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Neurochemical correlates of caudate atrophy in Huntington disease

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Abstract

BACKGROUND—The precise pathogenic mechanisms of Huntington disease (HD) are unknown, but can be tested in vivo using proton magnetic resonance spectroscopy (¹H MRS) to measure neurochemical changes.

OBJECTIVE—To evaluate neurochemical differences in HD gene mutation-carriers (HGMC) vs. controls, and to investigate relationships among function, brain structure and neurochemistry in HD. Since previous ¹H MRS studies have yielded varied conclusions about HD neurochemical changes, an additional goal was to compare two ¹H MRS data analysis approaches.

METHODS—HGMC with pre-manifest to early HD and controls underwent evaluation of motor function, MR imaging and localized ¹H MRS in caudate and frontal lobe. Analytical approaches tested included absolute quantitation (unsuppressed water signal as an internal reference) and relative quantification (calculating ratios of all neurochemical signals within a voxel).

RESULTS—We identified a suite of neurochemicals reduced in concentration proportionally to loss of caudate volume in HGMC. Caudate concentrations of NAA, creatine, choline, and caudate and frontal concentrations of glutamate+glutamine and glutamate correlated with caudate volume in HGMC subjects. The relative, but not the absolute quantitation approach revealed disease-related differences; the Glx signal was decreased relative to other neurochemicals in caudate of HGMC subjects vs. controls.

CONCLUSIONS—This is the first study to demonstrate correlation among structure, function and chemical measures in HD brain. Additionally, we demonstrate that a relative quantitation approach may enable magnification of subtle differences between groups. Observation of decreased glutamate-glutamine signals suggests that glutamate signaling may be disrupted relatively early in HD, with important implications for therapeutic approaches.

Keywords

Huntington disease; caudate; magnetic resonance spectroscopy; magnetic resonance imaging; glutamate

Introduction

The Huntington disease (HD) mutation, an unstable expansion of CAG trinucleotide repeats on the IT15 gene, produces a mutant protein (huntingtin) with expanded polyglutamine residue¹. Increased CAG-expansion length associates with earlier symptom onset² and more rapid progression of brain atrophy, particularly in caudate, which undergoes early and severe atrophy³. Although HD etiology is known, mechanisms by which HD mutation causes progressive brain atrophy and associated cognitive, affective and motor symptoms are poorly understood, hindering therapeutic development.

Major goals of HD research include development of mechanistic insights and identification of biomarker surrogates for clinical endpoints⁴. Since striatal volume loss occurs >10 years before onset of motor/cognitive symptoms, MRI-measured striatal volume is one promising biomarker⁵. Volumetric changes (reflecting neuron death, gliosis and other factors) are likely preceded by neurochemical changes⁶, which may be examined in vivo by MR spectroscopy (MRS). MRI- and MRS-based HD studies generally relate the primary measure (brain structure volume or neurochemical concentration) to functional variables (e.g., motor control, cognition). Interestingly, no human HD studies directly test relationships among all three (structural, neurochemical, functional) variables. One aim of this study was to address this knowledge gap.

Consensus on neurochemical changes is lacking among human HD studies⁷. Creatine (energy substrate), n-acetylaspartate (neuronal marker), and glutamate (excitatory neurotransmitter) have been reported to decrease or not change in HD^{7, 8}. Cholines (cell membrane constituents reflecting cell turnover) and combined glutamate-glutamine signal (termed Glx) are reported to increase, decrease, or not change^{7, 9-11}. Myo-inositol (glial marker and osmolyte) and lactate (end-product of glycolysis that increases with mitochondrial dysfunction) are reported to increase or not change¹¹⁻¹³. Differences in HD severity, brain region sampled, subject sample size, and acquisition/analytical approaches may in part explain the divergent results.

To address the problem of variability in HD ¹H MRS studies, we propose a modified data analysis approach. Roughly half of previous studies report “absolute” (molar) neurochemical concentrations (calibrating neurochemical peak area to total brain water peak area). This approach is widely considered the standard for ¹H MRS¹⁴, despite many technical difficulties and assumptions^{14, 15}. Other ¹H MRS studies report neurochemicals as ratios (relative to peak area of a reference neurochemical in the same voxel). Although a ratio approach may obscure interpretation of which neurochemical (numerator or denominator) has changed, and may complicate inter-study comparisons¹⁶, it offers two benefits: ease of implementation (no additional spectra to collect) and avoidance of partial-volume effects arising from varying CSF fractions in each voxel¹⁴. We propose that calculation of peak area ratios (of each neurochemical relative to *all* others in the spectrum) enables screening for patterns of HD-related changes. This approach avoids partial-volume effect complications and the potentially-problematic assumption that the reference peak is unchanged by disease.

Our primary aims were to investigate relationships among neurochemical, volumetric and clinical variables in HD, and to identify any HD-characteristic neurochemical change patterns in caudate (a primary site of HD pathophysiology) and frontal lobe (a region with substantial connectivity to caudate¹⁷). We also tested the hypothesis that ratio-based data analysis facilitates detection of subtle neurochemical differences between HD and control subjects.

Methods

Study design

Participants were recruited from the Seattle, WA area. Inclusion criteria included age 18-70 and HD mutation-positive (CAG repeat >39) history, including those with pre-manifest and those with neurologist-diagnosed early-stage manifest HD (collectively, HD gene mutation carriers; HGMC). Controls (no family history of HD or confounding neuromotor problems) were age- and gender-matched to HGMC. The University of Washington Institutional Review Board approved the study, and participants gave informed consent. All subjects

underwent clinical evaluation for motor impairment, then MRI/MRS during a subsequent session.

Demographics

Subjects ranged from age 19-66 (Table 1). Subjects with pre-manifest (n=6) and early (n=4) HD were included in the HGMC group; CAG repeats ranged from 40-48 and CAP score¹⁸, a measure of disease burden calculated as age $\times$ (CAG repeats-33.66), ranged from 222-494.

Motor function assessment

Motor function was evaluated by a trained clinician experienced in movement disorders (A.S.) using the motor section of the Unified Huntington Disease Rating Scale (UHDRS). For one pre-manifest and one manifest subject, motor scores were not obtained.

MR imaging and volumetric analysis

MR procedures utilized a Philips 3-Tesla Achieva whole-body scanner with standard transmit-receive head coil. Whole brain and caudate volumes were determined based upon T1-weighted magnetization-prepared rapid gradient echo (MPRAGE) high-resolution images: repetition time (TR)=7 ms, echo time (TE)=3.20 ms; flip angle=8°; matrix size=240×240, 160 sagittally-collected slices (1-mm thickness; ~20-min scan time). MPRAGE was resampled to 1×1×1-mm resolution, and volume measurements were performed with standardized manual tracing procedures¹⁹ implemented using MEASURE software²⁰. Volumes for left caudate (the site of the MRS measures) are reported.

MR spectroscopy

Voxels were centered on caudate and frontal lobe (Fig. 1A), based upon reconstructed MPRAGE images. From each 20×20×20-mm voxel, 64 spectra were collected (TR=2000 ms, TE=32 ms, 2048 complex samples) using point-resolved spectroscopy (PRESS) with water suppression. Additional PRESS spectra were collected from these voxels to determine glutamate concentrations²¹ (TR=2000 ms, TE=80 ms) and estimate water concentrations (T2-weighted water-unsuppressed scan, TR=8000 ms, TE=30 ms, 2 measurements, 2048 complex samples). The following neurochemicals (Fig. 1B,C) were evaluated with standardized model-fitting procedures (LCModel software²²): total NAA (NAA; *N*-acetyl aspartate+*N*-acetyl aspartylglutamate), total creatine (Cr; creatine+phosphocreatine), total choline (Cho; phosphocholine+glycerolphosphocholine), myo-inositol (m-Ins), glutamate (Glu), and Glx (glutamate+glutamine). Residual water signals were subtracted with a decomposition-fitting algorithm. Free induction decays were zero-filled, smoothed (1.1-Hz exponential-dampening filter), then zero- and first-order phase-corrected. Spectrum quality determinants included Cramér-Rao lower bounds (as % estimated concentration) <20%, signal-to-noise ratio ≥ 5 , and peak width ≤ 0.1 ppm²³. Gray and white matter and CSF volume estimates (FSL FAST segmentation; www.fmrib.ox.ac.uk/fsl/fast) were used only to correct for CSF partial-volume effects. Absolute concentrations were determined by scaling in vivo spectra to unsuppressed water peak.

Statistics

Statistical analyses were performed using SigmaStat software (version 3.5, Aspire Software International, Ashburn, VA, USA). Differences between controls and HGMC were evaluated using *t*-tests (or Mann-Whitney tests, where data were non-normally-distributed) and correlations were evaluated with linear regression. Although multiple statistical tests inherent in exploratory data-screening studies necessarily increase risk of type I errors, as with other exploratory studies of neurochemical changes in HD¹¹, corrections for multiple

testing were not performed to avoid inflation of type II errors²⁴. Appropriate caution should therefore be applied in interpretation of the statistical results of this exploratory study.

Results

Functional assessments

Motor function was impaired in HGMC relative to controls (Table 1), with scores ranging from 5-18. Among HGMC, motor impairment was negatively-correlated with caudate ($p<0.001$) and whole brain ($p=0.012$) volume, and positively-correlated with CAP score ($p=0.003$). Among neurochemicals measured in caudate, NAA correlated with motor score, and in frontal lobe, Glx and Glu concentrations correlated with motor score (Fig. 2).

Volumetry

Group-wise differences in brain volume were evident, with smaller whole-brain and left-caudate volumes in HGMC vs. controls (Table 1). Among HGMC, low caudate volume associated with decreased motor function, increased disease burden (CAP score; $p=0.022$) and decreased whole brain volume ($p = 0.003$). We observed reduced concentrations of several neurochemicals in association with HD atrophy; caudate NAA, creatine, choline, Glx, and glutamate correlated significantly with caudate volume in HGMC (Fig. 3A). Caudate volume, a well-characterized marker of disease progression, also correlated with glutamate and Glx concentrations in frontal lobe (Fig. 3B).

Neurochemical analysis

In addition to correlations with functional/structural measures, several neurochemicals varied with disease burden among HGMC. In caudate ($p=0.023$) and frontal lobe ($p=0.005$), NAA decreased with increasing CAP score. Frontal lobe Cho and Glu ($p=0.033$, $p=0.013$) also decreased with increased CAP score.

Group-wise differences in mean neurochemical concentrations between HGMC and controls were not observed in caudate or frontal lobe voxels with absolute quantitation (Table 1). Relative quantification, however, revealed several neurochemical differences between HGMC and controls. Group-wise differences in neurochemical ratios were calculated as below (sample calculation for NAA/glutamate ratio).

$$\text{neurochemical ratio difference} = \frac{\left[\frac{\text{NAA}}{\text{Glu}} \right]_{\text{HGMC}} - \left[\frac{\text{NAA}}{\text{Glu}} \right]_{\text{C}}}{\left[\frac{\text{NAA}}{\text{Glu}} \right]_{\text{C}}} \cdot 100\% \quad \text{Eq.1}$$

Results of this approach are compiled as a heat map (Fig. 4). In caudate, Glx relative to NAA, Cho and m-Ins was significantly decreased in HGMC vs. controls. Differences between HGMC and controls in NAA, Cr or Cho relative to each other were negligible, and trends toward increased m-Ins relative to all other neurochemicals were noted. In HGMC (relative to controls) a trend toward reduced glutamate was apparent, and Glu/m-Ins ratios were significantly decreased. In frontal lobe, no group-wise differences were observed, although trends toward increasing m-Ins and Glx, and decreasing Glu, were noted.

Primary findings

This first report of correlations between HD effects on brain volume and chemistry demonstrates that a suite of neurochemical concentrations in caudate (and glutamate and glutamate-glutamine in frontal lobe) are reduced in association with smaller caudate volume in HGMC. By comparing water-referenced vs. ratio-based analyses, we provide evidence

that some inconsistency among ^1H MRS HD studies may be due to analytical approach. This methodological comparison illustrates the potential utility of relative quantitation for amplifying patterns of neurochemical differences between HGMC and controls. We also report decreased relative Glx signal in caudate of HGMC vs. controls. Although the prevailing perception is that glutamate-glutamine is increased in HD²⁵, recent studies^{11, 26} support our finding of decreased brain glutamate. As our sample was small (10 HGMC, 10 controls) and included pre-manifest and early-HD subjects, findings from this exploratory study should be interpreted cautiously, and should be used to design a more highly-powered follow-up study.

Caudate volume correlates with neurochemical changes

Longitudinal atrophy is one of the earliest and best-described pathological changes during HD; neuronal loss and glial proliferation in striatum are defining features of pathological progression²⁷. Although genetic models have advanced understanding of HD pathology, it remains unclear when in human HD neurochemical changes occur, and whether qualitative neurochemical changes precede changes in cell numbers. MRS studies generally relate the time-course of HD neurochemical changes to motor function or disease burden, rather than striatal volume. Unschuld and colleagues²⁶ recently published the only MRS study that includes brain structure volumes, although correlations between volumetric and MRS measures were not reported. We report that caudate NAA, Cr, Cho, Glu, and Glx concentrations (corrected for CSF contamination, and thus, for percent tissue in the voxel) are reduced proportionally to caudate volume. These results indicate that HGMC caudate undergoes not only quantitative reduction (Table 1) but also qualitative changes consistent with loss of neuronal integrity (decreased NAA) and impaired cellular energetics (decreased Cr). Reports indicate decreased NAA and Cr in later-stage HD^{7, 9, 11, 28, 29}, and correlations between reduced NAA or Cr and worsening function or disease burden^{7, 9, 11}. Although we did not demonstrate decreases in these neurochemicals in HGMC vs. controls, caudate NAA was negatively-correlated with disease burden and motor impairment. Neurochemical concentrations in controls (Fig. 2 and 3, thick lines along axes) do not necessarily fall along the trajectory of neurochemical changes with HD progression; this is not unexpected, since mutant HD protein expression is constitutive (i.e., HGMC and control neurochemical concentrations may never match, even far in advance of manifest disease).

Based on postmortem observations of increased glial density in late-stage HD caudate²⁷, increased Cho with disease progression might be expected. Cho signal arises from choline-containing compounds (phosphocholine, glycerolphosphocholine, and phospholipid cell membrane constituents), and correlates with Ki-67, a cellular proliferation marker³⁰. One explanation for the observed association between low Cho and low caudate volume may be inter-subject variability in relative rates of glial proliferation and neuron loss. If glial proliferation outstrips neuron loss, higher Cho and caudate volume would occur; if glia proliferate at lower rates or later relative to neuronal loss, lower Cho and caudate volume would result. Reported choline changes in HD are substantially heterogeneous (decreased, increased, no change^{7, 8, 11}).

Low caudate volume in HGMC was also related to low Glu and Glx in caudate and in frontal lobe, where Glu and Glx were the only neurochemicals associated with volumetric or motor function measures. Conclusions regarding glutamate and glutamate-glutamine changes in HD have been somewhat heterogeneous (discussed below).

Group-wise neurochemical differences

Reduced caudate Glu and Glx ratios were the primary characteristic distinguishing HGMC from controls. This is consistent with our observation of lower Glu and Glx in caudate and

frontal lobe in association with smaller caudate volume (Fig. 3). Although other neurochemicals (NAA, Cho, Cr) also correlated with caudate volume, group-wise concentration differences were not observed due to greater variability inherent in group-wise comparisons. HGMC spanned a >2-fold range of CAP scores, likely reducing ability to detect group-wise differences. In contrast, heterogeneity in disease progression does not impede correlations among disease metrics; in fact, it provides a larger dynamic range over which to test relationships.

Reduced relative Glx in HGMC (vs. control) caudate (Fig. 4) reflects combined reduction of glutamate and glutamine; both signals overlap at TE=32. Although additional spectra were acquired at TE=80 to measure primarily glutamate²¹, signals from glutamate at TE=80 exhibited more variability than Glx at TE=32 (Table 1). This could reflect either greater variability in glutamate than glutamine concentrations or greater variability in the signal acquired at TE=80 vs. TE=32. Both Glx and glutamate were decreased (Fig. 4) relative to other neurochemicals in HGMC caudate. Disease-related differences in most Glx ratios were statistically verifiable, but differences in glutamate ratios (except glutamate/m-Ins) were not, due to variability in TE=80 signal. These observations point to a likely difference in glutamate concentrations between HGMC and controls.

Evidence for biphasic glutamate changes during HD progression

Postmortem studies indicate that up to 95% of striatal medium-spiny GABAergic neurons (MSN) die in advanced HD²⁷. Excitatory input to striatal MSN comes primarily from cortical and thalamic glutamatergic neurons; thus, corticostriatal glutamatergic neurotransmission has been a focus of investigation in HD.

Increases and decreases in glutamatergic neurotransmission (glutamate release, glutamate-receptor-mediated currents) are reported in HD. These apparently conflicting observations may reflect temporal and region-specific pathophysiological elements^{31, 32}. Prior to HD phenotype appearance, HD mutation-expressing mice exhibit increased striatal glutamate neurotransmission³¹, and intra-striatally-injected glutamate agonists elicit HD pathology^{33, 34}. Astrocytic glutamate clearance from the synaptic cleft (preventing postsynaptic excitotoxicity) is impaired in HD mutant mice^{35, 36}; impairment is ameliorated by increasing expression of astrocytic glutamate transporters³⁷. Conversely, in later stages of HD, decreased glutamate signaling is observed, with reduced glutamate concentrations^{38, 39} and receptor binding⁴⁰ in postmortem striatum. In HD mutation-carrying animals, excitatory postsynaptic currents^{31, 41}, sensitivity to excitotoxicity⁴², and glutamate release along the corticostriatal pathway³¹ are decreased upon HD phenotype onset.

Our observation of decreased Glx in caudate is surprising, given that reduced glutamatergic signaling is associated with later-stage HD. HGMC subjects were at a relatively early disease phase (60% not yet diagnosed with HD). Since the time-course of glutamate changes has largely been determined in animals, our observation of decreased glutamate in pre- to early-HD subjects may reflect a species-specific difference. Numerous differences between animal models and human HD are reported⁴³. Another potential explanation is that, although most HGMC were pre-manifest, motor function and caudate volume were notably affected (Table 1), perhaps consistent with phenotypes of later-stage HD models. A diffusion-tensor imaging study in these HGMC detected HD-related reductions in frontostriatal white matter integrity¹⁷. This finding, along with the observed relative decrease in caudate Glx, supports the idea that glutamatergic signaling is already down-regulated in these subjects.

Therapeutic implications

Decreased striatal glutamatergic transmission in subjects with pre-manifest- to early-HD has important implications for therapeutic intervention. Strategies focused on glutamate have sought to suppress signaling, based on early glutamate excitotoxicity observed in animal models. However, clinical trials of anti-glutamatergic agents (amantadine, riluzole, remacemide, ketamine) have been largely ineffective⁴⁴. The trials utilized changes in motor impairment as endpoints; these motor-impaired subjects may have (as in the current study) already progressed from increased to decreased glutamate signaling.

Methodological considerations

¹H MRS studies frequently report neurochemical concentrations relative to a single reference peak (e.g., creatine). Since creatine concentrations change in HD, it is unclear whether altered ratios indicate change in the neurochemical of interest or creatine. The current approach screened changes in ratios of all neurochemicals relative to each other to identify consistent trends (e.g., decreased Glx relative to NAA, Cho and m-Ins more likely reflects a decrease in Glx than simultaneous increases in all denominator neurochemicals).

Our detection of HD-related neurochemical differences by relative, but not absolute, quantification suggests that internal water signal-referencing methods may not be universally appropriate. One caveat¹⁵ for water-referencing approaches is that water densities and relaxation times of each water signal component must not differ between experimental groups. Total water signal reflects combined signals from gray and white matter, CSF, intra- and extracellular water; each exhibit different T2 relaxation times⁴⁵ that may change independently with disease⁴⁶. While our study was not designed to measure brain tissue water, such an investigation may be warranted.

Although no other MRS HD studies explicitly compare absolute vs. relative quantification, our ratio screening approach is testable with published data. For example, a recent study reported reduced caudate NAA and Cr in manifest-HD subjects vs. controls using absolute quantitation¹¹. Re-calculation of concentrations as ratios (Eq. 1, with variance calculated as in ⁴⁷) shows that Cr, Cho, NAA, and Glx do not differ relative to each other. Instead, m-Ins is increased relative to each, consistent with trends observed in our study. Although ratio-based analysis did not reveal HD-related Glx changes, this manifest-HD group was advanced in disease stage relative to our subjects (UHDRS=20.9 vs. 11.0). Manifest-HD subjects evidenced lower absolute caudate Glx, creatine, choline and NAA than controls (although only creatine and NAA reached statistical significance). A ratio approach may become less useful as neurochemical concentrations universally decrease with extensive neuronal death. Myo-inositol, however, could conceivably increase to maintain osmolarity during widespread cell destruction.

Summary

Analytical method can contribute significantly to variability in HD ¹H MRS results. Screening for HD-related changes in all neurochemical peaks, relative to each other, enables identification of patterns that likely indicate true disease-related changes rather than analytical artifacts. Decreased relative caudate Glx in pre-manifest- to early-HD suggests earlier dysregulation of corticostriatal glutamatergic neurotransmission than suggested by animal models. HD progression therefore may partially account for mixed results of anti-glutamatergic drugs. Careful targeting of this type of therapeutic approach to subjects far in advance of disease onset may be important.

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Fig. 1.

Representative illustration of voxel placement (white box) centered on caudate (upper panel) and frontal lobe (dorsolateral prefrontal cortex; lower panel) (A). The caudate voxel was placed to maximize coverage of the caudate and to minimize coverage of other nearby structures such as internal capsule and putamen as much as possible. For both the caudate and frontal lobe voxels, percentages of gray and white matter included in the voxel did not differ between HGMC and controls (*t*-test). Representative spectra obtained from the caudate voxels in control (B) and HGMC (C) subjects. Thick black line indicates fit obtained using LCModel, thick gray line indicates baseline. Labels indicate NAA, creatine (Cr), choline (Cho), myo-inositol (ml), glutamate (Glu), and glutamate+glutamine (Glx).

Fig. 2.

Relationships between motor function (UHDRS total motor score) and neurochemical measures in caudate and frontal lobe among HGMC subjects. Worsening motor function is indicated by an increased UHDRS score. Pre-manifest subjects are indicated with open circles, subjects with early HD with gray-filled circles. For two HGMC subjects, UHDRS scores were not obtained, and for three HGMC subjects, frontal lobe Glu did not meet spectrum quality determinants, and was excluded. Lines indicate the result of linear regression for caudate NAA ($p = 0.018$), frontal lobe Glx ($p = 0.008$) and frontal lobe Glu ($p = 0.014$). Thick lines along the axes indicate the mean $\pm$ SD for control subjects, provided for context.

A) Caudate

B) Frontal Lobe

Fig. 3.

Relationships between caudate volume, a well-characterized indicator of disease progression in HD, and neurochemical concentrations in caudate (A) and frontal lobe (B) among HGMC subjects. Pre-manifest subjects are indicated with open circles, subjects with early HD with gray-filled circles. Spectrum quality control determinants were not met for one HGMC subject's caudate Glu and three HGMC subjects' frontal lobe Glu; therefore these values were excluded. Lines indicate the result of linear regression for caudate NAA ($p = 0.006$), Cr ($p = 0.011$), Cho ($p = 0.003$), Glx ($p = 0.044$) and Glu ($p = 0.025$), and for frontal lobe Glx ($p = 0.028$) and Glu ($p = 0.012$). Thick lines along the axes indicate the mean $\pm$ SD for control subjects, provided for context.

Fig. 4.

Differences between HGMC and control subjects in terms of neurochemical ratios within the caudate and frontal lobe. Patterns of changes are emphasized by grayscale-coding: directionality of neurochemical ratio differences is indicated in black (negative) or white (positive) and the magnitude of the difference is represented by intensity. Significant differences between HGMC and controls are indicated (* $p < 0.05$, ** $p < 0.01$, t -test, or † $p < 0.05$ Mann-Whitney).

Table 1
Subject characteristics; mean (SD)

Subject Characteristics	Control (n=10)	HGMC (n=10)
Age, years	41.0 (12.9)	42.7 (14.1)
% Female	50	50
CAG repeats	n/a	42.6 (2.92)
CAP score ^a	n/a	356 (90.3)
Motor UHDRS score [‡]	3.70 (2.41)	11.0 (4.00) ***
Whole brain (mL)	1040 (83.1)	907 (96.6) **
Left caudate (mL)	4.23 (0.236)	3.32 (0.560) ***

	Caudate	Frontal Lobe	Caudate	Frontal Lobe
tNAA (IU)	8.60 (1.39)	12.2 (1.00)	9.51 (1.41)	12.1 (1.20)
tCr (IU)	7.50 (1.31)	9.38 (1.21)	7.89 (1.64)	9.36 (0.980)
tCho (IU)	1.87 (0.423)	2.47 (0.510)	2.10 (0.582)	2.40 (0.430)
Myo-I (IU)	4.21 (1.33)	6.42 (1.62)	5.52 (1.77)	7.00 (1.20)
Glx (IU)	13.4 (2.07)	13.2 (2.29)	12.7 (2.66)	14.9 (2.63)
Glu (IU)	7.33 (1.41)	10.29 (2.01)	6.54 (2.65)	10.1 (1.71)

* p < 0.05,

** p < 0.01,

*** p < 0.001; t-test between HD and control

^a A measure of disease burden calculated as a function of age and number of CAG repeats (Zhang et al., 2011)

[‡] Subjects scoring < 20 on UHDRS were excluded from the study due to risk of movement compromising MRS data quality.