Increased Basal Ganglia Iron Levels in Huntington Disease

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Objective: To quantify in vivo brain ferritin iron levels in patients with Huntington disease (HD) and normal control subjects.

Design and Subjects: A magnetic resonance imaging method that can quantify ferritin iron levels with specificity in vivo was employed to study 11 patients with HD and a matched group of 27 normal controls. Three basal ganglia structures (caudate, putamen, and globus pallidus) and 1 comparison region (frontal lobe white matter) were evaluated.

Results: Basal ganglia iron levels were significantly increased (P<.002) in patients with HD, and this increase occurred early in the disease process. This was not a generalized phenomenon, as white matter iron levels were lower in patients with HD.

Conclusions: The data suggest that increased iron levels may be related to the pattern of neurotoxicity observed in HD. Reducing the oxidative stress associated with increased iron levels may offer novel ways to delay the rate of progression and possibly defer the onset of HD.

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HUNTINGTON disease (HD) is a genetic disease caused by the repetition of a CAG trinucleotide sequence encoding for a polyglutamine tract at the N terminal of the gene coding for a protein of unknown function named huntingtin. Despite our understanding of the genetics of HD, the pathogenesis that results in the observed phenotypes remains puzzling, encumbering the search for therapeutic interventions.

Except for juvenile onset cases, where the disease may be more virulent, the rate of progression of HD is not affected by the CAG repeat number or the presence of a double dose of the gene in homozygotes. This suggests that the mutant genes contribute to reaching the threshold of neurotoxicity, and once the threshold is reached, the progression is dependent primarily on nongenetic factors. Supporting this contention are the observations that the defective gene and its protein product are expressed throughout development, are ubiquitously present in most brain areas as well as the periphery, and are not noticeably present at higher levels in cells that degenerate first. In contrast, the typical HD phenotype is characterized by middle to late age of onset and by early, specific neurodegeneration of striatal (caudate and putamen) neurons. These observations suggest that some of the nongenetic factors are specific to the central nervous system (CNS) and that, therefore, a CNS-specific risk factor such as a CNS-specific neurotransmission could be involved. In addition, the above observations suggest an age-related risk factor that may predispose the striatum to the earliest and most severe involvement. The striatum is a site of high iron concentrations that increase with age from very low levels at birth, and several postmortem studies have found increased iron levels in the striatum of patients with HD, suggesting a role for iron in the HD process.

Whether elevated levels of iron are present prior to death is an important scientific and treatment question. An association between high iron levels and CNS damage has been observed in a variety of neurodegenerative disorders, and iron involvement has been suggested as a common risk factor. Up to 90% of non-heme iron in the brain is in the iron storage protein ferritin. Magnetic resonance imaging (MRI) can measure tissue iron in vivo through its effect on transverse re-
SUBJECTS AND METHODS

SUBJECTS WITH HD

Seventeen patients with HD were referred from an urban university neurology clinic and, after being fully informed of the study, agreed to participate and gave written consent. Eleven white subjects (7 men and 4 women) completed the study. These subjects ranged in age from 25 to 67 years (mean age ± SD, 46.2 ± 11.9 years) and were symptomatic from .75 to 20 years (mean ± SD, 7.8 ± 6.3 years). Prior to referral into the study, the subjects with HD underwent complete clinical assessment and the diagnosis was confirmed by an experienced neurologist (S.P.) via a thorough review of the history and clinical evaluation. All subjects with HD had a family history and/or a genetic test positive for HD. The 9 subjects who agreed to undergo genetic testing had a positive test result, with expanded CAG trinucleotide repeat sequences ranging from 40 to 54 repeats (mean ± SD, 45 ± 4.6 repeats).

Severity of choreoathetoid movements was assessed with the Abnormal Involuntary Movement Scale, on which scores of subjects with HD ranged from 9 to 24 (mean ± SD, 17.1 ± 4.3), and severity of dementia was assessed with the Mini-Mental State Examination, on which scores ranged from 20 to 30 (mean ± SD, 26.7 ± 2.9).

NORMAL CONTROL SUBJECTS

To control for the effects of age on the dependent measures, we selected cases from a pool of normal control subject volunteers recruited from the community and hospital staff who were participating in an ongoing study of normal aging and were recruited through printed advertisements. Normal subjects were excluded if there was a family history of Alzheimer disease, HD, or other neurodegenerative disorders, or a history of head trauma resulting in loss of consciousness for longer than 15 minutes. Normal subjects were included if they fell in the demographic parameters (age range and race) of the HD group and none was excluded on the basis of MRI findings. These criteria reduced the sample of 76 normal controls available for the study to a final normal comparison group that included 27 subjects (23 men and 4 women) ranging in age from 26 to 69 years (mean age ± SD, 44.8 ± 16.2 years). The subjects with HD and control subjects did not differ in mean age either in the overall or in sex-specific groups (P > .5).

MRI PROTOCOL

The methods have been described in detail elsewhere and will only be summarized here since the principal differences consisted of the use of a new set of MRI instruments. All subjects were scanned using the same 2 MRI instruments (1.5 T and 0.5 T) (Picker Instruments, Cleveland, Ohio), and the 2 scans were done within 1 hour of each other using the same imaging protocol.

Two pilot sequences were obtained to specify the location and spatial orientation of the head and the position of the axial image acquisition grid. A coronal pilot spin echo image of 100/50/1 (repetition time [RT]/echo time [TE]/excitations), 10-mm thickness, was acquired and used to align the subsequent sagittal pilot images. The middle slice of the sagittal pilot images was aligned on the coronal pilot to obtain a true mid-sagittal image of the brain. After the sagittal pilot spin echo images (550/26/2, 5-mm thickness) were acquired, the mid-sagittal image was used to position the axial image acquisition grid. The axial image acquisition sequence acquired interleaved contiguous slices using a Carr Purcell Meiboom Gill dual spin echo sequence of 2500/20,90/2 (RT/TE/excitations), 3-mm slice thickness, 192 gradient steps, and 25-cm field of view.

The coronal and sagittal pilot scans obtained prior to the axial image acquisition were used to determine the alignment and accuracy of head repositioning in the second MRI instrument. To consistently position the actual image slices identically within the brain and thus sample the same volume of tissue, the axial slice-select grid was adjusted so that the anterior commissure was contained within the same slice.
in both high- and low-field instruments. For increased consistency, all subsequent measures were referenced to this slice.\textsuperscript{21}

**IMAGE ANALYSIS**

\(T_2\) was calculated for each voxel by an automated algorithm from the 2 signal intensities (echo times, 20 and 90) of the dual spin echo sequence to produce gray-scale-encoded \(T_2\) maps of the brain.\textsuperscript{21}

The \(T_2\) measures were extracted using a Macintosh-configured image analysis workstaton. A single rater, blind to clinical information, obtained all measurements. The image analysis software permitted the rater to delineate the region of interest (ROI) using a mouse. The contour of the entire cross-sectional area of the head of the caudate, putamen, globus pallidus, and a sample of suproralbital white matter was drawn manually by the rater using the gray/white matter contrast of the early echo (TE, 20) images and was then transferred onto the \(T_2\) maps.

To obtain \(T_2\) measures of homogeneous brain tissue, all pixels with \(T_2\) values that fell above the right side inflection point on the histogram distribution of the ROI were eliminated. This minimized the influence of voxels containing small partial volumes of cerebrospinal fluid, which can markedly increase the \(T_2\) of the voxel. Thus, the final measure was the average \(T_2\) for the remaining homogeneous region of brain tissue.\textsuperscript{21}

\(T_2\) data for each of the 4 ROIs were obtained from contiguous pairs of slices. The slice containing the anterior commissure and the slice immediately superior to it were used to obtain the putamen and globus pallidus \(T_2\) data. The third and fourth slices above the anterior commissure were used to obtain the \(T_2\) data for caudate nucleus, and the second and third slices superior to the orbitofrontal gray matter were used to obtain the frontal lobe white matter data. The \(R_2\) was calculated as the reciprocal of \(T_2\times 1000\). The average \(R_8\) of the 2 slices from both hemispheres were the final measures used in the subsequent analyses. The FDRI measure was calculated as the difference in \(R_2\) (high-field \(R_1\) - low-field \(R_2\)). Test-retest reliability for FDRI measures was very high with intraclass correlation coefficients ranging from 0.88 to 0.99 (\(P<.002\)).\textsuperscript{21}

**STATISTICAL METHODS**

The mean ages of subjects with HD and normal subjects were similar, but the age distributions of the 2 groups were different (there were few subjects with HD in the tails of the age distribution). To control for any confounding effects of age in the group comparisons, a combination of case matching and covariance analysis was used. Subjects with HD and normal control subjects were stratified into 5-year age intervals (aged 25-29 years, 30-34 years, and so on) separately by sex, and normal subjects who fell in age strata in which there were no subjects with HD were excluded from analyses. This rough matching procedure eliminated gross differences in the age distributions. To control for possible instrument drift or other temporal factors, normal scans were included only if done within 16 weeks of the scanning of a subject with HD. The resulting normal comparison group included 27 subjects.

The FDRI means in the normal and HD groups were then compared using a factorial analysis of covariance design. Because of the known increases in brain iron levels and FDRI with advancing age,\textsuperscript{6,21} and the possibility that sex may also affect age-related changes in FDRI (G.B., unpublished data, 1998), sex and age (a continuous variable) were included as covariates in the statistical designs. The inclusion of the covariates did not change the results substantively. Separate analyses were done in each of the 4 ROIs (caudate, putamen, globus pallidus, and white matter). In follow-up analyses, a test for the difference between correlated correlations was used to determine whether the standardized group differences in FDRI varied significantly across the 3 basal ganglia structures and the white matter region. Finally, the separate contribution of low- and high-field \(R_2\) to the discrimination of the 2 groups was examined in multiple discriminant (regression) models, again controlling for sex and age.

**Value** was significantly lower (\(F_{1,34} = 6.57, P = .015\)). The age- and sex-adjusted mean FDRI (and adjusted, within-group standard errors) in the 4 brain ROIs of subjects with HD and normal subjects are presented in Table 1. In further analyses, we evaluated whether the FDRI differences were present early in the disease by comparing the mean FDRI of the 3 subjects with HD (all men), whose symptom duration ranged from 9 months to 2 years, with normal control men. In the 3 basal ganglia regions, the differences were significant whether the HD group was compared with 3 normal controls matched in age within 1 year (\(P<.03\)) or when compared with the entire sample of 13 normal men with the same age range (25-45 years) (\(P<.001\)). The mean FDRI did not differ in the white matter (\(P>.28\)).

Pearson biserial correlations (partialing age and sex) between diagnosis and FDRI were computed in each region, yielding a standardized index of the size of the group difference, and pairwise comparisons among the 4 ROIs were performed using a test for the difference between correlated correlations.\textsuperscript{29} The diagnostic effect in the white matter was obviously different from the effects in the basal ganglia regions, being both statistically significant and opposite in direction (all \(t_{35}, 4.6-6.6, P<.001\)). The size of the diagnostic effects in the basal ganglia regions were not significantly different (all \(P>.10\)). Thus, increased FDRI was specific to the basal ganglia and similar in size in the caudate, putamen, and globus pallidus.

The diagnostic effects in \(R_2\) values obtained with the individual MRI instruments (high- and low-field) are summarized in Table 2 and Table 3. Multiple discriminant analyses that included both high-field and low-field \(R_2\) and controlled for age and sex confirmed that both field measures independently contributed to diagnostic discrimination in the caudate (high field, \(t = -5.76, P<.001\); low field, \(t = 5.26, P<.001\)) and putamen (high field, \(t = -4.57, P<.001\); low field, \(t = 5.68, P<.001\)). In the globus pallidus (high field, \(t = -3.92, P<.001\); low field, \(t = 1.06, P = .30\)) and white matter (high field, \(t = 3.29, P = .002\); low field, \(t = -1.71, P = .09\)), only high-field \(R_2\) was significant.
analyses confirm that combining the R2 measures through the high-field instrument alone. Multiple discriminant elses observed in postmortem studies of HD7-9 and the first is the first confirmation in vivo of the increased iron lev-
s in the white matter region. To our knowledge, this regions and is significantly different from the changes ob-
cur ferritin iron,12,17 which was a significantly better di-
agnostic discriminator in the caudate and putamen than
increase in ferritin iron levels is specific to the basal ganglia
gions and decreased in the frontal lobe white matter in HD.
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regions and is significantly different from the changes observed in
the white matter region. To our knowledge, this is the first confirmation in vivo of the increased iron levels observed in postmortem studies of HD12,19 and the first demonstration that the increased iron levels are present early in the disease process. The effect size obtained with the FDRI method is almost twice as great in the caudate and putamen regions that shown by the R2 measured with
the FDRI calculation yields a more specific measure of tis-
i facture damage has been an HD model of striatum cell death for over 20 years. The striatum receives dense excit-
atory glutaminergic input from the cortex. Glutamate, glutamate receptor agonists, and especially N-methyl-D-
aspartate (NMDA) receptor agonists produce toxic effects in the striatum very similar to the lesions seen in HD. However, NMDA receptors are equally dense in the neocortex (superficial layers), striatum, hippocampus, and other regions, while HD is localized primarily in the stria-
tum and leaves areas such as the hippocampus rela-
tively unaffected.11 If NMDA antagonism is involved in HD pathophysiology, another synergistic factor must also be involved in the toxic effects.

The striatum has relatively high iron levels, while the hippocampus does not, supporting the possibility that both NMDA and iron need to be elevated for the full neurotoxic effect to manifest. The globus pallidus, on the other hand, is an example of a structure that is less severely affected than the striatum, despite having higher iron concentrations16; however, this structure could be less sus-
rate components of the measure (high- and low-field R2) is also instructive. High-field R2 is sensitive not only to ferritin iron (which increases R2) but to field-independent tissue characteristics, such as the increased MRI-visible wa-
ter, that characterize tissue destruction and result in R2 de-
creases.12,18 The low-field R2 measure is much less sensitive to ferritin iron, and thus provides a “purier” measure of changes in MRI-visible water. Increased low-field R2 in the HD group can thus be interpreted as an indication of tissue destruction and is most pronounced in the caudate and putamen, the regions most affected by HD.

The CNS is at especially high risk for damage from oxidative free radical processes catalyzed by iron.30 Toxins that disrupt metabolism and result in increased lactate levels with decreasing pH will release iron from the ferritin stores.31 In addition, increases in reactive oxygen species may also release iron from ferritin31 as do other changes such as increased nitric oxide levels.32 Most hypo-
theses concerning HD pathogenesis have included a role for oxidative damage.33

Animal as well as human postmortem studies support the supposition of metabolic dysfunction with con-
comitant oxidative damage34-38 and/or oxidative damage secondary to excitotoxic neurotransmission.39,40 Excito-
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### Table 1. Mean FDRI* Adjusted for Age and Sex in Patients With Huntington Disease and Normal Control Subjects†

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>Patients With Huntington Disease, FDRI (SEM)</th>
<th>Normal Controls, FDRI (SEM)</th>
<th>F</th>
<th>P</th>
<th>d†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caudate</td>
<td>3.52 (0.20)</td>
<td>2.11 (0.16)</td>
<td>32.58</td>
<td>&lt;.001</td>
<td>2.11</td>
</tr>
<tr>
<td>Putamen</td>
<td>3.85 (0.22)</td>
<td>2.76 (0.17)</td>
<td>17.67</td>
<td>&lt;.001</td>
<td>1.55</td>
</tr>
<tr>
<td>Globus pallidus</td>
<td>6.00 (0.27)</td>
<td>4.53 (0.21)</td>
<td>20.40</td>
<td>&lt;.001</td>
<td>1.67</td>
</tr>
<tr>
<td>White matter</td>
<td>1.37 (0.07)</td>
<td>1.58 (0.05)</td>
<td>6.57</td>
<td>.015</td>
<td>−0.95</td>
</tr>
</tbody>
</table>

* Field-dependent relaxation rate (R2) increase (FDRI) is defined as the difference in the R2 values of each brain region obtained using high (1.5-T) and low (0.5-T) field-strength magnetic resonance imaging instruments.
† Eleven patients with Huntington disease and 27 normal control subjects (all df = 1.34).
‡ d indicates effect size index calculated as the difference in the covariate adjusted means divided by the root mean square error (covariance adjusted pooled within-cell SD).

### Table 2. Mean High-Field Relaxation Rate (HR2) Adjusted for Age and Sex in Patients With Huntington Disease and Normal Control Subjects*

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>Patients With Huntington Disease, HR2 (SEM)</th>
<th>Normal Controls, HR2 (SEM)</th>
<th>F</th>
<th>P</th>
<th>d†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caudate</td>
<td>16.31 (0.26)</td>
<td>15.30 (0.20)</td>
<td>10.72</td>
<td>.002</td>
<td>1.21</td>
</tr>
<tr>
<td>Putamen</td>
<td>17.43 (0.28)</td>
<td>16.86 (0.22)</td>
<td>2.78</td>
<td>.10</td>
<td>0.61</td>
</tr>
<tr>
<td>Globus pallidus</td>
<td>21.23 (0.35)</td>
<td>19.40 (0.27)</td>
<td>19.17</td>
<td>&lt;.001</td>
<td>1.62</td>
</tr>
<tr>
<td>White matter</td>
<td>15.45 (0.14)</td>
<td>16.10 (0.11)</td>
<td>14.83</td>
<td>&lt;.001</td>
<td>−1.42</td>
</tr>
</tbody>
</table>

* Eleven patients with Huntington disease and 27 normal control subjects (all df = 1.34).
† d indicates effect size index calculated as the difference in the covariate adjusted means divided by the root mean square error (covariance adjusted pooled within-cell SD).

### Table 3. Mean Low-Field Relaxation Rate (LR2) Adjusted for Age and Sex in Patients With Huntington Disease and Normal Control Subjects*

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>Patients With Huntington Disease, LR2 (SEM)</th>
<th>Normal Controls, LR2 (SEM)</th>
<th>F</th>
<th>P</th>
<th>d†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caudate</td>
<td>12.79 (0.12)</td>
<td>13.18 (0.09)</td>
<td>7.03</td>
<td>.012</td>
<td>−0.98</td>
</tr>
<tr>
<td>Putamen</td>
<td>15.58 (0.13)</td>
<td>14.11 (0.11)</td>
<td>10.57</td>
<td>.003</td>
<td>−1.20</td>
</tr>
<tr>
<td>Globus pallidus</td>
<td>15.23 (0.16)</td>
<td>14.87 (0.13)</td>
<td>3.52</td>
<td>.069</td>
<td>0.69</td>
</tr>
<tr>
<td>White matter</td>
<td>14.08 (0.15)</td>
<td>14.51 (0.12)</td>
<td>6.01</td>
<td>.020</td>
<td>−0.91</td>
</tr>
</tbody>
</table>

* Eleven patients with Huntington disease and 27 normal control subjects (all df = 1.34).
† d indicates effect size index calculated as the difference in the covariate adjusted means divided by the root mean square error (covariance adjusted pooled within-cell SD).

COMMENT

The data show FDRI is elevated in the 3 basal ganglia regions and decreased in the frontal lobe white matter in HD. Indeed, the increases in FDRI are considerably larger than those observed in other neurodegenerative disorders such as Alzheimer disease and Parkinson disease.12 The increase in ferritin iron levels is specific to the basal ganglia regions and is significantly different from the changes observed in the white matter region. To our knowledge, this is the first confirmation in vivo of the increased iron levels observed in postmortem studies of HD12,19 and the first demonstration that the increased iron levels are present early in the disease process. The effect size obtained with the FDRI method is almost twice as great in the caudate and putamen regions that shown by the R2 measured with the high-field instrument alone. Multiple discriminant analyses confirm that combining the R2 measures through the FDRI calculation yields a more specific measure of tissue ferritin iron.12,17 Which was a significantly better diagnostic discriminator in the caudate and putamen than either of its component measures alone.

Although the FDRI differences in the basal ganglia regions were comparable in size, interpretation of the sepa-
ceptible because it has minimal NMDA enervation. In addition, cortex iron levels are highest in the deeper layers, which is where neurodegeneration is most noticeable in HD; NMDA enervation is more abundant in superficial cortical layers. Finally, the involvement of iron deposition in HD is also supported by the fact that the manifestation of HD is age-dependent, and with advancing age, brain iron levels increase. If the striatum, this age-related deposition progresses in a dorsoventral, mediolateral, and posteroanterior direction (G.B., unpublished observations, 1997), which is similar to the progression of HD neurotoxicity.

Human studies have demonstrated mitochondrial defects in HD. Multiple animal models of HD use mitochondrial toxins that interfere with energy production. Reductions in oxidative phosphorylation tend to have a subcortical pattern of pathological development and induce age-dependent excitotoxic lesions with distribution similar to that of HD. Metabolic dysregulation of the mitochondria can result in increased free radical production and activation of NMDA receptors, which can magnify neurotoxicity at least in part through free radical mechanisms.

The destructiveness of free radicals is greatly enhanced by the catalytic effects of iron. If huntingtin is primarily a metabolic dysregulator, more severe dysregulation may occur when the CAG repeat number is large, as occurs in juvenile-onset HD. In these young individuals, the toxicity may be dependent primarily on the presence of iron, which is deposited earlier and reaches higher levels in the globus pallidus and substantia nigra than in the rest of the extrapyramidal system. Dysregulation of iron metabolism in these structures has been implicated in the pathophysiology of Parkinson disease, which often manifests with rigidity, and rigidity is preponderant in patients with juvenile-onset HD as opposed to the choreoathetoid movements observed most often in those with adult-onset HD.

Consistent with a possible role for iron as a risk factor in oxidative neurotoxicity, one human treatment study suggested that early in the course of HD, antioxidant treatment may slow the rate of motor dysfunction. In vivo brain iron quantification could lead to new treatments of HD that might slow its course. The current findings were obtained in affected individuals, some of whom were early in their illness, and it may reasonably be hypothesized that a relationship exists between premorbid iron levels and the onset and/or rate of progression of symptoms of HD. If that supposition can be confirmed in prospective studies, new avenues of treatment and prevention of the disease would be possible (e.g., iron lowering and/or antioxidant treatments). The treatment implications are especially relevant since iron chelator and antioxidant treatments are available, have been successfully used in other neurodegenerative disorders, and, with appropriate precautions, could be evaluated for efficacy in patients with HD and preclinical HD. Genetic testing makes it possible to identify at-risk individuals long before the appearance of symptoms and provides the opportunity for very early intervention.

Our observation of increased basal ganglia iron levels early in the disease process does not necessarily indicate that increased brain iron levels are an etiology of HD. Even if they are not, one may hypothesize that reducing iron levels could still be an important therapeutic intervention, given the known neurotoxic effects of increased iron levels. A clarifying analogy is Wilson disease, wherein it is therapeutically useful to treat an important factor of the pathogenesis (copper accumulation) while being unable to address the genetic etiology (defective ceruloplasmin).


