Decreased N-acetyl-aspartate/choline ratio and increased lactate in the frontal lobe of patients with Huntington's disease: a proton magnetic resonance spectroscopy study

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Abstract

Background—Both the effect of the mutation and the pathogenesis of Huntington's disease are unknown and a lack of biological markers for the natural history of the disease impedes the evaluation of novel therapeutic approaches.

Methods—Proton magnetic resonance spectroscopy was carried out on a frontal region of the cortex in 17 patients with clinically overt Huntington's disease and four asymptomatic gene carriers.

Results—Eight of 17 (47%) clinically affected patients with Huntington's disease and each of the asymptomatic carriers had lactate peaks in the frontal cortex which were not present in controls. The N-acetyl-aspartate/choline (NAA/Ch) ratio was significantly reduced in the symptomatic patients indicating the presence of neuronal loss. The reduction was related to the clinical severity of the disease and was absent in the asymptomatic carriers.

Conclusion—The finding of lactate peaks supports the hypothesis that disturbed cerebral energy metabolism contributes to the pathogenesis of Huntington's disease.

After an increased number of CAG repeats was shown to be the genetic defect in Huntington's disease,[1] efforts were concentrated on the functional consequences of the mutation, pathogenesis of the disease, and future neuroprotective strategies. There is evidence that impairment of production of chemical energy significantly contributes to the pathogenesis of striatal lesions in Huntington's disease,[2 5] in particular, because inhibition of cerebral oxidative phosphorylation results in a pattern of vulnerability closely resembling the disease.[6–8] As these lesions can be attenuated or prevented by antagonists to glutamate receptors, it has been postulated that the mechanism of "weak" excitotoxicity is a major contributing pathogenetic factor.[9] Such a hypothesis is supported by PET studies, which show reduced glucose and oxygen metabolism of the basal ganglia and cortex in patients with Huntington's disease.[10–12]

We have studied the frontal cortex for several reasons. Firstly, there is neuropathological evidence that this region is involved in Huntington's disease[13] but precise correlations with the development of the clinical features are as yet lacking. Secondly, recent PET studies have convincingly showed reduced glucose metabolism in various stages of clinical disease including mild and severe forms.[11 12 14] Finally, neuropsychological deficits have also been reported in Huntington's disease, which have been attributed to frontal lobe dysfunction.[15 16] For these reasons, we carried out lactate measurements and determined the N-acetyl-aspartate/choline (NAA/Ch) ratio by proton magnetic resonance spectroscopy in a selected volume of the frontal lobe.

Subjects and methods

We examined 26 patients with a definitive molecular genetic diagnosis of Huntington's disease; in five of these patients the spectra could not be evaluated as choreoathetotic movements impeded the acquisition of spectroscopic data. These patients were excluded from the study. Each patient was given a clinical examination by a neurologist (HM) and subsequently the severity of the condition was defined from the Shoulson score[17] and the motor score of the United Huntington's Disease Rating Scale (UHDRS).[18] Duration of disease was defined as the time which had elapsed since the first motor symptoms appeared as judged by the patient, relatives, and referring physicians. Genetic testing was performed by standard techniques[19 20] and showed that each of the patients and asymptomatic carriers had 39 CAG repeats or more (table).

From clinical criteria for severity of the disease, patients were divided into four groups.

Group I consisted of four asymptomatic gene carriers (one man, three women; mean age 43·5 (range 35–60)). Each asymptomatic gene carrier had a Shoulson score of 13 and a motor score of 0.

Seventeen patients with Huntington's disease were clinically symptomatic; nine of these patients were men, eight were women; their mean age was 46·1 (range 25–70) years.

Group II comprised six patients (five men, one woman, mean age 46·5, range 39–56) had a mean Shoulson score of 10·5 (range 10–11) and a motor score of 19·4 (range 15–24). They were classified as being "mildly" handicapped.

Group III patients (three men, three

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Characteristics, clinical severity, scale rating, medication, and NAA/Ch and lactate/Ch ratios of individual patients

Patient No	Sex (M/F)	Age (y)	Repeats	Duration (y)	Severity	Shoulson	UHRDS	Medication	NAA/Ch	Lactate/Ch
1	F	35	46	0	Asymptomatic	12	4	None	1·50	0·35
2	F	43	40	0	Asymptomatic	13	0	None	1·51	0·89
3	F	36	48	0	Asymptomatic	13	0	None	2·35	0·39
4	M	60	39	0	Asymptomatic	13	3	None	1·59	0·81
5	M	44	45	2	Mild	10	24	None	1·73	0·00
6	F	39	44	7	Mild	11	19	None	2·00	0·25
7	M	56	43	4	Mild	11	19	None	1·62	0·13
8	M	55	42	5	Mild	10	23	T	2·34	0·00
9	M	41	44	5	Mild	11	15	None	1·63	1·12
10	M	44	43	9	Mild	10	17	T	1·63	0·00
11	M	43	44	18	Moderate	6	37	T	1·50	0·00
12	F	42	44	5	Moderate	3	55	T	1·66	0·15
13	F	70	40	5	Moderate	3	39	T	1·23	0·00
14	F	34	55	10	Moderate	4	49	T	1·67	0·63
15	M	42	49	9	Moderate	4	44	None	1·11	0·00
16	M	47	45	6	Moderate	3	50	T, C, L	1·27	0·56
17	F	63	44	15	Severe	0	62	None	0·95	0·00
18	F	25	52	3	Severe	0	70	T, C	1·18	0·35
19	F	33	50	4	Severe	1	85	T	1·24	0·00
20	M	56	45	5	Severe	1	74	T	1·40	0·34
21	F	44	47	14	Severe	1	70	T	1·55	0·00

T = Tiaprid; C = clonazepam; L = levomepromazin.

women, mean age 46·3 (range 34–70) had a mean Shoulson score of 3·8 (range 3–6) and a mean motor score of 45·6 (range 37–55). They were classified as being in the "moderate" range of deficits.

Group IV (one man, four women, mean age 44·2 (range 25–63)) were classified as having "severe" deficits as they had a mean Shoulson score of 0·6 (range 0–1) and a motor score of 72·2 (range 62–85).

Group V consisted of 19 healthy controls. Eight subjects in this group were men, 11 were women; their mean age was 38·0 (range 22–68).

¹H magnetic resonance spectroscopy was performed on a 1·5 Tesla Gyroscan S 15 (Philips) system. Based on a transversal T2 SE weighted imaging (TE = 30; 90 ms, TR = 2000 ms) we selected a volume of 20 × 20 × 40 mm in a frontal area of the brain by a specially resolved spectroscopy (SPARS) sequence (fig 1).[21] Water suppression was achieved by selective inversion pulse. Spectra were obtained using a headcoil after shimming to minimise inhomogenities of the magnetic field. We chose the following parameters: TE = 136 ms, TR = 2000 ms, sample frequency 1 kHz, 1024 samples, 256 measurements. In the standard processing of spectra zero filling 4k, Fourier transformation, and phase correction were used. Evaluation of the ratios choline (Ch)/creatine (Cr), N-acetylaspartate (NAA)/Ch, and lactate/Ch by integration of the peaks allowed a quantitative description of metabolic alterations. The spectroscopic data were obtained and processed without knowledge of the clinical characteristics of the patient.

Spectroscopic findings were compared with clinical and molecular genetic data. Statistical comparisons were performed using the Kruskal-Wallis test, the Mann-Whitney U test, and the Wilcoxon rank test.

Figure 1 Volume evaluated in the left frontal cortex.

Results

The Ch/Cr ratios did not show any significant differences between groups (group I 1·06 (0·06), group II 0·95 (0·13), group III 0·97 (0·07), group IV 0·94 (0·11), group V 1·07 (0·28)). We tried to relate the NAA/Ch ratio—which is accepted to reflect the amount of the neuronal marker NAA[22]—to the clinical findings in our patients. Whereas these ratios were indistinguishable in asymptomatic gene carriers, mildly affected patients, and controls (group I 1·73 (0·41), group II 1·83 (0·29), group V 1·80 (0·37)) (fig 2), they were decreased in group III (1·41 (0·24)) and group IV patients (1·26 (0·23)). The differences between controls (group V) and groups III (P = 0·022) and IV (P = 0·008) were significant There was no significant relation between the reduction in NAA/Ch ratio and duration of the disease, age of the patients, and number of CAG repeats.

In the frontal cortex lactate peaks were present in eight of the 17 symptomatic patients and each of the four asymptomatic gene carriers but not in the controls (fig 3A-C; table). Nine of our patients did not have an increased lactate in the tissue volume examined. Although it seemed that duration and severity of the disease and low NAA were broadly pre-
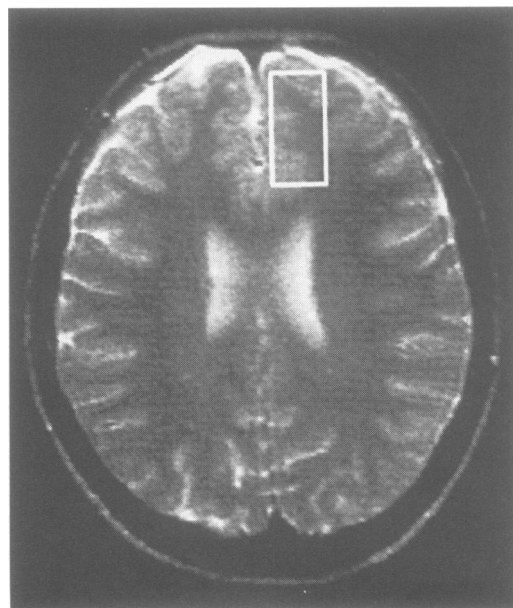
Figure 2 Distribution of NAA/Ch ratios in frontal cortex of patients with Huntington's disease and controls.

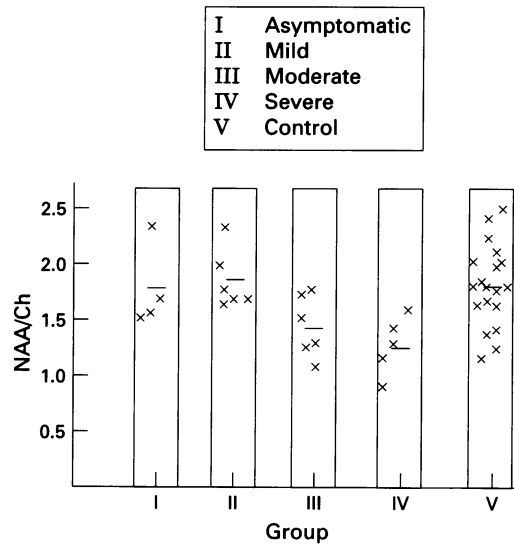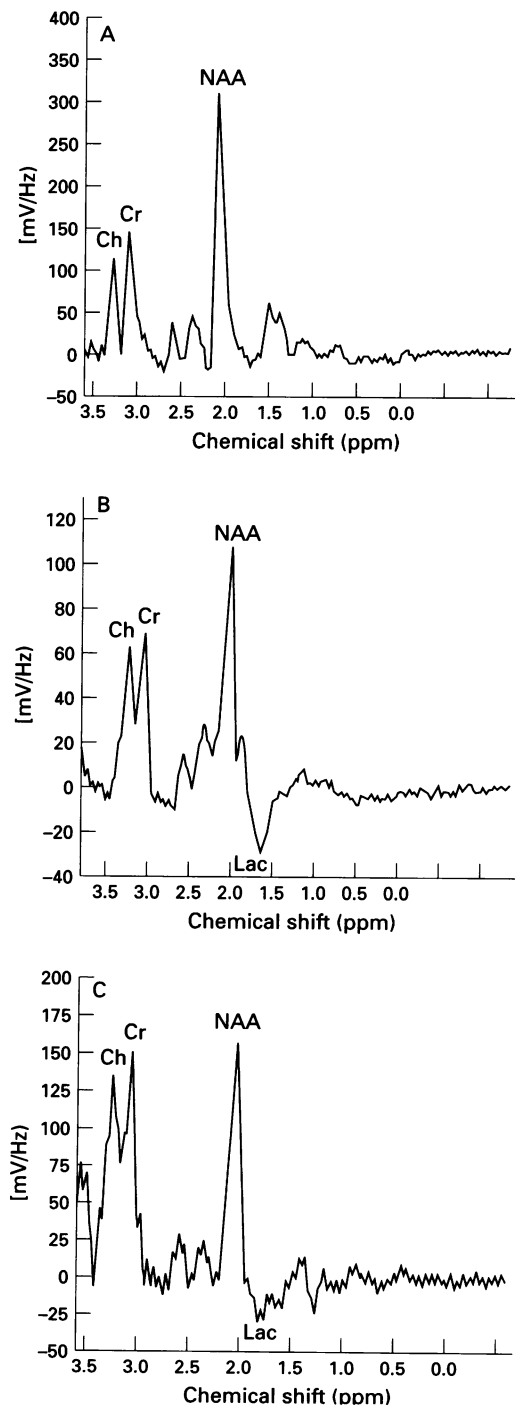| I	Asymptomatic
II	Mild
III	Moderate
IV	Severe
V	Control

Figure 3 (A) Normal magnetic resonance spectrum in a control without a lactate peak. (B) Example of increased cerebral lactate in an asymptomatic gene carrier (patient 4, table), and (C) in a symptomatic patient with a reduced NAA/Ch ratio (patient 18, table).

dictive of this result, exceptions obviously existed (see for example, patient 8 in the table). Results were apparently unrelated to medication as six of the patients did not receive any drug and yet lactate peaks were detected.

Discussion

We performed ^{1}H NMR spectroscopy on a well defined and identical frontal lobe region in 17 patients with clinically overt Huntington's disease and four asymptomatic gene carriers. In each of the asymptomatic carriers and in 47% of the symptomatic patients we detected cerebral lactate in the frontal cortex. This is consistent with the result reported by Jenkins et al[23] who found cerebral lactate in the occipital cortex of 16 patients with Huntington's disease. Our data suggest that the frontal cortex is involved early in the course of Huntington's disease. The presence of lactate concentrations supports the hypothesis that a defect of aerobic energy metabolism contributes to the pathogenesis of the disease. However, in our study cerebral lactate could be detected in only a proportion of those studied. This may be explained simply by the fact that the regions studied are of importance (frontal v occipital). This conclusion is supported by other findings of Jenkins et al,[23] that the same patients who had raised occipital lactate concentrations showed more heterogenous results in studies of the basal ganglia. We attempted to study the basal ganglia but could not obtain high quality signals. This is presumably due to artifacts caused by paramagnetic iron which can be detected in this area of the brain and influences the spectral lines.[24] Also at variance with the results of Jenkins et al,[23] is that the four asymptomatic gene carriers had greatly increased lactate peaks. In our opinion the small number of asymptomatic gene carriers studied (n = 4 v n = 2 in the study of Jenkins et al[23]) does not permit further conclusions. In our study there was a tendency for lactate peaks to occur early in the disease when there was minimal or no involuntary motor disturbance and for lactate to be absent in patients with a severe movement disorder. Therefore, the pattern of our findings excludes the possibility that movement alone—such as that in the basal ganglia after finger movements[25]—is likely to be responsible for the lactate peaks. The variability of the results could be a property of spectroscopic measurements, which have some uncertainty; however, the standard deviations of mean values for ratios evaluated in our normal control population were comparable with those reported in the literature.[23 26] We did not detect lactate in normal controls.

Older patients with longstanding and severe disease and a decrease of NAA/Ch ratios were more likely not to have lactate in the frontal cortex. This may be simply due to neuronal loss, which does not allow lactate to increase during later stages of the disease. The result also relates to recent rodent studies which showed that the capability to respond to meta-

bolic stress by anaerobic glycolysis is age dependent.[27] This decrease in the ability to adjust to increased demands for chemical energy is found in middle aged animals and may be partly genetically determined.[27] Therefore, the possibility exists that older people with Huntington's disease or patients who have had Huntington's disease for a longer time have less ability to respond to metabolic stress. Whether this idea is of relevance for the pathogenesis of Huntington's disease remains to be established.

A decreased NAA/Ch ratio indicates the presence of neuronal loss; therefore, our finding that NAA concentrations are reduced in later stages of Huntington's disease but normal in patients with less severe clinical deficits is consistent with the established knowledge that the frontal cortex is part of the degenerative process. Whether NAA can serve as a biological marker for the late disease must be shown in longitudinal studies. Davie *et al*[28] and Taylor-Robinson *et al*[29] detected increased glutamine/glutamate peaks during their magnetic resonance spectroscopic studies of symptomatic patients with Huntington's disease (n = 5 and n = 3 respectively); we did not find comparable peaks in this study.

In conclusion, this study shows that the neuronal marker NAA is preferentially reduced in late stages of Huntington's disease. Lactate can be detected in the frontal cortex of symptomatic and asymptomatic patients with Huntington's disease but a clear cut relation to the clinical course of the disease could not been shown by studies of this cortical region. Further studies should consider the natural course of alterations of aerobic metabolism in the frontal lobe of asymptomatic gene carriers. Although the presence of lactate concentrations supports the concept of a nuclear encoded energy deficit being part of the pathogenesis of Huntington's disease, our findings are less consistent than previously reported. This might be a secondary effect of reduced neuronal activity in the frontal cortex—or possibly a pathogenetic factor for the development of the disease specific lesions.

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