

# Integrated MR Imaging and Proton Nuclear Magnetic Resonance Spectroscopy in a Family with an X-Linked Spastic Paraparesis

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Seven members of a family with an X-linked spastic paraparesis syndrome were analyzed by MR imaging and stimulated echo, solvent-suppressed proton nuclear magnetic resonance spectroscopy. The MR scans of three symptomatic males and two asymptomatic females demonstrated abnormal signal in the supratentorial white matter. Each of these patients had a proton spectroscopic examination of a  $2 \times 2 \times 2$  cm voxel localized to the abnormal white matter of the centrum semiovale. The spectra demonstrated depression of *N*-acetyl aspartate/creatine, *N*-acetyl aspartate/choline, and creatine/choline ratios compared with normal control subjects. Additionally, these patients had abnormal elevations of amino acid resonances in the 2.1–3.0 ppm range. In a patient with symmetric white matter signal intensity abnormalities, an asymmetric spectroscopic study correlated with asymmetric symptoms. One asymptomatic family member with a normal MR study had abnormal metabolite ratio measurements. She was referred for further evaluation, since the proton spectrum suggested she may possess the affected gene.

If the findings in this study are duplicated in other cases of hereditary dysmyelinating syndromes, we believe the integrated MR/proton nuclear magnetic resonance spectroscopy examination will be of benefit in evaluating and counseling families with familial dysmyelinating disorders.

*AJNR* 12:785–789, July/August 1991

The clinical applications and technical aspects of MR spectroscopy have recently been reviewed in the literature [1, 2]. Over the past several years there has been increasing interest in using proton nuclear magnetic resonance spectroscopy (PMRS) to study the metabolism of the brain [3–8]. Instead of a conventional anatomic image of the brain, proton spectroscopy provides biochemical information concerning hydrogen-bearing non-water metabolites in the CNS, including *N*-acetyl aspartate, the creatine-phosphocreatine pool, and choline-containing compounds. The integration of MR imaging with spectroscopy offers the potential for a more comprehensive evaluation of pathologic conditions of the brain.

Patients with diffuse dysmyelinating disorders are ideal subjects for PMRS studies because the area of abnormality is easily sampled within the constraints of relatively large voxel sizes. Voxels that include white matter without significant gray matter or ventricular CSF contamination can be sampled with a high degree of accuracy and assurance. In order to determine the value of the integrated MR/PMRS examination in patients with hereditary white matter disorders, we examined seven members of a pedigree with a complicated X-linked hereditary spastic paraparesis (HSP) syndrome [9].

## Subjects and Methods

A family with five males in a single generation affected with a previously undescribed form of complicated hereditary spastic paraparesis has recently been reported [9]. This pedigree

Received October 10, 1990; revision requested January 17, 1991; revision received February 6, 1991; accepted February 20, 1991.

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0195–6108/91/1204–0785  
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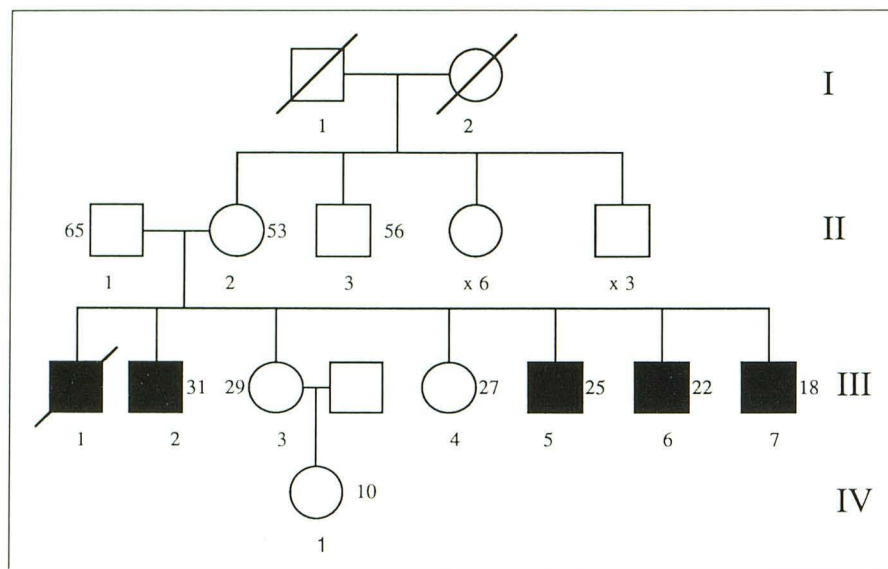


Fig. 1.—Pedigree of family with complicated hereditary spastic paraparesis. Males are represented by squares and females by circles. Open squares and circles represent asymptomatic individuals while closed squares and circles represent affected individuals. The ages of the individuals are displayed beside the circles or squares.

is illustrated in Figure 1. The disease is characterized by speech difficulties, lower limb spasticity and hyperreflexia, mental retardation, cerebellar ataxia, and tremor. The symptoms begin in the first two decades of life and progress for 3 to 6 years before stabilizing. Three of the four living males affected by the disease (III-2, III-5, III-7), as well as the asymptomatic mother (II-2), sister (III-3), maternal uncle (II-3), and niece (IV-1), were evaluated by MR and PMRS. A second asymptomatic sister (III-4) and the father (II-1) were evaluated by MR alone.

Standard MR examinations included sagittal short TR/TE (600/20/1) and axial long TR sequences (3000/35,90/1) performed on a 1.5-T GE Signa scanner. Gradient moment nulling (a flow-compensation technique), inferior presaturation pulses, and a variable bandwidth technique (4 KHz and 16 KHz to increase signal-to-noise ratio) were employed on the long TR images. In case III-5, gadopentetate dimeglumine (0.1 mmol/kg) was administered and postcontrast short TR/TE sequences with first-order gradient moment nulling techniques were performed.

MR images were read by two neuroradiologists. Clinical histories were not withheld. At the time of the MR interpretation, the PMRS data were not available to the reviewers.

The coordinates for sampling a  $2 \times 2 \times 2$ -cm volume-of-interest in the affected white matter of the centrum semiovale were determined from the MR images. Voxel localization was confirmed by a short TR (300/19) image of the  $2 \times 2 \times 2$ -cm voxel in the axial plane (with suppression of all signal outside the voxel and with no solvent suppression) [6, 10, 11]. The voxel's X, Y, and Z coordinates were verified on this scan and the localization of the sampled volume was further confirmed by adding the image of the voxel to the spin-echo image using an algorithm developed in house on the GE Signa scanner.

Seven family members underwent localized PMRS after giving informed consent. Gradient shimming (X, Y, Z) was accomplished on the  $2 \times 2 \times 2$  cm voxel and linewidths ranged from 3.5 to 6.0 Hz ( $<0.1$  ppm variation). Spectroscopic acquisitions were made by use of a stimulated echo technique (STEAM) preceded by chemical shift selective solvent suppression pulses centered on water followed by spoiler gradients (1 gauss/cm for 4–8 msec) performed in all three axes to further reduce contributions from the water protons [8, 12, 13]. TR was 2000 msec, TE was 19 msec, and TM (middle interval between second and third slice-selective pulses) was 8.6 msec. An

eight-step phase-cycling scheme was used for each  $90^\circ$  orthogonal pulse in the STEAM sequence and 256 scans were averaged. Scan time for the PMRS data collection was 4 min 36 sec. Attempts were made to place the voxel within areas of abnormal white matter without overlapping soft tissue, skull, ventricle, or gray matter. Two regions of interest were sampled in three of the patients in identical areas of the centrum semiovale on opposite sides of the brain without voxel overlap. Only one region of interest was obtainable in four patients owing to patient time constraints. Thus, 10 spectra from the family were available for analysis. Both sets of data in the three patients with two voxels were used in data analysis.

### Data Analysis

Spectral analysis was performed on an AT&T personal computer system using an analysis package developed in house. The spectra were evaluated without fitting routines applied. No baseline correction programs were used, and only first- and second-order phase corrections were used. In order to prevent bias in the analysis of the spectra, 28 spectra were submitted for analysis to three blinded examiners. These spectra included members of the family with the white matter disorder, normal control subjects, and patients with other CNS diseases. In five cases the data from the same spectra were submitted twice in order to determine the reproducibility of the examiners' results (three of these five were members of the HSP family).

The spectra were blindly and independently processed by two investigators, and the spectra were interpreted by three investigators. N-acetyl aspartate (NAA) to creatine/phosphocreatine (CR) ratios, NAA to choline-containing compound (CH) ratios, and CR/CH ratios were computed for each patient by each reviewer and the average values recorded (Table 1). To determine metabolite ratios, the peak heights were measured from baseline rather than from areas under curves, and the nonfitted spectra were used. Additionally, the reviewers were asked to evaluate the spectra for the presence of elevated amino acid peaks between the NAA (2.01 ppm) and CR (3.02 ppm) peaks.

To provide estimates of normal NAA/CR, NAA/CH, and CR/CH ratios, seven normal subjects also had PMRS, and the imaging, localization, and analysis techniques were identical to those used



**TABLE 1: Results of PMRS Studies in Seven Family Members and Seven Controls**

Patient/Findings	NAA/CR	NAA/CH	CR/CH	Elevated Amino Acids
III-2, III-5, III-7/Symptomatic, abnormal WM	1.0	1.3	1.3	Yes
II-2, III-3/Asymptomatic, abnormal WM	1.1	1.0	0.9	Yes
IV-1/Asymptomatic, normal WM	1.1	1.0	0.9	No
II-3/Asymptomatic, normal WM	1.4	1.8	1.3	No
Controls ( <i>n</i> = 7)	1.3	2.1	1.7	No
SD	.16	.17	2.3	

Note.—NAA = *N*-acetyl aspartate, CR = creatine/phosphocreatine, CH = choline-containing compounds, WM = white matter, SD = standard deviation.

with the patients. The normal volunteers had normal MR examinations and were without neurologic symptoms. The voxels analyzed were in the centrum semiovale at locations comparable to those taken in the HSP family. These volunteers were also analyzed for the presence of elevated amino acid peaks and their ratios were computed in an identical blinded fashion by the same three evaluators (Table 1).

## Results

### MR

Abnormal signal intensity on the long TR sequences was observed in the white matter of all three symptomatic patients, their mother, and two sisters. The white matter abnormality in the three affected patients consisted of a prominent diffuse confluent increase in signal intensity symmetrically distributed in all supratentorial white matter structures (Fig. 2). The abnormal white matter did not enhance in the one patient who received gadopentetate dimeglumine. The mother and sisters had white matter hyperintensity on the long TR images although limited to the centrum semiovale. The unaffected niece (IV-1) had normal-appearing myelin on all pulse sequences. The uncle (II-3) had focal areas of high signal intensity in the peripheral white matter, which was thought to represent small vessel ischemic changes in this 56-year-old with long-standing hypertension. His white matter appearance was markedly different from those of the affected patients. The father of the affected males had a normal-appearing MR examination except for minor small vessel ischemic changes. He refused spectroscopy.

### PMRS

The results of the PMRS spectra are given in Table 1. The members of the family with abnormal white matter signal (III-2, III-3, III-5, III-7, II-2) had depressed NAA/CH and CR/CH levels and slightly depressed NAA/CR ratios. Because of the small number of subjects, an assessment of statistically different ratio values between the family members and normal volunteers is not possible. However, a trend is seen in that the family members who presumably bore the abnormal white matter gene had mean values of NAA/CH, NAA/CR, and CR/CH that were beyond a standard deviation from normal volunteer values. This is particularly notable in the NAA/CH and CR/CH ratios (Table 1).

In all three affected symptomatic members of the family, elevated amino acid peaks were detected, whereas the asymptomatic family members were divided into those with

normal and abnormal MR studies. In asymptomatic patients with abnormal MR studies, the amino acid peaks were elevated. Thus, all patients with abnormal white matter by MR had elevated amino acid peaks. In patients with normal MR studies, the amino acids were normal in magnitude, even though the measured metabolite ratios were often low. In the asymptomatic niece (IV-1) the white matter, amino acid peaks, and neurologic examination were normal, but the PMRS spectral ratios were depressed. The non-gene-bearing uncle (II-3) had a normal NAA/CR ratio and minimally depressed NAA/CH and CR/CH ratios, probably due to a prominent choline peak.

The average differences between NAA/CR, NAA/CH, and CR/CH ratios in control subjects versus family members with the gene present were  $(1.3-1.1)/1.3 = 15\%$ ,  $(2.1-1.1)/2.1 = 48\%$ , and  $(1.7-1.0)/1.7 = 41\%$ , respectively.

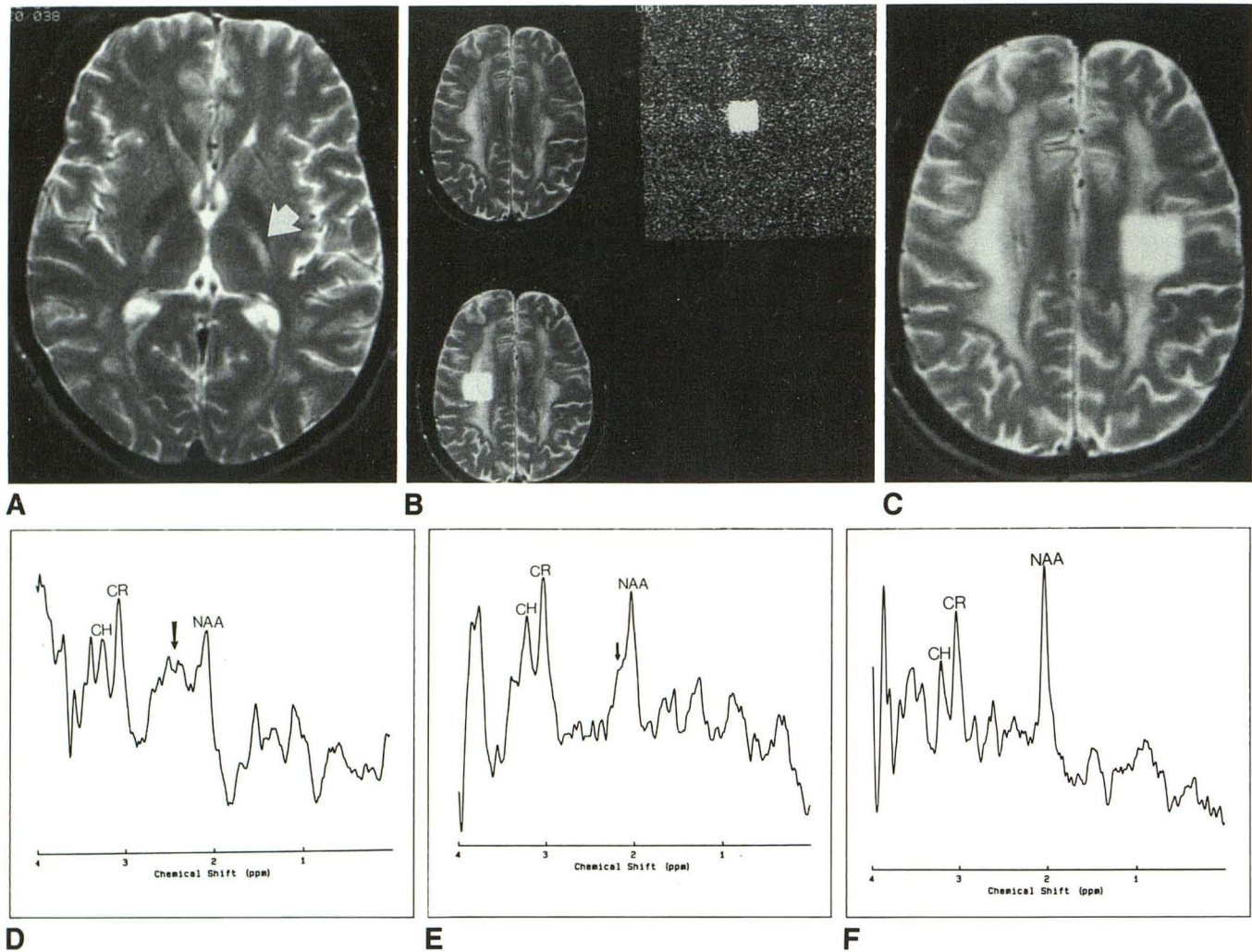
In one patient the MR results demonstrated symmetric white matter high signal on the long TR images, but the PMRS data showed that, while both sides of the brain demonstrated depressed NAA/CH and CR/CH peaks, the right side of the brain had lower values than the left (Fig. 2). The elevations of amino acid peaks were also more striking on the right side. The spectroscopic findings correlated well with increased left-sided spasticity and hyperreflexia noted on physical examination.

In all five patients with diffuse abnormal signal intensity of the white matter, the three evaluators agreed that elevated amino acid peaks in the 2.0–2.5 ppm range were present. All three evaluators agreed that the amino acid peaks were normal in height in six of seven control subjects. In one control subject, one of the three evaluators reported an elevated amino acid peak: the other two evaluators reported no such elevation.

## Discussion

By employing three orthogonal slice-selective pulses and spoiling all signal arising outside the intersection of these pulses, one can obtain a well-localized sampling volume for PMRS spectra [6, 10, 11]. Chemical suppression of the water proton contribution can result in a greater than 500-fold reduction in water-proton signal. Voxels as small as  $1.5 \times 1.5 \times 1.5$  cm have been employed at our institution; the main cost in sampling this small volume is the reduction in signal-to-noise ratio (SNR). This SNR reduction can be compensated for by increasing the number of sampling averages. While this solution is readily applicable to pathologic specimens and very





**Fig. 2.**—MR and PMRS images of male (III-2) with spastic paraparesis.  
**A**, Axial MR image (3000/90) through internal capsule demonstrates high signal in posterior limb of internal capsules bilaterally (arrow).  
**B**, MR image (3000/90) (top left) demonstrates abnormal intensity bilaterally in white matter of centrum semiovale. STEAM scan (300/19) (top right) of the  $2 \times 2 \times 2$  cm voxel selected for spectroscopy may then be superimposed on axial scan (bottom left) to ensure correct voxel localization.  
**C**, STEAM scan (300/19) of voxel in left centrum semiovale is superimposed on axial (3000/90) image. The voxel in **B** correlates with the spectrum in **D**, and the voxel in **C** produced the spectrum in **E**.  
**D–F**, Proton spectrum of right voxel (**D**) demonstrates depression of NAA/CH and CR/CH ratios. The values for the right voxel were 1.0 for NAA/CH and 1.2 for CR/CH, whereas on the left (**E**) the NAA/CH was 1.4 and the CR/CH was 1.4 as well. Note also the elevated amino acid peaks (arrows) most striking on the right (**D**) side. Compare with PMRS spectrum (**F**) of normal volunteer. All the spectra were acquired with 1000 Hz sweep width, 1024 complex points, 2-sec repetition times, and 256 averages (32 times through an eight-step phase-cycling procedure). The spectra were processed with 2-Hz line-broadening, zero-filling to 2048 points, Fourier transform, zero- and first-order phase correction.

cooperative volunteers, the drawback in potential motion as scanning times are extended becomes prohibitive. Because of this, we have selected an  $8\text{-cm}^3$  volume that allows good SNR with 256 sampling averages in under 5 min of scan time. This volume is well-suited for evaluating patients with demyelinating diseases, since the volume of white matter in the centrum semiovale allows adequate sampling without skull, ventricle, or gray matter contamination.

The use of short TE values in our STEAM sequence allows increased SNR and less sensitivity to T2 contributions to the signal. The increase in SNR allows reduction of scan averages (or sample size), thereby reducing scan times and subsequent patient motion. In addition, we believe that the use of short

TEs increases the sensitivity of the STEAM sequence to coupled spins (i.e., from protons of the  $-\text{CH}_2-$  groups of glutamine, gamma amino butyrate, valine, etc.) present in amino acids in the 2–3 ppm range. It may be useful to analyze these amino acids in certain CNS disorders such as amino acidurias, maple syrup urine disease, or mitochondrial myopathies. The drawback in using shorter TEs is increased noise and increased eddy current distortion. Baseline correction algorithms, curve fitting techniques, and smoothing programs all tend to obscure the critical but small amino acid peaks. For this reason, we tend to analyze the spectra in a more "raw" form. The spectra are not as attractive, and the baseline fluctuates, but essential data is not "smoothed" out.



Because the processing of PMRS spectra is an operator-dependent function in most institutions, we believe that the unbiased analysis of scan data, as in this study (with blinded, independent, and multiple evaluators) is essential to determine the validity of PMRS studies. This is especially true of clinical studies with a small sample size, such as have appeared in the spectroscopy literature to date.

The results of the PMRS study of this family raise several issues. The presence of elevated amino acid peaks in the 2.1–2.5 ppm range corresponds to resonances usually ascribed to glutamine (2.14, 2.45), keto-glutarates (2.44, 2.23), succinate (2.39), valine (2.27), GABA (2.25), and methionine (2.16, 2.14) [5]. Elevation of these peaks has also been seen in patients with active plaques of multiple sclerosis [12]. In previous work done in this institution we have found that plaques of multiple sclerosis that enhance on MR often show elevation of proton spectra peaks in the 2.1–2.3 ppm range (Grossman et al. Paper presented at RSNA, November 1989). These plaques are believed to represent areas of active blood brain barrier breakdown and active myelinolysis. The PMRS findings suggest that one may be sampling by-products of active myelin breakdown (Grossman et al. RSNA 1989) [12]. Such peaks were present in each family member who had abnormal white matter detected on the imaging studies (even in the women "carriers" who were asymptomatic). These peaks were not present in patients or control subjects who had normal-appearing white matter.

Analysis of the NAA/CR, NAA/CH, and CR/CH ratios is made difficult by residual water contamination, which may cause fluctuations in the baseline due to its overwhelming dominance of the MR signal. Although the small sample size inherent in analysis of a seven-member family group does not permit a rigorous statistical analysis, we believe there are differences between the family and the normal volunteers. We noted a trend that the NAA/CH ratio in patients with abnormal white matter was almost half that of the controls, and that the CR/CH ratio also appeared to be slightly depressed. Less definitive depression of the NAA/CR ratio was also present. Only through PMRS evaluation of a larger population of patients with dysmyelinating disorders and normal control subjects can we conclusively state whether these findings are typical of white matter disorders.

That the NAA values may be depressed is consistent with the belief that NAA arises in neurons, is essential for normal brain functioning, and depression of NAA correlates with brain tissue loss [12, 13]. Similar reductions in the absolute concentrations of choline and creatine would be expected in the diffuse neuroaxonal loss associated with white matter diseases.

The asymmetric exaggeration of PMRS abnormality in the patient who had symmetric white matter high signal abnormality on MR images suggests that PMRS may add information to conventional MR for the assessment of myelin abnormalities. This may be translated into more precise and meaningful clinical correlation. This patient's PMRS abnor-

mality more closely reflected his clinical symptomatology than did his MR study.

Another dilemma was raised with regard to the asymptomatic niece (IV-1) who had a normal MR study, normal amino acid peaks, but depression of NAA/CH and CR/CH ratios. The possibility that this woman carries the affected X gene but has not manifested signal intensity abnormality was suggested to the clinician dealing with this kindred. If this contribution of PMRS is verified in future cases and by chromosomal analysis, it will herald a more active role for PMRS in genetic counseling.

Patients with acquired or inherited disorders of myelin provide the neuroradiologist with an excellent opportunity to examine the full capability of combined MR and PMRS techniques. The large area of abnormality seen in the hereditary dysmyelinating disorders (Pelizaeus-Merzbacher, metachromatic leukodystrophy, adrenoleukodystrophy, Canavan disease, Alexander disease, etc.) readily lends itself to PMRS. An integrated MR/PMRS study may be helpful in the evaluation and subsequent genetic counseling of families affected by these disorders.

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