

CHAPTER 3.1

CHILDHOOD WHITE MATTER DISORDERS: QUANTITATIVE MR IMAGING AND SPECTROSCOPY

J. Patrick van der Voorn
Petra J. W. Pouwels
Augustinus A. M. Hart
Judith Serrarens
Michèl A.A.P. Willemsen
Hubertus P.H. Kremer
Frederik Barkhof
Marjo S. van der Knaap

RADIOLOGY. 2006 NOV;241(2):510-7

ABSTRACT

Purpose:

To prospectively investigate whether quantitative MR parameters, including magnetization transfer ratio (MTR), apparent diffusion coefficient (ADC), fractional anisotropy (FA) and MR spectroscopy (MRS) metabolite concentrations, allow discrimination of different types of pathology underlying white matter signal abnormalities.

Materials and methods:

Institutional review board approval and informed consent were obtained. We included 41 patients (19 male, 22 female; mean age, 15.4 years) and 41 controls (25 male, 16 female; mean age, 11.3 years). Of the patients, 12 had a hypomyelinating disorder, 14 a demyelinating disorder, 5 a disorder characterized by myelin vacuolation, and 10 a disorder characterized by cystic degeneration. Regions-of-interest, selected within the parietal white matter for MRS, were transferred to the corresponding slices of the generated ADC, FA and MTR maps to extract quantitative measurements. Linear discriminant analysis (LDA) and univariate analysis of covariance were used for statistical analysis.

Results:

LDA showed that 95% of all patients were classified correctly using total creatine (tCr), choline-containing compounds (Cho), myo-inositol (Ins), MTR and ADC. In the hypomyelination group all MR parameters were close to normal with the exception of elevated tCr ($p=0.03$) and Ins ($p<0.001$) and decreased MTR ($p<0.001$). In the demyelination group Cho ($p=0.02$) and Ins ($p<0.001$) were highly elevated. The myelin vacuolation and cystic degeneration groups showed high ADC ($p<0.001$) and variable decreases of all MRS-metabolites. MTR was markedly reduced ($p<0.001$) in the cystic degeneration group.

Conclusion:

Quantitative MR techniques can discriminate between different types of white matter pathology, and may classify white matter lesions of unknown origin with respect to underlying pathology.

INTRODUCTION

MR imaging is highly sensitive in the detection of white matter lesions (1). In contrast, it has a limited specificity with regard to the pathology underlying the white matter signal abnormalities. Highly variable pathologic changes may underlie white matter disorders on MRI, including hypomyelination, demyelination, gliosis, interstitial edema, myelin vacuolation with intramyelinic edema, cystic white matter degeneration and diffuse infiltration by tumor cells, such as in gliomatosis cerebri. In all types of pathology, the T1 and T2 relaxation times become longer, leading to non-specifically increased signal intensity on T2- and decreased signal on T1-weighted images (2).

Quantitative MR techniques, such as diffusion tensor imaging (DTI), magnetization transfer imaging (MTI) and proton MR spectroscopy (MRS), may provide more insight into the underlying white matter pathologic changes (2-5). The purpose of our study was to prospectively investigate whether quantitative MR parameters, including magnetization transfer ratio (MTR), apparent diffusion coefficient (ADC), fractional anisotropy (FA) and MR spectroscopy (MRS) metabolite concentrations, allow discrimination of different types of white matter pathology underlying white matter signal abnormalities.

MATERIALS AND METHODS

Patients and control subjects (Table 1)

This study was performed with informed consent of the patients and control subjects or the parents of either and approval of the institutional ethics review board.

Table 1: Characteristics of Patients and Controls

	HM	DM	MV	CD	Controls
No. subjects (M/F)	12 (7/5)	14 (7/7)	5 (1/4)	10 (4/6)	41 (25/16)
Mean age \pm SD	11.5 \pm 6.3	15.6 \pm 12.2	17.4 \pm 8.0	18.6 \pm 12.7	11.3 \pm 9.5
No. subjects MTR	10	14	5	9	30
No. subjects ADC	11	14	5	10	37
No. subjects FA	8	11	5	8	29
No. subjects MRS	12	14	5	10	17

Note.—Patient groups are indicated as HM (hypomyelination), DM (demyelination), MV (myelin vacuolation) and CD (cystic degeneration). SD, standard deviation. MTR, magnetization transfer ratio. ADC, apparent diffusion coefficient. FA, fractional anisotropy. MRS, magnetic resonance spectroscopy.

In a prospective study, 41 consecutive patients (19 male, 22 female; mean age: 15.4 years; age range: 8 months to 34 years) with a white matter disorder of known etiology underwent quantitative MRI and MRS at the VU University medical center between January 2000 and January 2005. Twelve patients had a hypomyelinating disorder. The initial diagnosis was based on MRI criteria (6): two MR examinations obtained at least 12 months apart showing an unchanged pattern of seriously deficient myelination in patients older than 18 months. In two of these patients Pelizaeus-Merzbacher disease (PMD) was diagnosed on the basis of demonstration of a mutation in the PLP gene. In two other patients who died during the study period autopsy confirmed the serious hypomyelination. Fourteen patients had a demyelinating disorder: metachromatic leukodystrophy (MLD, 10 patients) or globoid cell leukodystrophy (GLD, also called Krabbe disease, 4 patients). In all patients with MLD and GLD the diagnosis was proven by demonstration of deficient activity of the respective lysosomal enzymes. Five patients had a disorder characterized by myelin vacuolation: megalencephalic leukoencephalopathy with subcortical cysts (MLC). Ten patients had a white matter disorder characterized by rarefaction and cystic degeneration: vanishing white matter disease (VWM). In the patients with MLC and VWM, the diagnosis was genetically confirmed.

Forty-one control subjects (25 male, 16 female; mean age: 11.3 years; age range: 7 months to 36 years) were included in the study on the basis of their normal MRI. Seventeen subjects were healthy, adolescent or adult, volunteers (8 male, 9 female) with a similar age-range as the patients. Their health status was determined by evaluation of their medical history. Twenty-four pediatric subjects (17 male, 7 female) consecutively underwent MRI during the study period and no abnormalities were found. They all had a normal neurological examination. Most of them underwent MRI because of seizures.

MR imaging and Proton MR Spectroscopy

All examinations were performed on a 1.5 T MR scanner (Siemens Vision, Erlangen, Germany). The imaging protocol included sagittal T1-weighted images using a three-dimensional (3D) magnetization-prepared-rapid-acquisition-gradient-echo (MPRAGE) sequence (repetition time [TR] 15 ms, echo time [TE] 4 ms, 1 excitation), transverse T2-weighted spin echo images (TR 3000 ms, TE 22, 60 and 120 ms, 1 excitation), coronal or transverse FLAIR images (TR 9000 ms, TE 105 ms, inversion time [TI] 2200 ms, 1 excitation) and transverse diffusion weighted images using an echo-planar-imaging (EPI) sequence with b-values of 0, 500 and 1000 s/mm² (20 slices of 5 mm were acquired, with a 96x128 matrix, using TR 5100 ms, TE 137 ms). Automatically generated ADC maps were obtained.

DTI was performed with a multi-slice EPI-sequence with optimized gradients according to the method described by Jones et al. (7) using a reference b=0 s/mm² and 8 non-collinear gradient vectors with b=1044 s/mm². In transverse orientation, 16 slices of 5 mm were acquired, with a 128x128 matrix, using TR=3600 ms and TE=123 ms. The DTI

analysis included a correction of eddy-current induced distortion, and calculation of eigen-values of the diffusion tensor and FA map.

MTI was performed with a 3D FLASH sequence. Two sets of images were obtained with (M_s) and without (M_0) MT saturation pulse (7.68 ms Gaussian RF pulse, 1500 Hz off-resonance), using TR=23 ms, TE=4 ms, flip angle 20°, and a 3D-slab consisting of 54 transverse slices of 3 mm. MTR maps were created according to $MTR=1-M_s/M_0$.

MRS was performed by one of two authors (J.P.v.d.V and P.J.W.P., with 5 and 10 years of experience in MRS of the brain, respectively) using a short-echo time stimulated echo acquisition mode (STEAM) sequence (TR/TE/mixing-time=6000/20/10 ms, 64 accumulations). Single-acquisition reference measurements without water suppression were acquired additionally to enable eddy-current correction. The spectra were acquired from a single volume of interest (VOI) of parietal white matter (4-6 ml). The location of the VOI was selected by the spectroscopist as to avoid or minimize contamination by cerebrospinal fluid and gray matter. Furthermore, 10 single-acquisition STEAM measurements without water suppression, with TE values ranging from 20 to 1500 ms, and an inter-measurement delay of 10 s were obtained. These measurements can be used to determine the fractional free water of each MRS voxel through a two-component fit to the data (8). From these measurements only the shorter T2-component was included as a separate parameter for statistical analysis. Metabolite concentrations were calculated using LCModel (9) and expressed as mmol/L VOI. VOI concentrations were determined for a large number of metabolites (10), but in this study attention was focused on the metabolites total Cr (tCr; creatine and phosphocreatine), total NAA (tNAA; N-acetyl-aspartate and N-acetyl-aspartyl-glutamate), choline-containing compounds (Cho), myo-inositol (Ins), lactate (Lac), glutamate (Glu) and glutamine (Gln).

Regions-of-Interest (ROIs) corresponding to the MRS VOIs were transferred to the equivalent ADC, FA or MTR maps by one of two authors (J.S. and J.P.v.d.V.) and mean ADC, FA and MTR in these ROIs were determined.

Statistical Analysis

Mean values \pm standard deviations (SD) were determined for all groups. First, all 11 parameters (7 metabolite-concentrations, ADC, FA, MTR and T2) were compared between white matter pathology groups and controls using univariate analysis of covariance (ANCOVA) with a Bonferroni correction applied for multiple comparisons (the Bonferroni correction was applied by multiplying the raw P-values by a factor of 11: the number of statistical tests performed), with subject age and sex included as covariates in the statistical model if these had a significant effect (SPSS for Windows, version 9.0; SPSS, Chicago, IL). Based on a residual analysis from ANCOVA it was decided whether or not to use a logarithmic transformation, and if so, whether or not to add a small constant to all values before transformation. P-values of <0.05 are considered statistically significant.

Subsequently, based on either the original or log-transformed variables, and if necessary age and/or sex standardized, a Fisher's polytomous Linear Discriminant Analysis

(LDA) was applied (using S-PLUS® 6.2 for Windows professional edition [Copyright 1988, 2003 Insightful Corp.]) (11) to optimally separate the four white matter pathology groups. In nine patients FA was not measured; in three of these patients MTR was not determined and in one ADC was also not determined. For the LDA containing those parameters, these patients could not be included.

LDA resulted in posterior probabilities for a patient coming from a particular white matter pathology group, based on the MR parameters of that patient. These probabilities were calculated from discriminant functions of the (possibly log-transformed and standardized) parameters, one function per white matter pathology group. A patient was classified by LDA as having the diagnosis with the highest a-posteriori probability, or equivalently, with the highest discriminant score. The performance of the method was judged by misclassification rates calculated from leave-one-out cross-validation (LOOCV) (12).

A backward stepwise LDA (using SAS® 8.2 for Windows (Copyright 1999 SAS Institute Inc., Cary, NC, USA.) based on the Partial R-squared criterion (13) was used to examine which MR parameters contributed most to the correct classification. After each elimination, performance was again estimated by LOOCV. However, the ordering of the parameters for exclusion was not incorporated in the LOOCV and therefore the performance of the reduced models may have been overestimated. This part of the analysis has to be considered as exploratory.

RESULTS

MTR, ADC, FA

MTR, ADC and FA maps of patients from each group and of a control are shown in Figure 1. MTR was lower in the cystic degeneration group than in the hypomyelination, demyelination and myelin vacuolation groups ($p < 0.001$) (Figure 2A). ADC was higher in the cystic degeneration group than in the hypomyelination and demyelination groups ($p < 0.001$) and higher in the myelin vacuolation group than in the hypomyelination group ($p < 0.001$) (Figure 2B). FA was higher in the hypomyelination group than in the demyelination, myelin vacuolation and cystic degeneration groups ($p < 0.001$) (Figure 2C).

Metabolites, T2

Spectra of patients from each group and of a control are shown in Figure 1. The concentration of tCr was higher in the hypomyelination and in the demyelination group than in the myelin vacuolation and cystic degeneration groups (all $p < 0.001$) (Figure 3A). The concentration of tNAA was higher in the hypomyelination group than in the demyelination, myelin vacuolation and cystic degeneration groups ($p < 0.001$) (Figure 3B). The concentration of Cho was higher in the demyelination group than in the

hypomyelination, myelin vacuolation and cystic degeneration groups ($p < 0.001$) and lower in the cystic degeneration group than in the hypomyelination group ($p = 0.009$) (Figure 3C).

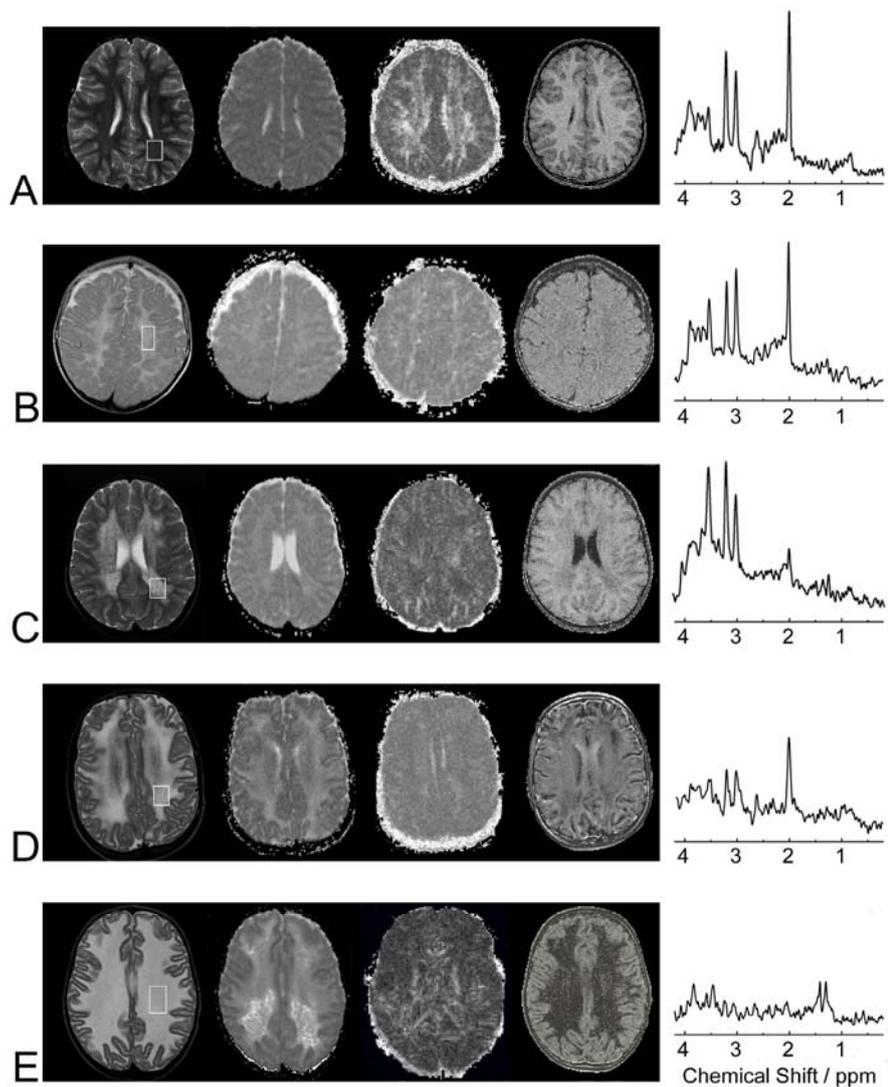


Figure 1. VOI localizations for MRS (STEAM TR/TE/TM = 6000/20/10 ms, 64 accumulations) on the transverse T2-weighted images (3000/120, 1 excitation), and equivalent transverse ADC, FA and MTR maps from left to right with corresponding spectra in a control subject and patients from each group, shown with the same vertical scaling: (a) a 5-year-old male control; (b) a 2-year-old male patient with hypomyelination; (c) a 6-year-old male patient with demyelination; (d) a 11-year-old female patient with myelin vacuolation and (e) a 3-year-old female patient with cystic degeneration. In the patient with hypomyelination group close to normal MR parameters are seen with the exception of increased tCr and Ins and decreased MTR. In the patient with demyelination a decrease in tNAA and increases in Cho and Ins are evident. The patients with myelin vacuolation and cystic degeneration show high ADC and variable decreases of all MRS-metabolites. MTR is markedly reduced in the patient with cystic degeneration.

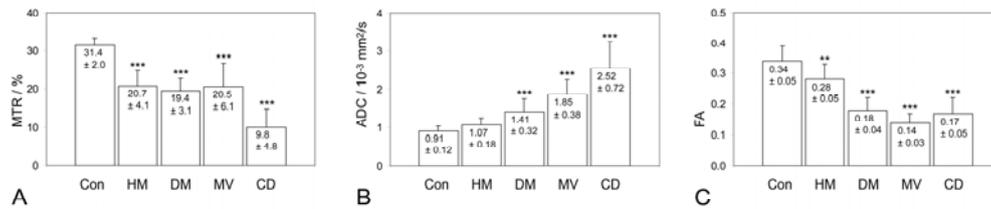


Figure 2. Graphs show mean and standard deviations for all four white matter pathology groups, which include hypomyelination (HM), demyelination (DM), myelin vacuolation (MV) and cystic degeneration (CD), and controls (con) for (a) MTR; (b) ADC and (c) FA. Error bars represent standard deviations. Significance levels are indicated for the differences with the control group: **P<0.01; ***P<0.001.

The concentration of Ins was higher in the demyelination group than in the myelin vacuolation and cystic degeneration groups ($p<0.001$), higher in the hypomyelination group than in the myelin vacuolation ($p=0.004$) and cystic degeneration groups ($p<0.001$), and higher in the myelin vacuolation group than in the cystic degeneration group ($p=0.003$) (Figure 3D). The concentration of Lac was higher in the demyelination group than in the hypomyelination group ($p=0.027$) (Figure 3E). No significant differences in Glu (Figure 3F) and Gln concentrations (Figure 3G) were found. T2 was higher in the cystic degeneration group than in the hypomyelination, demyelination ($p<0.001$) and myelin vacuolation groups ($p=0.004$) and T2 was higher in the myelin vacuolation group than in the hypomyelination ($p<0.001$) and demyelination groups ($p=0.001$) (Figure 3H).

LDA and LOOCV

The LDA revealed four discriminant functions (one per group) containing the observed MR parameters. These functions are given in the Appendix. Filling in the seven MR parameters MTR, ADC, tCr, tNAA, Cho, Ins and Lac for the analyzed patients in the discriminant functions resulted in correct classification of all patients, except for one patient with hypomyelination being classified as demyelination and one patient with demyelination being classified as hypomyelination. LOOCV resulted in the same two misclassifications. When MTR and ADC were excluded from the LDA, keeping the five metabolites of MRS only, LOOCV misclassification occurred in eight patients. The combination of tCr, Cho, Ins, MTR and ADC resulted in the same two misclassifications as using all seven MR parameters, while further elimination of ADC and Ins in that sequence added one misclassification each. Elimination of one more parameter (MTR, Cho or tCr) led to ten or more misclassifications. Backward stepwise LDA ordered the MR parameters in decreasing importance for classification of the white matter pathology groups as follows: tCr, Cho, MTR, Ins, ADC, Lac and tNAA.

Adding Glu, Gln and T2 to the LDA resulted in four LOOCV misclassifications. In the backward stepwise LDA they were eliminated as first (T2), fourth (Gln) and fifth (Glu) parameter. Adding FA to the LDA increased the number of LOOCV misclassifications from

two to four, while adding FA to tCr, Cho, Ins, MTR and ADC increased the number of LOOCV misclassifications from two to three.

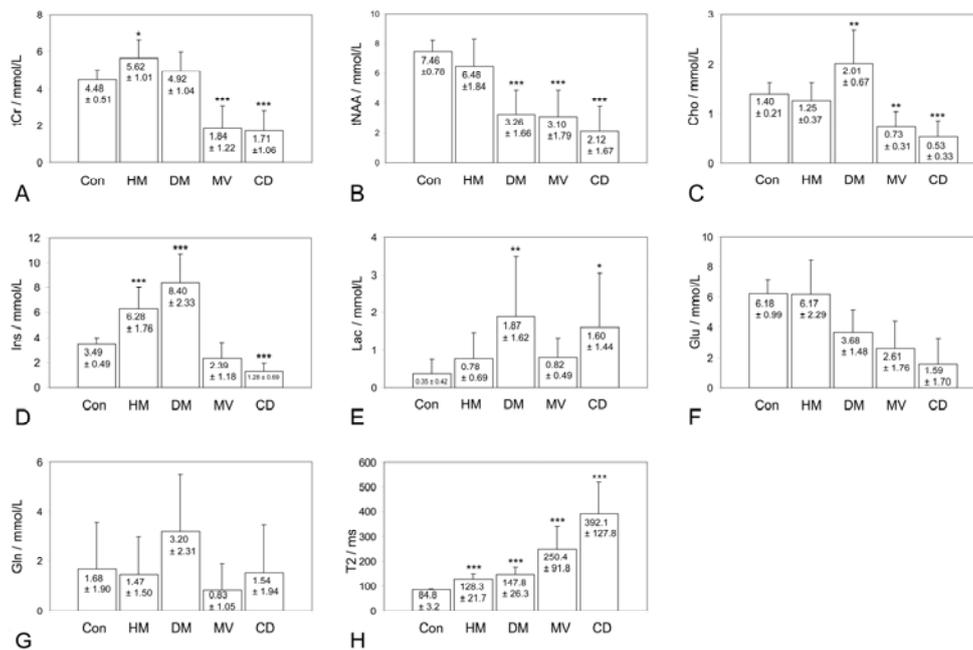


Figure 3. Graphs show mean and standard deviations for all four white matter pathology groups, which include hypomyelination (HM), demyelination (DM), myelin vacuolation (MV) and cystic degeneration (CD), and controls (con) for (a) tCr; (b) tNAA; (c) Cho; (d) Ins; (e) Lac; (f) Glu; (g) Gln and (h) T2. Error bars represent standard deviations. Significance levels are indicated for the differences with the control group: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

DISCUSSION

In our study clear differences in these MR parameters were found between patients and controls, and between patient groups. For the hypomyelination group all MR parameters were close to normal with the exception of elevated tCr and Ins levels and decreased MTR values. The most striking findings for the demyelination group were the highly elevated Cho and Ins levels. The findings for the myelin vacuolation and cystic degeneration groups tended to be similar with high ADC and variable decreases of most MRS metabolites, but were more pronounced in the cystic degeneration group. Especially MTR was markedly more severely reduced in the cystic degeneration group than in the myelin vacuolation group.

We wanted to determine whether, using these parameters, white matter abnormalities with a different underlying pathology could be discriminated. LDA showed that the combination of tCr, Cho, Ins, MTR and ADC resulted in only two

misclassifications (95% of all patients were classified correctly), and further elimination of ADC and Ins added one misclassification each.

The two consistently misclassified patients were a patient with demyelination classified as hypomyelination and a patient with hypomyelination classified as a demyelination. The first patient was the only MLD patient with stable disease. She had received bone marrow transplantation several years before participation in the study and had a stable disease since then, both clinically and on MRI. So, in fact she had no active demyelination, as was suggested by the LDA model. The parameters in the hypomyelination patient suggested progressive loss of myelin. In addition to the lack of myelination, demyelination and axonal degeneration can occur in hypomyelination patients (14-15). This was the only patient with hypomyelination in whom MRI showed evidence of further loss of the little myelin present, as was correctly picked up by the MR parameters.

DTI and MTR provide information about tissue microstructure. Within the abnormal white matter, ADC, a measure for isotropic water diffusivity, was increased in patients, and FA, a measure for the degree of diffusion anisotropy, was decreased, indicating enhanced mobility of water molecules in all directions as a result of damage to the tissue matrix. Water mobility was highest in the white matter of patients with myelin vacuolation and cystic degeneration reflecting the rarefied white matter in patients with cystic degeneration and spongiform white matter changes with numerous vacuoles in patients with myelin vacuolation, both resulting in reduced cellular density and increased water-filled spaces. In patients with hypomyelination diffusion anisotropy was only slightly decreased, which suggests that the diffusion anisotropy does not necessarily depend on myelinated fibers. Hypomyelinated white matter contains little or no myelin, but has normal axonal density, which is apparently sufficient to maintain close to normal diffusion parameters. The DTI results are in concordance with findings by others in several case reports (16-21).

The MTR in the white matter of patients with demyelination, hypomyelination and myelin vacuolation showed comparable decreases indicating a reduced capacity of the macromolecule-bound protons in brain tissue to exchange magnetization with the surrounding protons in free water, probably reflecting non-specific damage to myelin and axonal membranes. The decrease in MTR was most pronounced in the white matter of patients with cystic degeneration. At autopsy these patients reveal diffusely rarefied to cavitated white matter with profound losses of oligodendrocytes, myelin sheaths and axons, accompanied by a feeble astrogliosis and macrophage response (22). The extremely low MTR reflects the loss of all tissue structures (23).

Patients with myelin vacuolation and demyelination had similar MTR values, but the ADC observed in patients with demyelination was lower than that observed in patients with myelin vacuolation, which could be explained by dense accumulation of lipid-containing macrophages and glial cells in MLD and GLD white matter, which serves to hinder water diffusion, prior to the extensive demyelination. Low ADC within the unaffected, subcortical white matter has been observed in MLD patients (19).

Quantitative localized proton MR spectroscopy of white matter in patients with myelin vacuolation revealed marked decreases of tNAA, tCr and Cho with close to normal values for Ins consistent with axonal damage or loss and astrocytic proliferation. MRS of the white matter in patients with cystic degeneration showed a decrease of all normal signals and the presence of Lac, compatible with the presence of mainly cerebrospinal fluid and little brain tissue. In patients with hypomyelination tNAA was normal, whereas Ins and tCr were increased, reflecting normal axonal density and increased astrogliosis. In patients with demyelination, MRS showed decreased NAA, accompanied by elevated Cho, Ins, and Lac, indicating axonal damage or loss, enhanced membrane turnover or accumulation of myelin breakdown products, astrogliosis, and infiltrating macrophages, respectively. Our MRS observations are in agreement with previous studies that were performed on the disorders separately (14-17, 24-28). Interestingly, tCr was the most important MR parameter for classification of the white matter pathology groups, which demonstrates that this metabolite should not be used as an internal reference when white matter pathology is studied.

Our study confirms the limited value of T2 measurements, apart from the qualitative use in T2-weighted MRI to detect a white matter disorder as such. First, the difference in T2 between hypomyelination and demyelination patients was not significant. Secondly, T2 did not contribute to the discrimination of the four white matter pathology groups. Unfortunately, we did not measure T1, because we had to compromise when deciding on the MR protocol in order to limit the time for each patient in the magnet.

Although our study is one of the largest reported series of patients with a white matter disorder, a limitation of our study still is that the number of patients is rather small, which is related to the rarity of the diseases. This factor prevents us from taking a random sample from the population of these patients and perform a validation study in addition to the LOOCV provided in this study. Another limitation of our study is that some MR parameters, like MTR, depend on the details of the scanner used, and the implementation of the sequence. Thus the exact LDA functions used in our study are valid only for the patients examined on our scanner and not for other scanners.

In conclusion, the results of our study demonstrate that, in contrast to conventional MR techniques in which signal changes are not specific, quantitative MR techniques are useful in the discrimination of different white matter pathologies. As such, they may help to classify unknown white matter lesions into demyelinating and hypomyelinating disorders and disorders characterized by myelin vacuolation and characterized by rarefaction and cystic degeneration.

ACKNOWLEDGEMENTS

We thank the following physicians for referral of patients for this study: Dr. H. Stroink, Dr C.E. Catsman-Berrevoets, Dr W.E.J. Weber, Dr. J.S.H. Vles, Dr. E.A.J. Peeters, Dr. W.C.G. Overweg-Plandsoen, Dr. I.N. Snoeck-Streef and Dr. K.P.J. Braun. We are grateful to the parents and the patients for their participation in the study.

APPENDIX

The discriminant functions for the four white matter pathology groups (HM, hypomyelination; DM, demyelination; MV, myelin vacuolation; CD, cystic degeneration) containing all MR parameters except FA, Glu, Gln and T2 are:

$$\begin{aligned}
 \text{HM: } & -6.53 * \text{MTR} + 36.2 * \ln(\text{ADC}) - 55.0 * \text{tCr}_{\text{ss}} + 695 * \ln(\text{tNAA} + 10)_{\text{ss}} + 78.5 * \text{Cho}_{\text{as}} + 46.9 * \\
 & \ln(\text{Ins} + 1)_{\text{ss}} + 18.4 * \ln(\text{Lac} + 1)_{\text{as}} - 845 \\
 \text{DM: } & -6.20 * \text{MTR} + 49.7 * \ln(\text{ADC}) - 56.1 * \text{tCr}_{\text{ss}} + 688 * \ln(\text{tNAA} + 10)_{\text{ss}} + 85.4 * \text{Cho}_{\text{as}} + 50.3 * \\
 & \ln(\text{Ins} + 1)_{\text{ss}} + 16.9 * \ln(\text{Lac} + 1)_{\text{as}} - 847 \\
 \text{MV: } & -4.73 * \text{MTR} + 66.3 * \ln(\text{ADC}) - 60.5 * \text{tCr}_{\text{ss}} + 695 * \ln(\text{tNAA} + 10)_{\text{ss}} + 83.0 * \text{Cho}_{\text{as}} + 42.4 * \\
 & \ln(\text{Ins} + 1)_{\text{ss}} + 10.8 * \ln(\text{Lac} + 1)_{\text{as}} - 868 \\
 \text{CD: } & -5.97 * \text{MTR} + 58.3 * \ln(\text{ADC}) - 58.3 * \text{tCr}_{\text{ss}} + 703 * \ln(\text{tNAA} + 10)_{\text{ss}} + 84.7 * \text{Cho}_{\text{as}} + 36.6 * \\
 & \ln(\text{Ins} + 1)_{\text{ss}} + 13.6 * \ln(\text{Lac} + 1)_{\text{as}} - 864
 \end{aligned}$$

where :

$$\begin{aligned}
 \text{tCr}_{\text{ss}} &= \text{tCr for males, tCr} - 0.646 \text{ for females} \\
 \ln(\text{tNAA} + 10)_{\text{ss}} &= \ln(\text{tNAA} + 10) \text{ for males, } \ln(\text{tNAA} + 10) - 0.054 \text{ for females} \\
 \text{Cho}_{\text{as}} &= \text{Cho} + 0.0219 * (\text{age}_{\text{years}} - 12.78) \\
 \ln(\text{Ins} + 1)_{\text{ss}} &= \ln(\text{Ins} + 1) \text{ for males, } \ln(\text{Ins} + 1) - 0.172 \text{ for females} \\
 \ln(\text{Lac} + 1)_{\text{as}} &= \ln(\text{Lac} + 1) + 0.0154 * (\text{age}_{\text{years}} - 12.78)
 \end{aligned}$$

By filling in the observed MR parameters for a patient in the above formulae, the discriminant function with the highest value gives the white matter pathology group as predicted from the MRI data.

REFERENCES

1. Miller DH, Robb SA, Ormerod IEC, Pohl KRE, MacManus DG, Kendall BE, Moseley IF, McDonald WI. Magnetic resonance imaging of inflammatory and demyelinating white-matter diseases of childhood. *Dev Med Child Neurol* 1990; 32: 97-107
2. Van der Knaap MS. Magnetic resonance in childhood white-matter disorders. *Dev Med Child Neurol* 2001; 43: 705-712
3. Barker PB, Horska A. Neuroimaging in leukodystrophies. *J Child Neurol* 2004; 19:559-70
4. Horsfield MA, Jones DK. Applications of diffusion-weighted and diffusion tensor MRI to white matter diseases - a review. *NMR Biomed* 2002; 15:570-7.
5. Symms M, Jager HR, Schmierer K, Yousry TA. A review of structural magnetic resonance neuroimaging. *J Neurol Neurosurg Psychiatry* 2004; 75(9):1235-44.
6. van der Knaap MS, Naidu S, Pouwels PJW et al. New syndrome characterized by hypomyelination with atrophy of the basal ganglia and cerebellum. *AJNR* 2002; 23:1466-74
7. Jones DK, Horsfield MA, Simmons A. Optimal strategies for measuring diffusion in anisotropic systems by magnetic resonance imaging. *Magn Reson Med* 1999; 42: 515-525
8. Ernst T, Kreis R, Ross BD. Absolute quantification of water and metabolites in the human brain. I. Compartments and water. *J Magn Reson* 1993; 102:1-8
9. Provencher SW. Estimation of metabolite concentrations from localized in vivo proton MR spectra. *Magn Reson Med* 1993; 30: 672-679
10. Pouwels PJW, Brockmann K, Kruse B, Wilken B, Wick M, Hanefeld F, Frahm J. Regional age dependence of human brain metabolites from infancy to adulthood as detected by quantitative localized proton MRS. *Pediatr Res* 1999;46:474-85.
11. Venables WN, Ripley BD. *Modern Applied Statistics with S*. Fourth edition. Springer, 2002.
12. McLachlan GJ. *Discriminant Analysis and Statistical Pattern Recognition*. New York: John Wiley & Sons, Inc, 1992.
13. Jennrich R.I. Stepwise Discriminant Analysis. In: *Statistical Methods for Digital Computers*, eds. Enslein K, Ralston A, Wilf H, New York: John Wiley & Sons, Inc, 1977.
14. Plecko B, Stockler-Ipsiroglu S, Gruber S, et al. Degree of hypomyelination and magnetic resonance spectroscopy findings in patients with Pelizaeus Merzbacher phenotype. *Neuropediatrics* 2003; 34:127-36.
15. Pizzini F, Fatemi AS, Barker PB. Proton MR spectroscopic imaging in Pelizaeus-Merzbacher disease. *AJNR* 2003; 24:1683-9.
16. Sijens PE, Boon M, Meiners LC, Brouwer OF, Oudkerk M. 1H chemical shift imaging, MRI, and diffusion-weighted imaging in vanishing white matter disease. *Eur Radiol*. 2005 (epub)
17. Brockmann K, Finsterbusch J, Terwey B, Frahm J, Hanefeld F. Megalencephalic leukoencephalopathy with subcortical cysts in an adult: quantitative proton MR spectroscopy and diffusion tensor MRI. *Neuroradiology* 2003; 45:137-42.

18. Engelbrecht V, Scherer A, Rassek M, Witsack HJ, Modder U. Diffusion-weighted MR imaging in the brain in children: findings in the normal brain and in the brain with white matter diseases. *Radiology* 2002; 222:410-8.
19. Oguz KK, Anlar B, Senbil N, Cila A. Diffusion-weighted imaging findings in juvenile metachromatic leukodystrophy. *Neuropediatrics* 2004;35:279-82.
20. Guo AC, Petrella JR, Kurtzberg J, Provenzale JM. Evaluation of white matter anisotropy in Krabbe disease with diffusion tensor MR imaging: initial experience. *Radiology* 2001 Mar;218(3):809-15.
21. Ono J, Harada K, Sakurai K et al. MR diffusion imaging in Pelizaeus-Merzbacher disease. *Brain Dev* 1994 ;16:219-23.
22. van der Knaap MS, Barth PG, Gabreels FJ et al. A new leukoencephalopathy with vanishing white matter. *Neurology* 1997; 48:845-55.
23. van Waesberghe JH, Kamphorst W, de Groot CJ et al. Axonal loss in multiple sclerosis lesions: magnetic resonance imaging insights into substrates of disability. *Ann Neurol* 1999; 46:747-54
24. Brockmann K, Dechent P, Wilken B, Rusch O, Frahm J, Hanefeld F. Proton MRS profile of cerebral metabolic abnormalities in Krabbe disease. *Neurology* 2003; 60:819-25.
25. Kruse B, Hanefeld F, Christen HJ, Bruhn H, Michaelis T, Hanicke W, Frahm J. Alterations of brain metabolites in metachromatic leukodystrophy as detected by localized proton magnetic resonance spectroscopy in vivo. *J Neurol* 1993; 241:68-74
26. De Stefano N, Balestri P, Dotti MT, Grosso S, Mortilla M, Morgese G, Federico A. Severe metabolic abnormalities in the white matter of patients with vacuolating megalencephalic leukoencephalopathy with subcortical cysts. A proton MR spectroscopic imaging study. *J Neurol* 2001;248:403-9.
27. Zarifi MK, Tzika AA, Astrakas LG, Poussaint TY, Anthony DC, Darras BT. Magnetic resonance spectroscopy and magnetic resonance imaging findings in Krabbe's disease. *J Child Neurol* 2001;16:522-6.
28. Spalice A, Popolizio T, Parisi P, Scarabino T, Iannetti P. Proton MR spectroscopy in congenital Pelizaeus-Merzbacher disease. *Pediatr Radiol* 2000;30:171-5.