

# Persistent functional deficit in multiple sclerosis and autosomal dominant cerebellar ataxia is associated with axon loss

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## Summary

Proton magnetic resonance spectroscopy (MRS) and MRI were carried out in 11 patients with multiple sclerosis who had clinical evidence of severe cerebellar involvement, 11 multiple sclerosis patients (of similar age and disease duration) who had minimal or no signs of cerebellar disease, eight patients with autosomal dominant cerebellar ataxia (ADCA) and 11 healthy controls. In all subjects MRS was localized to cerebellar white matter (volumes of interest 3–6 ml). Apparent metabolite concentrations were calculated using the fully relaxed water spectrum as an internal standard of reference. The patients also underwent MRI to assess cerebellar volume and (in the two multiple sclerosis groups) lesion volume within the posterior fossa. Magnetic resonance spectroscopy from cerebellar white matter showed a highly significant reduction in the concentration of N-acetyl groups (NA) [which consists predominantly of N-acetylaspartate (NAA), a neuronal marker] in the multiple sclerosis group with cerebellar deficit compared with the multiple sclerosis

group with minimal or no signs of cerebellar involvement, and healthy controls. Follow-up MRS performed in six of the multiple sclerosis patients 9 months later showed no change in the median NA concentration. The ADCA group showed a significant reduction of NA from a region of cerebellar white matter and also a reduction in the concentration of choline-containing compounds. The multiple sclerosis group with severe cerebellar deficit and the ADCA group both had significant cerebellar atrophy (suggesting nerve cell body and axon loss) compared with the multiple sclerosis patients with minimal or no signs of cerebellar deficit and healthy controls. The multiple sclerosis patients with cerebellar deficit had a significantly greater lesion volume in the posterior fossa, although the proportion of the spectroscopic voxel occupied by lesions was small, suggesting that axonal loss from normal appearing white matter also contributes to the observed reduction in NA. These results support the hypothesis that axonal loss is important in the development of persistent clinical disability in multiple sclerosis.

**Keywords:** MRS spectroscopy; N-acetyl aspartate; hereditary ataxia; multiple sclerosis

**Abbreviations:** ADCA = autosomal dominant cerebellar ataxia; Cr = creatine; EDSS = extended disability status score; MRS = magnetic resonance spectroscopy; NA = N-acetyl containing compounds; NAA = N-acetyl aspartate; TE = echo time; TR = repetition time

## Introduction

Irrecoverable neurological deficit in multiple sclerosis depends ultimately on loss of the ability of axons to conduct. The mechanisms that produce this are not fully understood (McDonald *et al.*, 1992). The histology of chronic lesions suggests two possibilities. First, conduction block is due to persistent demyelination. This possibility is not straightforward; evoked potential studies have shown that acute conduction block with its consequent functional loss which

characterizes the new lesion is usually reversible, probably due to an increase in the number of sodium channels from the nodes into the internodal axon (Black *et al.*, 1991; Moll *et al.*, 1991). Remyelination may contribute to the reversibility of functional loss but it is not essential since good recovery of vision may occur despite persistent and often marked delays in the visual evoked potentials (Halliday *et al.*, 1972). The failure of these compensatory mechanisms (perhaps as

a result of repeated episodes of demyelination in the same fibres) would contribute to irreversible functional loss. Whether and how this occurs is unknown.

A second possibility is axonal loss, a feature of the lesion known since the days of Charcot (1868) but frequently ignored. It is seen to some extent in all lesions (Lassmann *et al.*, 1994) and is extensive in many chronic lesions at post-mortem (Allen, 1991).

Although conventional MRI is sensitive in detecting the lesions of multiple sclerosis, it reveals neither demyelination nor axon loss *per se*. Proton MRS, however, has the potential to detect the latter non-invasively during life. The normal proton spectrum is dominated by NA (the sum of *N*-acetyl aspartate and *N*-acetylaspartylglutamate). The predominant component of the NA peak is NAA, an amino acid of unknown function (Birken and Oldendorf, 1989), which has been shown in experimental studies on neonatal rats to be contained almost exclusively within neurons (Urenjak, 1993). A loss of neurons (cell bodies and axons) would be predicted to cause a persistent reduction in the concentration of NAA. In order to test the hypothesis that irrecoverable neurological deficit in multiple sclerosis is associated with axon loss, we have compared the apparent concentration of NA in the cerebellum of patients with severe chronic ataxia to that in patients who are not ataxic and in two groups of controls: healthy individuals and a group of patients with ADCA, a condition which is characterized pathologically by extensive loss of neurons (Schut and Haymaker, 1951; Hoffmann *et al.*, 1971; Dürr *et al.*, 1995).

## Material and methods

Patients with clinically definite multiple sclerosis (Poser, 1983) and ADCA (Harding, 1982) were recruited from the National Hospital for Neurology and Neurosurgery. The study was approved by the Joint Ethics Committee at the Institute of Neurology and the National Hospital for Neurology and Neurosurgery, London. Informed consent was obtained from all patients prior to each study.

### Patient groups

Each patient underwent a full neurological examination and Kurtzke cerebellar functional system and extended disability status scores (EDSSs) (Kurtzke, 1983) were obtained for the multiple sclerosis patients.

#### Group 1

Eleven multiple sclerosis patients with marked upper limb cerebellar ataxia and/or gait ataxia were in this group. They were classified on the Kurtzke functional cerebellar scale between 3 and 5. All patients in this group had had evidence of marked cerebellar deficit for 1 year or more. They had a median age of 37 years (range 25–44 years) and a median disease duration of 6 years (range 2–34 years).

#### Group 2

Eleven multiple sclerosis patients with minimal or no signs of cerebellar deficit were in this group. They were classified on the Kurtzke functional cerebellar scale as 0 or 1. Their median age was 32 years (range 22–56 years) and median disease duration, 6 years (range 3–20 years).

#### Group 3

Eight patients with ADCA were in this group. Five had type 1 ADCA, two with the SCA1 mutation (Orr *et al.*, 1993; Giunti *et al.*, 1994), two with the SCA3 mutation (Kawaguchi *et al.*, 1994) and one with no known mutation. Three had the type 3 ADCA with a pure cerebellar syndrome, where the mutation is also unknown, using the classification devised by Harding (1982). The median age in this group was 46.5 years (range 25–65 years) with a median disease duration of 6 years (range 3–22 years).

#### Controls

Eleven healthy controls with a median age of 31 years (range 24–48 years) were studied.

### Magnetic resonance imaging and spectroscopy

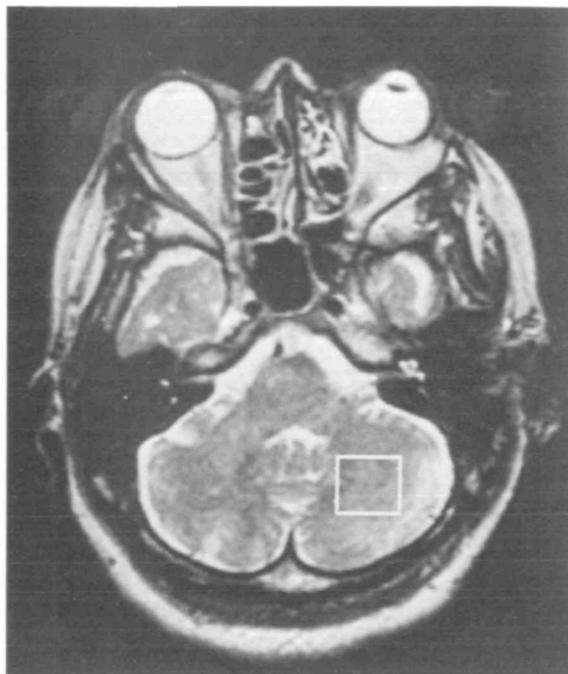
Both MRI and MRS were carried out on a Signa 1.5 Tesla system (General Electric, Milwaukee, Wis., USA) without contrast enhancement.

#### MRI–sagittal images

T<sub>1</sub>-weighted 3 mm contiguous slices covering the posterior fossa were collected, [repetition time (TR) 640 ms, echo time (TE) 11 ms].

#### MRI–axial images

A proton density weighted scan [TR 5000 ms, effective TE (TE<sub>ef</sub>) 18/78 ms] (5 mm slices, 2.5 mm interspace, 256×256 matrix, echo train length 8) was collected. Finally, contiguous axial slices were collected through the posterior fossa (TR 2000 ms TE<sub>ef</sub> 17 ms) (3 mm, 256×256 matrix, echo train length 8). The axial posterior fossa scans of the two multiple sclerosis groups were analysed by one of the authors (D.H.M.) who was blinded to the clinical details. All intrinsic lesions in the posterior fossa were documented. These were divided into anatomical site: cerebellar hemispheres and peduncles, medulla and pons and midbrain. Lesions that fell within the spectroscopic volume of interest were also recorded. A lesion load for the individual sites and total posterior fossa lesion load were then determined using a semi-automated contouring programme which segmented lesions ('Dispimage', D. L. Plummer, UCL, London, UK). The same contouring programme which allowed exclusion of CSF between the folia



**Fig. 1** A proton density weighted scan [TR 5000 ms, effective TE ( $TE_{eff}$ ) 18/78 ms] showing a volume of interest localized to the cerebellar white matter in an MS patient with no cerebellar deficit.

was then used on the  $T_1$ -weighted sagittal slices through the posterior fossa to determine the cerebellar volume. This was carried out by one of the authors (C.A.D.) who was blinded to patient identity at the time of analysis.

### Magnetic resonance imaging

After imaging, a volume of interest, ranging in size from 3.5 to 6 ml, localized to the cerebellar white matter, was prescribed (Fig. 1). In the patient groups the cerebellar hemisphere ipsilateral to the more ataxic side was chosen. The size and shape of each volume was adjusted to avoid inclusion of grey matter and CSF. In both multiple sclerosis groups, the region of interest included lesions (when present) and normal appearing white matter. An MRI of the voxel was then obtained to ensure accurate localization. Water suppressed  $^1H$  spectra were obtained using a stimulated echo-acquisition mode sequence (Frahm *et al.*, 1987, 1989, 1990). Acquisition parameters were TR 2000 ms, mixing time 12 ms, TE 135 ms; 384 averages were collected using an eight-step phase cycle in ~12 min; 1024 points were collected, with a spectral width of 750 Hz. Data processing included 1 Hz line broadening for filtering, Fourier transformation and zero order phase correction. Peak areas were determined using a line-fitting programme ('SA/GE', G.E. Milwaukee, Wis., USA). Peaks were fitted to a Gaussian line shape using a Marquardt fitting procedure. Absolute concentrations for the metabolites were calculated using the fully relaxed water signal as an internal standard of reference (Christiansen *et al.*,

1993). Metabolite concentrations [met] were calculated from the equation

$$\text{met} = [H_2O] \times 2/PI \times T_{1\text{ corr}} \times T_{2\text{ corr}} \times S_{0\text{ met}}/S_{0\text{ H}_2\text{O}} \times 1/2^R$$

where  $S_{0\text{ met}}$  and  $S_{0\text{ H}_2\text{O}}$  denote the signal intensities for metabolites and water, respectively,  $[H_2O]$  is the brain water concentration from the volume of interest. The water concentration from the voxel of interest was calculated by comparing the signal intensity from the proton density weighted images in the putamen (where no lesions were visible) with the signal intensity from the region of interest in the cerebellum. The putamen was chosen since this region of the basal ganglia is not affected by heavy metal deposition in healthy controls (Olanow, 1992). Furthermore, a recent study by Grimaud *et al.* (1995) has shown no evidence of hypointensity on  $T_2$ -weighted images to suggest increased heavy metal deposition in the putamen of multiple sclerosis patients.

The water concentration of normal appearing grey matter has been taken as 45.5 M (Norton *et al.*, 1966). In the control groups the concentration of water in white matter has been taken as 39.75 M (Norton *et al.*, 1966).  $T_{1\text{ corr}}$  and  $T_{2\text{ corr}}$  are  $T_1$  and  $T_2$  correction values based on published values from the cerebellum for the metabolites studied (Frahm *et al.*, 1989). Since absolute  $T_1$  and  $T_2$  values were not calculated for patients and controls the term 'apparent concentration' is used.  $2/PI$  is the proton index and refers to the number of protons in each metabolite [three for NAA, three for creatine (Cr) and nine for choline-containing compounds]. The value of 2 represents the two protons in  $H_2O$ .  $R = R_{\text{metabolite}} - R_{\text{water}}$  and accounts for different receive attenuator settings.

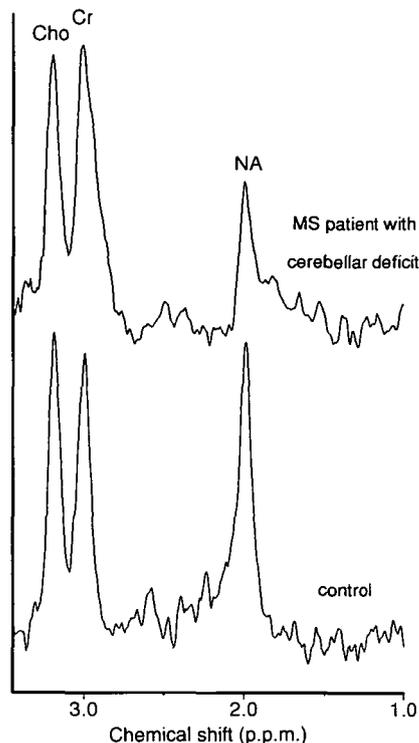
Statistical analysis was performed with a Mann-Whitney confidence interval and test. Results are expressed as a median value together with the range and  $P$  value. A Spearman's rank correlation test was also used and results are expressed as an  $r$  value together with levels of significance.

### Results

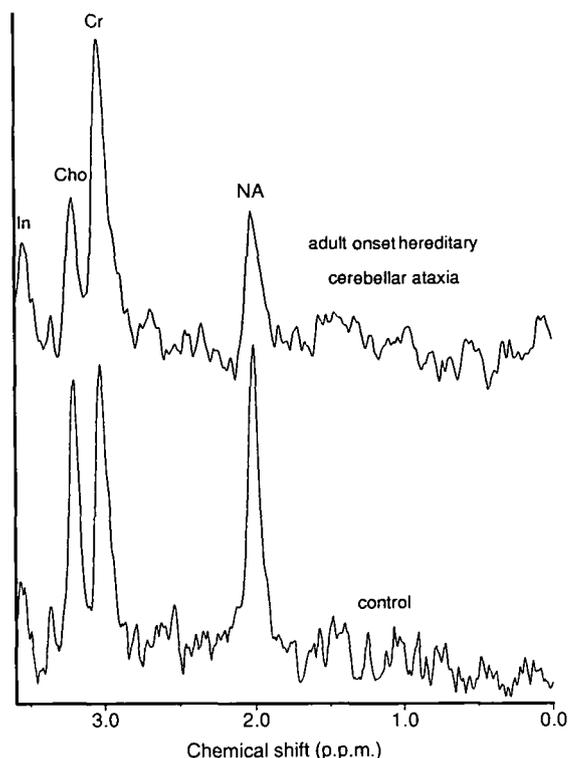
The three resonances visible at TE 135 ms have been assigned as follows (Frahm *et al.*, 1989b; Behar and Ogino, 1991): NA at 2.02 p.p.m. and 2.6 p.p.m., Cr/phosphocreatine at 3.04 p.p.m. and choline-containing compounds at 3.2 p.p.m.

### Magnetic resonance spectroscopy

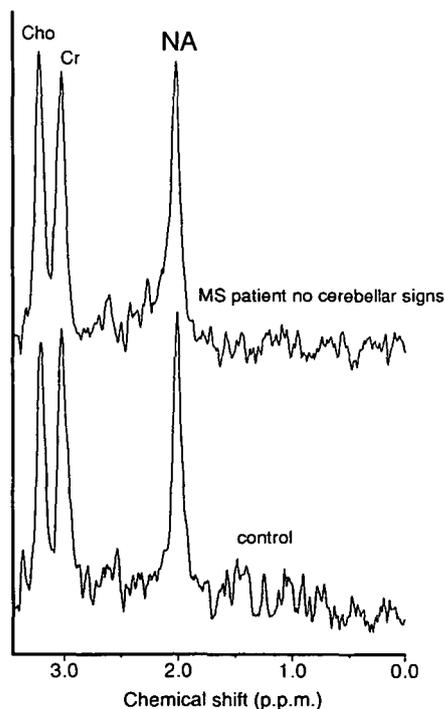
In the multiple sclerosis patients with severe ataxia, there was a highly significant reduction in the median apparent concentration of NA (6.7 mM, range 4.58–8.93 mM) in the cerebellar white matter compared with the controls (9.6 mM, range 8.3–10.8 mM,  $P = 0.0002$ ) (Fig. 2). In contrast, the apparent concentration of NA in the multiple sclerosis patients without cerebellar deficit (median 9.74 mM, range 9.25–11 mM) showed no significant difference from the control group ( $P > 0.4$ ) (Fig. 3). The patients with hereditary ataxia also showed a significant reduction in the median



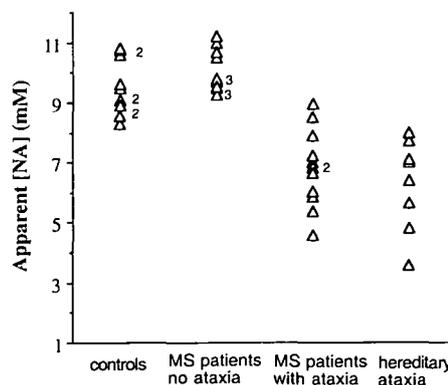
**Fig. 2** Magnetic resonance spectroscopy (TE 135 ms, TR 2.135 s) from cerebellar white matter (volume 3.5 ml) in multiple sclerosis patient with severe cerebellar deficit (ataxia) compared with healthy control.



**Fig. 4** Magnetic resonance spectroscopy (TE 135 ms, TR 2.135 s) in 45-year-old patient with Type 1 ADCA (SCA1 mutation) compared with healthy control.



**Fig. 3** Magnetic resonance spectroscopy (TE 135 ms, TR 2.135 s) in multiple sclerosis patient with no cerebellar deficit (no ataxia) (volume 3.7 ml) compared with healthy control.



**Fig. 5** Apparent concentration of NA in multiple sclerosis patients and controls.

apparent NA concentration from cerebellar white matter (6.82 mM, range 3.55–7.96 mM,  $P = 0.0003$ ) compared with the control group (Fig. 4). The NA values for the four groups are shown as a scatter graph (Fig. 5). The choline concentration showed no significant change in either multiple sclerosis group compared with the controls. The ADCA group, however, showed a significant median reduction in the apparent concentration of choline-containing compounds (median 1.0 mM, range 0.44–1.90 mM) compared with the control group (median 1.56 mM range 1.2–3.15 mM,  $P = 0.04$ ) (Fig. 4). There was no significant correlation between

concentrations of choline-containing compounds and NA, cerebellar volume or disease duration.

There was no significant difference between the median apparent concentration of Cr in the cerebellar white matter from the control group (9.05 mM, range 7.8–11.6 mM) compared with the multiple sclerosis patients with cerebellar deficit (median 9.82 mM, range 7.65–11.7 mM,  $P = 0.2$ ) nor indeed the multiple sclerosis group without cerebellar deficit (median 9.8, range 7.98–11.0 mM,  $P = 0.2$ ). The ADCA group also showed no significant difference in the median apparent concentration of Cr (9.76 mM, range 8.6–11.8 mM) compared with the healthy controls.

Since transient reductions in the NAA/Cr ratio have been observed in acute multiple sclerosis lesions (Arnold, 1992; Davie *et al.*, 1994) we repeated the MRS study in six of the 11 ataxic multiple sclerosis patients after an interval of 9 months. The median apparent concentration of NA at follow-up (median 7.42 mM, range 5.04–8.73 mM) showed no significant difference from the first study (median 7.45 mM, range 5.88–8.93 mM,  $P > 0.4$ ) (Table 1).

### Magnetic resonance imaging

The cerebellar volumes for the multiple sclerosis patient groups are shown in Tables 1 and 2. In the ataxic multiple sclerosis group, there was a highly significant reduction in the median cerebellar volume (35 955 mm<sup>3</sup>, range 33 858–43 807 mm<sup>3</sup>) compared with the control group (44 001 mm<sup>3</sup>, range 40 426–47 926 mm<sup>3</sup>,  $P = 0.0005$ ). In contrast, the median cerebellar volume of multiple sclerosis patients who were clinically unaffected (median 42 679 mm<sup>3</sup>, range 37 336–45 250 mm<sup>3</sup>) showed no significant difference from the control group ( $P > 0.05$ ). The patients with ADCA also showed a significant reduction in the median cerebellar volume (31 379 mm<sup>3</sup>, range 20 532–38 478 mm<sup>3</sup>,  $P = 0.0003$ ) compared with the control group.

The posterior fossa lesion loads in the two multiple sclerosis groups are shown in Table 1 and 2. The median voxel lesion load in the multiple sclerosis group with

cerebellar deficit (median 157 mm<sup>3</sup>, range 74–260 mm<sup>3</sup>) was increased compared with the multiple sclerosis group without cerebellar deficit (median 117 mm<sup>3</sup>, range 0–263 mm<sup>3</sup>,  $P < 0.04$ ). When corrected for voxel size, the percentage of voxel occupied by abnormal signal was found to be moderately higher in the multiple sclerosis group with cerebellar deficit (median 9%, range 5.2–15.7%) compared with the patient group with no cerebellar deficit (median 5.9%, range 0–15.3%,  $P < 0.05$ ). There was also a significantly greater lesion volume in the total ipsilateral cerebellar hemisphere ( $P < 0.008$ ) and within the brainstem ( $P < 0.009$ ) in the multiple sclerosis patients with cerebellar deficit compared with the multiple sclerosis patients with no clinical cerebellar involvement.

Taking all multiple sclerosis patients together, there was a modest though significant negative rank correlation between the concentration of NA from the cerebellum and both the Kurtzke EDSS ( $r = -0.517$ ;  $0.02 > P > 0.01$ ) (Fig. 6) and the Kurtzke functional cerebellar score ( $r = -0.49$ ;  $0.05 > P > 0.02$ ) (Fig. 7). There was also a negative correlation between the concentration of NAA and lesion volume within the voxel ( $r = -0.527$ ;  $0.02 > P > 0.01$ ). Additionally, there was a positive correlation between NA concentration and cerebellar volume ( $r = 0.438$ ;  $0.05 > P > 0.02$ ). There was no rank correlation between lesion volume in the posterior fossa and Kurtzke score, though there was a significant correlation between the total posterior fossa lesion volume and the cerebellar functional score ( $r = 0.536$ ;  $0.02 > P > 0.01$ ). No significant correlation was found between disease duration and apparent NA concentration.

### Discussion

Our study has demonstrated a reduction in the apparent concentration of NA in the cerebellum in patients with ADCA, a condition in which there is loss of neurons (nerve cell bodies and axons) (Schulz and Haymaker, 1951; Hoffmann *et al.*, 1971). This finding is in keeping with similar reductions

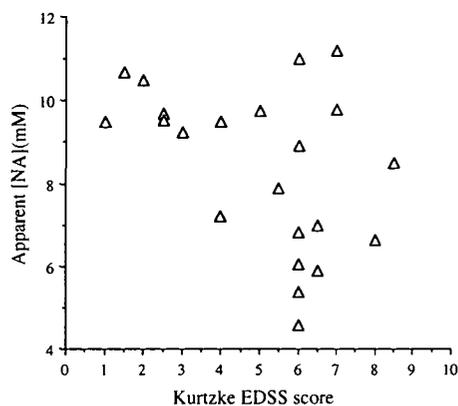
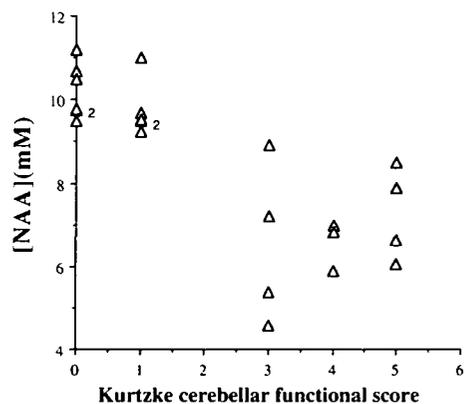
**Table 1** MS patients with cerebellar deficit

	Cerebellar volume (mm <sup>3</sup> )	[NAA] (mM)		Voxel lesion load (mm <sup>3</sup> )	Posterior fossa lesion load (mm <sup>3</sup> )	Ipsilateral cerebellum (mm <sup>3</sup> )	Pons/midbrain (mm <sup>3</sup> )	EDSS score	Cerebellar functional score
		1st study	Follow-up						
(1)	34 659	5.88	5.89	157	600	157	212	6.5	4
(2)	43 807	7.9	8.0	187	1573	256	1072	5.5	5
(3)	34 199	6.05	5.04	174	1390	332	581	6.0	5
(4)	34 700	7.0	7.49	243	2563	285	1441	6.5	4
(5)	35 170	8.5	8.73	191	773	225	305	8.5	5
(6)	35 995	8.93	7.36	74	562	173	171	6.0	3
(7)	33 858	6.82	ND	140	967	248	425	6.0	4
(8)	37 700	7.22	ND	137	644	193	402	4.0	3
(9)	42 218	5.39	ND	260	2150	596	544	6.0	3
(10)	40 818	6.65	ND	134	1066	250	660	8.0	5
(11)	40 100	4.58	ND	117	1808	286	897	6.0	3

ND = not determined.

**Table 2** MS patients without cerebellar deficit

	Cerebellar volume (mm <sup>3</sup> )	[NAA] (mM)	Voxel lesion load (mm <sup>3</sup> )	Posterior fossa lesion load (mm <sup>3</sup> )	Ipsilateral cerebellum (mm <sup>3</sup> )	Pons/midbrain (mm <sup>3</sup> )	EDSS score	Cerebellar functional score
(1)	40 995	9.25	141	890	205	168	3.0	1
(2)	42 679	9.74	263	856	379	290	5.0	0
(3)	37 336	9.7	45	320	45	177	2.5	1
(4)	45 250	9.48	117	713	140	235	1.0	1
(5)	44 830	11	88	101	88	13	6.0	1
(6)	43 840	9.5	121	748	204	439	4.0	0
(7)	40 027	9.78	0	0	0	0	7.0	0
(8)	37 991	11.2	35.1	910	172	307	7.0	0
(9)	42 734	9.52	152	346	164	63	2.5	1
(10)	44 702	10.48	0	116	0	22	2.0	0
(11)	40 340	10.67	127	841	173	175	1.5	0

**Fig. 6** Scattergraph of apparent concentration of NA versus Kurtzke EDSS score in multiple sclerosis patients.**Fig. 7** Scattergraph of NA concentration versus Kurtzke cerebellar functional score in multiple sclerosis patients.

observed in other diseases characterized by neuronal degeneration including Alzheimer's disease (Miller *et al.*, 1993), Huntington's disease (Jenkins *et al.*, 1993) and multiple system atrophy (Davie *et al.*, 1995). Since NAA is likely to be largely confined to neurons in the adult brain and is the predominant component of the *N*-acetyl signal, we conclude that the decrease in the apparent concentration of this amino acid reflects a decrease in the population of neurons.

We observed a similar reduction in the cerebellum of ataxic patients suffering from multiple sclerosis, but not in multiple sclerosis patients without ataxia. It could be argued that the reduction of NA we have observed is simply the result of a change in the chemical environment altering the  $T_2$  relaxation time rather than an absolute reduction in concentration of NA. This is unlikely for a number of reasons. First, if a change in  $T_2$  were to occur, one would anticipate that the relaxation time would increase rather than decrease because of the greater water content in acute and chronic multiple sclerosis lesions (Barnes *et al.*, 1991). If one assumes a  $T_2$  value of 300 ms (Frahm *et al.*, 1989b) from cerebellar white matter, it would require a decrease of ~150% in the  $T_2$  value to account for our findings. Such a change is improbable and a spectroscopic study by Christiansen *et al.* (1993) has shown no significant change in the  $T_1$  and  $T_2$  relaxation times of NA from acute or chronic multiple sclerosis lesions compared with controls.

Other workers have observed a qualitative reduction in the NAA/Cr ratio in lesions (Arnold *et al.*, 1989, 1992; Miller *et al.*, 1991; Davie *et al.*, 1994; Husted *et al.*, 1994) and normal appearing white matter (Davie *et al.*, 1994a; Husted *et al.*, 1994). A recent post-mortem study by Davies *et al.* (1995) showed an absolute reduction of NA and Cr from multiple sclerosis lesions studied 48 h after death, whereas areas of normal appearing white matter studied showed normal metabolite levels. In the present study, the cerebellar regions studied with spectroscopy in the multiple sclerosis patients contained predominantly normal appearing white matter which may explain why we failed to observe any difference in the apparent concentration of Cr between the patient groups and healthy controls.

Previous investigators have failed to show a correlation between a reduction in NAA and disability (Arnold, 1994), probably because they have, to date, concentrated on lesions and normal appearing white matter in the periventricular areas which are not clinically eloquent (Phadke and Best, 1983) except in relation to cognitive function (Rao *et al.*, 1989; Feinstein *et al.*, 1993).

A partially reversible decrease in NAA/Cr ratio has also

**Table 3** Autosomal dominant cerebellar ataxia patients

	Age (years)	Disease duration (years)	Classification	Mutation	Cerebellar volume (mm <sup>3</sup> )	[NAA] (mM)	[Cho] (mM)
(1)	48	3	Type 1	SCA 1	36 611	6.44	0.94
(2)	45	5	Type 1	SCA 1	29 841	5.65	1.36
(3)	65	5	Type 1	SCA 3	35 320	7.1	1.29
(4)	25	5	Type 1	SCA 3	38 478	7.96	1.90
(5)	38	8	Type 1	Not known	23 204	3.55	1.00
(6)	50	22	Type 3	Not known	20 532	7.0	0.44
(7)	52	20	Type 3	Not known	22 487	4.78	0.75
(8)	24	6	Type 3	Not known	27 392	7.7	1.86
Control (median)	31				44 001	9.6	1.56

Cho = choline-containing compounds.

been observed in acute multiple sclerosis lesions (Arnold, 1992; Davie *et al.*, 1994a). Similar changes in acute relapsing experimental allergic encephalomyelitis can be accounted for by reversible changes in mitochondrial function and do not necessarily imply neuronal degeneration (Brenner *et al.*, 1993b; Heales *et al.*, 1995). In all six of our 11 multiple sclerosis patients with ataxia, followed up after 9 months, the decrease in apparent concentration of NA persisted, indicating a long-lasting change. While we cannot exclude the possibility of a prolonged inhibition of mitochondrial synthesis of NAA, neuronal degeneration provides a more plausible explanation since cerebellar atrophy was found in both ataxic groups. Similarly, it is unlikely that demyelination alone would account for our findings since we and others have shown normal apparent levels of NA in adult patients with phenylketonuria (Davie *et al.*, 1994b; Kreiss *et al.*, 1994) a condition in which dysmyelination and demyelination may occur in the absence of axonal loss (Malamud, 1966).

The hypothesis that axonal loss is associated with persistent functional deficit is consonant with the increasing *in vivo* evidence for loss of axonal integrity in multiple sclerosis. Ophthalmoscopically visible defects in the retinal nerve fibre layer (Frisén and Hoyt, 1974) which are attributable to axon loss are common in the disease and may be seen after a single attack of optic neuritis (W. I. McDonald, unpublished observation); chronic lesions of long (>2 years) duration frequently show a biexponential transverse magnetization decay curve attributable to expansion of the extra cellular space as a result of axon loss (Barnes *et al.*, 1991); atrophy of the cervical spinal cord correlates with the severity of disability and the pyramidal score of the Kurtzke scales (Kidd *et al.*, 1993); a low magnetization transfer ratio (providing evidence for tissue disorganization to which both axon degeneration and demyelination probably contribute) correlates with the EDSS (Gass *et al.*, 1994).

While the evidence points to a clear association between neurological impairment and neuronal loss, a firm connection remains to be established by showing a graded relationship between the two. Since, however, the capacity for recovery after nerve fibre loss is limited, it seems likely that, to the extent that it occurs in multiple sclerosis lesions, neural loss

will contribute to the irrecoverable component of clinical deficit.

The question arises whether the MRS findings in this study are simply due to lesions within the regions studied. This is unlikely for two reasons: first, we were only able to detect a modest increase in voxel lesion load in the patients with multiple sclerosis with cerebellar deficit compared with the non-ataxic group; secondly, the median percentage of voxel occupied by abnormal signal in the ataxic patients was only 9%, compared with a median reduction in concentration of NAA of 30%. Such a disproportionate reduction of NAA may reflect axonal loss within the normal appearing white matter in the ataxic group (Davie *et al.*, 1994a). There was a significantly greater lesion volume in adjacent regions of the cerebellum and brainstem in these patients and it is therefore possible that the decrease in NAA is a consequence of axonal damage in lesions located outside the spectroscopic volume of interest which has resulted in secondary degeneration of axons and nerve cell bodies. The mechanism of neuronal disruption in multiple sclerosis is not established but it could be mediated by nitric oxide (Mitrovic *et al.*, 1994; Heales *et al.*, 1995). There is *in vitro* evidence that  $\gamma$ -interferon (which is released in immune mediated inflammation) can stimulate astrocytes and microglia to increase the formation of nitric oxide (Bolaños *et al.*, 1994), and it has been shown recently that there is increased nitric oxide production in multiple sclerosis (Bolaños *et al.*, 1994; Johnson *et al.*, 1995). Although astrocytes are relatively resistant to the toxic effects of nitric oxide, neurons are particularly sensitive to it (Mitrovic *et al.*, 1994).

An unexpected finding in this study was the reduction of apparent concentration of choline-containing compounds from cerebellar white matter in a number of patients with ADCA: concentration of choline-containing compounds was below the control range in four of the eight patients (Table 3). This was not associated specifically with any mutation or type of ADCA or clearly correlated with apparent concentration of NA, disease duration or cerebellar volume. However, although the patient group was small, there was a tendency for the patients with the lowest values of apparent concentration of

choline-containing compounds to have the longest disease duration and greatest degree of cerebellar atrophy.

The resonance observed at 3.2 p.p.m. in the proton spectrum is not one compound but the combination of signal from a number of metabolites. These include free choline, phosphocholine, glycerophosphocholine, acetylcholine, phosphatidylcholine, sphingomyelin and betaine (McIlwain *et al.*, 1985; B. L. Miller, 1991; Brenner *et al.*, 1993). Immunocytochemical studies have provided evidence for the existence of cholinergic transmission in the human cerebellum, with the detection of muscarinic receptors in the mossy fibres that terminate in the granular layer (Casanova *et al.*, 1990). Mossy fibres account for about two-thirds of the myelinated axons underlying the cerebellar cortex. It is conceivable that degeneration of these axons may explain the reduction of concentration of choline-containing compounds that we have observed in some patients with ADCA. Kish *et al.* (1987) have shown a significant reduction of choline acetyltransferase from the cerebral cortex in patients with ADCA, suggesting a loss of cholinergic neurons projecting from the nucleus basalis. It is not yet known whether cholinergic neurons are lost from the cerebellum in ADCA. Occasional patients with degenerative cerebellar disease have been treated successfully with choline chloride, although overall a therapeutic response is rare in this context.

The present investigation provides the strongest evidence so far to support the hypothesis that axonal loss contributes to the development of persistent neurological impairment in multiple sclerosis. If this is correct, MRS may have a role in monitoring treatment, though in the immediate future it is likely to be confined to special centres with facilities for acquiring spectroscopic data from large areas of the brain in an acceptable time span (Arnold, 1992; Husted *et al.*, 1994).

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