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*AJNR Am J Neuroradiol* 1996, 17 (6) 1013-1024

<http://www.ajnr.org/content/17/6/1013>

This information is current as  
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# Proton MR Spectroscopy of Childhood Adrenoleukodystrophy

V. Rajanayagam, J. Grad, W. Krivit, D. J. Loes, L. Lockman, E. Shapiro, M. Balthazor, D. Aeppli, and A. E. Stillman

**PURPOSE:** To determine the potential of proton MR spectroscopy to monitor patients with childhood-onset cerebral adrenoleukodystrophy (COALD). **METHODS:** Single-voxel MR spectroscopy was performed in 16 children with COALD (24 examinations) who had had no treatment and in 7 children (13 examinations) who had had bone marrow transplantation. **RESULTS:** In the untreated children with clinically active COALD, the metabolite ratios *N*-acetyl-aspartate (NAA)/creatinine (Cr) and NAA/choline (Ch) were decreased while Ch/Cr was increased. This trend agrees well with those reported by other researchers, although different experimental sequences and parameters were used in our study. Comparison of these ratios with those from a control group yielded significant differences in the occipital region. In the children who were clinically stable after bone marrow transplantation, the mean levels of the three ratios were between those of the control subjects and the patients with untreated COALD; the differences in these ratios approached significance. In patients who had been monitored periodically, MR spectroscopy metabolite ratios correlated well with the dementia rating score, reflecting clinical status. **CONCLUSION:** There is good correlation between MR spectroscopy metabolite ratios and a patient's clinical status. MR spectroscopy appears to be a useful, noninvasive tool to monitor patients with adrenoleukodystrophy, and it increases the overall sensitivity of MR techniques in clinical applications.

**Index terms:** Adrenoleukodystrophy; Children, diseases; Magnetic resonance, spectroscopy

*AJNR Am J Neuroradiol* 17:1013-1024, June 1996

Adrenoleukodystrophy (ALD) is a disorder characterized by elevated levels of very long chain fatty acids in the plasma and a variable phenotype; the gene has been localized to Xq28 (1-5). There are several well-recognized forms: adult-onset adrenomyeloneuropathy, adrenal insufficiency in isolation, and childhood-onset cerebral adrenoleukodystrophy (COALD). In patients with COALD, the mean age of disease onset is 7 years, demyelination is rapid, and deterioration leading to death occurs over an average of 3½ years. There is no laboratory test, including levels of very long chain fatty acids, that can distinguish among these three

forms of the disease or that can indicate the degree of deterioration (1, 6).

Advances in human genetics have not only resulted in the identification of the genetic abnormalities associated with many neurodegenerative diseases but have also offered hope for treatment. A dietary approach has been attempted (2). In COALD patients, however, this dietary treatment has had little impact on the progression of the disease (7, 8). Bone marrow transplantation, on the other hand, has been successful in stopping the progression of demyelination and neurologic symptoms, provided the disease is diagnosed and treated early (2, 9-12).

Initial diagnosis of COALD is made with magnetic resonance (MR) imaging together with evidence of neuropsychological and clinical deterioration. About 80% of children with COALD show increased signal on T2-weighted MR images in the posterior white matter and visual pathways, 15% show increased signal in anterior white matter, and 5% show brain stem and internal capsule abnormalities. These MR abnormalities correlate well with

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Received June 1, 1995; accepted after revision January 16, 1996.

Funded in part by National Institutes of Health/National Institute of Neurological Disorders and Stroke grant NS 29-099-04.

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*AJNR* 17:1013-1024, Jun 1996 0195-6108/96/1706-1013

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measured behavioral and neuropsychological deficits (13–18). Although MR imaging has proved critical in the initial diagnosis, it has not been useful in monitoring the course of the disease (19).

Recently, MR spectroscopy has been used to identify levels of cerebral metabolites in ALD patients (20–22) (J. Vion-Dury, B. Chabrol, S. Confort-Gouny, F. Nicoli, and P. J. Cozzone, "Localized Proton MR Spectroscopy of the Brain in X-Linked Adrenoleukodystrophy," presented at the meeting of the Society of European Leukodystrophy, Germany, 1992). Levels of *N*-acetyl-aspartate (NAA), creatine and phosphocreatine (Cr), choline-containing compounds (Ch), and lactate have been found to differ between healthy subjects and patients with white matter lesions. We hypothesized that changes in metabolites may be sensitive to changes in disease severity. Thus, we investigated the potential of MR spectroscopy for use in monitoring patients with COCALD so as to identify changes in metabolites that correlate with disease severity.

## Subjects and Methods

Our study group consisted of 23 children, 5 to 15 years old, with the biochemical defect for ALD. Proton MR spectroscopy was performed as an additional sequence to the standard MR imaging protocol used to detect the presence of cerebral effects of ALD. Sixteen children who had not been treated (24 examinations) and 7 children (13 examinations) who had undergone bone marrow transplantation were studied. Six of the untreated children and 5 of the children with bone marrow transplants had more than 1 examination. The mean age of these 2 groups of patients at each MR examination was  $9 \pm 4$  years and  $11 \pm 3$  years, respectively.

Informed consent was obtained from the parents prior to the examination, and institutional guidelines were followed. Generally, two regions of interest (ROIs) were examined in each patient. Most children were sedated (2 to 6 mg/kg pentobarbital sodium) and monitored using pulse oximetry and an apnea monitor during the MR examination.

Combined MR imaging and spectroscopic studies were done on a Siemens (Erlangen, Germany) 1.5-T whole-body scanner using the quadrature head coil. A double spin-echo sequence (23) was used to obtain single-voxel ( $2 \times 2 \times 2$  cm<sup>3</sup>) water-suppressed spectra from both the occipital and the frontal regions. If the lesions were evident on MR images, then the voxels were placed in those regions. Water suppression was done by applying frequency-selective pulses followed by gradient spoiling of the excited magnetization. This was followed by localization

using the double spin-echo sequence with parameters of 1600/135/256 (repetition time/echo time/excitations), 1000-Hz sweep width, and 1024 complex points. A Gaussian pulse of 60-Hz bandwidth was used for water suppression. The longer echo time of 135 milliseconds enables the detection of the lactate resonance and also simplifies spectral overlap. The ROIs in the diseased regions were defined graphically from T1-weighted axial, coronal, and sagittal images, which were obtained as a part of the routine MR examination. After global and localized shimming, the water suppression was optimized and, thereafter, both water-suppressed and unsuppressed spectra were obtained for each ROI. In follow-up examinations, efforts were made to localize the same ROI as in the earlier examination.

The residual eddy current-induced phase modulation was corrected with the reference water signal (24) and then apodized and zero-filled to 2048 points before Fourier transformation. The spectra were first phase-corrected (zero-order) and then cubic spline baseline-corrected. The three prominent resonances were identified as NAA at 2.02 ppm, Cr at 3.03 ppm, and Ch at 3.21 ppm, according to published criteria (25), and the areas were determined by routine spectral integration of the metabolite resonances that were later used to calculate the ratios. No correction for T1 and T2 was made in our studies. Examinations were considered unsuccessful if the patient moved during data acquisition and/or if we had difficulty measuring peak areas, and these data were omitted from the analysis.

Descriptive statistics, including mean and standard deviations, were calculated for each group of patients on the basis of the visible and invisible lesions on the MR images. Repetitive measurements in the same patients were weighted according to their inverse variances and mean weighted values were determined. The variances were based on a minimum correlation of 0.5 between observations within a subject, with correlations increasing to 1.0 when the same regions with signs of disease were measured at the same time. The data were then evaluated by means of weighted multivariate analysis of variance to determine the differences between the groups of patients in both the occipital and frontal regions. All statistical evaluations were done using an analysis package (version 6) from SAS Institute, Inc (Cary, NC). Differences were considered significant if the *P* values were less than .05. For some comparisons, sample sizes and, thus, the power are low so that the null hypothesis cannot be rejected even though the means may suggest a difference.

Symptoms indicating the presence of cerebral disease are quite subtle, with some of the prime indicators being declining school performance, memory loss, and behavioral problems (26). Information obtained from histories, interviews, and neurologic examinations was used to determine the clinical status of the patients, and degree of dementia was graded from 0 to 4 on a clinical dementia rating scale (DRS) (27). A score of 0 indicates biochemical abnormality with or without Addison disease but no neurologic, cognitive, behavioral, or academic difficulties.

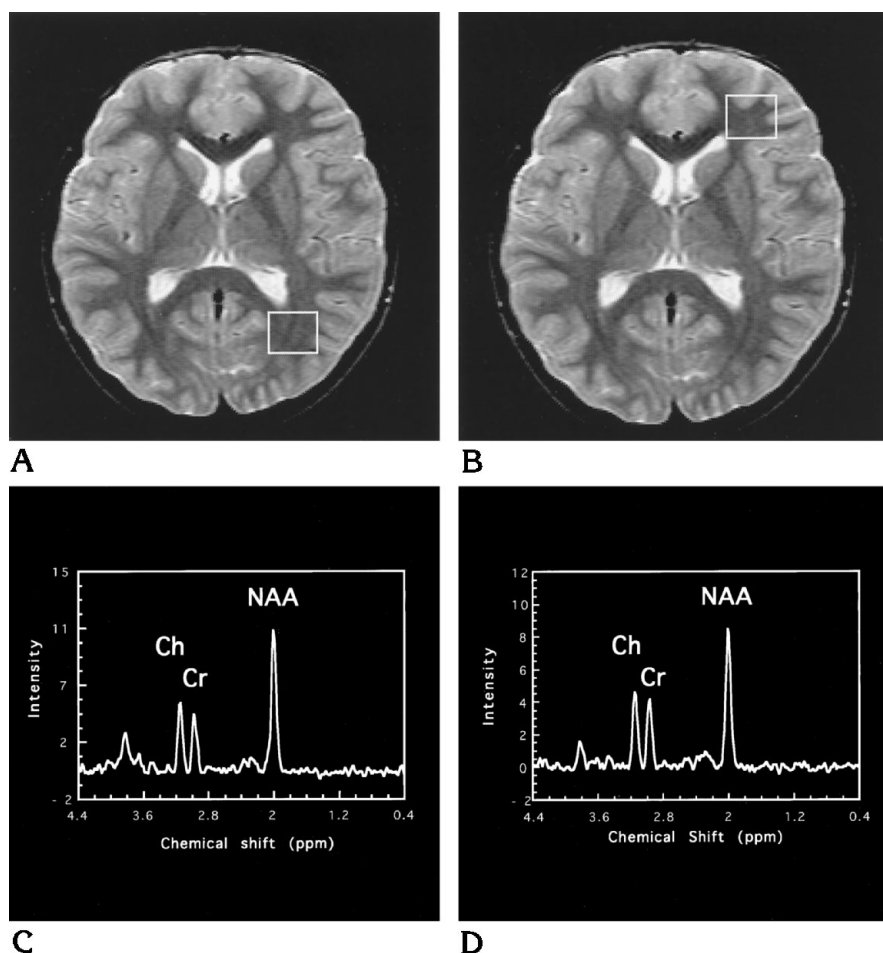


Fig 1. T2-weighted axial images (2500/90) (A and B) and localized spectra (1600/135) (C and D) from the occipital and frontal regions, respectively, of a healthy 8-year-old boy. The metabolite ratios for the occipital and frontal regions, respectively, are  $\text{NAA/Cr} = 2.54, 2.07$ ;  $\text{NAA/Ch} = 2.17, 1.89$ ;  $\text{Ch/Cr} = 1.17, 1.10$ .

A score of 1 indicates new-onset mild cognitive or learning problems discerned by parents or teachers and/or incoordination but no focal neurologic findings. A score of 2 indicates significant school difficulties, a need for special help in school, sensory abnormalities, and/or focal neurologic problems. A score of 3 indicates loss of mental ability, disorganization and confusion, a need for special education, significant neurologic abnormality, but perhaps normal reading and vocabulary. A score of 4 indicates significant dementia with loss of cognition in most areas, lack of orientation, loss of language, gross motor impairment, and a need for help in all activities.

MR images were scored on a point system, described in a recent report (28), derived from the appearance of the disease on MR images. This scale ranges from 0 to 34: the higher the score, the more severe the disease. The reader is referred to the original report for a detailed description of the scoring method.

## Results

### Metabolite Ratios in Normal Cerebral Cortex

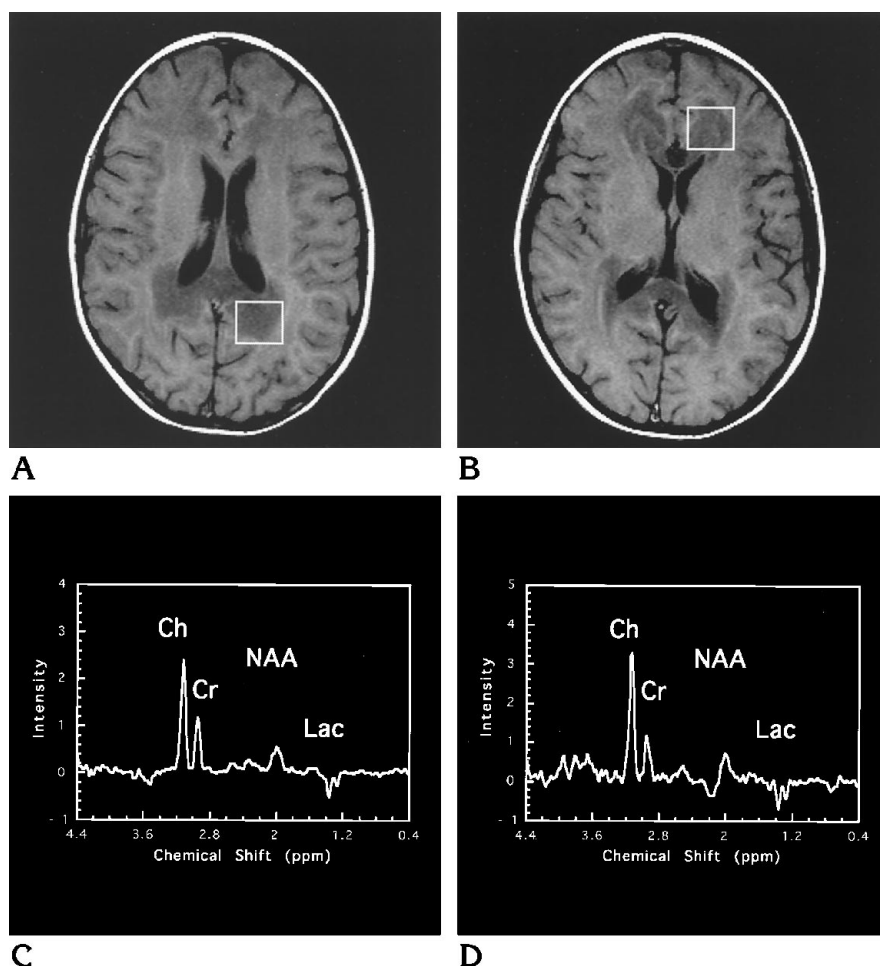
Reference axial images and localized spectra from the occipital and the frontal regions of a

healthy 8-year-old boy are illustrated in Figure 1. The ROIs are marked for reference. Note the characteristic intensity pattern of the three prominent resonances in the spectra in Figure 1C and D; Ch and Cr are almost of equal intensity while NAA is almost twice as intense as Ch and Cr. The metabolite ratios are listed in Table 1. Slight differences can be seen between the two regions, with the frontal regions generally having slightly reduced values, consistent with observations of others (29).

### Metabolite Ratios in Patients with Untreated COCALD

Patients with untreated COCALD showed an increased MR signal intensity on both the T2-weighted and proton density-weighted images and decreased intensity on the T1-weighted images in affected areas (13, 14). Representative T1-weighted images as well as localized spectra from the occipital and frontal regions from an 11-year-old patient are shown in Figure 2. Ev-

Fig 2. T1-weighted axial images (600/15) (A and B) and localized spectra (1600/135) (B and C) from the occipital and frontal regions, respectively, of an 11-year-old patient with COCALD. The diseased areas appear hypointense on the images. Note the intensity differences of NAA and Ch peaks and the presence of lactate (Lac). The metabolite ratios for the occipital and frontal regions, respectively, are NAA/Cr = 0.75, 0.72; NAA/Ch = 0.34, 0.28; Ch/Cr = 2.21, 2.53.



idence of the disease is clearly visible in both the frontal and occipital regions on the MR images. In the localized spectra, the intensity distributions of the metabolites are different from the healthy control subject; the intensity of NAA is reduced while that of Ch is increased. In addition, the characteristic doublet pattern of the lactate resonance is clearly seen at 1.33 ppm in both regions. In this patient, the NAA/Cr, NAA/Ch, and Ch/Cr ratios are 0.76, 0.34, and 2.21 for the occipital region and 0.72, 0.28, and 2.53 for the frontal region, respectively.

In the patients with untreated COCALD, we found that the ratios from the affected lesions in the occipital region followed the same pattern as in the above patient; NAA/Cr and NAA/Ch were decreased and Ch/Cr was increased. The weighted mean ratios as well as the standard deviations for this group of patients are listed in Table 1. The number of patients and the number of successful examinations are given in parentheses.

Although the number of patients with frontal disease studied is small, similar trends in the metabolite ratios were evident. As seen in Table 1, NAA/Cr and NAA/Ch ratios were decreased and the Ch/Cr ratio was increased. This pattern of changes in the metabolite ratios agrees well with the earlier reports on ALD (20, 21) despite the use of different repetition times and echo times and different spatial localization sequences in this study.

#### *Metabolite Ratios in Patients with COCALD Who Had Bone Marrow Transplantation*

Figure 3 illustrates axial T2-weighted images (A and B) and localized spectra from the occipital (C) and frontal (D) regions of a 12-year-old patient with COCALD examined 3 years after bone marrow transplantation. Characteristic regions of hyperintensity are absent in these images. In the localized spectra, however, the intensity distribution of the resonances differs

**TABLE 1: Metabolite ratios from control subjects and from lesions in patients with childhood adrenoleukodystrophy**

	NAA/Cr	NAA/Ch	Ch/Cr
Healthy 8-year-old boy			
Occipital region	2.54	2.17	1.17
Frontal region	2.07	1.89	1.10
Untreated COCALD patients			
Occipital region	1.26 ± 0.36 (11/20)	1.08 ± 0.44 (11/20)	1.49 ± 0.23 (11/20)
Frontal region	1.01 ± 0.17 (5/5)	0.72 ± 0.25 (5/5)	1.92 ± 0.31 (6/6)
COCALD patients treated with bone marrow transplantation			
Occipital region	1.51 ± 0.23 (7/12)	1.63 ± 0.30 (7/12)	0.95 ± 0.17 (7/13)
<i>P</i> values*	0.44	0.17	0.014
Frontal region	1.99 ± 0.26 (2/3)	2.03 ± 0.48 (2/3)	1.08 ± 0.15 (3/4)
<i>P</i> values†	0.08	0.13	0.14
Control subjects‡			
Occipital region	2.21 ± 0.42 (152)	2.75 ± 0.61 (152)	0.82 ± 0.16 (152)

Note.—Weighted mean values ± 1 SD are given. *P* values have been obtained from a weighted multivariate analysis of variance of the two groups of patients. Numbers in parentheses indicate the number of patients/successful examinations.

\* Comparison between the COCALD patients with and without bone marrow transplantation for occipital regions only.

† Comparison between the COCALD patients with and without bone marrow transplantation for frontal regions only.

‡ Metabolite ratios of the reference control group obtained from a multicenter study (Sauter [30]).

from that of both the healthy control subject and the untreated COCALD patients. The differences in Ch and Cr intensities are minimal and the signal intensity of NAA is relatively higher. The NAA/Cr, NAA/Ch, and Ch/Cr ratios for this patient are 2.26, 1.79, and 1.26 for the occipital region and 1.77, 1.74, and 1.02 for the frontal region, respectively. The intensity pattern of the spectral peaks as well as the metabolite ratios differ from those of the patients without bone marrow transplantation.

As seen in Table 1, in the occipital region of patients who have had bone marrow transplantation, the weighted mean ratios of NAA/Cr and NAA/Ch are increased to  $1.51 \pm 0.23$  ( $P = .44$ ) and  $1.63 \pm 0.30$  ( $P = .17$ ), respectively, while Ch/Cr is decreased to  $0.95 \pm 0.17$  ( $P = .01$ ) as compared with the untreated patients. Thus, the Ch/Cr ratio may be a useful marker to characterize disease treatment.

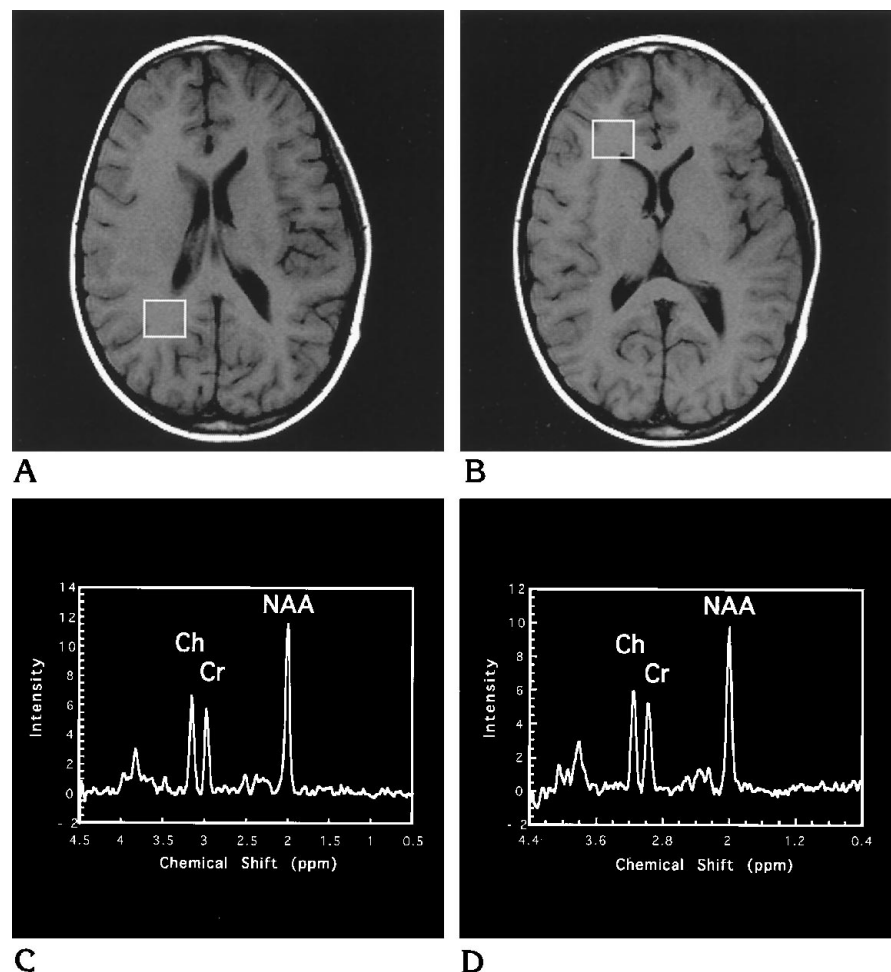
Figure 4 illustrates the three metabolite ratios from the occipital region of COCALD patients with and without bone marrow transplantation.

As explained previously, some of the patient data were omitted from the analysis because of patient movement and/or poor-quality spectra. Eleven children who had no treatment (20 examinations) and 7 children with bone marrow transplants (13 examinations) were included in the final analysis for the occipital region. The regions in which the metabolite ratio was normal as well as the 95% confidence interval are identified by the shaded area. There is a clear pattern of differences, with the majority of the NAA/Ch and Ch/Cr ratios from untreated patients falling outside the 95% confidence interval. Table 1 also shows these normal metabolite ratios from the occipital region of patients in the control group, which were obtained from a Siemens multicenter study in which MR imaging was performed on 1.5-T scanners with the same experimental parameters and the same spectral localization sequence as used in our study (30). This group included 202 healthy volunteers, 18 to 80 years old (mean,  $31 \pm 10$  years).

Our data from the occipital region of the healthy 8-year-old boy, indicated as a broken line in Figure 4, falls within the normal range of values of the control group. Only minor differences can be found between the ratios obtained in the control group and that of this subject. This observation is consistent with the finding that the primary changes in these metabolites occur predominantly in the first 2 years of life (31). Thus, for statistical purposes, the comparison of data from the occipital region of our patients with those of the control group can be justified. It is also clear from Figure 4 that the ratios from the COCALD patients exhibit similar patterns of differences from those of the healthy boy.

Comparison of the weighted mean patient metabolite ratios with those from the control group yielded significant differences ( $P < .005$ ) for the untreated patients. In the case of the patients with bone marrow transplantation, the mean values of the metabolite ratios are between those of the control group and the untreated patients, all showing borderline significant differences from the control group, with  $P = .05$ . Although comparisons were from different patient populations, the differences in ratios between these two groups indicate that MR spectroscopy might serve as a useful tool for monitoring disease treatment. Systematic MR spectroscopy examinations before and after bone marrow transplantation are now underway to investigate sensitivity to change.

Fig 3. T1-weighted axial images (600/15) (A and B) and localized spectra (1600/135) (C and D) from the occipital and frontal regions, respectively, of a 12-year-old patient with COCALD who had undergone bone marrow transplantation. Note the absence of hypointense regions in the images, but the spectral distribution is different from those of the control subject shown in Figure 1C and D. The metabolite ratios for the occipital and frontal regions, respectively, are NAA/Cr = 2.26, 1.77; NAA/Ch = 1.79, 1.74; Ch/Cr = 1.26, 1.02.



We saw similar patterns in the frontal regions; however, the differences between the patients with and without treatment were not statistically significant owing to the smaller number of patients with frontal disease lesions (Table 1). Moreover, no comparisons with the control group were made because control values for the frontal region were unavailable.

#### *Metabolite Ratios in Monitoring Patients with COCALD*

To monitor disease progression, some patients were studied more than once. A total of 10 patients, both treated and untreated, were monitored using MR spectroscopy; 8 patients were studied twice and 2 patients were studied 3 times. To check data reproducibility, a healthy adult was scanned on 4 different occasions and the measured values exhibited variations amounting to 8.0%, 4.1%, and 7.4% for the ratios NAA/Cr, NAA/Ch, and Ch/Cr, respectively. This gives a good representation of the mea-

surement system variability, and changes on the order of 10% can therefore be considered noticeable differences. Furthermore, an increase in the NAA/Cr and NAA/Ch ratios and a decrease in the Ch/Cr ratio indicate an improvement in clinical status.

The changes in the three metabolite ratios, the clinical status, and the MR imaging data from these studies were classified into three groups on the basis of MR spectroscopy and MR imaging characteristics. Group 1 comprised patients exhibiting minimal or no changes (<10%) in metabolite ratios and MR imaging scores. Group 2 patients showed changes in MR spectroscopy but no changes in MR imaging scores. Group 3 patients exhibited changes in both MR spectroscopy and MR imaging scores. These data are listed in Table 2.

#### *Group 1 Patients*

This group consisted of patients whose metabolite ratios showed minimal or no change,

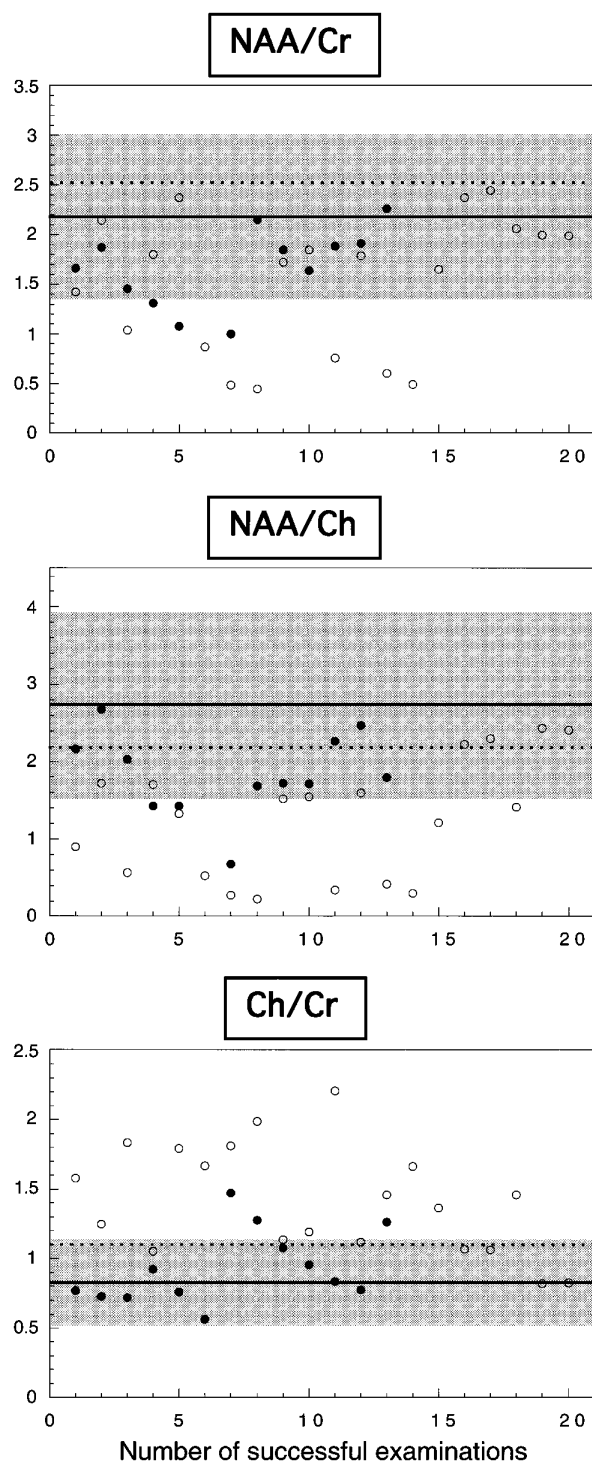


Fig 4. Individual metabolite ratios from the occipital region of the treated (filled circles) and untreated (open circles) COCALD patients. The mean values of the metabolite ratios from the control group (30) are plotted (solid line) together with the 95% confidence intervals (shaded area). The data from the healthy 8-year-old boy are also shown (broken line), and it is clear that these data lie within the 95% confidence intervals of the control group. Note the patterns of distribution of the patients' metabolite ratios with respect to those of the control subjects.

consistent with a lack of change in both MR imaging and clinical scores.

In patients A and B, the trends in MR spectroscopy ratios, clinical status, and MR imaging scores agree well over time. While the MR imaging score agrees with MR spectroscopy and clinical status for patient A, patient B, who was also clinically stable, had a higher MR imaging score, indicating more disease involvement in the brain.

Patient C, who was 10 years old and initially studied 1 year after bone marrow transplantation and again 9 and 21 months later, showed a stable distribution of metabolite ratios for the first two visits. The initial changes were all less than 10% and this finding correlates well with stability in both the clinical status and the MR imaging scores. During the third visit, the ratios showed a decrease in NAA/Ch and a slight increase, within the normal range, in Ch/Cr. The change in NAA/Cr ratio was minimal. Both the clinical status and MR imaging data, however, were stable.

#### Group 2 Patients

These patients exhibited changes in MR spectroscopy data but no changes in MR imaging scores or clinical status. In patient D, increases in NAA/Cr (25%) and NAA/Ch (17%) ratios indicated an improvement on the second visit, 13 months later. There was no change in the Ch/Cr ratio. On the third visit, 20 months later, NAA/Cr and NAA/Ch increased further, by 19% and 63%, respectively, and Ch/Cr decreased by 27%. Clinical status and MR imaging scores were unchanged, but normal (ie, zero) throughout. This case shows a difference between MR spectroscopy and other measures. MR spectroscopy appears to be sensitive to small differences that may not be measurable on MR images or on the DRS.

Patient E was clinically stable as were the MR imaging scores, which were only mildly abnormal. The metabolite ratios differed from the normal values with improvement in the Ch/Cr and NAA/Ch metabolite ratios. Ch/Cr has decreased by 41% and NAA/Ch has increased by 29%. However, the 24% decrease in NAA/Cr is inconsistent with the changes in the other ratios.

These two cases, in which there are no clinical symptoms, may illustrate developmental changes as well as sensitivity to abnormalities not yet expressed clinically or on MR images.



**TABLE 2: Monitoring disease progression in patients with childhood adrenoleukodystrophy: proton MR spectroscopy metabolite ratios of the white matter, clinical status, and MR imaging scores**

Patients	Age, y	Treatment?	NAA/Cr	NAA/Ch	Ch/Cr	Clinical Status*	MR Score†
<b>Group 1</b>							
A (1,2)	8	No	1.84 ± 1.72	1.55 ± 1.52	1.19 ± 1.14	1 ± 1	2 ± 2
B (1,2)	15	No	1.99 ± 1.99	2.40 ± 2.43	0.83 ± 0.82	1 ± 1	5 ± 5
C (1,2)	10	Yes	1.91 ± 1.89	2.47 ± 2.26	0.77 ± 0.83	1 ± 1	8 ± 8
C (2,3)	11	Yes	1.89 ± 1.63	2.26 ± 1.71	0.83 ± 0.96	1 ± 1	8 ± 8
<b>Group 2</b>							
D (1,2)	6	No	1.65 ± 2.06	1.21 ± 1.41	1.36 ± 1.46	0 ± 0	0 ± 0
D (2,3)	7	No	2.06 ± 2.44	1.41 ± 2.30	1.46 ± 1.06	0 ± 0	0 ± 0
E (1,2)	5	No	2.37 ± 1.80	1.32 ± 1.70	1.79 ± 1.06	0 ± 0	0.5 ± 0.5
F (1,2)	13	Yes	1.66 ± 1.87	2.17 ± 2.68	0.77 ± 0.73	1 ± 1	12 ± 12
<b>Group 3</b>							
G (1,2)	6	Yes	2.15 ± 1.00	1.68 ± 0.68	1.28 ± 1.47	2 ± 3	10.5 ± 8.5
H (1,2)	11	Yes	1.31 ± 1.46	1.42 ± 2.03	0.92 ± 0.72	1.5 ± 1	10 ± 8
I (1,2)	6	No	0.49 ± 0.87	0.27 ± 0.52	1.81 ± 1.67	4 ± 4	12 ± 13.5
J (1,2)	9	No	0.49 ± 0.61	0.30 ± 0.42	1.66 ± 1.46	4 ± 4	14 ± 18

Note.—The numbers in parentheses indicate the visits. Changes in metabolite ratios amounting to less than  $\pm 10\%$  are considered not significant.

\* Clinical status is based on a dementia rating scale described in the "Subjects and Methods" section and by Shapiro and Klein (27).

† MR score is based on the method described by Loes et al (28).

Patient F showed a slight improvement in MR spectroscopy ratios, which was correlated with a slight improvement in his clinical status, although not enough to move him from category 1 to 0. No change was seen in the high MR scores.

Findings in both group 1 and group 2 patients illustrated that MR spectroscopy data may be more sensitive to the disease process than MR imaging scores. Regions with no visible abnormality or with minimally visible abnormalities on MR images exhibit abnormal metabolite ratios. This may reflect early manifestations of the disease. Furthermore, the high MR score in patients C and F (see Table 2) do not reflect the clinical status of the disease (DRS rating of 1 for each).

### Group 3 Patients

Group 3 was made up of patients who exhibited changes in MR spectroscopy and MR imaging scores. Patient G, a 6-year-old, was studied 11 months apart and is an interesting case. First-echo (echo time, 45) axial images from the same location are shown in Figure 5A and B, respectively, for the two visits. The changes in the MR image intensities of the disease regions were minimal, although markedly abnormal. However, in the corresponding localized spectra from the occipital region, marked differences in the intensity distributions of the resonances were seen (Fig 5C and D).

In this patient, the metabolite ratios NAA/Cr and NAA/Ch decreased by 53.5% and 59.5%, respectively, and the Ch/Cr ratio increased by 14.8%. These changes are significant and suggest disease progression. This observation correlates well with the clinical status of the patient (DRS score changed from 1 to 3). However, the MR imaging data were inconsistent with both MR spectroscopy and clinical data. The MR imaging score was reduced in the second visit, indicating an improvement in brain involvement.

Patient H showed a clear trend toward improvement in the metabolite ratios, clinical status, and MR imaging scores. All three measures correlate well.

Patients I and J were at a severe stage of the disease, which is apparent from the low NAA/Cr and NAA/Ch ratios and high Ch/Cr ratio. This agrees well with the high DRS rating and high MR imaging score. The slight improvement in MR spectroscopy ratios does not reflect clinical or MR imaging status and may reflect a burn-out of metabolites.

In this group of patients, although good agreement was found between MR spectroscopy and the clinical status, MR imaging does not always accurately reflect the clinical status, as illustrated by patient G. On the other hand, when metabolite ratios are greatly abnormal, as illustrated by patients I and J, changes in their values do not correlate with the clinical status.

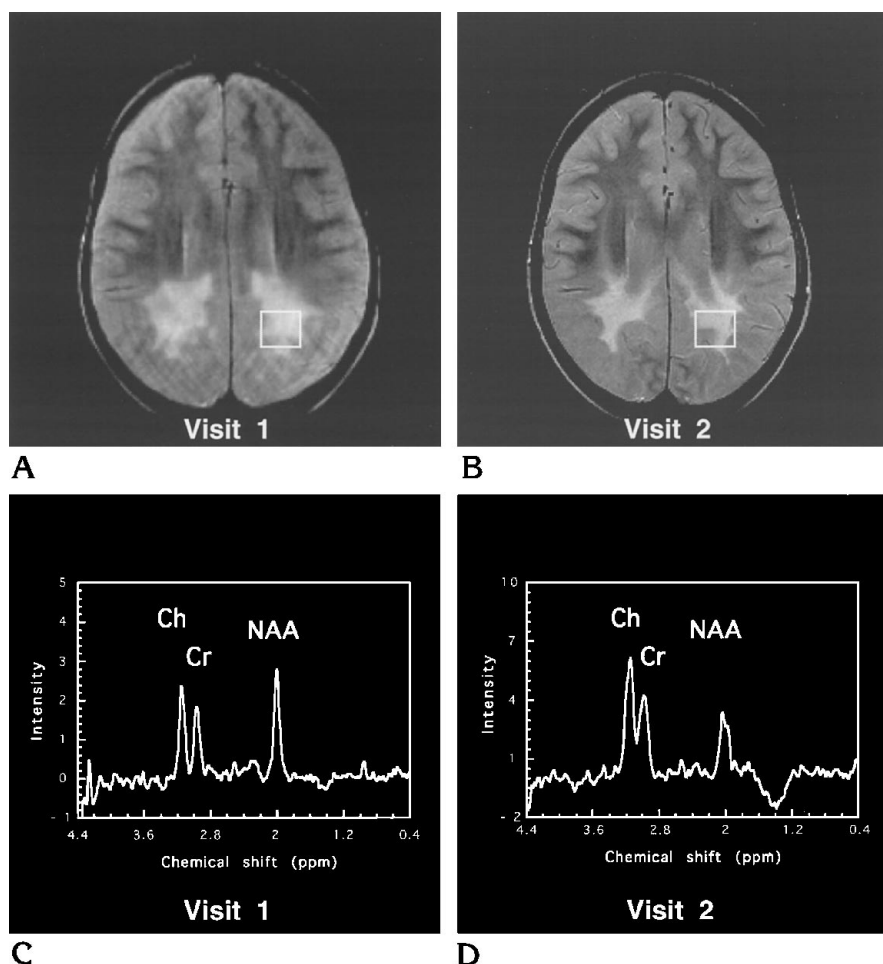


Fig 5. T2-weighted axial images (2500/90) (A and B) and localized spectra (1600/135) (C and D) from the occipital regions of a 6-year-old patient with COCALD and bone marrow transplant obtained from two visits, 11 months apart. Note the relative decrease in the NAA peak in the second visit (D), indicating disease progression.

Thus, in this case, the MR spectroscopy changes may not reflect clinical status.

## Discussion

Proton MR spectroscopy has been used previously to show the differences in the three prominent metabolites in regions of abnormality on MR images of the brain in patients with ALD (20–22). We observed similar differences in our study. Moreover, we found differences between patients with clinically active disease and those who have been successfully treated with bone marrow transplantation. In the small group of COCALD patients who have been followed up serially, it appears that MR spectroscopy findings correlate well with the clinical severity of the disease.

In the COCALD form, demyelination occurs in specific tracts and areas of the brain. Posterior demyelination occurs in about 80% of the cases and frontal demyelination in about 15% (27). Most of our patients had posterior demy-

elination, and in some patients the disease had spread to the frontal regions as well. Figure 2 illustrates one such case. The affected areas appear hyperintense on the T2-weighted images and hypointense on the T1-weighted images, and the localized spectra from these regions exhibit decreased NAA/Cr and NAA/Ch ratios and an elevated Ch/Cr ratio. The extreme values of these ratios indicate the severity of the disease. The decrease of NAA/Cr is presumably due to decreased NAA levels. NAA is understood to be a neuronal marker (32, 33) and a decrease in NAA is due to loss of neurons or neuronal activity as a result of myelin breakdown. However, there are metabolically active cells still present, as indicated by the presence of Cr. It is not clear whether there is a decrease of Cr in the affected regions, although it has been assumed to be constant in healthy adults (34) and in ALD patients (21). An increase in the Ch signal is thought to be an indicator of the active demyelination process (35). The Ch/Cr ratio is elevated in affected areas and this may

be due to elevated levels of Ch in the form of phosphatidylcholine in myelin (21).

Lactate was visible in a number of patients, including the ones who had undergone bone marrow transplantation. The lactate probably arises from lymphocytes, known to be preferentially glycolytic (36). Lymphocytic infiltration is present in active areas of disease. In addition, histochemical evidence shows an increase in tissue necrosis surrounding the affected regions (37). Under this circumstance, lactate could remain in the necrotic tissue and leave the diseased area only very slowly by passive diffusion (36).

Metabolite ratios in patients who have undergone bone marrow transplantation are between those of untreated patients and healthy subjects. It is known that bone marrow transplantation has been successful in treating ALD patients provided the neurologic symptoms are diagnosed early (2, 9–12). The progressive changes in the metabolite ratios in the patients who have had bone marrow transplantation indicate either an arrest of the disease process or its reversal: the number of neurons or the neuronal activity does not decrease further and the excess Ch is metabolized. However, it is not clear at this stage why the lactate peak is still seen in some patients who have had bone marrow transplantation. It could well be that the necrotic tissue still contains some lactate. Studies monitoring the same patients before and after bone marrow transplantation are ongoing and these factors may be clarified in the future.

It seems unlikely that the improved NAA/Cr and NAA/Ch ratios seen in the bone marrow transplantation patients are due to the recovery of neurons. Evaluation of the MR images of these patients showed that there had been contracture of the white matter (atrophy or volume loss), a phenomenon that has been reported recently (19). Hence, a more likely explanation for the improved NAA/Cr and NAA/Ch ratios may be that this volume loss has caused an increase in the gray matter contribution to the measurement voxel in MR spectroscopy.

This study focused on obtaining MR spectroscopy data from abnormal regions on MR images. All ROIs were placed in the white matter regions of the occipital and frontal lobes. As mentioned, slight differences between the two regions have been found in healthy control subjects. Others have also reported this (29), but no explanation is apparent at this time.

Currently, our protocol involves the acquisition of spectra from two ROIs from both the occipital and frontal regions, even in patients in whom the disease is confined to just one region. Our experience suggests that early signs of demyelination are more sensitive to MR spectroscopy and precede morphologic evidence, which appears later with characteristic intensity patterns on MR images. This, however, requires verification with a larger number of patients, and is an ongoing objective in our laboratory.

Our measurements of metabolite ratios confirm the potential utility of MR spectroscopy. Disease progression can be monitored by using MR spectroscopy, as the numbers given in Table 2 demonstrate. Inferences may be difficult when simultaneous changes occur in the numerator and the denominator of our ratios. However, when changes are consistent across all three metabolite ratios (decreases in NAA/Cr and NAA/Ch and an increase in Ch/Cr), greater disease involvement can be inferred.

In general, good agreement between MR spectroscopy, MR imaging scores, and clinical status was found. The trends in all three were in agreement for patients in group 1. In group 2 patients, MR spectroscopy appears to be more sensitive than MR imaging, and it matches more closely the clinical status of the patients with respect to the degree of abnormality. In group 3 patients, deterioration in function appears to be reflected in abnormal levels of MR spectroscopy ratios and higher MR imaging scores. One patient in group 3 was correctly identified as having disease progression on MR spectroscopy but not by MR imaging scores.

Changes in MR spectroscopy do not correlate well with clinical status when the clinical status is poor. Improvements in MR spectroscopy are not always reflected in changes in clinical status. It may be that changes in clinical status when the status is poor are not sensitively reflected in the DRS rating. However, no evidence can be found in clinical records to indicate improvement in clinical status that correlates with MR spectroscopy improvement.

We did not make any corrections for differences in relaxation times among these metabolites. The repetition time of 1600 used in this study is greater than the T1 relaxation times of the three major metabolites. The T1 relaxation times of NAA, Cr, and Ch are 1370, 1170, and 1290, respectively, at the clinical field strength of 1.5 T (38). Discrepancies exist in the pub-

lished T2 relaxation times, with values ranging from 240 to 388, 160 to 240, and 270 to 395 for NAA, Cr, and Ch, respectively (28, 38–42). It may be that the T2 decay causes almost equal contributions to the metabolites. However, these observations are valid only for healthy subjects and it is not known whether pathologic processes affect relaxation times enough to cause variations in signal intensities at these experimental parameters of 1600/135. Measurement of relaxation times for each patient is not practical with the present measurement setup. Longer scan times are not tolerated by patients. We have seen changes in metabolite ratios amounting to less than 14% in healthy control subjects and less than 11% in ALD patients, with a repetition time of 5000 implying that the selection of a longer time is not critical, particularly when measuring ratios (34, 39). The fact that the pattern of changes in ALD patients is similar in different studies with different repetition time/echo time values suggests that even if there are some differences in the peak intensities, the relative changes appear to be quite reproducible and reliable. This, therefore, enhances the usefulness of metabolite ratio measurements.

Measurement of absolute concentrations rather than ratios is desirable. However, no generally accepted method of quantitation exists. As a result, a range of concentration values with large errors has been reported by a number of different groups for the primary metabolites, even in the normal brain (28, 38–41). Therefore, at present, metabolite ratios are preferable for monitoring patients.

In conclusion, we have described a noninvasive method for studying patients with CO-CALD. Metabolite ratios of the patients who have had bone marrow transplantation were in between those of untreated patients and the control group. Disease progression or improvement can also be monitored by measurements of metabolite ratios using localized proton MR spectroscopy. The ratios provide in vivo insight of metabolic processes and correlate well with brain MR data and clinical status. In some cases, MR spectroscopy promises to be a more sensitive tool for monitoring these patients. Studies are continuing to assess the clinical potential of MR spectroscopy with a larger group of patients.

## Acknowledgments

We gratefully acknowledge the care of and assistance to patients provided by the nurses and technicians of the MR imaging unit of the Department of Radiology, University of Minnesota. We also extend our thanks to H. W. Moser for his helpful comments.

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