

Preliminary Study of the Association of White-Matter Metabolite Concentrations With Disease Severity in Patients With Huntington's Disease

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Proton magnetic resonance spectroscopy is used to measure several metabolites in cortical gray and white matter in patients with Huntington's disease. The preliminary results show that CAG-repeat length correlates with white-matter N-acetylaspartate concentrations, and disease severity correlates with several metabolites.

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Huntington's disease (HD) is a devastating neurodegenerative disorder that is characterized by cognitive impairment, psychiatric/behavioral disturbance, and motor abnormalities including chorea and rigidity. Initial onset of physical symptoms occurs in middle age and slowly progresses to death over an average period of 15–20 years. Disease severity is correlated with over-expansion of CAG repeats on the IT15 gene,¹ which encodes the huntingtin protein.²

Degeneration of the striatum (caudate nucleus and putamen) in HD has been well documented, but evidence has accumulated characterizing abnormalities

in brain regions outside of the striatum. Morphometric analyses have shown atrophy in almost all brain structures, including the entire cerebral cortex.^{3,4} Studies have also demonstrated relationships between structure abnormalities and disease symptoms.⁵ Halliday et al. found that the severity of cortical atrophy correlates with the atrophy observed in the striatum and with the pathological grade of the disease, and the rate of cortical atrophy correlated with CAG-repeat numbers.⁶ Beglinger et al. showed that in early-stage HD subjects, the volume of the cerebral cortex was not significantly different from that of controls, whereas the cerebral white-matter (WM) volume was robustly decreased.³ WM volume has also been significantly associated with cognitive performance, showing a structure–function relationship outside the striatum.³ Other investigators have found significant changes in WM by use of diffusion tensor imaging (DTI).^{7,8} Taken together, the literature indicates that changes in cortical WM are of interest in understanding the pathophysiology of HD.

Proton magnetic resonance spectroscopy (1H-MRS) can provide information on brain pathophysiology by quantifying the concentrations of monitor brain metabolites such as N-acetylaspartate (NAA), creatine (Cr), choline (Cho), and myo-Inositol (Ins). Lower concentrations of NAA have been interpreted to reflect neuronal loss, metabolic dysfunction, or myelin repair.¹² Changes in the Cho signal have been proposed to reflect membrane damage or repair, and Ins is an important regulator of brain osmotic balance.¹² 1H-MRS has been used to investigate preclinical HD, with the main focus being the striatum. Sturrock et al. found decreases in NAA and increases in Ins in the putamen correlated with motor symptoms and disease severity in HD.⁹ Several 1H-MRS studies of cortical areas in prodromal HD have found Cho to increase with disease severity.¹⁰ However, they have been limited to single voxel studies, did not

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distinguish between changes in WM and gray matter (GM), and did not evaluate the relationship between metabolite concentration and cognition. 1H-MRS has been used to show that concentration of metabolites may correlate with cognitive functioning in healthy subjects.¹¹

In the present study, we used 2D multivoxel 1H-MRS to measure several metabolites in cortical GM and WM regions superior to the lateral ventricles in patients with HD. We then examined the relationships between these metabolite concentrations and measures of disease severity. Because previous research has found WM structural abnormalities that correlate with CAG-repeat lengths and disease severity, we hypothesize that WM metabolite concentrations in 1H-MRS would also correlate in a similar manner.

METHODS

The protocol and all study procedures were approved by the University of Iowa Institutional Review Board. All participants provided written informed consent and received financial compensation. Participants had either diagnosed HD (i.e., positive family history in the presence of characteristic motor abnormalities diagnosed as HD by an independent neurologist) or a positive genetic test for the HD gene expansion, with at least some motor abnormalities as defined by the Unified Huntington's Disease Rating Scale (UHDRS) motor exam (i.e., Diagnostic Confidence Level >1). The subjects were participating in a multisite, prospective, randomized, clinical trial of citalopram or placebo, examining symptomatic treatment for early executive dysfunction in HD. All participants at the University of Iowa site were offered participation in an add-on imaging study, and a subset of six nonclinically depressed subjects with HD who had complaints of reduced cognition completed spectroscopy scans at their baseline visit before receiving drug. Patients were screened for depression with a clinician-administered depression rating scale (Hamilton Rating Scale for Depression [Ham-D]) and interviewed by the study investigator. Patients with active depression were excluded. All participants had a positive test for the HD gene expansion (CAG >39). A battery of measures of functional and motor symptoms (Unified HD Rating Scale [UHDRS]), Total Functional Capacity (TFC), and Total Motor Score [TMS]), as well as neuropsychological tests (Symbol Digit Modalities Test [SDMT],

Psychomotor Speed and the Trail-Making Test Part B [TMTB], Executive Functioning) were administered on the same day as the MR scans.

MRI and multivoxel 1H-MRS experiments were performed on a Siemens 3T scanner. T1-MPRAGE images (TR/TI/TE: 2530/800/3.18 msec) were obtained, with a voxel size of 0.8 mm³. T2 turbo spin echo images were obtained for accurate placement of the 1H-MRS grid. 1H-MRS was performed with a phase-encoded version of a point-resolved spectroscopy sequence (PRESS) with and without water suppression (TR/TE: 1500/40 msec, slice thickness: 15 mm; FOV: 220×220 mm; total scan time: 582 sec). The 1H-MRS volume of interest was selected in cortical GM and WM regions superior to the lateral ventricles and parallel to the AC–PC axis.

The 1H-MRS data were processed with the software package LCModel. The T1-MPRAGE images were the basis for tissue classification of GM, WM, and CSF in the 1H-MRS volume of interest. Tissue water was used as a concentration reference and for partial volume correction, as previously described.¹³ The 1H-MRS signals examined included several metabolites whose spectrums overlap and therefore are quantified together. The NAA signal represents N-acetylaspartate and N-acetylaspartylglutamate signals, dominated by N-acetylaspartate. The Cr signal combines creatine and phosphocreatine signals. The Cho signal consists of contributions from glycerophosphocholine, phosphocholine, and, typically, a small amount of free choline. Also analyzed in this experiment was the Ins signal. Statistical analyses were performed with SPSS (<http://www.spss.com/>).

RESULTS

All participants had mild-to-moderate HD (TFC range: 7–13; TMS mean: 17.2 [20.0]) and evidence of cognitive decline relative to their estimated premorbid IQ, particularly on psychomotor speed (Symbol Digit Modalities Test mean: 1.2 standard deviations (SD) below normative controls). In an exploratory bivariate correlation analysis uncorrected for multiple comparisons, the CAG-repeat length correlated significantly only with WM NAA concentrations (Pearson $r = -0.822$; $p = 0.045$), and several clinical measures of disease severity consistently demonstrated strong and significant correlations with WM NAA, Cho, and/or Ins (Table 1). In follow-up multivariate regression analyses with patient age as

TABLE 1. Pearson Correlation Coefficients (two-tailed p values)

	TFC	CAG	TMS	SDMT	TMTB
GM NAA	-0.776 (0.069)	0.619 (NS)	0.737 (NS)	-0.581 (NS)	-0.769 (0.074)
WM NAA	0.964 (0.002)	-0.822 (0.045)	-0.973 (0.005)	0.811 (0.050)	0.872 (0.024)
GM Cr	-0.456 (NS)	0.394 (NS)	0.599 (NS)	-0.339 (NS)	-0.565 (NS)
WM Cr	0.584 (NS)	-0.801 (0.055)	-0.442 (NS)	0.419 (NS)	0.658 (NS)
GM Cho	-0.344 (NS)	0.089 (NS)	0.686 (NS)	-0.576 (NS)	-0.202 (NS)
WM Cho	-0.861 (0.028)	0.650 (NS)	0.934 (0.020)	-0.941 (0.005)	-0.649 (NS)
GM Ins	0.131 (NS)	-0.489 (NS)	0.146 (NS)	-0.019 (NS)	0.249 (NS)
WM Ins	-0.858 (0.029)	0.610 (NS)	0.941 (0.017)	-0.955 (0.003)	-0.645 (NS)

N=6, except for Total Motor Score (TMS; N=5).

TFC: Total Functional Capacity; SDMT: Symbol Digit Modalities Test; TMTB: Trail-Making Test, Part B; GM: gray matter; WM: white matter; NAA: N-acetylaspartate; Cr: creatine; Cho: choline; Ins: myo-inositol.

a covariate, the correlations between WM metabolites and disease measures remained strong and significant, except for the correlation of NAA with CAG ($p=0.068$).

DISCUSSION

The directions of the correlations of metabolites NAA, Cho, and Ins in WM with subject SDMT score were in the expected directions (i.e., NAA correlated positively with motor and cognitive tests for which higher scores reflected better performance [TFC and standardized SDMT scores]) and negatively with tests for which lower scores reflected better performance (TMS). Since higher Cho and Ins have been shown to reflect pathology, the correlations involving these metabolites were expected to be in the opposite direction. However, none of the measured GM metabolite concentrations correlated with either CAG-repeat length or other disease measures.

The findings of abnormal metabolite concentrations in cortical WM support the previous research that HD disease pathology extends beyond the striatum. DTI has been used to investigate WM microstructure in HD pathology.^{7,8,14} Rosas *et al.* compared preclinical HD subjects with normal-controls and found decreased fractional anisotropy (FA) within the corpus callosum and subcortical structures.⁸ Magnotta *et al.* extended that research and found a relationship between DTI scalar measures in WM and the proximity to disease diagnosis.⁷ Kloppel *et al.* used fiber tracking and FA measures to conclude that WM changes may be distributed throughout the brain, and they found a correlation between WM changes, atrophy, microstructural, and

cognitive measures.¹⁴ Similarly, there is accumulating evidence of cortical-striatal pathology at the cellular level. Gu and colleagues¹⁵ proposed a pathogenic model of HD stemming from dysfunctional cortical and striatal circuitry. Specifically, they showed that expression of the mutant huntingtin protein in cortical interneurons was critical for cortical pathogenesis and that this interneuron dysfunction occurs at an early stage, which, in turn, leads to neuropathogenesis in the cortex and striatum.¹⁵ The findings in our study point to a WM metabolic perturbation in patients with HD related to disease severity and consistent with evidence of WM pathology detected by past morphologic, DTI, and cellular studies.

Our preliminary findings show evidence of cortical WM pathology by use of an imaging modality, multi-voxel 1H-MRS, not previously reported, and findings converge with results from other imaging techniques pointing to cortical WM abnormalities in HD. Although no significant correlations with cortical GM metabolite concentrations were observed, these results do not preclude the possibility of altered GM metabolism in the sample relative to subjects without HD. The preliminary findings are limited because of the small sample, the exploratory bivariate correlation uncorrected for multiple comparisons, and lack of control group. A larger, longitudinal study is needed to confirm the role of metabolite concentrations in WM and GM as biomarker for HD disease severity and progression.

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