1H magnetic resonance spectroscopy in preclinical Huntington disease


Huntington disease (HD) is a hereditary brain disease, causing progressive deterioration after a preclinical phase. The pathophysiology of early brain abnormalities around disease onset is largely unknown. Some preclinical mutation carriers (PMC) show structural or metabolic changes on brain imaging but the most sensitive imaging modality has not been determined. $^1$H magnetic resonance spectroscopy (MRS) studies in PMC have reported conflicting results. We studied 19 PMC and 8 controls with MRS and determined relative metabolite peak areas for choline, creatine and N-acetyl aspartate (NAA) in putamen and thalamus. We found no significant differences in metabolite signals between PMC and controls. Decreases in the NAA concentration ratio of putamen relative to thalamus correlated weakly ($R^2 = 0.22$, $p = 0.04$) with increases in the product of CAG repeat length and age, a predictor of striatal damage. Since other brain imaging methods have shown changes in these study subjects, MRS is not a very sensitive detector of early HD brain pathology.
INTRODUCTION

Huntington disease (HD) is a progressive neurodegenerative disease, caused by a CAG trinucleotide expansion in the IT15 gene on chromosome 4\(^1\). Mutation carriers will develop progressive cognitive, behavioural and motor abnormalities at some point in their lives. A practical tool for detecting changes in brain of preclinical mutation carriers (PMC) is needed\(^2\), both for clarifying the initial pathological changes preceding clinically manifest HD and for use as a biomarker to facilitate evaluation of putative neuroprotective therapeutic agents for this incurable disease.

During the preclinical phase, some PMC show changes in brain structure or metabolism measured by anatomical MRI\(^3\) and PET scans\(^4\).

MR spectroscopy (MRS) has advantages over PET in availability and cost, but studies in PMC have found conflicting results. Proportions of abnormal results ranged from none to all in small series\(^5\)-\(^7\). The largest studies reported abnormal metabolite levels in 15 of 17 PMC\(^8\) and abnormal frontal lobe spectra with normal basal ganglia spectra in 17 PMC\(^9\). Different patterns of striatal metabolic alterations have been noted, stressing elevated lactate levels\(^5\), decreased \(N\)-acetylaspartate\(^6\) (NAA) or heterogeneity of results\(^8\).

We studied different imaging modalities in the preclinical phase of HD\(^10\) and report our findings with MRS in this article.

RESULTS

The relative metabolite peak areas in putamen and thalamus in PMC and controls were comparable (Table 1). The trend that NAA metabolite signal was lower in PMC than in controls is illustrated for putamen in Fig. 1 and for the ratio of putaminal over thalamic NAA in Fig. 2. Three subjects had subtle motor abnormalities on follow-up but showed metabolite values around the mean without a distinctive pattern (data not shown). No significant correlation between NAA, choline (Cho) or creatine (Cr) metabolite concentrations with the product of age and CAG repeat length\(^11\) (\(\text{AxCAG}\)) was found in putamen or thalamus. However, the putaminal/thalamic ratio of NAA concentration decreased with increasing disease burden (AxCAG) \((R^2 0.22; p = 0.04\) for linear fit; Fig. 3).

DISCUSSION

We studied basal ganglia metabolite levels in 19 well-characterized PMC, with investigators
blinded for gene status. Mutation-free siblings participated as controls and were specifically scanned for this study. Previously we reported MRI and PET changes in other neuroimaging modalities in PMC as compared to controls. We also found a correlation of these changes with AxCAG, a measure of cumulative exposure to mutant huntingtin. In the current study, no significant differences were found between PMC and controls, or between a subset of subjects with subtle motor abnormalities and other mutation carriers. Because emphasis in the early stages of HD pathophysiology lies on striatal structures, we calculated the metabolite levels in putamen relative to thalamus, another “deep” gray matter area. Here, we found a weak correlation suggesting decreasing striatal NAA content with increasing AxCAG numbers (P=0.04). To our knowledge, a correlation of MR spectra with a measure of mutant protein exposure in preclinical HD has not been reported before, possibly due to smaller sample sizes in other reports. Sanchez-Pernaute found decreased levels of striatal NAA and, to a lesser extent, of Cr in 4 PMC. This could reflect different methods of data analysis, or a more advanced state of biological damage in their set of PMC. However, our study subjects did show significant changes in striatal PET and MRI values, proving that at least some mutation related neuronal changes have occurred in many of our study subjects.

Jenkins et al. found a significant correlation of NAA loss with an index of age and CAG repeat length in clinical HD patients, but reported data on only 4 PMC. In an MRS study with a number of PMC comparable to ours, results more than 1 SD off the mean were defined abnormal. In this study, a heterogeneous profile was found with more pronounced abnormalities in the dominant hemisphere. In our PMC group, results are similar in that we have not found significant changes in MRS spectra compared to controls. We did not consider handedness but our data indicated lack of significant left-right differences in the group as a whole as well as in individual subjects. In a recent large study, Gomes-Anson and co-workers described a frontal decrease of choline, but did not observe differences in the basal ganglia between PMC and controls.

### Table 1 – Relative peak areas in preclinical mutation carriers and controls

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>PMC (n=19)</th>
<th>Controls (n=9)</th>
<th>Statistics (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Putamen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NAA</td>
<td>40.6±9.3</td>
<td>46.6±7.3</td>
<td>0.12</td>
</tr>
<tr>
<td>Cho</td>
<td>39.6±11.9</td>
<td>39.4±6.7</td>
<td>0.97</td>
</tr>
<tr>
<td>Cr</td>
<td>35.0±5.9</td>
<td>35.7±4.5</td>
<td>0.78</td>
</tr>
<tr>
<td>Thalamus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NAA</td>
<td>47.9±7.1</td>
<td>46.2±6.3</td>
<td>0.56</td>
</tr>
<tr>
<td>Cho</td>
<td>35.2±5.0</td>
<td>36.0±5.3</td>
<td>0.73</td>
</tr>
<tr>
<td>Cr</td>
<td>31.2±4.7</td>
<td>32.1±2.4</td>
<td>0.62</td>
</tr>
<tr>
<td>Putaminothalamic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ratio</td>
<td>0.858±0.21</td>
<td>0.990±0.23</td>
<td>0.14</td>
</tr>
<tr>
<td>NAA</td>
<td>1.13±0.28</td>
<td>1.10±0.15</td>
<td>0.79</td>
</tr>
<tr>
<td>Cho</td>
<td>1.14±0.23</td>
<td>1.12±0.17</td>
<td>0.85</td>
</tr>
</tbody>
</table>

Data are mean±SD, left and right values averaged. PMC, preclinical mutation carriers; NAA, N-acetyl aspartate; Cho, choline; Cr, creatine.
Fig. 1 – Relative metabolite peak areas for putaminal NAA in preclinical mutation carriers (PMC) and controls.

Fig. 2 – Putamen/thalamus ratio of metabolite peak areas for NAA in preclinical mutation carriers (PMC) and controls.

Fig. 3 – Correlation of NAA putamen/thalamus ratio with the product of age and CAG repeat length in preclinical mutation carriers (PMC), linear fit.
Some limitations to our “negative” cross-sectional study make it impossible to conclude that MRS is an unsuitable technique to detect abnormalities in preclinical HD. In a chronic disease like HD, follow-up studies are preferable because they allow intra-individual comparisons which can detect small changes, especially if different patterns of neuronal damage in HD would exist. Possibly, using other MRS parameters metabolites (e.g. myoinositol) or comparing to other brain regions (e.g. white matter) would have shown different results. Clearly, to detect subtle changes more readily, increased MRS sensitivity is needed and this is influenced by several factors. The first is spatial resolution versus the signal-to-noise ratio. We used MRS voxels of 2 cm$^3$ as compared with 2.25 to 8 cm$^3$ in the studies of Jenkins$^5$, Reynolds$^8$ and Gomes-Anson$^9$. The use of larger voxels to achieve higher spectral signal-to-noise ratios per unit of measuring time would have resulted was prohibited by the small dimensions of the brain areas studied (thalamus, putamen). The benefit of using even smaller CSI voxels in spectroscopy of the putamen and thalamus regions would be a reduction of partial volume effects, but this would be at the cost of spectral signal-to-noise ratios. The only factor likely to improve sensitivity in future MRS studies of HD as compared with our present study and those cited above would be magnetic field strength. At 3.0T as compared with 1.5T the MRS signal-to-noise-ratios roughly double, with an improving spectral resolution that will increase the detectability of amino acids, for example.

Control subjects were slightly older than PMC and theoretically, age related changes in controls could obscure similar, but HD related, changes in PMC. According to a recent review focussing on aging of the brain, there have been nine reports of decreases of NAA or NAA/Cr ratios with aging as opposed to five reports denying changes of NAA or NAA/Cr with aging$^{12}$. This indicates that the slightly lower age of PMC compared with controls can not be held responsible for NAA reductions in PMC, and, if anything, may have diminished the significance of our findings.

Since changes in other neuroimaging modalities have been found in our study subjects, these negative results suggest that MRS is not a very sensitive imaging technique for studying early changes in preclinical HD.

**EXPERIMENTAL PROCEDURES**

**Study population**

Selection of study subjects has been described previously$^{10}$. Briefly, subjects had completed the standardized procedure for presymptomatic genetic testing for the HD mutation at either Groningen or Leiden University Medical Center. They were asked to participate in a prospective imaging study with MRI and PET, either as a PMC (over 35 CAG repeats, n=27) or as a control (n=14). A movement disorder neurologist who was blinded to gene status
rated all subjects using the motor part of the Unified Huntington’s Disease Rating Scale (UHDRS) at baseline and follow-up, establishing preclinical disease state at study entry. The last 30 study subjects at follow-up were also studied with MRS. Therefore data for MRS is cross-sectional only. Three subjects were excluded because of technical problems or misplacement of volume of interest. PMC (n=19) tended to be slightly younger than controls (n=8; Table 2).

Table 2 – Age and CAG repeat length of preclinical mutation carriers and controls

<table>
<thead>
<tr>
<th>Variable</th>
<th>PMC (n=19)</th>
<th>Controls (n=8)</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>42.8±7.3</td>
<td>47.8±10.1</td>
<td>NS</td>
</tr>
<tr>
<td>Sex, female</td>
<td>63%</td>
<td>50%</td>
<td>NS</td>
</tr>
<tr>
<td>CAG repeat length</td>
<td>43.3±2.6</td>
<td>19.0±1.9</td>
<td></td>
</tr>
</tbody>
</table>

Data are mean±SD.

PMC, preclinical mutation carriers.

At the follow-up visit, three subjects had developed motor abnormalities that may be HD signs (level of confidence: 3 on a 0-5 scale). Their UHDRS motor scores were 8, 12 and 16. These were mostly oculomotor signs, and chorea scores never exceeded score 1 (slight/intermittent). These 3 subjects were all mutation carriers. Mean UHDRS motor scores were 2.9 (range: 0-16) for PMC and 0.5 (0-2) for controls. Most items scored in both groups were oculomotor signs. Investigators were blinded to mutation state until the scan data analysis was completed.

**Magnetic resonance spectroscopy and data analysis**

The standard birdcage head coil of a single Magnetom Sonata system (Siemens AG, Erlangen, Germany) was used for MRI and 1H MRS at 1.5 Tesla. Point resolved spectroscopy 2D-chemical shift imaging (CSI) measurements with a repetition time of 1500 ms and an echo time of 135 ms were performed. In our experience, the loss of signal-to-noise ratio of metabolite signals due to T2 relaxation during the comparatively long echo time interval of 135 ms is more than offset by the improved baseline definition resulting in quantified metabolite peak areas of a better reproducibility than can be achieved with the use of very short echo times. Furthermore, at an echo time of 135 ms the main lipid resonance at 1.3 ppm can be differentiated from the 1.32 lactate doublet being out-of-phase at that particular echo time. In retrospect, the latter argument has turned out to be of no value because neither lipid nor lactate signals were detected in this study. An axial MRI series was used as guidance for defining a volume of interest, a 2 cm thick slab containing the putamen and thalamus regions, for MRS. Fig. 4 is an example showing putamen and thalamus regions in this 8x8 MRS matrix. In practice, multiple retrospective positionings of the 8x8 matrix (step size 1 mm in the RL and AP directions) were applied for fitting all these anatomic regions into appropriate MRS voxels/voxel pairs. The CSI defined volume of interest was approximately 8x8 cm² subdivided in 64 voxels. The number of peaks fitted included the chemical shift ranges of 3.1-3.3 ppm for Cho,
2.9-3.1 ppm for Cr, 2.2-2.4 ppm for glutamate/glutamine (Glx) and 1.9-2.1 for NAA and their line widths and peak intensities were unrestricted. Fig. 5 shows a representative example of spectra in a PMC and a control subject.

Using standard post-processing protocols, the raw data were processed automatically, allowing for operator independent quantifications. Due to limited patient examination times, absolute quantification requiring additional CSI measurements without water suppression was not an option. We used an alternative method to facilitate tissue signal comparisons by expressing the left and right side putamen (1 voxel) and thalamus (2 voxels) metabolite signals in percents of mean voxel peak area. The sum of the Cho, Cr and NAA peak areas in the inner 6x6 voxels of each spectral map are normalized to a value of 3600% of the mean total metabolite signal (Cho+Cr+NAA) in the 36 voxels. The advantage of expressing the metabolite signals of all voxels in the same reference area is that the signals of Cho, Cr and NAA in putamen and thalamus can be compared directly. As a consequence the sum of the Cho, Cr and NAA signals in each voxel can be higher or lower than 100% (the average). We use relative metabolite concentrations rather than metabolite ratios, to prevent any index of early pathology to be compromised by independent variables contained in both the numerator and the denominator of MRS outcomes. Using non-normalized metabolites as in the current study, early changes can perhaps be defined for specific metabolites which have pathological significance (e.g. creatine).

Note that in this procedure and in our data presentation we have ignored the Glx signals which are minor at the echo time used (135 ms) and turned out to be similar in the different brain structures of patients and controls. The left and right Cho, Cr and NAA peak areas were similar both in the putamen and in the thalamus region (data not shown). The left and right relative metabolite peak areas were therefore averaged for putaminal and thalamic values. Putaminal over thalamic metabolite ratios were also calculated. Even with retrospective positioning, it was often not possible to obtain MRS voxels filled entirely with white matter. Therefore, results for white matter are not presented.

**Ethical approval and consent**

The ethical board of both Groningen and Leiden University Medical Center approved this study and all participants gave written informed consent.

**Statistical analysis**

Mean relative concentrations in putamen and thalamus were compared between PMC and controls (ANOVA). The product of age and CAG-repeat length (AxCAG) was calculated as a measure of cumulative exposure to mutant huntingtin. Correlations (linear fit) between relative concentrations and AxCAG were calculated. \( p \) values <0.05 were considered statistically significant.
ACKNOWLEDGMENT

This work was financed by the Prinses Beatrix Fonds, Project nr. 99-0209
We thank the participants in this study for their effort and cooperation.
REFERENCES


