Reduced basal ganglia blood flow and volume in pre-symptomatic, gene-tested persons at-risk for Huntington’s disease

Gordon J. Harris,1,2 Ann Marie Codori,3 Robert F. Lewis,1,2 Eike Schmidt,2 Asheesh Bedi2 and Jason Brandt3

1Department of Radiology, Harvard Medical School, Massachusetts General Hospital, 2Department of Psychiatry, Tufts University, New England Medical Center, Boston and 3The Department of Psychiatry and Behavioral Sciences, The Johns Hopkins Medical Institutions, Baltimore, USA

Correspondence to: Gordon J. Harris, Radiology Computer Aided Diagnostics Laboratory, Gray 2 Room B-285, Massachusetts General Hospital, 55 Fruit Street, Boston, MA 02114, USA
E-mail: harris@helix.mgh.harvard.edu

Summary
The aim of this study was to examine basal ganglia volumes and regional cerebral blood flow in asymptomatic subjects at-risk for Huntington’s disease who had undergone genetic testing. We determined which measures were the best ‘markers’ for the presence of the mutation and for the onset of symptoms. Twenty subjects who were Huntington’s disease gene mutation-positive and 24 Huntington’s disease gene mutation-negative participants, all of whom had a parent with genetically confirmed Huntington’s disease, and were therefore 50% at-risk for inheriting the Huntington’s disease gene mutation, were included in the study. To evaluate basal ganglia structure and function, MRI and single photon emission computed tomography (SPECT) were used. Quantitative measures of regional volumes and relative measures of regional perfusion were calculated. SPECT and MRI scans were co-registered so that MRI anatomy could be used accurately to place SPECT regions. Estimated years-to-onset in the mutation-positive subjects was calculated based on a regression formula that included gene (CAG)n repeat length and parental age of onset. Changes in imaging measures in relation to estimated years-to-onset were assessed. The imaging measure that was most affected in mutation-positive subjects was putamen volume. This was also the measure that correlated most strongly with approaching onset. In subjects ≥7 years from estimated onset age, the putamen volume measures were similar to those of the mutation-negative subjects. However, in subjects ≤6 years from estimated onset age, there were dramatic reductions in putamen volume, resulting in >90% discrimination from both the far-from-onset and the mutation-negative subjects. Caudate volume and bicaudate ratio also showed a significant decline in the close-to-onset subjects, although to a lesser degree than putamen volume reductions. Furthermore, SPECT basal ganglia perfusion deficits were observed in mutation-positive subjects. Imaging markers of neuropathological decline preceding clinical onset are important for assessing the effects of treatments aimed at slowing the course of Huntington’s disease. The current study suggests that quantitative assessment of basal ganglia may provide a means to track early signs of decline in individuals with the Huntington’s disease gene mutation prior to clinical onset.

Keywords: MRI; single photon emission computed tomography (SPECT); Huntington’s disease; pre-symptomatic; gene-testing.

Abbreviations: AC = anterior commissure; MMSE = Mini-Mental State Examination; PC = posterior commissure; PCR = polymerase chain reaction; QNE = Quantified Neurological Examination; rCBF = regional cerebral blood flow; SPECT = single photon emission computed tomography; TE = echo time; TR = repetition time

Introduction
Huntington’s disease is an autosomal dominantly inherited, currently fatal, neurodegenerative disorder. Persons who inherit the abnormal, mutated gene develop progressive motor, cognitive and emotional abnormalities (Bruyn, 1968; Folstein, 1989). In 1993, a large, multicentre effort identified the mutated Huntington’s disease gene, named IT15, which is located on chromosome 4 and contains an expanded and unstable trinucleotide (CAG)n repeat (Huntington’s Disease Collaborative Research Group, 1993). The Huntington’s disease allele has 40 or more CAG repeats, while repeat
lengths of 36–39 indicate a Huntington’s disease allele with reduced penetrance: some subjects with alleles in this range live to old age without developing symptoms, while others develop clinical Huntington’s disease. Normal \( IT15 \) genes that do not lead to development of Huntington’s disease have repeat lengths of 35 or less. Subjects with alleles of 27–35, while normal, have mutable alleles which may produce longer Huntington’s disease alleles in offspring (ACMG/ASHG Huntington’s Disease Genetic Testing Working Group, 1998). The discovery of the Huntington’s disease gene mutation, and the ability to test for its presence, make possible the investigation of brain structure and function, using neuroimaging techniques, in pre-symptomatic subjects known to harbour an \( IT15 \) gene with 40 or more repeats.

The current study used both quantitative MRI and single photon emission computed tomography (SPECT) to measure basal ganglia size and blood flow in offspring of Huntington’s disease patients who have been tested for the Huntington’s disease gene mutation. While abnormalities of brain structure and function in clinical Huntington’s disease cases are well established, it is less clear how early the characteristic brain changes occur. The primary goal of this study was to understand better the course of this devastating illness by examining in vivo brain changes in Huntington’s disease gene mutation-positive subjects relative to estimated years to clinical symptom onset. A further goal was to evaluate cross-sectionally which imaging measures represent the best potential markers of approaching illness onset. Such neuroimaging markers could be used as objective indicators of approaching clinical onset, and could be used further to evaluate future clinical trials of treatments aimed at delaying onset or slowing progression of Huntington’s disease.

The characteristic neuropathological features of Huntington’s disease are neostriatal atrophy and neuronal loss (Roos et al., 1985; Vonsattel et al., 1985; Carrasco and Mukherji, 1986; Hedreen and Folstein, 1995). Neuroimaging studies can be used to quantify striatal atrophy, but were themselves free of the illness. Thus, their genetic
documentation and genetically confirmed Huntington’s disease, All persons in this study were offspring of parents with documented and genetically confirmed Huntington’s disease, but were themselves free of the illness. Thus, their genetic

Several studies have evaluated striatal structure and function in subjects at-risk for Huntington’s disease. Structurally, MRI volumetric differences were observed in basal ganglia between subjects with the Huntington’s disease gene mutation (or with the linked DNA markers of the gene prior to the discovery of the gene) compared with subjects without the gene. The genetically positive subjects were far from being diagnosable, but they had significantly higher Quantified Neurologic Examination (QNE) scores, indicating minor neurological symptoms (Ayward et al., 1994, 1996). These reports demonstrated that basal ganglia structural abnormalities are present even before a clinical diagnosis can be made.

Most prior Huntington’s disease pre-symptomatic functional imaging studies with PET and SPECT evaluated at-risk subjects as a single group. In general, they reported the percentage of at-risk subjects whose caudate activity was below the normal range. Age of at-risk subjects is likely to be an important factor in interpreting these studies (Mazziotta et al., 1987a). In one study, the mean age was 28 years, and caudate hypometabolism was not observed in any of the subjects (Young et al., 1987), while in another study, the mean age was 31 years and 10% of the subjects had decreased caudate function (Ichise et al., 1993). In several studies, the mean age was 35–36 years and caudate hypometabolism was reported in 22–33% of subjects (Mazziotta et al., 1987b; Grafton et al., 1990; Baxter et al., 1992; Kuwert et al., 1993a, b). Thus, in studies with younger subjects, fewer individuals had abnormal caudate function, probably because deficits had not yet developed in many of the subjects. Other researchers evaluated subjects whose groupings were based on testing for linked DNA markers prior to the discovery of the gene itself. They reported that gene linkage testing increased gene group classification considerably (Hayden et al., 1987; Grafton et al., 1990). Furthermore, subjects who tested gene marker-positive evidenced caudate functional decline over time compared with gene marker-negative subjects (Grafton et al., 1992).

We report here the first study of both functional and structural brain changes in subjects who have been tested for the Huntington’s disease gene mutation, but are not yet experiencing clinical symptoms. These imaging measures were examined in relation to predicted years to clinical onset. Neuroimaging measurements may be sensitive and objective markers of approaching disease onset, and thus could be used to monitor illness onset and progression, and potentially be used to monitor the effectiveness of clinical interventions as they are developed.

Methods
Subjects
All persons in this study were offspring of parents with documented and genetically confirmed Huntington’s disease, but were themselves free of the illness. Thus, their genetic
Table 1 Demographic and clinical information by Huntington’s disease gene status

<table>
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<th>Gene-positive (n = 20)</th>
<th>Gene-negative (n = 24)</th>
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<tbody>
<tr>
<td>Number of CAG repeats</td>
<td>43.8 ± 2.4 (40–50)</td>
<td>19.8 ± 4.1 (10–30)</td>
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<tr>
<td>Age (years)</td>
<td>37.4 ± 9.1 (28–68)</td>
<td>39.9 ± 8.7 (20–59)</td>
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<tr>
<td>Gender (M/F)</td>
<td>12/8</td>
<td>11/13</td>
</tr>
<tr>
<td>Education (years)</td>
<td>16.0 ± 2.4 (12–20)</td>
<td>15.4 ± 2.6 (12–20)</td>
</tr>
<tr>
<td>Race</td>
<td>20 Caucasian</td>
<td>23 Caucasian/1 Black</td>
</tr>
<tr>
<td>QNE</td>
<td>5.4 ± 3.9 (1–13)</td>
<td>4.5 ± 2.2 (0–9)</td>
</tr>
<tr>
<td>MMSE</td>
<td>29.2 ± 1.3 (25–30)</td>
<td>29.6 ± 0.8 (27–30)</td>
</tr>
</tbody>
</table>

Mean ± SD; range in parentheses. There was no overlap between groups on the number of CAG repeats. There were no significant between-group differences on age, gender, education, race, QNE or MMSE scores.

SPECT and MRI in pre-symptomatic Huntington’s disease

risk for Huntington’s disease, uncorrected for age, was 50%. Participants in the Baltimore Huntington’s Disease Presymptomatic Testing Program at the Johns Hopkins University School of Medicine, who had received genetic test results and are being followed-up longitudinally, were given the opportunity to participate in the current imaging study. Exclusion criteria were a history of neurologic illness, including head trauma causing unconsciousness of >1 h, QNE (Folstein et al., 1983) score of 16 or higher, or acute psychiatric disorder at the time of predictive testing (assessed using the SADS-L: Spitzer and Endicott, 1978). Subjects who satisfied these criteria were asked to participate in the brain imaging protocol, and only those who completed both the SPECT and MRI scans were included in this study. Of the 44 subjects enrolled, 20 had tested positive for the expanded Huntington’s disease gene (mutation-positive) and 24 were Huntington’s disease mutation-negative. The mutation-negative group had 30 or fewer trinucleotide (CAG)$_n$ repeats for the longer allele, and the mutation-positive subjects had 40 or more repeats. Demographic and clinical information by gene status group are presented in Table 1. The two groups did not differ in age, gender, education or race. Furthermore, the groups did not differ significantly ($P > 0.30$) on the QNE, a reliable and validated standardized assessment of motor systems (Folstein et al., 1983), or on the Mini-Mental State Examination (MMSE) (Folstein et al., 1975). This indicates that mutation-positive subjects did not suffer significant neurological or gross cognitive deficit compared with the mutation-negative group.

Approximately half the individuals in the current study appeared in our earlier MRI report of gene-tested subjects (Aylward et al., 1996). This project was approved by the Johns Hopkins Joint Committee on Clinical Investigations. Informed consent was obtained from all participants.

Genetic testing

Subjects were tested for the number of (CAG)$_n$ trinucleotide repeats present in their IT15 genes. DNA analyses were performed by the Genetic Core Facility and the Neurogenetic Research Laboratory at the Johns Hopkins Hospital. The DNA was isolated by standard phenol–chloroform extraction. The (CAG)$_n$ triplet repeat of IT15 was amplified by the polymerase chain reaction (PCR). PCR products were resolved on 6% denaturing polyacrylamide gels using a Model 373 gene scanner coupled with Genescan 2500 software (Applied Biosystems). Repeat length was determined using an internal lane standard labelled with red (ROX) coloured fluorescent dye. Data were presented in peak and tabular form using Genotyper software (Applied Biosystems). The number of repeats for the larger of the two alleles ranged from 10 to 30 in the mutation-negative subjects, and from 40 to 50 in the mutation-positive group.

SPECT and magnetic resonance image acquisition

For SPECT scanning, all subjects were scanned on the same Trionix Triad three-headed rotating Anger camera, high-resolution (6 mm full-width half-maximum resolution) SPECT system. Slant-hole collimation was used to minimize the distance between the camera and the head. Patients were injected with 20 mCi of [Tc-99m]HMPAO 5 min prior to scanning in a quiet, darkened room to minimize cerebral sensory activation. HMPAO is used frequently as a SPECT cerebral blood flow tracer, due to its high first-pass uptake and stable in vivo distribution for several hours post-injection (Sharp et al., 1986). Axial images were reconstructed, with 64 × 64 voxel matrix and 3.56 mm isotropic voxel size.

For MRI, all subjects were scanned with the same 1.5 Tesla General Electric Signa MRI scanner. Image acquisitions included the entire cerebral cortex and cerebellum. A high-resolution spoiled gradient in the steady state coronal acquisition [repetition time (TR) = 35, echo time (TE) = 5, flip angle 45, 1.5 mm slice thickness] was used for all image registration and regional measurements. A dual-echo axial series was also acquired for each subject (TR = 3000, TE = 30/100, nex = 1, contiguous 3 mm thick images).

Image co-registration and spatial normalization

To sample subcortical regions of interest in SPECT images, it is more reliable to use the structural clarity of MRI images from the same patient to guide SPECT region placement. This is particularly important when the regions of interest are pathologically affected by the disease under study, as is the case with the basal ganglia in Huntington’s disease (Harris and Pearlson, 1993). Therefore, we spatially registered the MRI images from each patient with the SPECT images prior to regional analyses.

Images were co-registered and spatially normalized both within and across subjects by converting all SPECT and MRI...
images into the spatial domain of the Talairach atlas (Talairach and Tournoux, 1988) using the ‘SN’ software developed by the Research Imaging Center at University of Texas Health Science Center at San Antonio (Lancaster et al., 1995). Images were reformatted in this orientation, parallel to the anterior commissure (AC)–posterior commissure (PC) plane. The reformatted images were sampled with 2 mm thick slices and 1 mm × 1 mm pixel size for both the SPECT and MRI data sets. This step achieved three purposes: first, the SPECT and MRI images from each patient were co-registered; secondly, the MRI images were spatially normalized to account for head size differences across subjects; and thirdly, by spatially normalizing all images in the Talairach domain, subject group images were averaged within subject groups and compared across subject groups.

Image intensity normalization

SPECT images were normalized by adjusting the image intensity according to the cerebellar mean value for each acquisition series. The cerebellum was chosen as the reference region because it is thought to be less affected than the cerebral cortex in Huntington’s disease (Kuwert et al., 1990; Martin et al., 1992). The cerebellum mean value was measured on the first slice inferior to the cerebellar peduncles. This slice was selected because it is near the mid-level of the cerebellum, while avoiding the slices where the peduncles enter and contribute a large proportion of white matter tracts to the cerebellum. The cerebellum normalization method was standardized to minimize variability in the measure between subjects as follows. First, a rough estimate was made by thresholding the cerebellum with an auto-thresholding tool, and the image intensity of the subject’s entire three-dimensional data set was divided by the cerebellum mean value and multiplied by 100. Thus, a value of 100 in the SPECT images corresponds to 100% of the cerebellum mean value. A second normalization step was then performed to finely adjust the cerebellum measure in a uniform manner across all scans. The cerebellum was re-thresholded at a uniform cut-off intensity across all scans to outline the cerebellum (any brainstem inclusions were edited out of the region manually). This cerebellum mean was then calculated and the images readjusted as described above. Regional measures of any brain structure from these normalized images have a mean value that is a direct percentage of the cerebellum mean value.

MRIs were also normalized to have a uniform image signal intensity so that averaging across subjects would have equal weighting for each scan. To normalize the MRIs for signal intensity, the slice 10 mm above the AC–PC line was selected, the brain edge was defined and the ventricles were thresholded out. The mean signal intensity of the brain tissue was used for normalizing the MRIs by dividing the entire multi-slice image data set by this value and multiplying by 100. Thus, a value of 100 in the MRIs corresponds to the mean brain value.

Regional measurements

All measurements from the SPECT images and MRIs were made blind to gene status. Measures were computed first on the MRIs. Volumes were determined for the head of the caudate, putamen, globus pallidus, thalamus and lateral ventricles. Lateral ventricle volumes were calculated by thresholding the ventricles at a cut-off level of 50% of the mean brain value. Lateral ventricles were measured from 2 to 26 mm superior to the AC–PC plane. For all subcortical structures, measurement began 2 mm superior to the AC–PC plane, where the caudate and putamen were clearly separated by the internal capsule, and continued superiorly to 14 mm superior to the AC–PC plane, above which the head of the caudate merged with the caudate body. The borders of the caudate were delimited by the anterior limb of the internal capsule laterally, and the frontal horn or body of the lateral ventricle medially. The putamen was circumscribed laterally by the external capsule and medially by the globus pallidus and the internal capsule. The globus pallidus was bordered laterally by the putamen and medially by the posterior limb of the internal capsule. Structures were outlined manually by a single rater of demonstrated reliability (the intra-class correlation coefficients for inter- and intra-rater reliability were >0.9 for all measures), and areas were summed across slices and multiplied by slice thickness to compute volumes. All volume measures were calculated at 4 mm intervals. Bicaudate ratios, defined as the distance between the heads of the caudate nuclei at their narrowest separation as a percentage of the width of the brain along the same line, were also measured on the slice 10 mm superior to the AC–PC line (Barr et al., 1978; Harris et al., 1992).

Image volumes defined on the MRIs were then transferred directly onto the SPECT images. Since the images were co-registered, the regions were in the same spatial locations on the SPECT images as on the MRIs. The mean SPECT image values for each region were then calculated. As described above, these measures correspond to values normalized as a percentage of cerebellar mean value.

Predicted years-to-onset estimation

For mutation-positive subjects, a formula was used to calculate predicted age at onset. This formula is based on the subject’s trinucleotide repeat length and the age at Huntington’s disease onset of his/her affected parent. The prediction equation was derived from stepwise multiple regression analysis of data from a sample of 50 symptomatic parent–child pairs in the Johns Hopkins Huntington’s Disease Clinic, and is summarized in Equation (1) as follows:

\[
\text{age at onset} = (-0.81 \times \text{repeat length}) + (0.51 \times \text{parental onset age}) + 54.87
\]

For these 50 patients, this equation yielded a multiple \( r \) of 0.74 (\( P = 0.001 \)). Neither the sex of the affected parent nor the parent’s repeat length significantly added to the equation’s
predictive power, and so were not included. Parental age at onset was the age at which abnormal movement was first observed in the parent, as documented by a structured interview and review of records. Years-to-onset was calculated by subtracting the age at scan time from the estimated onset age as calculated by the equation above. Mean years-to-onset for 18 mutation-positive subjects was 6.07 ± 6.21 years (reliable parental onset age was unavailable for two subjects). The mutation-positive subjects were split into two subgroups based on the years-to-onset: a close-to-onset group with years-to-onset less than the group mean (years-to-onset ≤ 6 years; n = 12), and a far-from-onset group with years-to-onset greater than the group mean (years-to-onset ≥ 7 years; n = 6).

**Statistical analyses**
Significance testing was carried out using two-tailed t-tests (n = 44; d.f. = 43 for all t-tests unless otherwise specified) to evaluate between-group differences in imaging measures. Linear regression analysis was used to determine which structural or functional imaging measures best correlated with predicted years-to-onset, QNE scores, number of (CAG)$_n$ trinucleotide repeats and age. One-way ANOVA (analysis of variance) with Scheffe post hoc analysis was used to examine differences among the three subgroups: mutation-positive subjects close to and far from onset, and mutation-negative subjects. Discriminant function analysis was used to evaluate how well the brain volume and blood flow measures enabled classification of subjects into subgroups.

**Results**
Differences between the two gene status groups were greatest in the putamen (Table 2 and Fig. 1). Huntington’s disease mutation-positive subjects had lower putamen measures than mutation-negative control subjects for both SPECT regional cerebral blood flow (rCBF) and for MRI volume. There were no significant correlations in the mutation-negative subjects between any of the SPECT or MRI measures of the caudate and putamen, and 12% (3 out of 24) of the mutation-negative subjects or mutation-positive subjects far from onset. Among the ≤6 years-to-onset mutation-positive subjects, 92% (11 out of 12) had ‘small’ putamena (volume >1 SD below the mutation-negative mean). In contrast, only one of the six subjects (17%) estimated to be ≥7 years to onset had similarly ‘small’ putamen volume, and 12% (3 out of 24) of the mutation-negative subjects were in this range. Although these subgroups did not differ in age [F(2,41) = 2.77; P > 0.07], education [F(2,41) = 0.84; P > 0.43] or MMSE [F(2,41) = 0.67; P > 0.51], the close-to-onset subgroup had higher QNE scores than did the far-from-onset subgroup (‘close’ = 6.67 ± 4.27; ‘far’ = 2.67 ± 1.97) [F(2,41) = 4.09; P < 0.03], although neither of these subgroups had QNE scores that differed significantly from the mutation-negative group (4.54 ± 2.25).

**Correlation between imaging measures and predicted years-to-onset**
Predicted years-to-onset correlated most strongly with putamen volume [r(17) = 0.77; P < 0.0001] as shown in Table 3 and Fig. 2. There was a clear demarcation among mutation-positive subjects depending on predicted years-to-onset. Assessment of the subgroups separated by a split at the mean years-to-onset indicated that putamen volume was sharply reduced in subjects ≤6 years from predicted onset (‘close-to-onset’) compared both with mutation-positive participants ≥7 years or more from onset (‘far-from-onset’), and with mutation-negative subjects. Among the ≤6 years-to-onset mutation-positive subjects, 92% (11 out of 12) had ‘small’ putamena (volume >1 SD below the mutation-negative mean). In contrast, only one of the six subjects (17%) estimated to be ≥7 years to onset had similarly ‘small’ putamen volume, and 12% (3 out of 24) of the mutation-negative subjects were in this range. Although these subgroups did not differ in age [F(2,41) = 2.77; P > 0.07], education [F(2,41) = 0.84; P > 0.43] or MMSE [F(2,41) = 0.67; P > 0.51], the close-to-onset subgroup had higher QNE scores than did the far-from-onset subgroup (‘close’ = 6.67 ± 4.27; ‘far’ = 2.67 ± 1.97) [F(2,41) = 4.09; P < 0.03], although neither of these subgroups had QNE scores that differed significantly from the mutation-negative group (4.54 ± 2.25).

**Comparison of MRI measures among years-to-onset subgroups and controls**
The mutation-positive subgroups did differ from the control groups on several MRI measures (Table 4). Putamen and caudate volumes were smaller and the bicaudate ratio was greater in the close-to-onset group than in the far-from-onset and mutation-negative subjects, again with the greatest reduction in putamen volume. Comparisons between the far-from-onset mutation-positive and the mutation-negative groups revealed no volume differences.

Discriminant function analysis was effective at classifying subjects by group when mutation-positive subjects in the close-to-onset (≤6 years) subgroup were compared with mutation-negative subjects or mutation-positive subjects far from onset. MRI putamen volume correctly classified 94.4% of subjects in the close and far from onset subgroups (12 out of 12 mutation-positive close-to-onset, 5 out of 6 mutation-positive far-from-onset). Putamen volume correctly classified 91.7% of close-to-onset mutation-positive subjects and mutation-negative subjects (11 out of 12 mutation-positive close-to-onset, 22 out of 24 mutation-negative). This discrimination improved to 94.4% when SPECT and MRI measures of the caudate and putamen were included in the discriminant function analysis (11 out of 12 mutation-positive close-to-onset, 23 out of 24 mutation-negative).
Images from each subject were normalized spatially into the coordinate system of the Talairach atlas (Talairach and Tournoux, 1988), then averaged within subject subgroups. The top set of images were averaged from 12 subjects, who were within 6 years from estimated onset age. The bottom row represents averaged scans from the six subjects who were >7 years from anticipated clinical onset. Displayed are MRI (left), SPECT images (right) and combined MRI and SPECT overlay images (centre), located 10 mm superior to the AC–PC line. The brain outline (yellow) was defined on the top left MRI and copied to all other images in the figure to demonstrate accuracy of registration among group-averaged images. Also note the anatomical clarity within the averaged images, indicating excellent alignment among individual scans. The putamen (red) and caudate (green) were outlined on the MRIs and transferred to the SPECT scans. Note the visibly smaller putamen in the close-to-onset subjects (top). The bicaudate ratios (red lines) and their respective values are displayed (~7 for the far-from-onset subjects compared with 12 for the close-to-onset cases). The SPECT basal ganglia rCBF values were similar between the close-to-onset and far-from-onset groups. However, the gene-positive subjects overall displayed decreased putamen rCBF compared with the gene-negative control subjects.

Correlation between imaging and clinical measures
Correlations between imaging measures and QNE were observed in those same regions that had correlated with predicted years-to-onset described above; namely QNE correlated with MRI measures of the bicaudate ratio, and volumes of the caudate and putamen. However, while putamen volume correlated most strongly with years-to-onset, the bicaudate ratio had the strongest correlation with QNE \( r(19) = 0.57; P = 0.009 \) as shown in Fig. 3 and Table 3. There was a trend towards correlation between years-to-onset and QNE \( r = -0.42; P = 0.08 \). MRI measures did not correlate with \((CAG)\_n\) repeat length or with age.

SPECT imaging measures
SPECT putamen rCBF was the measure that showed the greatest decline in the mutation-positive subjects compared with the mutation-negative control subjects (Table 2). The caudate showed a trend toward decreased rCBF in mutation-positive subjects \( t(42) = 1.88; P = 0.07 \). These SPECT perfusion decreases were similar in both the close-to-onset and far-from-onset subgroups. This relationship between approaching onset and SPECT basal ganglia perfusion differed from the MRI observations reported above, where only the close-to-onset subgroup demonstrated neuroanatomical changes compared with mutation-negative control subjects. Thus, while SPECT decreases were not
Fig. 2 Relationship of the putamen volume to predicted years-to-onset in mutation-positive subjects ($n = 18$), with gene-negative subjects ($n = 24$) shown to the right (predicted years-to-onset was undetermined for two mutation-positive subjects whose parental age of onset was unknown). As mutation-positive subjects approached the predicted onset age, the putamen volume declined [$r(17) = 0.77; P < 0.0001$]. Mean and standard deviation bars for the gene-negative group are shown. A horizontal line is displayed at 1 SD below the mutation-negative mean. This putamen volume cut-off was used post hoc to divide the mutation-positive group into those close-to-onset ($\leq 6$ years) and those far-from-onset ($\geq 7$ years), as displayed by a vertical line. Those in the far-from-onset group are similar to the gene-negative subjects, while those in the close-to-onset group have markedly reduced basal ganglia volumes.

Correlation between imaging and clinical measures

SPECT measures, unlike MRI measures, did not correlate with predicted years-to-onset or with QNE scores (Table 3). However, in mutation-positive subjects, triplet repeats correlated inversely with basal ganglia SPECT perfusion in the caudate, putamen and globus pallidus (Table 3). In mutation-positive subjects only, putamen and globus pallidus SPECT perfusion values correlated positively with age. These regions were also correlated with trinucleotide repeat length. This increase in SPECT perfusion with age in mutation-positive subjects is probably a spurious effect resulting from the inverse correlation between SPECT measures and repeat length, and the inverse relationship between repeat length and age. Age was inversely correlated with predicted years-to-onset ($r = -0.58; P = 0.01$) and (CAG)$_n$ repeat length ($r = -0.52; P = 0.02$). Since longer repeats are associated with younger onset, older pre-symptomatic subjects typically have shorter repeat lengths, and are also closer to onset than younger subjects.

Discussion

Prior studies have indicated severe structural abnormalities in the striatum in early Huntington’s disease (Harris et al., 1992, 1996), and even pre-clinically (Aylward et al., 1994, 1996). The results of the current study, together with similar studies of patients with early Huntington’s disease symptoms (Harris et al., 1992, 1996), begin to shed some light on the relationship between onset of clinical symptoms and neuropathological deficits in Huntington’s disease. None of the subjects in the current study were clinically symptomatic for Huntington’s disease. There was a trend towards QNE scores increasing as subjects approached clinical onset, however, which suggests that minor neurological signs and symptoms gradually develop over several years prior to clinically diagnosable illness onset.

The current study demonstrates that the putamen, as measured by MRI volume, begins to atrophy several years prior to onset of symptoms, and its pre-symptomatic decline is consistent with prior published findings that the putamen...
Table 2: Mean ± SD for regional imaging measurements by gene status group

<table>
<thead>
<tr>
<th></th>
<th>+ HD gene (n = 20)</th>
<th>− HD gene (n = 24)</th>
<th>t value (d.f. = 42)</th>
<th>P value</th>
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<td><strong>SPECT (% cerebellum)</strong></td>
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<tr>
<td>Caudate</td>
<td>85.2 ± 7.5</td>
<td>89 ± 6.0</td>
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<tr>
<td>Putamen</td>
<td>100.5 ± 5.9</td>
<td>104.3 ± 5.3</td>
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<td>0.03</td>
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<td>Globus pallidus</td>
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<td>96.6 ± 6.0</td>
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<td>0.55</td>
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<tr>
<td>Thalamus</td>
<td>98.8 ± 6.0</td>
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<td><strong>MRI (volume, ml)</strong></td>
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<tr>
<td>Caudate</td>
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<td>Putamen</td>
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<td>9.90 ± 0.71</td>
<td>2.17</td>
<td>0.04</td>
</tr>
<tr>
<td>Bicaudate ratio</td>
<td>11.4 ± 3.3</td>
<td>9.5 ± 2.5</td>
<td>1.61</td>
<td>0.12</td>
</tr>
<tr>
<td>Lateral ventricle</td>
<td>14.4 ± 6.6</td>
<td>12.2 ± 7.1</td>
<td>1.05</td>
<td>0.30</td>
</tr>
</tbody>
</table>

+ HD gene = positive for the Huntington’s disease gene mutation, − HD gene = negative for the Huntington’s disease gene mutation.

Table 3: Regression coefficients (r) and statistical significance (P value) of predicted years-to-onset, QNE, number of CAG repeats and age with MRI and SPECT measurements

<table>
<thead>
<tr>
<th></th>
<th>Years-to-onset</th>
<th>QNE</th>
<th>CAG repeats</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MRI</strong></td>
<td>r</td>
<td>P</td>
<td>r</td>
<td>P</td>
</tr>
<tr>
<td>Caudate</td>
<td>0.54</td>
<td>0.02</td>
<td>-0.50</td>
<td>0.02</td>
</tr>
<tr>
<td>Putamen</td>
<td>0.77</td>
<td>&lt;0.0001</td>
<td>-0.54</td>
<td>0.01</td>
</tr>
<tr>
<td>Globus pall.</td>
<td>0.37</td>
<td>NS</td>
<td>-0.07</td>
<td>NS</td>
</tr>
<tr>
<td>Thalamus</td>
<td>0.12</td>
<td>NS</td>
<td>-0.05</td>
<td>NS</td>
</tr>
<tr>
<td>BCR</td>
<td>-0.56</td>
<td>0.02</td>
<td>0.57</td>
<td>0.009</td>
</tr>
<tr>
<td><strong>SPECT</strong></td>
<td>r</td>
<td>P</td>
<td>r</td>
<td>P</td>
</tr>
<tr>
<td>Caudate</td>
<td>0.13</td>
<td>NS</td>
<td>-0.14</td>
<td>NS</td>
</tr>
<tr>
<td>Putamen</td>
<td>0.20</td>
<td>NS</td>
<td>-0.05</td>
<td>NS</td>
</tr>
<tr>
<td>Globus pall.</td>
<td>0.26</td>
<td>NS</td>
<td>-0.12</td>
<td>NS</td>
</tr>
<tr>
<td>Thalamus</td>
<td>0.17</td>
<td>NS</td>
<td>-0.11</td>
<td>NS</td>
</tr>
</tbody>
</table>

BCR = bicaudate ratio, Globus pall. = Globus pallidus. NS = not significant, P > 0.05. Data are presented for mutation-positive subjects (n = 20 for repeat length, QNE and age; n = 18 for years-to-onset with two missing estimates). For gene-negative subjects (n = 24), there were no significant correlations between any imaging measure and QNE, repeat length or age.

Table 4: One-way ANOVA with mean ± SD for regional imaging measurements by gene status group

<table>
<thead>
<tr>
<th></th>
<th>+ HD gene</th>
<th>+ HD gene</th>
<th>− HD gene</th>
<th>F value (d.f. = 2,41)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SPECT (% cerebellum)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caudate</td>
<td>84.2 ± 6.8</td>
<td>85.4 ± 9.8</td>
<td>89.0 ± 6.0</td>
<td>2.25</td>
<td>0.12</td>
</tr>
<tr>
<td>Putamen</td>
<td>99.4 ± 4.5*</td>
<td>99.8 ± 7.1</td>
<td>104.3 ± 5.3</td>
<td>4.11</td>
<td>0.02</td>
</tr>
<tr>
<td>Globus pallidus</td>
<td>94.0 ± 4.6</td>
<td>95.7 ± 7.0</td>
<td>96.6 ± 6.0</td>
<td>0.83</td>
<td>0.44</td>
</tr>
<tr>
<td>Thalamus</td>
<td>98.2 ± 5.1</td>
<td>97.7 ± 7.1</td>
<td>100.6 ± 5.5</td>
<td>1.12</td>
<td>0.34</td>
</tr>
<tr>
<td><strong>MRI (volume, ml)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caudate</td>
<td>3.42 ± 0.41* †</td>
<td>4.04 ± 0.38</td>
<td>4.01 ± 0.51</td>
<td>6.81</td>
<td>0.003</td>
</tr>
<tr>
<td>Putamen</td>
<td>5.76 ± 0.59* †</td>
<td>7.67 ± 0.88</td>
<td>7.67 ± 0.92</td>
<td>22.35</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Globus pallidus</td>
<td>1.81 ± 0.29</td>
<td>2.03 ± 0.20</td>
<td>2.06 ± 0.36</td>
<td>2.55</td>
<td>0.09</td>
</tr>
<tr>
<td>Thalamus</td>
<td>9.45 ± 0.50</td>
<td>9.50 ± 0.42</td>
<td>9.90 ± 0.71</td>
<td>2.54</td>
<td>0.09</td>
</tr>
<tr>
<td>Bicaudate ratio</td>
<td>11.7 ± 2.4* †</td>
<td>7.3 ± 2.3</td>
<td>9.1 ± 1.8</td>
<td>10.30</td>
<td>0.0003</td>
</tr>
</tbody>
</table>

The mutation-positive subjects were subgrouped by predicted years-to-onset: the close-to-onset group had 6 or less years-to-onset (≤ 6 YTO) and the far-from-onset group had 7 years or more to onset (≥ 7 YTO). P < 0.05 difference from Huntington’s disease gene mutation-negative group on Tukey post hoc analysis; †P < 0.05 difference from ≥ 7 YTO mutation-positive group on Tukey post hoc analysis.
reaches 50% of normal volume early in the illness (Harris et al., 1992). In close-to-onset subjects in the current study, as in mildly symptomatic Huntington’s disease patients in prior reports, the putamen volume shows the greatest deficits. Furthermore, the MRI putamen volume displayed the strongest relationship with predicted years-to-onset. MRI caudate volume and bicaudate ratio also correlated with years-to-onset. As a result, close-to-onset subjects with ≤6 years-to-onset had structural deficits in these measures compared with both the far-from-onset and mutation-negative groups, while far-from-onset individuals were similar to mutation-negative subjects. In subjects predicted to be within 6 years-to-onset, imaging measures were very effective at classifying subjects into gene status groups. The MRI putamen volume (the most affected measure) was entered into discriminant function analysis and correctly classified 92% of the close-to-onset participants from the mutation-negative subjects. Between close-to-onset and far-from-onset subjects, 94% were correctly classified by discriminant function analysis of the putamen volume. These data suggest that the MRI putamen volume is a sensitive measure of structural brain changes within ~6 years of predicted clinical onset.

The bicaudate ratio correlated most strongly with QNE in pre-symptomatic gene carriers in the current study. The bicaudate ratio had also been observed previously to correlate most strongly with QNE in symptomatic Huntington’s disease patients (Harris et al., 1992, 1996). It is interesting that the putamen volume correlated most strongly with years-to-onset, while the bicaudate ratio correlated most strongly with QNE. However, this discrepancy makes sense in light of the specificity of each measure. In subjects without severe atrophy, the bicaudate ratio reflects frontal horn enlargement, and therefore generalized subcortical atrophy, rather than caudate volume loss specifically (Aylward et al., 1991). Thus, the bicaudate ratio is a broad measure of subcortical tissue loss. The QNE is also a broad measure that incorporates several domains of diverse neurological symptoms. Therefore, it is not surprising that a non-specific subcortical atrophy measure such as the bicaudate ratio would correlate best with a multifaceted neurological examination such as the QNE.

In contrast, clinical onset is usually defined according to onset of motor symptoms. The finding that the putamen volume correlates best with approaching clinical onset is in accordance with studies indicating that a variety of motor circuits involve the putamen but not the caudate, while cognitive circuits are thought to involve the caudate but not the putamen (Alexander et al., 1986; DeLong et al., 1990). Furthermore, the putamen volume demonstrated the most significant changes and was the best group discriminator, both pre-symptomatically and in early, mild Huntington’s disease patients (Harris et al., 1992, 1996; Aylward et al., 1994, 1996), while the bicaudate ratio had more overlap between groups. Therefore, putamen volume measures are preferable as a marker of pre-clinical or early Huntington’s disease, despite the high correlation of bicaudate ratio with QNE.

SPECT rCBF was decreased in the putamen, and there was a trend towards decreased caudate rCBF in asymptomatic individuals who carry the expanded Huntington’s disease allele compared with mutation-negative individuals. These
SPECT results are consistent with prior SPECT and PET studies in patients with symptomatic Huntington’s disease that also showed basal ganglia perfusion deficits (Hayden et al., 1986; Hasselbalch et al., 1992; Harris et al., 1996). In the current study, the rCBF measures were uncorrelated with years to expected onset, unlike the structural measures. The caudate and putamen SPECT perfusion measures were very similar between the close-to-onset and far-from-onset subgroups of mutation-positive subjects. This may indicate that, while perfusion measures begin to show effects earlier than the structural measures, rCBF deficits do not decline as rapidly as the MRI measures of volume loss.

While the putamen decline was the most severe effect observed pre-symptomatically with both SPECT and MRI, there were some striking differences between SPECT and MRI measures in the relationship between imaging changes and estimated years to illness onset, and clinical measures. Although the current study was cross-sectional, and longitudinal studies are needed to detail the true time course of perfusion and volume changes pre-clinically, some observations are suggested by the current study of the relative time course of SPECT and MRI changes prior to clinical onset. Pre-symptomatic mutation-positive subjects demonstrated MRI atrophy only in close-to-onset subjects, while far-from-onset participants had SPECT measures of putamen and caudate perfusion that were more similar to those of close-to-onset subjects than to the mutation-negative group. These divergent observations suggest that basal ganglia cells may be dysfunctional, with hypoperfusion likely to be indicative of hypometabolism, prior to cell death and atrophy. Thus, hypoperfusion visible on SPECT is present prior to atrophy. After some period of physiological deficit, cell loss and atrophy begin and continue for several years prior to onset of clinical illness. During this pre-clinical atrophy period, however, the remaining cells demonstrate the hypoperfusion as a relatively consistent effect. Only after substantial subcortical atrophy occurs does clinical onset ensue. It is difficult with SPECT to parse the effects of atrophy from true perfusion changes. However, there are several factors to suggest that there are perfusion abnormalities caused by Huntington’s disease that are not solely atrophy effects. First, the careful image co-registration of the current study was designed to minimize the impact of atrophy on the SPECT measures. Secondly, there are similar perfusion deficits in both mutation-positive subgroups, even though there are no signs of atrophy in the far-from-onset subgroup. Thirdly, a recent study demonstrated blood flow velocity decreases during cognitive stimulation in Huntington’s disease patients, while normal controls showed increases during the same task (Deckel et al., 1998). While atrophy might cause less observed activation in Huntington’s disease patients than in control subjects, actual haemodynamic decreases suggest a true dysfunction. Furthermore, SPECT perfusion values correlated with triplet repeat length, but the MRI measures did not. If the IT15 gene mutation in Huntington’s disease causes metabolic/haemodynamic abnormality, this may explain why only the SPECT measures correlated with triplet repeat length. However, these correlations should be replicated, and these conclusions must be regarded as speculative.

With the recent discovery of the Huntington’s disease gene mutation, imaging technologies are no longer needed to confirm Huntington’s disease diagnosis in early, mildly symptomatic patients with an unclear family history. However, there are clinically important concerns driving further investigations of imaging in Huntington’s disease. Neuroimaging can provide objective and quantitative measures of striatal damage prior to, and coincident with, definite clinical onset. This could prove critically important in drug trials for pre-symptomatic carriers of the Huntington’s disease gene mutation, and for assessing trials of treatments aimed at slowing the course of this devastating illness. A number of treatment strategies have been suggested based on the glutamate hypothesis of Huntington’s disease pathogenesis, including treatment with such relatively benign agents as d-α-tocopherol (Peyser et al., 1995). Trials in pre-symptomatic subjects carrying the Huntington’s disease mutation may occur within a decade. Because signs and symptoms are subtle early in the illness and develop gradually, it will be clinically useful to track putamen volume decline and compare this with normal values and with the expected trajectory of decline as onset approaches. Treated pre-symptomatic subjects could then be compared with untreated subjects, and neuroimaging measures could be used to evaluate objectively whether treatment is slowing the progression of degeneration.

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References


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