Quantitative proton MRS of Pelizaeus–Merzbacher disease

Evidence of dys- and hypomyelination

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Abstract—*Background:* Pelizaeus–Merzbacher disease (PMD) is a rare X-linked recessive neurologic disorder caused by a mutation in the proteolipid protein (PLP) gene on chromosome Xq22. The associated depletion of PLP and severe reduction of other major myelin proteins results in dysmyelination. MRI reveals loss of T1 contrast between gray and affected white matter and T2 hyperintensities of white matter due to elevated water content. *Methods:* In vivo proton magnetic resonance spectroscopy (MRS) was used to determine cerebral metabolite patterns in five patients with genetically proven PMD. Absolute metabolite concentrations were obtained in cortical gray matter, affected white matter, and basal ganglia and compared to age-matched control values. *Results:* In comparison to age-matched controls, MRS of affected white matter resembled the metabolite pattern of cortical gray matter, as indicated by increased concentrations of *N*-acetylaspartate and *N*-acetylaspartylglutamate (tNAA), glutamine (Gln), *myo*-inositol (Ins), and creatine and phosphocreatine. Most remarkably, the concentration of choline-containing compounds was reduced. Parietal gray matter and basal ganglia appeared normal but showed a tendency for elevated tNAA, Gln, and Ins. *Conclusions:* Magnetic resonance spectroscopy (MRS)–detected alterations are consistent with enhanced neuroaxonal density, astrogliosis, and reduction of oligodendroglia. These disturbances in cellular composition are in close agreement with the histopathologic features characteristic of dys- and hypomyelination. The proton MRS profile of Pelizaeus–Merzbacher disease (PMD) differs from the pattern commonly observed in demyelinating disorders and allows PMD to be distinguished from other leukodystrophies.

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Pelizaeus–Merzbacher disease (PMD, MIM #312080) is an X-linked recessively inherited leukodystrophy caused by mutation of the proteolipid protein (*PLP*) gene on chromosome Xq 22. *PLP* encodes the two major myelin proteins of the CNS, PLP and its isoform DM 20. Gene duplication is the most common mutation in PMD, but missense mutations, insertions, and deletions have been identified as well. *PLP* mutations result in dysmyelination, a lack of properly formed myelin, and in this respect PMD is different from other leukodystrophies.[1,2]

Neuropathologic characteristics of PMD comprise i) a reduction or even absence of myelin sheaths in large areas of the white matter, predominantly in periventricular regions; ii) well-preserved neurons and axons; and iii) relatively preserved islets of myelin giving white matter a patchy "tigroid" appearance without active demyelination.[3,4] A close correlation between the degree of dysmyelination and the clinical severity was found.[3] The classification suggested by Seitelberger[3,4] according to clinical and pathologic features is now largely reduced to two subtypes, the classic and connatal forms. Onset of the classic form is in the first year of life with muscular hypotonia, nystagmus, and delay in motor development. Nystagmus may disappear and spasticity and atactic-dystonic movements appear later. Slow developmental progress is often observed in the first decade of life, until relentless deterioration occurs with death in mid-adulthood. Tigroid dysmyelination is found in neuropathologic investigation. In contrast, patients with the rare and more malignant connatal form show little developmental progress. Severe neurologic symptoms include feeding problems, stridor, and marked spasticity resulting in multiple contractures. Epileptic seizures may occur. Patients usually die within the first decade of life. Pathologic examination shows complete lack of myelination in the entire brain.[1-3]

MRI studies of PMD show early on a diffuse hyperintensity or patchy changes of white matter on T2-weighted images and poor if any contrast between white and gray matter on T1-weighted images. It was proposed to divide the MRI changes into

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Table 1 *Clinical, genetic, and neuroradiologic, features of five patients with Pelizaeus–Merzbacher disease*

Feature	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Sex	M	M	M	M	M
PLP mutation	Duplication	Duplication	Duplication	Duplication	Duplication
Onset	Neonatal	Neonatal	Neonatal	Infantile	Neonatal
Current age, y	5	14	8	6	14
Age at MRI/MRS, y	0.6	1.3, 4.6, 5.6*	1.6*, 3.6, 4.7	2.9	6.8
Infantile hypotonia	+	+	+	+	+
Nystagmus	+	+	+	+	+
Best motor skill achieved (at age, y)	None	None	Walking	Sitting (5)	Walking with support (4)
Head circumference	< P3	n	n	n	< P3
Epilepsy	+	−	−	−	−
BERA abnormal†	+	+	+	+	+
MRI subtype‡	I	I	I	I	I

* Magnetic resonance spectroscopy (MRS) examination selected for the group analysis.
† Brainstem evoked response audiometry (BERA) with normal wave I and absent waves II to V.
‡ According to Nezu et al.[5]

+ = present; − = absent; N = normal.

three subtypes according to extent and severity of white matter alterations, with the most severe type I indicating diffuse alteration in the hemispheres and corticospinal tracts.[5]

Proton magnetic resonance spectroscopy (MRS) has been used to characterize the neurochemical disturbances in a large variety of brain disorders in childhood and, in particular, identify a consistent pattern of active demyelination in a variety of leukodystrophies of known and unknown origin.[6,7] Unfortunately, applications of proton MRS to PMD have led to conflicting results such as increased, decreased, or unchanged metabolite levels,[8-16] which at least in part may be explained by technical limitations.

The purpose of this work was to determine absolute metabolite concentrations in cerebral gray and white matter of genetically proven PMD patients using fully relaxed, short-echo time single-voxel proton MRS. We hypothesized that quantitative proton MRS will allow us to distinguish metabolite abnormalities in PMD from the metabolite patterns of both controls and patients with other leukodystrophies. Part of this work was presented previously in abstract form.[17]

Methods. *Patients.* Five boys with clinical and MRI features consistent with PMD were included in this study. Their ages at MRS ranged from 7 months to 6.8 years (mean ± SD 3.5 ± 2.6). Table 1 summarizes their clinical features. The diagnosis of PMD was confirmed by mutation analysis of the PLP gene in all five patients. Patients 2 and 3 were investigated three times, with follow-up MRS performed 1 to 3 years after the initial examination. In all patients, MRI revealed diffuse white matter signal alterations in the hemispheres and corticospinal tracts, according to subtype I as defined by the classification proposed recently.[5]

MRS and MRI. Written informed consent was obtained from the parents. MR examinations were performed at 2.0 T (Siemens Magnetom SP4000 and Siemens Magnetom Vision, Erlangen, Germany) using the standard imaging head coil or, for children with a body weight less than 10 kg, the extremity coil (Patients 1 and 3, first examination). Volumes-of-interest (VOI) for single-voxel proton MRS were selected from T1-weighted images (three-dimensional fast low angle shot) and T2-weighted images (fast spin echo). Locations were placed in parietal gray matter (8 to 12.5 mL in a paramedian position), affected white matter (4 to 6 mL in parieto-occipital and frontal lobes), and basal ganglia (4 to 6 mL) as indicated in figures 1 and 2. Fully relaxed, short-echo time proton MR spectra were recorded using a STEAM (STimulated Echo Acquisition Mode) localization sequence (TR/TE/TM = 6,000/20/30 msec for Magnetom SP4000, TR/TE/TM = 6,000/20/10 msec for Magnetom Vision, 64 accumulations) as described previously.[18]

Data analysis. Absolute metabolite concentrations (expressed in mmol/L VOI) were obtained with LCModel, a user-independent fitting routine, which is based on a library of model spectra of individual metabolites.[19] Metabolites routinely identified by this procedure are the neuroaxonal markers *N*-acetylaspartate and *N*-acetylaspartylglutamate (tNAA),[20,21] creatine and phosphocreatine (tCr) involved in energy metabolism,[22] choline-containing compounds (Cho) connected to membrane turnover,[23] the glial (astrocytic) marker *myo*-inositol (Ins),[24,25] the excitatory neurotransmitter glutamate (Glu) and its amidated form glutamine (Gln), and lactate as an indicator of nonoxidative metabolism. Absolute metabolite concentrations from patients with PMD were compared to those of a group of age-matched controls specifically assembled from our subject pool reported previously.[26] Statistical evaluation was performed using a two-tailed Student *t* test assuming unequal variances with a significance level of $p < 0.05$.

Results. MRI typical for PMD is shown in figure 1 for Patient 4. At the age of 2.9 years, the abnormal stage of myelination is readily observable. In T1-weighted MRI (figure 1, A and C), the contrast between gray and white matter is missing, and the thin corpus callosum is hardly myelinated. Hyperintensities in T2-weighted images (figure 1, B and D) are consistent with lack of normal myelin in white matter, which reverses the normal T2 contrast between gray and white matter at this age. Despite marked alterations in myelination as indicated by MRI, the metabolite patterns detected by MRS appear comparatively normal. However, whereas cortical gray matter and basal ganglia (figure 1, A and D) reveal the familiar metabolite pattern of healthy controls, MRS of affected parieto-occipital and frontal white matter (figure 1, B and C) unravels clear disturbances that are most easily recognized by the abnormally low Cho signal relative to that of

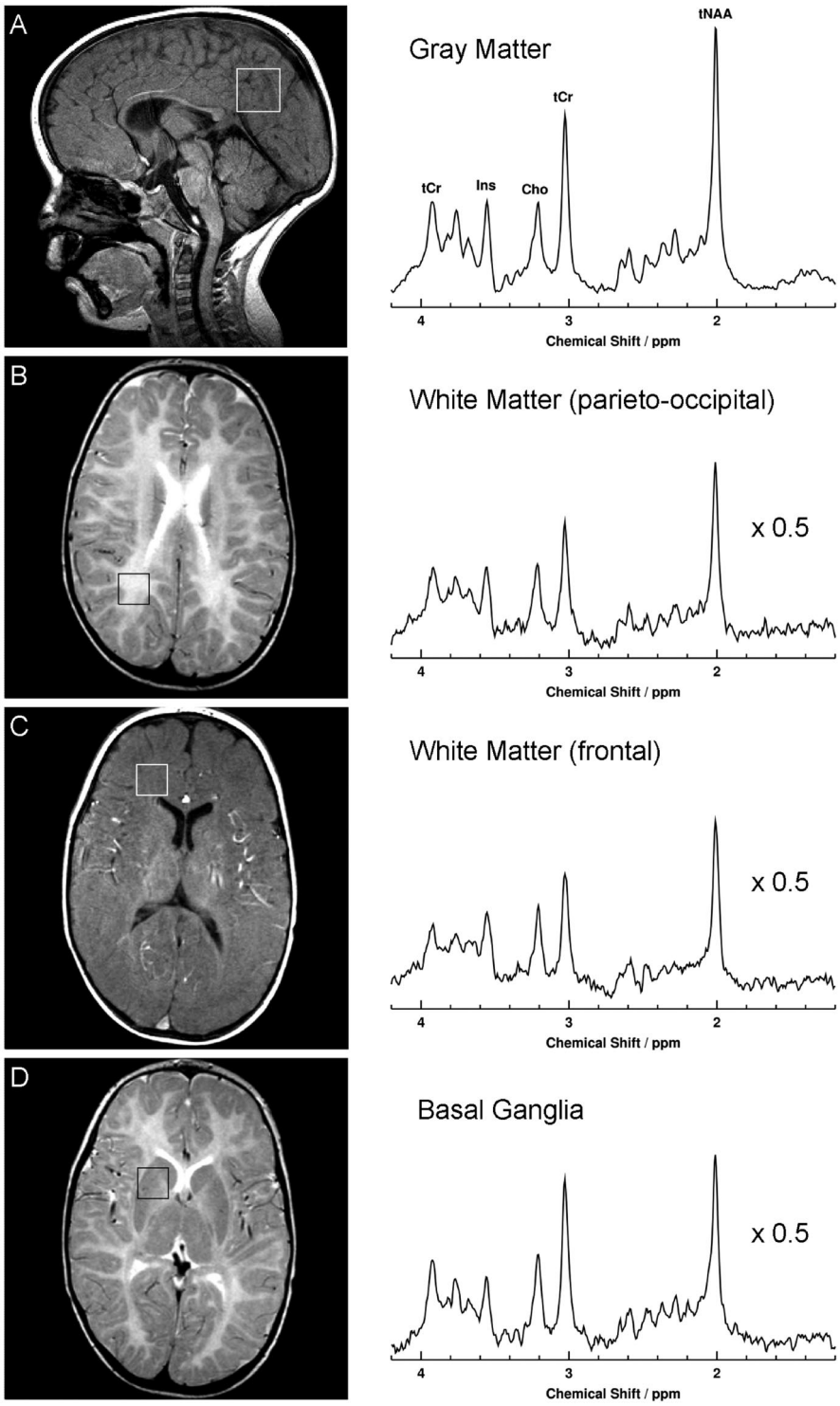

Figure 1. MRI and localized proton magnetic resonance spectroscopy (MRS) (boxes) of Patient 4 at the age of 2.9 years. (A) T1-weighted MRI and proton MRS of paramedial parietal gray matter. (B) T2-weighted MRI and proton MRS of right parieto-occipital white matter. (C) T1-weighted MRI and proton MRS of right frontal white matter. (D) T2-weighted MRI and proton MRS of right basal ganglia. While MRS of cortical and deep gray matter appears normal, MRS of affected white matter reveals a spectral pattern similar to that of cortical gray matter. The spectra of white matter and basal ganglia are scaled by a factor of 2 relative to cortical gray matter. tNAA = N-acetylaspartate and N-acetylaspartylglutamate; tCr = creatine and phosphocreatine; Cho = choline-containing compounds; Ins = myo-inositol.

tCr. Parallel to T1-weighted MRI, the resulting metabolite pattern in white matter resembles the MRS characteristics of cortical gray matter.

These observations are confirmed by a quantitative analysis of absolute metabolite concentrations as summarized in table 2. When averaged across all patients and compared to age-matched controls, affected parieto-occipital white matter shows a significantly altered metabolite pattern comprising increased concentrations of tNAA (+29%), Gln (+50%), Ins (+89%), and tCr (+30%) as well as decreased Cho (−17%). Even though statistically not significant, a tendency for elevated Glu was observed (+23%). In addition, the proton MR spectra of affected white matter reveal much smaller resonance line widths than those of age-matched children with normal myelination.

Although relatively normal concentrations are found in cortical gray matter and basal ganglia, the data show a similar tendency as in white matter for elevated tNAA (+14% in cortical gray matter and +12% in basal ganglia), Gln (+26% and +9%), and Ins (+20% and +39%).

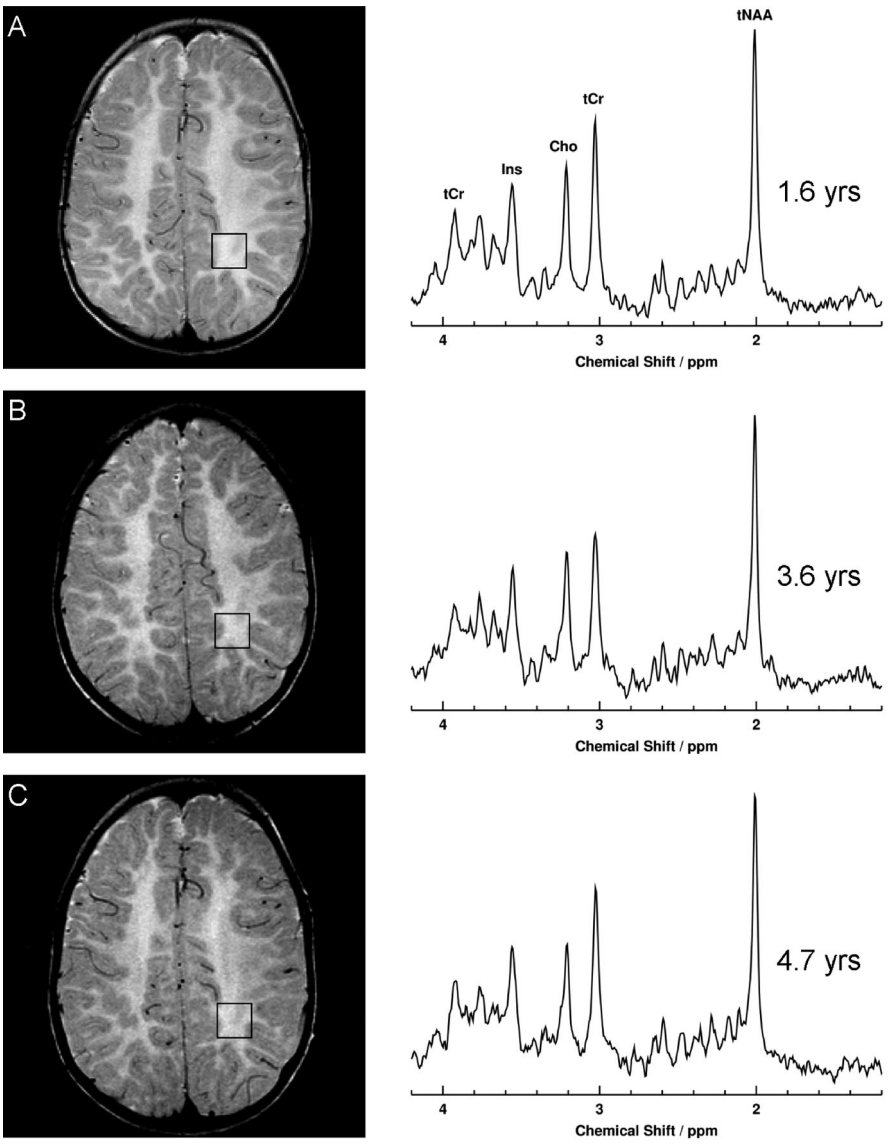

Figure 2. T1-weighted MRI and localized proton magnetic resonance spectroscopy (boxes) of Patient 3 at ages 1.6 (A), 3.6 (B), and 4.7 (C) years. Over the course of 3 years, the spectral pattern remains largely unchanged. tNAA = N-acetylaspartate and N-acetylaspartylglutamate; tCr = creatine and phosphocreatine; Cho = choline-containing compounds; Ins = myo-inositol.

Table 2 *Absolute concentrations (mmol/L) of brain metabolites in five patients with PMD and age-matched controls (mean ± SEM)*

	Gray matter		White matter (parieto-occipital)		Basal ganglia	
	PMD, n = 5	Control, n = 36	PMD, n = 5	Control, n = 22	PMD, n = 5	Control, n = 26
tNAA	8.8 ± 0.5	7.7 ± 0.2	8.8 ± 0.3*	6.8 ± 0.1	8.5 ± 0.6	7.6 ± 0.2
tCr	6.6 ± 0.4	6.3 ± 0.1	6.5 ± 0.1*	5.0 ± 0.1	8.5 ± 0.4	7.7 ± 0.1
Cho	1.3 ± 0.05	1.2 ± 0.03	1.5 ± 0.1†	1.8 ± 0.05	1.8 ± 0.1	1.9 ± 0.03
Ins	5.5 ± 0.4	4.6 ± 0.1	6.8 ± 1.0†	3.6 ± 0.1	5.3 ± 0.6	3.8 ± 0.1
Glu	10.1 ± 0.6	9.0 ± 0.2	8.0 ± 0.7	6.5 ± 0.2	8.3 ± 0.9	8.2 ± 0.2
Gln	5.4 ± 0.3†	4.3 ± 0.2	5.1 ± 0.6†	3.4 ± 0.2	6.0 ± 1.3	5.5 ± 0.3
FWHM‡	2.8 ± 0.1	2.8 ± 0.1	2.5 ± 0.1†	2.8 ± 0.1	3.5 ± 0.2	3.2 ± 0.1

PMD = Pelizaeus–Merzbacher disease; tNAA = *N*-acetylaspartate and *N*-acetylaspartylglutamate; tCr = creatine and phosphocreatine; Cho = choline-containing compounds; Ins = *myo*-inositol; Glu = glutamate; Gln = glutamine.

* Significant differences compared to controls (two-tailed Student *t* test, $p < 0.005$).
† Significant differences compared to controls (two-tailed Student *t* test, $p < 0.05$).
‡ Full width at half maximum (FWHM) of the creatine and phosphocreatine signal at 3.02 ppm in hertz.

Elevated Lactate was observed only once in white matter of Patient 1, yielding 3.3 mmol/L as compared to control values less than 1 mmol/L.

Follow-up examinations of Patient 3 are shown in figure 2. Over a period of 3 years, the spectral pattern and absolute concentrations of metabolites in parieto-occipital white matter remained largely unchanged.

Discussion. In five patients with genetically confirmed PMD, a neurochemical assessment by proton MRS revealed largely unchanged metabolite concentrations in cortical and subcortical gray matter. In contrast, affected white matter as identified by MRI was characterized by a reduction of Cho as well as elevated concentrations of tNAA, Gln, Ins, and tCr.

Cho mainly represents contributions from phosphorylcholine and phosphorylethanolamine as precursors for membrane synthesis as well as from the corresponding membrane degradation products glycerophosphorylcholine and glycerophosphorylethanolamine.[23] The elevation of Cho in demyelinating leukodystrophies is attributed to increased membrane turnover. In our patients, the reduction of Cho (−17%) in white matter may be assigned to the severe reduction of choline-containing myelin proteins and lipids caused by the lack of normally functioning oligodendrocytes.

MRS measurements of tNAA have been validated as an axon-specific monitor of white matter.[21] A close correlation between neuronal loss and a decrease in tNAA has been demonstrated in various cerebral disorders.[27] Marked elevation of tNAA as detected by MRS is highly characteristic of Canavan disease due to deficiency of aspartoacylase activity. The moderate increase of tNAA (+29%) observed in our patients with PMD probably reflects the elevated density of axons in white matter lacking the oligodendrocytic tissue and normal myelin sheaths between axons. Even though statistically not significant, the tendency for elevated tNAA, Gln, and Ins in cortical and subcortical gray matter hints at a similar effect in cortex and basal ganglia. In fact, the occurrence of an enhanced neuroaxonal density is further supported by the simultaneous observation of slightly increased levels of the excitatory neurotransmitter Glu (+23% in white and +12% in cortical gray matter), which is synthesized in neurons.

Ins is a key precursor of membrane phosphoinositides and phospholipids and involved in cell membrane structure.[28] The Ins signal detected by MRS reflects free Ins,[24] which has been recognized as the most important nonnitrogenous organic osmolyte in mammalian brain tissue.[29] For proton MRS of the brain, Ins has been validated as a glia-specific marker with particularly high concentrations in astrocytes.[25] Therefore, the increase of Ins (+89%) in affected white matter is consistent with an astrocytic proliferation. This notion is further supported by the observed increase of Gln (+50% in white and +26% in cortical gray matter), which is mainly present in astrocytes.

tCr is involved in energy metabolism and a constituent of both neuronal and glial cells. In rats, higher tCr concentrations have been found in astrocytes than in neurons.[22] Accordingly, the combination of a high neuroaxonal density with an increased number of astrocytes should result in a higher concentration of tCr, which is indeed observed (+30%).

Taken together, the pronounced metabolite alterations in PMD white matter are in line with enhanced neuroaxonal density, astrogliosis, and reduced oligodendroglia. These changes in cellular composition are in close agreement with the histopathologic alterations typically seen in PMD. The histologic hallmark that is the marked reduction or even complete lack of myelin results in a higher concentration of axons, while areas devoid of myelin show paucity or absence of oligodendroglia and a diffuse moderate proliferation of astroglia.[30] Whether the cerebral cortex is spared by the disease as previously suggested[30] remains an open question. It deserves further investigation in view of the enhanced neuroaxonal and astrocytic density (elevated tNAA and Ins) found in both cortical and deep gray matter.

The metabolite abnormalities in PMD are complemented by the unexpected finding of markedly reduced resonance line widths in proton MRS of white matter. Because the spectral resolution is directly proportional to the magnetic field homogeneity, smaller line widths indicate a better homogeneity. In terms of structure, this may be related to the absence of myelin sheaths that allow for a more homogeneous tissue composition with markedly reduced structural boundaries than in normal white matter. In addition, the lack of oligodendrocytes may contribute to the field homogeneity as oligodendrocytes contain a considerable amount of paramagnetic iron.[31]

Recent MRS studies of patients with PMD yielded conflicting results despite the fact that PMD was genetically proven. Apart from the possible influence of differences in patients' age or disease stage, the reported increases and decreases in ratios of Cho/tCr,[11-13] decreases in tNAA/tCr[8,9,11-13] and tNAA/Cho,[8,12] increases in Ins/tCr[13] as well as decreases and increases in the tNAA concentration[9,15] may reflect the use of different techniques ranging from chemical shift imaging to single-voxel MRS. Furthermore, the discrepancies imbed the uncertainties introduced by the use of ratios of T1- and T2-weighted resonance intensities, which are sensitive to putative alterations of T1 and T2 relaxation times even in the absence of a true metabolic change. Here, the observation of a reduction of the Cho concentration emerges as a novel spectroscopic hallmark of affected white matter in PMD. So far, this finding has only been reported based on metabolite ratios in a single patient with genetically confirmed PMD[32] and in two patients with connatal PMD but an absence of a mutation of the PLP gene.[14]

The metabolite abnormalities detected by in vivo proton MRS in white matter of patients with genetically proven PMD comprise enhanced neuroaxonal

density, astrogliosis, and reduced oligodendroglia. This profile clearly differs from the spectral alterations seen in leukodystrophies with predominant demyelination. MRS of white matter in demyelinating disorders like adrenoleukodystrophy is characterized by a marked reduction of tNAA, indicating loss of vital neuroaxonal tissue, in conjunction with elevation of Cho and Ins, indicating demyelination and glial proliferation.[33] Nevertheless, the cellular alterations demonstrated here are unlikely to be specific for PMD. Instead we presume that other conditions with hypo- and dysmyelination will present with a similar metabolite profile. In fact, hypomyelination with atrophy of the basal ganglia and cerebellum (H-ABC) revealed a corresponding metabolite pattern except for a normal Cho level, which is tentatively ascribed to compensation by strong glial proliferation.[34] Further studies of hypomyelinating disorders are needed to validate the common features of hypomyelinating disorders. These investigations should include patients with the recently identified PMD-like disease caused by mutations in the gene encoding gap junction protein alpha 12 (connexin 46.6).[35]

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