalis of thepons (Koeppen et al., 1989). Diffuse intranuclear accumulation of polyglutamine stretches is prominent in the dorsal brainstem at all levels (Yamada et al., 2000).

The oculomotor deficit in Huntington’s disease is not limited to saccadic slowing. There is an increased latency of
saccadic initiation, particularly apparent on an anti-saccade task, where errors are also frequent (Lasker and Zee, 1997). Perhaps this finding can be related to converging evidence for a third site of focal pathology in the parietal lobe in Huntington’s disease. Recent functional imaging data suggest that anti-saccades activate an inferior parietal network centred in the intra-parietal sulcus involved in covert attention (Connolly et al., 2000). Notably, attentional shifts are impaired in early Huntington’s disease (Lawrence et al., 1996). However, this area is also activated more generally in voluntary saccades (Müri et al., 1996; Luna et al., 1998) suggesting a common location of systems directing attention and eye movements (Corbetta, 1998). Alternatively, perhaps the intra-parietal sulcus carries out the spatial transformation inherent in the anti-saccade task (Connolly et al., 2000). Visuospatial deficits are also noted in early Huntington’s disease (Lawrence et al., 2000), particularly an inability to perform spatial transformations on fragmented figures (Gómez-Tortosa et al., 1996). Considerations such as these prompted a pathological study of the adjoining angular gyrus, which revealed striking neuronal loss (Macdonald et al., 1997).

If Huntington’s disease is indeed a ‘polytopic process’ (Lange et al., 1976) as these studies suggest, then any imaging analysis technique limited to a small number of structures and metrics will fail to characterize the distribution of pathology appropriately. In the present study, we used voxel-based morphometry (VBM) (Ashburner and Friston, 2000) to locate regionally specific differences in relative grey and white matter volumes within and between gene status groups previously identified by predictive testing. In contrast to techniques relying on inspection and manual demarcation of structures, VBM is unbiased toward particular regions. All of the stages of image processing are automated, and the software is widely available (SPM99, Wellcome Department of Imaging Neuroscience, London, UK: http://www.fil.ion.ucl.ac.uk/spm/). Statistical analysis is within a flexible General Linear Model framework, which allows effects to be partitioned between several explanatory variables in a principled manner. In particular, the framework allowed us to address questions of relevance to future clinical trials:

(i) Whether changes in structural imaging in early Huntington’s disease can be predicted from detailed clinical and neuropsychological assessment;
(ii) Whether there are changes in local tissue volume that have a non-linear relationship to CAG repeat number; and
(iii) Whether there are progressive structural changes over time, specific to the Huntington’s disease genotype.

Subjects
The disease and control groups were selected from a population who had undergone predictive genetic testing through the New South Wales (Australia) genetics service. According to standard guidelines (International Huntington Association and the World Federation of Neurology Research Group on Huntington’s Chorea, 1994), all predictive tests were preceded by a neurological assessment by a specialist neurologist who had concluded that there was no clinical evidence of Huntington’s disease. The study was publicized by the NSW Huntington’s Disease Association and by genetic service’s social worker (F.R.), who had remained in contact with many affected families since predictive testing. Subjects chosen were at 50% risk of Huntington’s disease prior to genetic testing on the basis of a single affected parent, and remained asymptomatic at the time of the current study. The criteria for exclusion from the study included symptoms suggesting Huntington’s disease onset, history of other neurological illness or uncontrolled psychiatric disorder, alcohol intake of >4 units daily, or head trauma causing loss of consciousness. Clinical signs suggestive of Huntington’s disease onset since predictive genetic testing were not exclusion criteria. Of 37 subjects selected initially, two were excluded due to dyslexia (which had preceded genetic testing), and one due to excess alcohol intake. Of the remaining 34 subjects, 16 had <36 repeats in the longest allele (mean repeat number 20.38, SD ± 3.70) and were classified as gene-negative (Rubinsztein et al., 1996; McNeil et al., 1997). Twelve subjects had ≥40 repeats and five had between 36 and 39 repeats (at risk of Huntington’s disease but with reduced penetrance). In one subject, the exact number of repeats could not be ascertained for technical reasons but was >35. For the purposes of analysis, all those subjects with ≥36 repeats were classified as gene-positive (mean repeat number of longest allele 41.06, SD ± 2.70). In total, there were 18 subjects in the gene-positive group. The range of repeat lengths within this group (38–46) is consistent with other studies of adult predictive test populations (Aylward et al., 1996; Harris et al., 1999). The distribution of repeat lengths in 33 subjects is plotted in Fig. 1. Age, sex and handedness characteristics of the two gene status groups, as well as estimated parental age of onset and inheritance pattern, are given in Table 1. Although age and sex were not exactly matched across groups, the potential confounding effects were controlled for in both clinical and imaging analyses (see below).

Clinical
Clinical examination
Each subject underwent formal motor examination by one of three neurologists (M.J.T., E.M., N.M.) experienced in the clinical assessment of Huntington’s disease who were blinded to genetic status. In addition to this bedside examination, subjects underwent more rigorous assessment for subtle

Methods
The Western Sydney Area Health Service ethics committee approved the project. Informed consent was obtained from all participants according to the Declaration of Helsinki (International Committee of Medical Journal Editors, 1991).
chorea given the importance of the identification of this sign in the diagnosis of early Huntington’s disease (McCusker et al., 2000). For this assessment, a video recording of foot movement was made while subjects were stressed using a mental distraction task. Two clinicians (E.M., N.M.) independently assessed the video for chorea without being able to identify the subject featured. Scores from the two observers were then combined. On the basis of all of these assessments, subjects were scored according to the motor component of the United Huntington’s Disease Rating Scale (UHDRS). This is a standardized test of motor function that includes quantification of chorea, bradykinesia, rigidity, motor impersistence, motor sequencing, ocular movements and gait. Scores range from 0–128, higher scores representing greater motor impairment. When applied to the whole spectrum of gene-positive subjects, the scale is internally consistent, has interobserver reliability and is useful for tracking disease progression (Huntington Study Group, 1996).

Cognitive function was assessed according to the UHDRS specifications by a phonetic verbal fluency test, symbol digit modalities test, Stroop word, Stroop colour and Stroop colour-word test (Huntington Study Group, 1996). Results from the six scores (one motor and five cognitive) were expected to be closely correlated.

Clinical score analysis

Matlab5.3 (Mathworks, Natick, MA, USA) was the platform for all clinical and imaging analyses. Using a General Linear Model, the variance in each score (‘data variable’) was partitioned into: variance attributable to Huntington’s disease gene status; variance attributable to confounding effects of age, sex and education; and residual variance. Subtracting the rank of the design matrix from the number of observations (one for each subject) left 29 degrees of freedom (DF). The least squares solution of such a model is a vector of parameter estimates. A contrast of gene-positive and gene-negative parameter estimates revealed the difference in score attributable to Huntington’s disease gene status (the ‘main effect’) for each data variable, controlled for the confounding effects mentioned above. The variance of the contrast was estimated using the residual variance, allowing a two-tailed t-test (on 29 DF) for the significance of the main effect.

In order to test the hypothesis that structural changes in Huntington’s disease always have a clinical correlate evident on thorough clinical testing, it was necessary to generate a single ‘optimal clinical score’ that would best discriminate the gene-positive and gene-negative subjects in our sample. The optimal clinical score would then be entered as a nuisance covariate in the imaging analysis to determine whether there would remain a significant main effect of Huntington’s disease gene status alone. The optimal clinical score would also be a principled measure of clinical stage, which might allow stratification within the gene-positive group.

The optimal clinical score was chosen to maximize differences between gene-positive and gene-negative groups, while minimizing the variability within groups. Formally, the

Table 1 Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>Number (n)</th>
<th>Mean age (SD) (years)</th>
<th>Sex ratio (male : female)</th>
<th>Handedness (left : right)</th>
<th>Inheritance (paternal : maternal)</th>
<th>Parental age of onset (SD) (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All subjects</td>
<td>34</td>
<td>40.97 (11.91)</td>
<td>19 : 15</td>
<td>1 : 4</td>
<td>1 : 1</td>
<td>46.67 (12.93)</td>
</tr>
<tr>
<td>Gene-negative</td>
<td>16</td>
<td>38.04 (11.43)</td>
<td>5 : 3</td>
<td>1 : 6**</td>
<td>4 : 3*</td>
<td>44.77 (13.19)*</td>
</tr>
<tr>
<td>Gene-positive</td>
<td>18</td>
<td>43.58 (12.02)</td>
<td>1 : 1</td>
<td>1 : 3**</td>
<td>4 : 5*</td>
<td>48.43 (12.93)**</td>
</tr>
</tbody>
</table>

*Missing data for 1 subject; **2 subjects ambidextrous; ***missing data for two subjects, two others with gene-positive but as yet asymptomatic parent.
optimal clinical score was defined as that linear combination of mean-corrected motor and cognitive variates that maximized the component of the variance attributable to gene status, while minimizing residual variance. This combination was found by linear discriminant analysis (Healy, 1986). First, a General Linear Model (similar to that described above) was solved for the entire data matrix. Secondly, a fitted data matrix was generated for the effect of gene status. Covariance matrices of the fitted data and residuals were then entered into a generalized eigenvalue equation. The generalized eigenvector with the highest eigenvalue was a vector of weights, which when multiplied on the left by the original data matrix generated the optimal clinical score.

**Imaging**

**Image acquisition**

Whole brain structural MRI was performed on the same 1.5 Tesla scanner (Siemens Magnetom Vision Plus, Erlangen, Germany) for all subjects. Images were acquired in the sagittal plane, with isotropic 1 mm³ voxel size. A high-resolution 3D T₁-weighted MP-RAGE sequence was chosen with the following parameters: T₁/TE (echo time): 9.7/4, flip angle 15°, matrix 256 × 256, FOV (field of view) 250 mm².

**Voxel-based morphometry (VBM)**

VBM is a fully automated, unbiased, whole brain morphometric technique that detects regional structural changes on a voxel-wise basis between groups of subjects. An optimized VBM protocol (Good et al., 2001a) was employed within SPM99. Generally this involves a number of fully automated pre-processing steps including extraction of brain, spatial normalization into stereotactic space, segmentation into grey, white matter and CSF compartments, modulation for volume changes induced by spatial normalization and, finally, smoothing with a 10 mm FWHM (full width half maximum) isotropic Gaussian kernel. These various pre-processing steps have been described in detail elsewhere (Ashburner and Friston, 2000; Good et al., 2001a, b). After smoothing, each voxel represents the local average amount of grey (or white) matter in the surrounding region, the size of which is defined by the smoothing kernel. We used customized grey and white matter templates for spatial normalization, created from the study group in order to provide templates that were age, sex, disease and scanner matched to the group. The rationale for customized templates has been discussed previously (Ashburner and Friston, 2000; Good et al., 2001a, b).

**Statistical inference**

The smoothed grey and white matter and CSF segments were analysed using SPM99 employing the framework of the General Linear Model. Regionally specific structural differences were assessed statistically using two-tailed t-tests for increases or decreases in grey (or white) matter.

Several different General Linear Models were used to address different questions. In each case, a design matrix was constructed with one or more conditions or covariates of interest and several nuisance covariates as columns, and with rows specifying subject-specific levels of each explanatory variable. The nuisance covariates modelled potential confounding effects on the data, including age, sex or global voxel intensity. Covariates were mean-corrected, a column of ones modelling the mean of the data across subjects. The choice of age and sex as nuisance covariates was based on previous studies demonstrating regional anatomical differences in normals (Pfefferbaum et al., 1994; Xu et al., 2000; Good et al., 2001a, b). We chose not to include parental age of onset as a nuisance covariate, since subject recollection was often vague and estimates in most cases could not be corroborated with supportive evidence. We did not include inheritance (paternal or maternal) since there is evidence that anticipation of the Huntington’s disease phenotype with paternal inheritance is directly attributable to repeat expansion (Kremer et al., 1995; Ranen et al., 1995) explicitly modelled in the analyses discussed.
below. A conceptual diagram of a simple design matrix is shown in Fig. 2.

For each of the grey, white and CSF tissue segmentations, the pre-processed data yielded 34 observations (one for each subject) at each voxel. For each tissue, the General Linear Model was solved at each voxel to generate a vector of parameter estimates (see discussion of clinical score analysis above). Inferences were based on contrasts of parameter estimates, chosen to highlight main effects or interactions of interest, using two-tailed \( t \)-statistics. \( P \)-values were corrected for multiple comparisons across the whole brain, according to the theory of Gaussian fields. When a prior hypothesis about regional pathology existed (see Introduction), correction was limited to a small volume around this location of a size appropriate to the specificity of the hypothesis (the radius of a sphere used for the small volume correction is given in Table 3). In most cases, reported results satisfy a corrected significance threshold of \( P < 0.05 \). Trends of lesser significance are reported when they are considered to be of particular interest, but these results require confirmation in future work.

The simplest analyses tested for the main effect of gene status (Fig. 2). Here, genetic predisposition was treated as a categorical variable: variability of genotype within the gene positive group was neglected. For grey and white matter, a contrast tested for regions of significant reduction in tissue volume in gene-positives. In the CSF segmentation, the opposite contrast tested for a reciprocal increase in CSF volume. To assess whether significant local effects reflected changes in global tissue quantities, analyses were performed both with and without global voxel intensity as a nuisance covariate. Analyses were also performed with and without an optimal clinical score nuisance covariate to test the pre-clinical pathology hypothesis.

The potential dependence of brain structure on differences in repeat number within the gene-positive and gene-negative categories was investigated with an extension of the basic design to include longest allele repeat length covariates for each gene status condition, corrected for condition mean. The shorter allele repeat length was included as an extra nuisance covariate. Contrasts tested for greater loss of grey or white matter tissue volume with increasing repeats in the gene-

### Table 2 Average clinical scores

<table>
<thead>
<tr>
<th></th>
<th>UHDRS motor</th>
<th>Verbal fluency</th>
<th>Symbol digit</th>
<th>Stroop colour</th>
<th>Stroop word</th>
<th>Stroop colour-word</th>
<th>Optimal clinical score</th>
</tr>
</thead>
<tbody>
<tr>
<td>All subjects</td>
<td>1.24</td>
<td>40.03</td>
<td>52.06</td>
<td>76.62</td>
<td>100.85</td>
<td>47.32</td>
<td>0</td>
</tr>
<tr>
<td>Gene-negative*</td>
<td>−1.11</td>
<td>+4.16</td>
<td>+4.07</td>
<td>+4.13</td>
<td>+10.40</td>
<td>+4.18</td>
<td>+0.16</td>
</tr>
<tr>
<td>Gene-positive*</td>
<td>+0.99</td>
<td>−3.70</td>
<td>−3.61</td>
<td>−3.67</td>
<td>−9.24</td>
<td>−3.71</td>
<td>−0.14</td>
</tr>
<tr>
<td>Weights</td>
<td>−0.0423</td>
<td>+0.0007</td>
<td>+0.0029</td>
<td>−0.014</td>
<td>−0.0132</td>
<td>+0.0034</td>
<td></td>
</tr>
</tbody>
</table>

*Subgroup averages are relative to group averages for each score.

### Table 3 Imaging results

<table>
<thead>
<tr>
<th>Segmentation, MNI coordinates (mm)</th>
<th>Z score</th>
<th>( P ) corrected (SVC, mm)*</th>
<th>Region</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>x</td>
<td>y</td>
<td>z</td>
</tr>
<tr>
<td>Gene status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grey matter</td>
<td>−12</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>−15</td>
<td>9</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>−22</td>
<td>9</td>
<td>0</td>
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<td>−12</td>
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</tr>
<tr>
<td></td>
<td>−9</td>
<td>33</td>
<td>−24</td>
</tr>
<tr>
<td>Age × gene status interaction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White matter</td>
<td>−12</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td>Age, gene-positives</td>
<td>−22</td>
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<td>Age, gene-negatives</td>
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<tr>
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<td>−18</td>
<td>14</td>
</tr>
<tr>
<td>Repeat × gene status interaction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grey matter</td>
<td>39</td>
<td>10</td>
<td>21</td>
</tr>
</tbody>
</table>

*The small volume correction (SVC) indicated is the radius of a sphere; where no SVC is indicated, a whole brain correction has been used.
positive relative to the gene-negative group. Classification into gene status categories was also derived from the repeat number, so this condition-by-covariate interaction effectively tested for a non-linear parametric effect of genetic load on brain structure.

Although this study could not investigate progression over time within subjects, age might reflect the duration of the pathological process between gene-positive subjects. Regional loss in grey and white matter volume with age has been demonstrated in normal subjects (Good et al., 2001a), so it was necessary to test for an age-by-condition interaction. The design matrix in this analysis was similar to the repeats analysis described above, with age covariates for each gene status condition, and with sex and global voxel intensities as nuisance variables.

In addition to the repeat and age interaction designs mentioned above, we also analysed gene-positive and gene-negative groups separately in analyses that included both age and repeat number as covariates of interest.

Results

Clinical score

Mean UHDRS motor and cognitive scores for the gene-positive and negative groups are shown in Table 2. When controlled for the confounding effects of age, sex and education, the Huntington’s disease genotype was associated with a significantly higher UHDRS motor score \( P < 0.016 \), Fig. 3), and a significantly lower Stroop word score \( P < 0.009 \), Fig. 3). There was a non-significant trend toward lower scores attributable to the Huntington’s disease genotype on the other cognitive tests (Fig. 3). The optimal clinical score for each subject was generated by linear combination of the six scores according to the weights in Table 2.

Effect of gene status on grey matter volume

The distribution of grey matter volume loss in Huntington’s disease gene-positive subjects is depicted in Fig. 4, rendered on the mean of normalized grey matter images from all 34 subjects. Local maxima for the main effect of gene status are tabulated in Table 3. Small volume corrections have been used in the case of a regional prior hypothesis. Significant grey matter volume reduction was identified in the left head and body of caudate, putamen and globus pallidus (Fig. 4A, I and K). Ventrally, this was continuous with volume loss in the nucleus accumbens and orbito-frontal cortex, particularly the left olfactory sulcus (Fig. 4B). On the right, volume loss in the caudate nucleus reached only borderline significance (Fig. 4H and L). There was loss of grey matter volume in the tectum of the midbrain (Fig. 4B, C, F and K), on the left extending into the medio-dorsal thalamic nucleus. Volume loss in the dorsal medulla and pons (Fig. 4C and J) did not survive whole brain correction (in the absence of a specific prior hypothesis).

Cortically, there was bilateral grey matter loss in the posterior insula, on the left extending ventro-medially into the amygdala (Fig. 4G and K). There was bilateral volume loss in the posterior intra-parietal sulcus, reaching corrected significance on the right (Fig. 4D, E and L). A white matter gene status analysis revealed a reciprocal significant increase in

![Fig. 3 Differences in score attributable to the Huntington’s disease genotype for the UHDRS motor and five cognitive tests controlled for the confounding effects of age, sex and education. Contrasts of parameter estimates for gene-positive minus gene-negative conditions are plotted, with 95% confidence intervals. Gene-positive subjects tend to have higher motor and lower cognitive scores.](image)
white matter volume in gene-positive subjects beneath the right intra-parietal sulcus (not shown). There were no other significant changes in the white matter or CSF gene status analyses.

When the grey matter gene status analysis was performed without global voxel intensity as a nuisance covariate, the distribution and magnitude of the effects remained unchanged. This suggested that local effects did not reflect changes in global grey matter volume. When the optimal clinical score was included as a nuisance covariate, grey matter volume loss was similarly distributed, and remained significant in the head of the left caudate nucleus (Fig. 7 and Table 3).

Effect of CAG repeat number
A significant interaction of CAG repeat number with gene status condition was found only in the right inferior frontal sulcus (Fig. 6); this survived whole brain correction (Table 3). This reflected a decrease in local grey matter volume (and a reciprocal increase in white matter volume) with increasing repeats in the gene-positive group, but also an increase in volume in the gene-negative group. There was no interaction in regions identified as significant in the gene status analysis.

Discussion
The principal regional prior hypothesis in this study was that caudate and putaminal volume would be reduced in subjects carrying the Huntington’s disease triplet repeat expansion relative to controls of normal genotype. While this was confirmed, the asymmetry of the volume loss was unexpected. Unilateral clinical presentations of Huntington’s disease have not been reported. A previous magnetic
resonance spectroscopy finding of higher lactate levels in left relative to right striatum was attributed to increased motor activation in the dominant hemisphere, and particularly increased glutamatergic synaptic input to striatal cells (Jenkins et al., 1998). Even if impairment in mitochondrial function were symmetric, differential oxidative stress might then lead to greater neuronal loss in the left striatum. In a murine model of Huntington’s disease, manipulation of sensory input and motor activity does indeed alter striatal volume (van Dellen et al., 2000). In our study, the majority of subjects were right-handed (Table 1). Unfortunately, numbers were insufficient to detect a different pattern of volume loss in left-handed subjects.

Fig. 5 Statistical parametric map of the interaction of age and gene status on local white matter volume, rendered on the mean normalized white matter image. This contrast identifies regions of loss of white matter with age in gene-positive relative to gene-negative subjects. The threshold for display is $P < 0.005$ (uncorrected). Z-scores are indicated by colour temperature according to the scale (D). MNI coordinates (mm) of section (A) $x = -23$, (B) $y = 26$, (C) $z = 23$.

Fig. 6 Statistical parametric map of the interaction of repeat length and gene status on local grey matter volume, rendered on the mean normalized grey matter image. This contrast identifies regions of greater loss of grey matter tissue volume with increasing repeats in the gene-positive relative to the gene-negative group. This condition-by-covariate interaction effectively tests for a non-linear parametric effect of genetic load on brain structure. The threshold for display is $P < 0.005$ (uncorrected). Z-scores are indicated by colour temperature according to the scale (D). MNI coordinates (mm) of section (A) $x = 39$, (B) $y = 10$, (C) $z = 21$.

Fig. 7 Regions of significant main effect of Huntington’s disease gene status on local grey matter volume, rendered on the mean normalized grey matter image. The threshold for display is $P < 0.005$ (uncorrected). Regions marked in red are those identified in the simple gene status analysis as in Fig. 4. Regions marked in yellow remain significant when the optimal clinical score is included as a nuisance covariate in the gene status analysis. The latter demonstrates that within the gene-positive group there is significant structural neuropathology, which has no clinical correlate apparent on thorough testing. MNI coordinates (mm) of section (A) $x = 27$, (B) $y = 12$, (C) $z = -3$. 

Huntington’s disease neuropathology
Another surprising feature of the striatal volume loss was that it appeared to be concentrated rostrally and ventrally—thus confounding the predictions of Vonsattel and colleagues (Vonsattel et al., 1985). A previous histopathological study of a pre-clinical case had identified degenerative changes only in the dorsal striatum (Gutekunst et al., 1999), thus supporting the idea that there is a dorsal to ventral gradient of neuronal loss with advancing disease. The reason for this discrepancy with our results is unclear. Involvement of ipsilateral orbito-frontal cortex, ventral pallidum and medio-dorsal thalamus (which together with the ventral striatum constitute the orbito-frontal basal ganglia loop) militates against our findings being artefactual. Interruption of this ‘limbic loop’ in the ventral striatum results in impulsivity in rats (Cardinal et al., 2001), mimicking the behavioural disturbance of established Huntington’s disease. The orbito-frontal cortex is regarded as important in decision-making (Bechara et al., 1998; Rogers et al., 1999) and reversal learning (Watkins et al., 2000). However, subjects with early Huntington’s disease seem to be unimpaired in these functions, but instead fail tests of planning such as the Tower of London (Watkins et al., 2000) and make perseverative errors in attentional set switching paradigms (Lawrence et al., 1996). These deficits are usually attributed to malfunction of the dorso-lateral prefrontal cortex or interruption of its efferent connections in the dorsal caudate nucleus. It is notable that grey matter loss was not identified anywhere in the dorso-lateral prefrontal cortex in our study, nor in the premotor cortex or frontal eye fields that provide input to the ‘motor’ and ‘oculomotor’ basal ganglia loops, respectively. If future work is to resolve this apparent conflict between clinical and imaging data, it must correlate regional volumes with differences in scores between subjects. The problem will require more focused clinical testing and larger numbers of subjects, but will be tractable with the VBM approach.

Our three prior hypotheses of regional grey matter loss outside the basal ganglia were all confirmed by the current study. Bilateral volume loss in the insular cortex would seem a plausible substrate for the loss of recognition of the facial expression of disgust in a similar cohort of pre-clinical gene-positive subjects (Gray et al., 1997). These subjects were unimpaired in the recognition of fearful expressions—a function previously attributed to the left amygdala (Morris et al., 1996)—yet in our study, the loss of grey matter in the insula seemed to extend into part of the left amygdala (Fig. 4G). Again, demonstration of a subject-specific clinico-pathological correlation would be of interest for future work.

Gene-positive subjects in the current study had very early Huntington’s disease. Nevertheless, the distribution of regional volume loss at this stage of disease provides an insight into the pathophysiology underlying clinical deficits apparent later in the course. For example, we suggest that involvement of the intra-parietal sulcus and midbrain tectum might be the anatomical substrate for the eye movement abnormality of Huntington’s disease. Delay in initiation of voluntary saccades in Huntington’s disease is more commonly attributed to disruption of descending input to the superior colliculus from the frontal eye fields (Lasker and Zee, 1997). The superior colliculus also receives inhibitory input from the oculomotor basal ganglia loop, disruption of which is thought to underlie an inability to suppress reflexive saccades. An alternative explanation is suggested by the marked loss of midbrain grey matter volume in our study, which may involve the superior colliculus directly. The burst neurones that generate the immediate premotor commands for vertical saccades are located nearby in the rostral interstitial nucleus of the medial longitudinal fasciculus, allowing a single site of midbrain pathology to explain both the initiation deficit and saccadic slowing. Another possibility, suggested by the current study, is that grey matter loss in the parietal eye fields may impair saccadic eye movement, particularly in the context of the anti-saccade task where deficits are most apparent in Huntington’s disease (Lasker and Zee, 1997).

Our finding of significant pathology beyond that which could have been predicted from thorough testing confirms the commonly held belief that the pathological changes of Huntington’s disease develop before clinical onset. The time course of this process remains unknown. One influence on the rate of pathological progression before clinical onset must be the CAG repeat length—greater numbers of repeats resulting in earlier clinical onset (Duyao et al., 1993; Brinkman et al., 1997). However, increasing CAG repeat length has not consistently been associated with reduced striatal volume in previous studies (Sieradzan et al., 1997; Harris et al., 1999). In our study, the only area of significant volume change with increasing repeats specific to the gene-positive group was in the right inferior frontal sulcus (Fig. 6). However, the range of repeat lengths in the gene-positive group was narrow since all had reached adulthood without symptoms. Moreover, the right frontal change partially reflects an increase in local grey matter volume with increasing repeats in gene-negatives, the functional significance of which remains obscure.

There is some evidence that repeat expansion in the Huntington’s disease gene alters regional cerebral development before it results in progressive neurodegeneration. In particular, the cellular composition of the caudate in pre-clinical gene-positive subjects is suggestive of abnormal morphogenesis (Gómez-Tortosa et al., 2001). It seems more likely, though, that the reduction in striatal grey matter volume in the gene-positive condition (identified in the gene status analysis) is the result of progressive neuronal loss. Paradoxically, in the age by gene status interaction, the only structural change with age specific to the gene-positive group was white matter loss around the lateral ventricles, not caudate atrophy. Perhaps the explanation lies in the differing time course of volume change in different tissues. If a PET finding in the Huntington’s disease caudate of constant risk of cell death over a three year period (Kremers et al., 1999; Clarke et al., 2000) can be extrapolated to a lifetime, an exponential decay in cell numbers with age would be
anticipated. In support of this finding, the rate of decline of caudate and basal ganglia volume measured by conventional techniques is greater in younger subjects (Aylward et al., 1997). Once clinical signs are apparent, however, their duration (Halliday et al., 1998) and severity (Harris et al., 1999) are better predicted by white matter atrophy, particularly around the frontal horns. Perhaps then cumulative damage results in an increasing risk of axonal loss in these areas with age. One caveat to be considered here is that our gene-positive group may not be uniform. The predicted age of clinical onset must be greater in older subjects who remain asymptomatic. Our findings of progressive white matter atrophy over time would require confirmation by serial scanning of gene-positive and negative cohorts, with analysis of repeated measures within each subject.

The inclusive approach to volumetric analysis adopted in this study is vindicated by the demonstration of pathological changes in widely distributed cortical and subcortical regions. Conventional morphometric techniques, limited to a small number of structures and metrics, would inevitably have missed many of these areas. Statistical inference using VBM and the General Linear Model is unbiased and objective, such that results are potentially reproducible by different operators. In contrast, the validity of measurement by inspection and manual demarcation is dependent on the skill of the observer and inter-observer reliability. With these conventional methods, the influence of the observer’s pre-conceptions on image interpretation is ill defined. In the VBM approach, regional prior hypotheses can be accommodated in a principled way by limiting the correction for multiple comparisons to voxels within a small volume of interest. Importantly, by introducing appropriate nuisance covariates into the General Linear Model, we have been able to control for potential confounds and to identify several different effects of interest on regional volumes within and between gene status groups. The conventional approach is more restrictive: precise matching of disease and control groups for all potential confounding variables is necessary if group means are to be compared by a simple $t$-test, but this inevitably renders the design inflexible for testing multiple hypotheses or attributing variance among a number of continuous and discrete explanatory variables.

The VBM approach could readily be extended to answer more subtle questions in Huntington’s disease. Imaging of large numbers of pre-clinical subjects may in the future allow stratification into prognostic groups, allowing prediction of the timing of clinical onset in individual cases. Pre-clinical subjects approaching disease onset are likely to be targeted in preliminary trials of neuroprotective agents. Evaluation of such agents by their effect on timing of clinical onset alone would be time-inefficient and insensitive to subtle modulation of the pathological process. Clearly, what is needed is a reliable measure of pathological burden in the pre-clinical group and particularly a measure of the progression of pathology within the time window of a realistic clinical trial (Hughes and Olson, 2001). Our results suggest that VBM may provide a surrogate marker of disease progression before clinical onset.

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