Early Reduction of Total *N*-Acetyl-Aspartate-Compounds in Patients With Classical Vanishing White Matter Disease. A Long-Term Follow-Up MRS Study

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ABSTRACT: The neuropathology of vanishing white matter (VWM) disease is characterized by a loss of white matter (WM). Although recent histopathological studies suggest a primary glial dysfunction, the purpose of this work was to assess the extent of axonal involvement in VWM using long-term follow-up proton MR spectroscopy. White and gray matter of nine children with genetically proven VWM and late infancy/early childhood onset were investigated with short-echo time, single-voxel proton MR spectroscopy over up to 8 years starting as early as less than 2 years after the onset of symptoms (5 patients). Total *N*-acetyl-aspartate (−51% from normal control), creatine and phosphocreatine (−47%), and *myo*-inositol (−49%) were reduced in WM at early disease stages. Choline-containing compounds were less severely decreased (−31%). Follow-up investigations revealed progressive reduction of all metabolites in WM. In gray matter, no distinct changes were detected at early stages. Later total *N*-acetyl-aspartate decreased slightly (−22%). Assuming the metabolite alterations to primarily reflect changes in cellular composition, the observed pattern indicates early axonal involvement or loss as well as relatively enhanced turnover of myelin. These early stages are followed by a complete cellular loss in cerebral WM. (*Pediatr Res* **63: 444–449, 2008**)

Vanishing white matter (VWM) disease (MIM 603836)—also known as myelinopathia centralis diffusa and childhood ataxia with diffuse central nervous system hypomyelination—was first separated from the vast group of unknown leukoencephalopathies in the early 90s (1,2). The MR imaging (MRI) and MR spectroscopy (MRS) features of VWM are unique and therefore considered diagnostic. MRI characteristically reveals bilaterally symmetric, diffuse involvement of cerebral white matter (WM) at the onset of symptoms and progressive cystic degeneration into an appearance like cerebrospinal fluid (CSF) over the course of the disease (3). Proton MRS shows a reduction of all WM metabolites except for the presence of lactate (Lac) and glucose (Glc) in concentrations typical for CSF (1,4,5).

Several phenotypic variations have been described encompassing i) rapidly fatal variants with antenatal onset and involvement of multiple organs, ii) onset in early infancy before 2 years of age (6–10), iii) a classical form with onset in late infancy/early childhood followed by a relapsing-remitting/chronic progression over half a decade (1,2,11–15), iv) juvenile onset cases with a protracted progression over 1–2 decades (3), and v) mild adolescence/adult forms, in females often associated with ovarian failure (16–22).

The mode of inheritance is autosomal recessive. Mutations in each of the five subunits $\alpha–\epsilon$ of the eucaryotic translation initiation factor 2B (eIF2B) have been found to cause the disease in most patients (23). eIF2B plays an essential role for initiation and regulation of cell protein synthesis. There is growing evidence that the mutations cause a decrease in eIF2B activity by different mechanisms and to a different extent. They may impair the ability of the subunits to form holocomplexes, and diminish the nucleotide exchange activity, or reduce the ability of the catalytic domain of the ϵ subunit to bind to the substrate, or actually enhance the binding (24). Another group reported a compromised generation of astrocytes positive for glial fibrillary acidic protein, thus raising the possibility of a functional deficiency (25,26). Certainly, the pathogenesis of VWM is still not fully understood (27). Early histopathological work discussed hypomyelination *versus* demyelination (1,3,28), while the observation of a commensurate degeneration of axons and myelin sheaths in cerebral WM prompted the hypothesis of a primary axonopathy (3). More recent studies, however, point toward a primary glial dysfunction which predominantly affects astrocytes and the myelin-producing oligodendrocytes (27,29,30). Phosphorous MRS of patients with childhood ataxia with diffuse central nervous system hypomyelination revealed abnormal cerebral concentrations of high-energy phosphates

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Abbreviations: Cho, choline-containing compounds; **CSF,** cerebrospinal fluid; **eIF2B,** eucaryotic translation initiation factor 2 B; **Glc,** glucose; **GM,** grey matter; **Ins,** *myo*-inositol; **Lac,** lactate; **MRS,** magnetic resonance spectroscopy; **tCr,** creatine and phosphocreatine; **tNAA,** *N*-acetyl-aspartate and *N*-acetyl-aspartyl-glutamate; **VOI,** volume of interest; **WM,** white matter; **VWM,** vanishing white matter

suggesting an impairment of myelin membrane synthesis or transport (31).

Histologically, the disease is characterized by cystic cavitations of the deep cerebral WM due to diffuse loss of both myelin and axons accompanied by a variable but generally meager astrogliosis and macrophage response (32). Regions of relatively preserved WM tissue may be adjacent to the cavitations. Although these regions often exhibit a paradoxically increased oligodendrocytic density, abundant cytoplasm, and vacuolation gives many of them a foamy appearance. Also dysmorphic astrocytes have been observed (29,30). Axonal involvement has been reported to vary highly from a complete loss in cavitations to a more variable loss in less involved areas to being relatively spared in other areas and cases (32).

The purpose of this study was to elucidate the extent of axonal involvement at early stages in infantile and early childhood forms of VWM and monitor the time course of progression as observable by single-voxel proton MRS with absolute quantification of metabolite concentrations.

PATIENTS AND METHODS

Patients. We investigated nine patients (five females) with a genetically proven diagnosis of VWM. The age at entering the study ranged between 20 mo and 26 y (mean 8 ± 6.3 y). Table 1 summarizes the clinical features of all the patients. The age at disease onset ranged between 1.6 and 4.5 y (mean 2.9 ± 0.9 y). In eight patients, the onset of symptoms was provoked by a minor trauma ($n = 3$) or a febrile infection ($n = 5$). In patient 6, no provoking event could be ascertained. Genetic analysis as described by Ohlenbusch *et al.* (33) yielded mutations in one of the five subunits of eIF2B in all nine patients. The histopathological findings of patient 1 have been published previously (34).

MRI/MRS. All the patients underwent combined MRI and localized proton MRS of cerebral WM and cortical gray matter (GM). Multiple follow-up studies were performed in three patients covering a period of 6 mo to 8 y. In five of the nine patients, the first measurement was obtained within 2 years after the onset of symptoms, ranging between 1 and 17 mo (mean 8 ± 6.2 mo). The studies were approved by the local Ethics committee. Before each examination informed written consent was obtained from the parents. If necessary, children were sedated and monitored by pulse oximetry.

A total of 20 MRS investigations were carried out at 2 T (Magnetom SP and Vision, Siemens, Erlangen, Germany) using the standard imaging head coil. Fully relaxed short-echo time proton MR spectra were acquired using a single-voxel stimulated echo acquisition mode sequence (64 accumulations, repetition time = 6000 ms, echo time = 20 ms, middle interval = 30 ms for SP and 10 ms for Vision) as described previously (35,36). Volumes of interest

(VOI) were chosen from T1-weighted three-dimensional fast low-angle shot images and T2-weighted (fast spin echo) images with sizes ranging from 3.9 to 18 mL in WM and 4.9 to 18 mL in GM. VOI locations included frontal and parieto-occipital WM and paramedian parietal GM (Fig. 1D).

Spectral evaluation and quantification of absolute metabolite concentrations were accomplished by fitting a linear combination of calibrated spectra of individual compounds using LCModel (37). Metabolite concentrations are expressed in mmol per liter VOI without corrections for CSF contributions and residual relaxation effects. A two-sided *t* test was performed and *p* values <0.01 were regarded as significantly different from a group of age-matched controls (38,39).

RESULTS

MRI. In all the patients, MRI revealed bilaterally symmetric and diffuse homogeneous signal alterations in hemispheric WM leading to hyperintensities on fluid-attenuated inversion-recovery (FLAIR) images (Fig. 1A–C) and T2-weighted images (Fig. 1D–E) at early stages of the disease. The vanishing of the WM and its replacement by CSF-like fluid could be best appreciated on FLAIR images, as shown in Fig. 1A–C for patient 9, where the initial high intensity of the WM signal gradually decreased during disease progression. At the final stage, only a faint ventricular lining and a meshwork of tissue strands extending between the ventricular lining and the cortex remained discernable. In the course of the disease, other structures such as the internal capsule and subcortical U-fibers became involved in the degenerative process. In all the patients, the central tegmental tract showed pathologic signal intensity. MRI of the two oldest patients 4 and 5 revealed T2-weighted signal attenuation in the putamen. In all patients, except for patients 3 and 7, the cerebellar WM displayed a progressive T2-weighted signal increase indicating disease involvement. After the application of a contrast agent (patients 2, 3, and 9), no enhancement could be detected.

MRS. Figure 2A shows a WM spectrum of patient 2 (early childhood form) obtained 11 mo after the onset of disease. It reveals a significant reduction of *N*-acetyl-aspartate and *N*-acteyl-aspartyl-glutamate (tNAA), creatine and phosphocreatine (tCr), and *myo*-inositol (Ins) compared with the spectrum of an age-matched normal control (Fig. 2B). Choline-containing compounds (Cho) are less severely affected and

Table 1. *Summary of clinical, neurological, and genetic findings*

| Patient	1	2	3	4	5	6	7	8	9
Gender	Male	Male	Male	Female	Female	Female	Male	Female	Female
Ability to walk (mo)	16 with support	NA	15 unstable	14	9	15 unstable	NA	12	13 clumsy
Initial mental development	Normal	Normal	Delayed	Normal	Normal	Normal	Normal	Speech delay	Normal
Symptoms onset (y)	1.6	2.3	4	2.5	4.5	2.5	2.8	2.3	3.6
Provoking event:						None			
Trauma		+	+						+
Infections	+			+	+		+	+	
Loss of walking (y)	1.6	10.3	None	2.5	4.5	3.5	3.5	3.3	4.2
Age at MRS (y)	1.8	3.3, 3.9, 4.2, 5.7, 6.2, 6.9, 8.1, 9.7, 10.8	4.6	21.1	26.4	9.5, 10.4, 16.7	3.4	6.8	4.1, 4.6
Familial occurrence	2nd cousins affected (2)	No	No	Yes (patient 5)	Yes (patient 4)	No	NA	No	No
Mutation:	*EIF2B5/*	*EIF2B3/*	*EIF2B2/*	*EIF2B2/*	*EIF2B2/*	*EIF2B4/*	*EIF2B3/*	*EIF2B5/*	*EIF2B3/*
Gene/protein level	p.Arg269Gly p.Arg269Gly	p.Val30Gly p.Ala87Val	Pro196Ser Glu213Gly	Glu213Gly	Glu213Gly	p.Leu269Arg p.Arg374Cys	Arg225Gln	p.Ala74Thr/ p.Cys335Arg	Arg226Gln
Present age (age at death) (y)	(1.8)	15.5	10.6	25.4	30.6	21.4	14	NA	8.8

NA indicates not available.
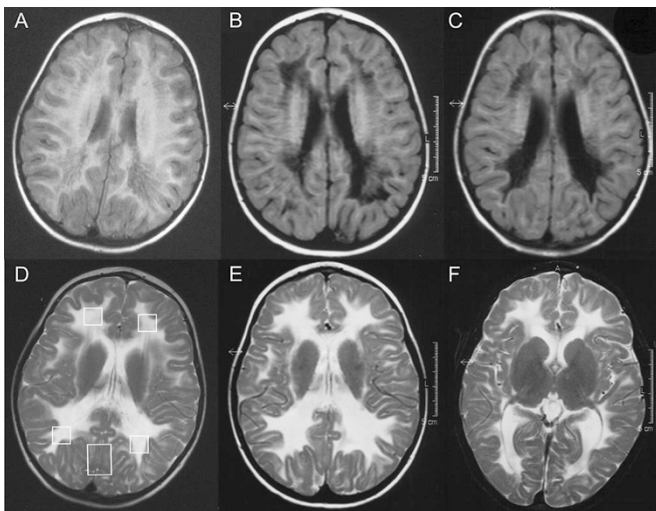
Figure 1. Representative FLAIR (*A–C*) and T2-weighted (*D–F*) images of patient 9 at (*A,D*) onset of symptoms as well as (*B,E*) 10 mo and (*C,F*) 4.6 y later. In FLAIR images, the hemispheric WM has abnormally high signal intensity at disease onset, whereas bilateral parieto-occipital areas already presented as hypointense. WM intensities further decrease with progressive cystic degeneration. In T2-weighted images, WM presented as hyperintense throughout the entire time of study. Image (*D*) depicts typical VOI locations chosen for MRS.
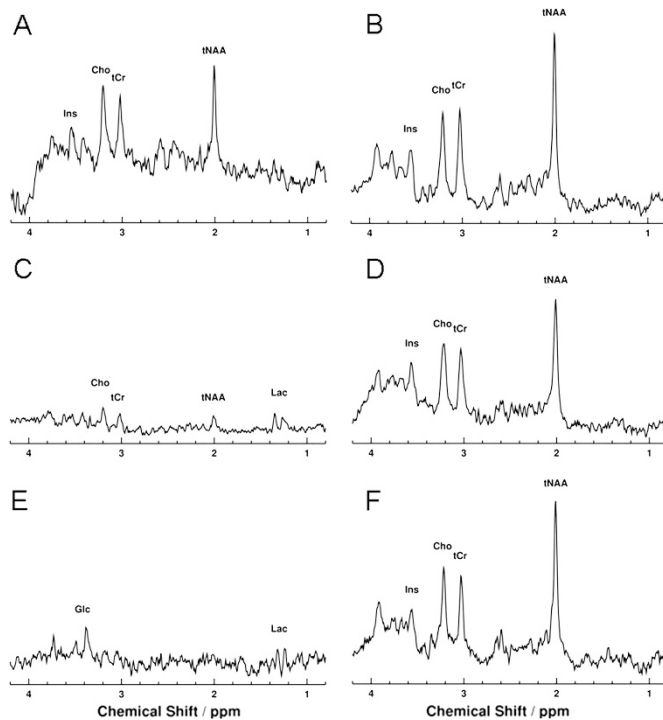

Figure 2. Proton MR spectra of WM of patient 2 obtained at (*A*) 11 mo (age 3.3 y), (*C*) 4.5 y (age 6.9 y), and (*E*) 8.6 y (age 10.8 y) after the onset of symptoms in comparison to age-matched controls (*B*, *D*, and *F*). The reduction of metabolite signals in early phases of the disease is followed by a further decrease during disease progression. In advanced stages, only Glc and Lac remain discernable closely resembling the pattern found for CSF.

show a milder signal decrease. Figures 2*C* and *E* demonstrate the alterations over time with progressive decrease of all metabolites until they are indistinguishable from the noise level. This process is accompanied by the simultaneous rise of

Lac and Glc. Figures 2*D* and *F* show proton MR spectra of WM of age-matched controls.

Table 2 summarizes the absolute concentrations of tNAA, tCr, Cho, Ins, Glc, and Lac of all the patients over the period of observation. In the first MRS obtained within 24 mo after onset of symptoms tNAA ($p = 10^{-6}$), tCr ($p = 0.9 \times 10^{-4}$), and Ins ($p = 0.9 \times 10^{-6}$) were significantly reduced, whereas Cho ($p > 0.05$) showed only a tendency for reduced levels in comparison with controls. Over the later course, the concentrations of all metabolites decreased further (tNAA $p = 0.2 \times 10^{-7}$; tCr $p = 0.6 \times 10^{-6}$; Cho $p = 0.5 \times 10^{-6}$; Ins $p = 0.1 \times 10^{-9}$). Six patients (patients 1, 2, 6, 7, 8, and 9) presented with increased Glc concentrations in at least one of the studies covering a period from 1 mo to 14.1 y after symptom onset. Elevated Lac could be observed in all the patients except patient 3 at various time points of the disease between 1 mo (patient 1) and 22 y (patient 4) after onset of symptoms.

Figure 3 depicts the individual time courses of all WM metabolites for all the patients. Despite the heterogeneity of the clinical courses, all tNAA concentrations throughout the observation periods as well as most tCr and Ins concentrations were below the normal range here defined as 2 standard deviations (SD) below (or above) the respective mean concentrations of controls (*horizontal lines* in Fig. 3). Patient 2 followed a relapsing-remitting course where the clinical remission was paralleled by a temporary increase of tNAA and tCr, while Cho and Ins continued to decrease. Patient 6 showed a fast progression of symptoms over 2 years yielding undetectable major metabolites. Patient 5 had a slow continuous progression over almost 2 decades that led to significantly lowered metabolite concentrations, except for Ins. Lac and Glc of patients 2, 5, and 6 did not vary according to the clinical pattern.

In patients with the early childhood form (patients 2 and 4–9) MRS of GM was essentially normal as shown for patient 2 in Fig. 4*A* and *B* obtained 11 mo and 8.6 y after the onset of symptoms (GM spectra of patient 3 were not available due to technical reasons). A group comparison of GM metabolite concentrations measured over time (Table 3) demonstrated only a slight decrease of tNAA ($p = 0.5 \times 10^{-3}$) concentration compared with controls. Elevated Lac was found in only 3 of 20 GM spectra obtained in later stages of the disease at 4 and 7 y after onset of symptoms (patient 2: 1.7 mM, patient 6: 3.3 mM, patient 8: 2.7 mM). Glc was increased in only one case (patient 2: 1.9 mM) 1.6 y after onset of symptoms.

Patient 1 with the late infantile form of VWM was the only one with GM alterations qualitatively similar to but less pronounced than those in WM. Concentrations of tNAA (3.3 mM, −6 SD from controls), tCr (4.3 mM, −3.8 SD), Ins (2.7 mM, −2.7 SD), and Cho (0.8 mM, −1.9 SD) were significantly reduced 1 mo after the acute onset of symptoms. Lac was slightly elevated (up to 1.3 mM) and Glc was not detectable. The patient did not regain consciousness and died only 1 mo later.

DISCUSSION

The present MRS findings for nine VWM patients with an infantile and early childhood variant are in line with literature reports pointing to a generalized vanishing of WM. In agreement with the characteristic MRI features of VWM, the

Table 2. *Absolute metabolite concentrations in cerebral white matter of all VWM patients*

	Patients 1–3, 7, 9; first MRS <2 y after onset of symptoms ($n = 5$)	Patients 2, 4–6, 8; all MRS >2 y after onset of symptoms ($n = 5$)	Controls
tNAA	3.3* ± 1.1	1.5* ± 1.5	6.9 ± 0.6 ($n = 17$)
tCr	2.6* ± 1.3	1.3* ± 1.3	4.9 ± 0.4 ($n = 17$)
Cho	1.1 ± 0.5	0.4* ± 0.4	1.6 ± 0.3 ($n = 17$)
Ins	1.9* ± 0.6	0.3* ± 0.6	3.7 ± 0.6 ($n = 17$)
Glc	4.2 ± 1.1	4.2 ± 0.7	0.7 − 1.2 ($n = 11$)
Lac	2.1 ± 0.1	2.2 ± 0.7	0.4 − 0.6 ($n = 11$)

* $p < 0.01$ (two sided t test); concentrations are given in mmol/L VOI and represent mean values (± SD) averaged across subjects and respective observation periods; control values were obtained from age-matched subjects (age range 5–10 y) (38) except for Glc and Lac (39).
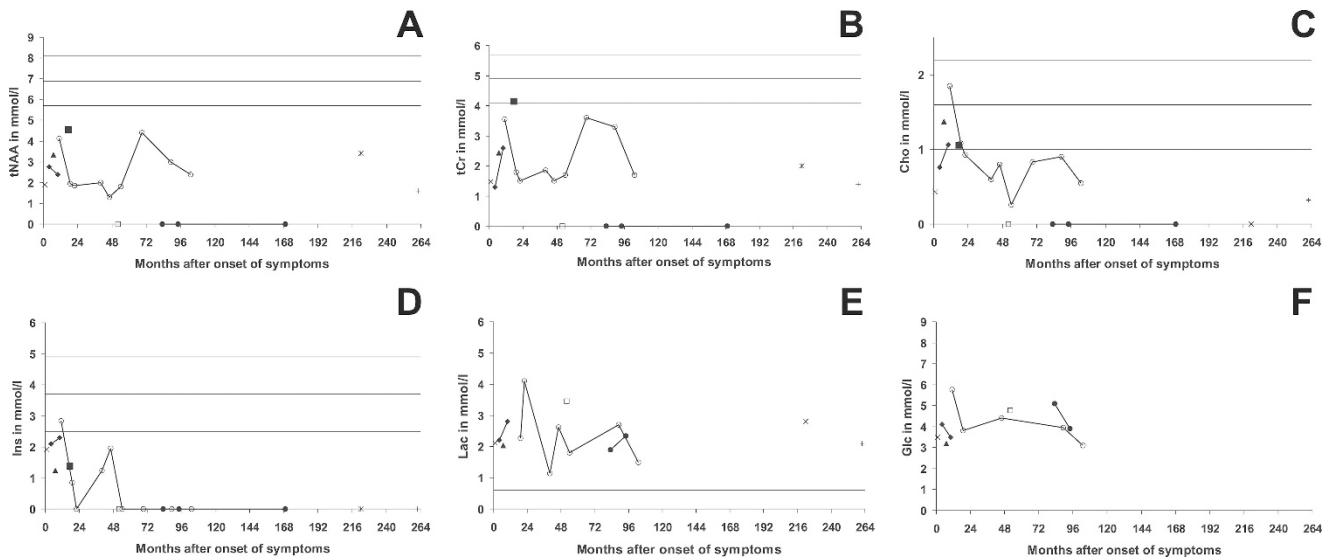

Figure 3. Absolute metabolite concentrations of (*A*) tNAA, (*B*) tCr, (*C*) Cho, (*D*) Ins, (*E*) Lac, and (*F*) Glc in WM of patient 1 (×), 2 (○), 3 (■), 4 (*), 5 (+), 6 (●), 7 (▲), 8 (□), and 9 (◆) over the whole observation period (connected data points refer to individual follow-up studies). tNAA is already reduced at early disease stages. Horizontal lines indicate mean values ± 2 SD of age-matched controls.

cerebral metabolite levels were most markedly altered in WM. When followed up to over 8 years of the disease course, proton MRS revealed a depletion of all major metabolites at later stages reaching a CSF-like appearance. The finding of elevated concentrations of Glc and Lac in all the patients at all disease stages indicates enhanced nonoxidative glucose consumption. This observation might reflect the anaerobic metabolism of macrophages involved in the clearance of cellular debris from ongoing neurodegeneration. Conversely, and except for the patient with late infantile onset, the metabolite levels in GM remained remarkably unaltered throughout the observation period.

Significant WM alterations already occurred at an early stage of the disease within the first 2 years after the onset of symptoms. This period is characterized by a significant reduction of tNAA, tCr, and Ins, and a milder decrease of Cho. Assuming these findings to primarily reflect changes in cellular composition rather than intracellular metabolism, the observed tNAA reduction refers to a significant loss of viable axons already at early disease stages in infantile and early childhood form of VWM. This is supported by the fact that previous studies not only demonstrated tNAA to be predominantly localized within neurons and axons but also validated tNAA as an axon-specific marker of cerebral WM (40–42). At later disease stages, a loss of vital neuro-axonal tissue in cavitated deep cerebral WM areas is unquestionable. How-
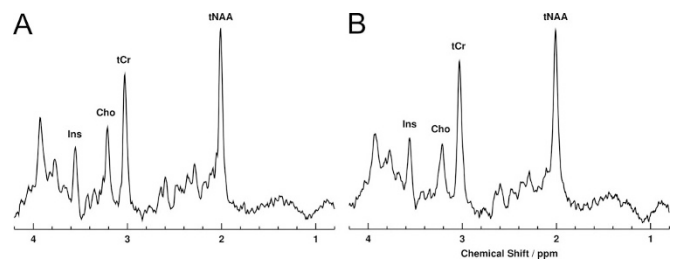

Figure 4. Proton MR spectra of GM of patient 2 obtained at (*A*) 11 mo and (*B*) 8.6 y after the onset of symptoms in early childhood. Except for a slight decrease of tNAA, both spectra reveal essentially normal metabolite signals.

Table 3. *Absolute metabolite concentrations in cerebral grey matter of VWM patients with the early childhood form*

	Patients 2, 4–9 ($n = 7$)	Controls ($n = 17$)
tNAA	6.4* ± 1.2	8.2 ± 0.8
tCr	5.7 ± 1.1	6.2 ± 0.5
Cho	1.1 ± 0.2	1.2 ± 0.2
Ins	3.8 ± 0.7	4.6 ± 0.7

* $p < 0.01$ (two sided t test); concentrations are given in mmol/L VOI and represent mean values (±SD) averaged across patients and the observation period; control values were obtained from age-matched subjects (age range 5–10 y) (38). For Glc and Lac see Results section.

ever, in less affected WM and/or at intermediate disease stages the axonal involvement is known to be variable up to relative preservation (2,43). The assumption that axonal loss solely

emerges as a secondary process—similar to other classic leukodystrophies—should yield a slower decline of tNAA than observed here, where tNAA concentrations were markedly reduced as early as 4–7 mo after the onset of symptoms. Of course, because all MRS studies were performed after the onset of symptoms, one cannot speculate about presymptomatic metabolite alterations and the causal nature of the present observations. At least one patient has been reported to exhibit only mildly reduced NAA levels soon after the onset of symptoms (4). However, as we cannot compare the severity of clinical courses, such findings might be attributed to individual variations in axonal involvement (32).

The Ins signal detected by MRS arises from free *myo*-inositol, which is considered the most important nonnitrogenous organic osmolyte in brain tissue and has been found in high concentrations in astrocytes (44). Similar to tNAA, its low concentration may be interpreted as a loss of respective cells in agreement with the disproportional feeble astrogliosis in VWM (27). Together, the lowered number/density of axons and astrocytes is perfectly in line with the proportional decrease of tCr as creatine and phosphocreatine are constituents of both neuronal and glial cells (45).

The reduction of the Cho concentration was less pronounced. The MRS-detectable Cho resonance comprises phosphorylcholine and phosphoryl-ethanolamine as precursor molecules for membrane synthesis as well as glycerophosphorylcholine and glycerophosphorylethanolamine as the corresponding membrane degradation products. Elevated Cho concentrations may thus either arise from enhanced membrane turnover [*e.g.*, in young infants (38)] and cell proliferation [*e.g.*, in brain tumors (46)] or from accumulation of myelin breakdown products as typically seen during demyelination (47). On the other hand, and apart from an end-stage scenario, a diminished Cho level has only been described in cases of hypomyelination such as Pelizaeus-Merzbacher disease (48).

Despite the increased oligodendrocyte cell count in better-preserved WM regions, the amount of stainable myelin has been found to be decreased in VWM disease. Up to this point it remains unclear if the myelin deficit reflects true hypomyelination and whether this is due to oligodendrocytic immaturity or mere dysfunction (29). However, regardless of the precise origin, insufficient myelination in the presence of some degree of deymelination as well as enhanced turnover of myelin may explain the low but—in comparison with tNAA and Ins levels—relatively high Cho concentration.

In patients with the early childhood form, the absence of essential metabolite alterations in GM is in line with its striking preservation on MRI. Over time, only a slight decrease of tNAA was observable which may indicate a subtle neuronal atrophy. In contrast, the one patient who presented with a disease onset in early infancy and a severe subacute clinical progression showed similar though less pronounced GM alterations than in WM. In view of the rapidly fatal course of this patient, these results might be attributed to an acute encephalopathy with severe metabolic perturbation.

A comparison of WM and GM metabolites supports the assumption of VWM being a disease of glial cells. Oligodendrocytes seem to be particularly vulnerable to reduced eIF2B

activity which is assumed to impair the cellular response to conditions of stress-like fever (32,49). This in turn might contribute to activation of cell-death signaling pathways.

In conclusion, this follow-up quantitative proton MRS study adds valuable information about the time course of the axonal and glial involvement in infantile and early childhood variants of VWM. During early stages of up to 2 years after the first onset of symptoms, the cerebral metabolite pattern is characterized by a pronounced reduction of tNAA. Ins and tCr are similarly reduced but to a more variable extent, whereas Cho is only slightly decreased. These observations may be understood as a partial loss of both glial cells and axons already at early disease stages. The reduced but relatively high level of Cho would be in line with insufficiently myelinated axons possibly in the presence of ongoing demyelinating processes.

Although there is mounting evidence for a primary myelin defect in VWM, the early and invariable tNAA decrease points to a severe axonal involvement already at disease onset—possibly one of the factors responsible for the clinical symptoms. On the other hand, due to the lack of data from presymptomatic stages, conclusions about the initial (and perhaps causative) events cannot be drawn by *in vivo* MRS.

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