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 \(^1\) or the presence of a double dose of the gene in homozygotes. \(^2\) 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. \(^3\) 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. \(^4\) 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. \(^5\) 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, \(^6\) 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. \(^7,8\)

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. \(^9-12\) Up to 90% of nonheme iron in the brain is in the iron storage protein ferritin. \(^6,13\) 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 7.5 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 difference 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 midsagittal 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.

RESULTS

The 2 groups differed in mean FDRI in all 4 ROIs. Controlling for the effects of age and sex, the mean FDRI was significantly higher among patients with HD in the caudate (F1,34 = 32.58, P < .001), putamen (F1,34 = 17.67, P < .001), and globus pallidus (F1,34 = 20.40, P < .001). The opposite was true in the white matter, where the HD mean...
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 workstation. 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 supraorbital 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 might fall above the right side inflection point on the histogram distribution of the ROI. The mean \( T_2 \) in the normal and HD groups were then compared using a factorial analysis of covariance design. The diagnostic effects in \( T_2 \) 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 \( T_2 \), 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 \( T_2 \) was significant.

**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.

**Table 1**

<table>
<thead>
<tr>
<th>Effect</th>
<th>Caudo-Putamen</th>
<th>Globus Pallidus</th>
<th>White Matter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age 25-29</td>
<td>0.80</td>
<td>0.79</td>
<td>0.82</td>
</tr>
<tr>
<td>Age 30-34</td>
<td>0.78</td>
<td>0.77</td>
<td>0.81</td>
</tr>
</tbody>
</table>

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 is the first confirmation in vivo of the increased iron lev-
regions and is significantly different from the changes ob-
crface in ferritin iron levels is specific to the basal ganglia
regions were comparable in size, interpretation of the sepa-
ragy components of the measure (high- and low-field R2)
is also instructive. High-field R2 is sensitive not only to fer-
titin 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 sensi-
tive to ferritin iron, and thus provides a “purer” 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 Tox-
ins that disrupt metabolism and result in increased lакtate levels with decreasing pH will release iron from the
ferritin stores.31 In addition, increases in reactive oxy-
genesis species may also release iron from ferritin31 as do other
changes such as increased nitric oxide levels.32 Most hy-
potheses 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 damage44-38 and/or oxidative damage
secondary to excitotoxic neurotransmission.39,40 Excito-
toxic damage has been an HD model of striatum cell death
for over 20 years. The striatum receives dense excita-
atory glutaminergic input from the cortex. Glutamate,
glutamate receptor agonists, and especially N-methyl-D-
apartate (NMDA) receptor agonists produce toxic ef-
cfects 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.41 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 neu-
rotoxic 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 con-
centrations50; however, this structure could be less sus-

**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>
</tbody>
</table>
| White matter      | 1.37 (0.07)                                 | 1.58 (0.05)               | 6.57  | .015   | -.95|"
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. 6,32 In 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. 34,38 Multiple animal models of HD use mitochondrial toxins that interfere with energy production. 33,36,37,60 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. 33,36 Metabolic dysregulation of the mitochondria can result in increased free radical production 30,36 and activation of NMDA receptors, which can magnify neurotoxicity at least in part through free radical mechanisms. 33,39

The destructiveness of free radicals is greatly enhanced by the catalytic effects of iron. 30 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. 8 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. 47 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 (eg, 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, 36,38 and, with appropriate precautions, 35 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. 30,32-34 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).

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REFERENCES


