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Quantitative proton MRS of cerebral metabolites in laminin $\alpha 2$ chain deficiency

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Abstract

Congenital muscular dystrophy (CMD) due to merosin (laminin $\alpha 2$ chain) deficiency is an autosomal recessively inherited disorder characterized by severe muscular weakness and hypotonia from birth on. Brain involvement is the rule and characterized by variable T2 hyperintensities of white matter which appears swollen on cranial MRI. The pathophysiology of these white matter changes is not clear. In five patients with laminin $\alpha 2$ deficient CMD we performed short-echo time localized proton MRS with determination of absolute metabolite concentrations in grey and white matter. In affected white matter, a consistent pattern of metabolites was detected comprising reduced concentrations of *N*-acetylaspartate and *N*-acetylaspartylglutamate, creatine, and phosphocreatine, and to a milder degree of choline-containing compounds. In contrast, concentrations of *myo*-inositol were in the normal range. Spectra of cortical and subcortical grey matter were normal. The observed metabolite profile is consistent with white matter edema, that is reduced cellular density, and relative astrocytosis. This interpretation is in line with the hypothesis that laminin $\alpha 2$ deficiency results in leakage of fluids across the blood–brain barrier and a histopathological report of astrocytic proliferation in CMD.

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1. Introduction

Laminin, a complex glycoprotein found extracellularly in the basement membranes, consists of three different polypeptide chains, a heavy alpha chain and

two light chains, β and γ . They are bound to each other by disulfide bonds forming a cross-shaped molecule. The alpha-2 ($\alpha 2$) chain is a subunit of laminin-2 (merosin) and laminin-4 (s-merosin). Laminin is thought to mediate the attachment, migration, and organization of cells into tissues during embryonic development by interacting with other extracellular matrix components. Tissues containing laminin comprise placenta, striated and cardiac muscle, peripheral nerve, meninges, chorioid plexus, and other regions of the brain [1]. In 1994, a lack of merosin, the laminin $\alpha 2$ chain, was demonstrated in patients with the classical non-Fukuyama type congenital muscular dystrophy (CMD) [2]. Subsequently, mutations in the *LAMA2* gene on chromosome 6q22–q23 were detected

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in patients with laminin $\alpha 2$ deficient CMD (MDC1A, MIM #607855) [3].

Clinical features in complete deficiency of laminin $\alpha 2$ include severe muscular hypotonia and weakness in the first weeks of life with slow improvement, but inability of independent ambulation in most cases [4]. Congenital contractures are common, feeding difficulties and respiratory problems may occur. Creatine kinase levels are markedly raised. Extramuscular involvement includes demyelinating peripheral neuropathy and striking white matter changes on cranial MRI. Widespread diffuse T2 hyperintensity of swollen appearing white matter is a characteristic finding after the first months of life [5]. Some of the patients have macrocephaly. However, clinical symptoms of cerebral involvement are usually rather mild. Intelligence is normal in most children, and epilepsy may occur in about 30% of patients [4]. In few patients, a range of structural malformations was demonstrated comprising occipital agyria, polymicrogyria, and hypoplasia of pons and vermis clinically accompanied by more severe mental and motor disability [5–8]. Recent reports described an expanding phenotype of laminin $\alpha 2$ deficiency with later onset which shows slowly progressive weakness of limb-girdle muscles. At least 25% of these patients achieve independent ambulation and several patients with *LAMA2* mutations reveal normal MRI [9].

In general, the striking discrepancy between marked white matter MRI signal alterations of cerebral white matter and only subtle clinical signs of brain dysfunction is well recognized. To date, the pathophysiology of the white matter changes remains unclear. In this study, we used localized proton magnetic resonance spectroscopy (MRS) with absolute quantification of cerebral metabolites to further characterize cerebral tissue abnormalities in patients with MDC1A.

2. Patients and methods

We studied five boys with MDC1A whose ages ranged from 10 months to 14 years at MRS. Table 1 summarizes the clinical, neuroradiological, and histological features. In patients 1, 2, and 4, diagnosis of complete or near-complete laminin $\alpha 2$ deficiency was based on immunohistochemical investigations of a biopsy from the quadriceps muscle. Muscle biopsies in patients 3 and 5 had been performed before immunohistochemistry for laminin $\alpha 2$ was available. In patient 3, laminin $\alpha 2$ deficiency was demonstrated in an additional skin biopsy. Immunocytochemical studies were performed using antibodies to the 80 kDa fragment of merosin (Anti-Human Merosin, Gibco-BRL, Berlin, Germany) in patients 1, 2, 3, and 4. Additionally, a second antibody (NCL Merosin, Novocastra, Newcastle-upon-Tyne, UK) was used in patients 1, 2, and 3. Laminin $\alpha 2$ deficiency was complete in patients 2 and 4, and near-complete in patients 1 and 3. Patient 5 was included in this study as his clinical, neuroradiological, and histological features clearly pointed to MDC1A. Mutation analysis of the *LAMA2* gene was not performed in our patients, as this assay currently is not readily available.

MRI revealed signal alterations of supratentorial white matter in all patients to a varying degree. Diffuse extensive widespread T2 hyperintensity of white matter was present in patients 1 (Fig. 1), 4, and 5. White matter appeared swollen in these patients, and head circumference was at the 90th, 98th, and above the 98th centile, respectively. Macrocephaly in patient 5 was present at birth already. MRI in patients 2 and 3 showed patchy T2 hyperintensities in supratentorial white matter that were extensive and widespread in patient 2 (Fig. 2) and confined to small frontal and occipital areas in patient

Table 1
Clinical, immunohistochemical, and neuroradiological features in five patients with laminin $\alpha 2$ chain deficiency

Patient	1	2	3	4	5
Sex	M	M	M	M	M
Affected sibling	–	–	–	–	–
Age at onset	Birth	Birth	Birth	Birth	Birth
Max. motor milestone	Walking	Sitting	Sitting	Sitting	Sitting
Contractures	+	+	+	+	+
Head circumference (centile) at birth	75	n.a.	n.a.	n.a.	>98
Head circumference (centile) at MRS	90	75	>98	98	>98
Learning difficulties	–	–	+	–	–
Epilepsy	–	+	–	–	–
Nerve conduction velocity	n	n	n.a.	Reduced	Reduced
Highest CK level	↑	↑	↑↑	↑	↑
Age at biopsy	3w	1y, 9y	3y	2m, 10y	6y
Laminin $\alpha 2$ deficiency demonstrated in	Muscle	Muscle	Skin	Muscle	n.a.
Degree of laminin $\alpha 2$ deficiency	Near-comp.	Complete	Near-comp.	Complete	n.a.
MRI WM changes	C	P	P	C	C
Age at MRS	10m, 2y7m	7y2m	9y6m	10y3m	14y4m

M, male; CK, creatine kinase; ↑, >100 U/L; ↑↑, >1000 U/L; WM, white matter; +, present; –, absent; n, normal; n.a., not available; near-comp., near-complete; C, confluent; P, patchy.

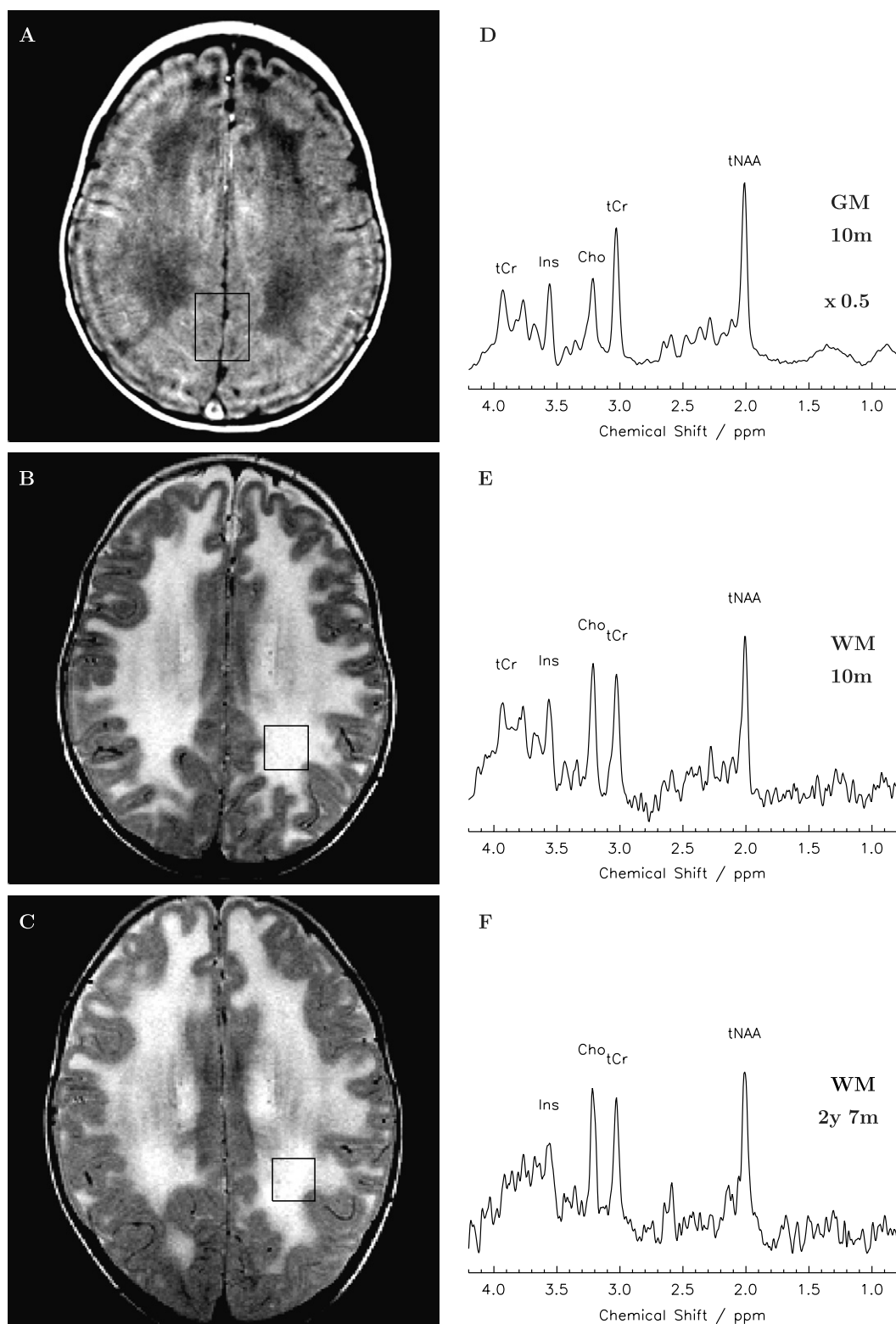


Fig. 1. Cerebral MRI and proton MRS of patient 1 with laminin $\alpha 2$ CMD at 10 months and 2 years 7 months of age. (A and D) T1-weighted MRI and MRS (STEAM, TR/TE/TM = 6000/20/10 ms) of paramedian parietal gray matter, (B and E) T2-weighted MRI and MRS of left parieto-occipital white matter at 10 months of age and (C and F) at 2 years and 7 months of age, respectively.

3 (Fig. 3). Head circumference was at the 75th and above the 98th centile, respectively. No overt cortical dysplasia was present in any of the patients.

Written informed consent was obtained from the patients' parents. Combined MRI/MRS studies were carried out at 2.0 T (Siemens Magnetom Vision,

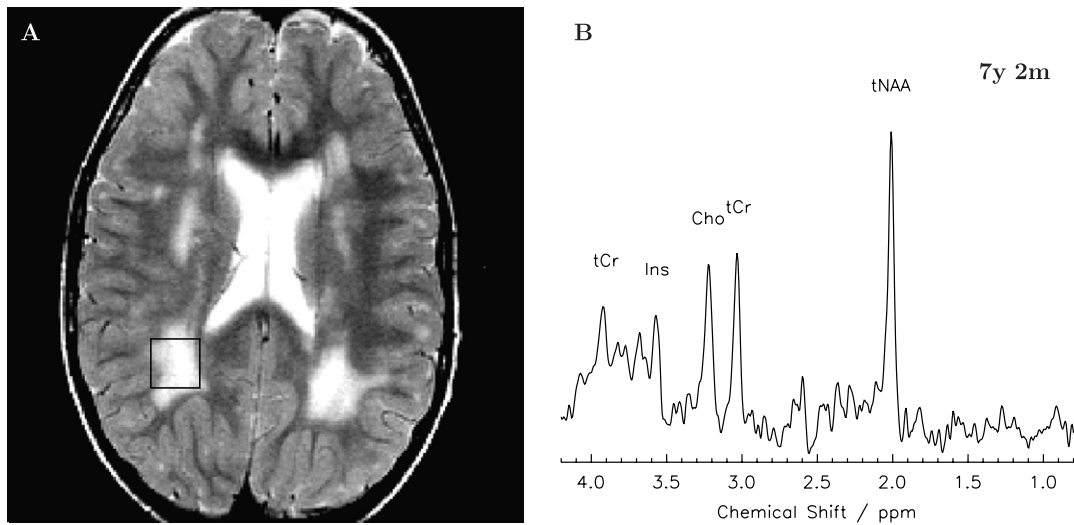


Fig. 2. Cerebral MRI and proton MRS of patient 2 with laminin $\alpha 2$ CMD at 7 years 2 months of age. (A) T2-weighted MRI and (B) MRS of right parieto-occipital white matter.

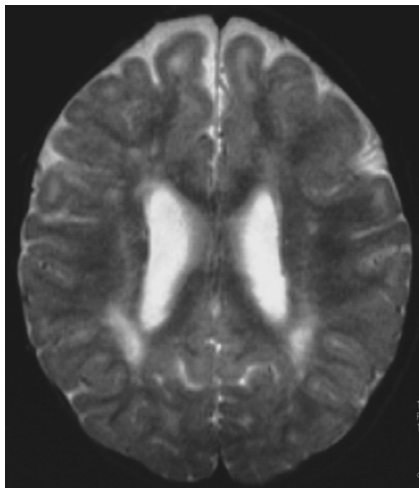


Fig. 3. T2-weighted MRI (1.5 T) of patient 3 at 4 years of age showing patchy white matter alterations.

Erlangen, Germany) using the standard imaging head-coil. Fully relaxed short-echo time proton MR spectra were acquired by means of a single-voxel stimulated echo acquisition mode (STEAM) localization sequence (TR/TE/TM = 6000/20/10 ms, 64 accumulations) as described previously [10]. Volumes of interest (VOI) were selected from T1-weighted (3D FLASH) and T2-weighted (FSE) images, with sizes ranging from 4.1 to 18 ml. VOI locations included one or two regions in affected white matter (frontal, parieto-occipital) and paramedian parietal grey matter in all patients. In addition, basal ganglia were examined in patient 4.

Spectral evaluation and quantification of absolute metabolite concentrations were accomplished with use of LCModel [11]. The approach represents a user-independent fitting routine based on a library of calibrated model spectra of individual compounds and

therefore avoids bias due to manual post-processing. Because of the use of an external reference, the method is free from putative changes of the internal water proton signal. Moreover, the quantification is neither compromised by T1 saturation (very long repetition time) nor markedly affected by T2 attenuation (very short echo time). Metabolite concentrations are therefore expressed in mM, i.e., mmol per liter VOI, without corrections for CSF contributions and residual T2 relaxation effects. Major detectable metabolites include the neuroaxonal markers [12] *N*-acetylaspartate and *N*-acetylaspartylglutamate (tNAA), creatine, and phosphocreatine (tCr) as ubiquitous compounds linked to energy metabolism [13], choline-containing compounds (Cho) involved in membrane turnover [14], the glial (astrocytic) marker [15] *myo*-inositol (Ins), and lactate (Lac) as the intermediate product of nonoxidative glucose consumption. Previous studies of regional age dependencies of cerebral metabolites provided age-matched controls for the evaluation of individual pathological alterations [16]. Values different from the mean normal value by ≥ 2 standard deviations (SD) were regarded as abnormal. Further details of MRS data acquisition and spectral evaluation have been described elsewhere [17].

3. Results

Fig. 1 shows representative MR images and spectra of grey and white matter of patient 1 with extensive diffuse leukoencephalopathy at 10 months and 31 months of age. Fig. 2 shows a complementary image and white matter spectrum of patient 2 with patchy white matter alterations. Quantitative analyses of absolute metabolite concentrations in white matter are summarized in Table 2. Patient 3, who showed the

Table 2
Cerebral metabolite concentrations (mM) in white matter and aged-matched controls [16]

Patient	Age	Region ^a	tNAA	tCr	Cho	Ins	Lac
1	10m	LPO	3.6*	3.9*	1.3*	3.8	0.9
		RPO	4.3*	3.9*	1.3*	3.9	1.0
Control (<1 y, n = 10)			6.2 ± 0.8	5.4 ± 0.5	2.0 ± 0.2	4.0 ± 0.5	–
	2y7m	LPO	4.3**	3.1**	1.3*	3.3	–
		RF	3.5**	3.3**	1.3*	3.8	–
Control (2–<5 y, n = 12)			7.2 ± 0.7	5.0 ± 0.4	1.9 ± 0.2	3.7 ± 0.6	–
2	7y2m	LF	5.4*	4.3	1.5	3.8	–
		RPO	5.3*	3.9*	1.5	3.6	–
3	9y6m	RPO	6.5	5.1	1.2	3.4	–
Control (5–<10 y, n = 7)			6.9 ± 0.6	4.9 ± 0.4	1.6 ± 0.3	3.7 ± 0.6	–
4	10y3m	LPO	5.0**	3.2**	1.0*	3.5	–
		RPO	5.6*	3.4*	1.2*	2.5	1.8
5	14y4m	LPO	5.3**	2.9**	1.0*	2.2	–
		RPO	4.8**	2.2**	0.8**	1.8	–
Control (10–18y, n = 12)			7.9 ± 0.6	4.8 ± 0.4	1.6 ± 0.2	3.9 ± 1.2	–

* ≥ 2 SD from control; ** ≥ 4 SD from control.

^a L, left; R, right; F, frontal; P, parietal; O, occipital.

Table 3
Cerebral metabolite concentrations (mM) in grey matter and aged-matched controls [16]

Patient	Age	Region ^a	tNAA	tCr	Cho	Ins	Lac
1	10m	PMP	7.7*	6.7	1.4	5.3	–
Control (<1 y, n = 6)			6.1 ± 0.7	5.5 ± 0.7	1.4 ± 0.3	4.8 ± 0.7	–
	2y7m	PMP	8.5	6.3	1.2	5.0	–
Control (2–<5 y, n = 16)			8.2 ± 0.9	6.4 ± 0.7	1.1 ± 0.2	4.7 ± 0.4	–
2	7y2m	PMP	8.4	6.5	1.2	4.7	–
3	9y6m	PMP	8.2	6.5	1.0	4.0	0.8
Control (5–<10 y, n = 13)			8.2 ± 0.9	6.2 ± 0.5	1.2 ± 0.2	4.6 ± 0.7	–
4	10y3m	PMP	8.5	7.1	1.0	4.5	–
5	14y4m	PMP	9.1	6.1	1.2	4.9	–
Control (10–18y, n = 22)			9.0 ± 0.8	6.5 ± 0.5	1.1 ± 0.2	4.3 ± 0.6	–
4	10y3m	BG	6.4	6.8	1.2	3.8	–
Control (10–18y, n = 5)			8.0 ± 0.9	7.7 ± 0.9	1.8 ± 0.4	3.3 ± 0.8	–

* ≥ 2 SD from control.

^a PMP: paramedian parietal, BG: basal ganglia.

mildest T2 signal alterations of all five patients, was the only one with all metabolites within the normal range.

In general, metabolic changes in white matter showed a characteristic pattern consisting of a significant reduction of tNAA and tCr as well as a milder decrease of Cho and normal Ins values. This profile was found in at least one white matter region investigated in four of five patients. The decreases of tNAA ranged from 2.2 to 5.3 SD of mean control, and tCr was reduced by 3 to 6.5 SD. A significant reduction of Cho by 2 to 4 SD was found in three patients, whereas patients 2 and 3 had a decrease of Cho by only 0.3 to 1.3 SD. In contrast, Ins was normal in all patients in all 11 white matter regions investigated. A slight increase of Lac was observed in three of the examined white matter regions.

Quantitative MRS of cortical grey matter (all patients) and basal ganglia (patient 4 only) revealed metabolite concentrations within the normal range for all metabolites (Table 3). The only exception was an increased concentration of tNAA in cortical grey matter of patient 1 at the time of his first investigation (age 10 months). At follow-up (2 years 7 months) the tNAA level was within the normal range.

4. Discussion

Using quantitative MRS in five patients with MDC1A we detected most pronounced changes of metabolites in cerebral white matter in those patients with more extensive widespread and diffuse white matter

signal alterations on MRI (patients 1, 4, and 5). In contrast, MRS was normal (patient 3) or revealed merely mild metabolite abnormalities (patient 2) in both patients with only mild and patchy T2-hyperintensities. On the other hand, MRS abnormalities did not correlate to the severity of muscular weakness, signs of cerebral dysfunction, or degree of laminin $\alpha 2$ deficiency, as evident from Table 1. In four patients with white matter alterations on T2-weighted MRI a consistent pattern of metabolite changes was detected comprising decreases of tNAA, tCr, and to a milder degree also of Cho. In contrast, Ins concentrations were in the normal range. There are two possible explanations for this metabolic profile. First, the alterations are consistent with, but not specific for neuroaxonal loss as suggested by the low levels of tNAA and tCr. Alternatively, the same pattern may be the result of a dilution of all metabolites, or more precisely of a generally reduced cellular density, which is accompanied by a relative astrocytic proliferation as indicated by the preserved level of Ins.

The pathophysiology of white matter alterations in MDC1A is not completely understood. Two lines of evidence suggest an important role for laminin $\alpha 2$ in CNS myelination and stability of cerebral white matter. First, a leakage of water across the blood–brain barrier has been presumed to result from laminin $\alpha 2$ deficiency. In a study combining immunohistochemical and specific electron microscopy techniques laminin $\alpha 2$ was localized in the normal human brain to the basal lamina of all cerebral blood vessels, whereas choroid plexus and meningeal blood vessels showed no immunoreactivity [18]. This observation led to the hypothesis, that laminin $\alpha 2$ may be important for the selective filtration capacity of the blood–brain barrier. Subsequently, it has been postulated, that the lack of laminin $\alpha 2$ in cerebral vessels of children with MDC1A may cause impaired selective filtration, leading to leakage of plasma components [18] and that the increased T2 relaxation time in MRI may be attributed to increased water content in white matter [19].

Second, recent investigations of oligodendrocyte differentiation and myelin membrane formation in oligodendrocytes grown in cell culture showed that laminin-2 substrates dramatically enhance myelin membrane formation. This effect is probably mediated via $\beta 1$ integrins [20]. It has been concluded that laminin $\alpha 2$ acts to enhance the production and spreading of the developing myelin sheath along and around the axon [20]. A critical role for laminin $\alpha 2$ in CNS myelination has been demonstrated in laminin $\alpha 2$ deficient mice, where a reduced thickness of the corpus callosum, a reduction in the concentration of mature oligodendrocytes without a change in total glia cell concentration, and defects in myelin lamellar compaction and sheath thickness have been demonstrated [21].

To our knowledge, histopathological investigations of cerebral white matter lesions in immunohistochemically proven MDC1A of humans are confined to two reports. In an older study, a brain biopsy of a 14-year old girl with a condition consistent with MDC1A showed intact myelin sheaths but obvious astrocytic proliferation [22], in line with the present MRS observations. An autopsy study of a 4-month-old infant with merosin-deficient CMD revealed polymicrogyria and occipital agyria with marginal neuroglial heterotopia, but normal pattern of myelination consistent with early age [8].

There are only two previous reports on the use of MRS for a characterization of white matter alterations in MDC1A, both based on a determination of metabolite ratios. In a 4-year-old girl with MDC1A MRS of affected white matter revealed a normal NAA/Cr ratio and increased Cho/Cr and Ins/Cr ratios [23]. Similar results may be obtained for the present study when the quantitative metabolite concentrations are used to calculate ratios thereof. For example, the mean tNAA/tCr concentration ratio of patients 4 and 5 in right and left white matter is 1.77 close to 1.65 obtained for age-matched controls (compare Table 2). In a more recent study using diffusion-weighted MRI and proton MRS of altered white matter in nine children with MDC1A the authors concluded that free-water concentrations are abnormally high in this condition [24]. This finding was based on reduced ratios of NAA/H₂O, Cr/H₂O, Cho/H₂O, and Ins/H₂O as well as on increased values of the apparent diffusion coefficient. Again in agreement with our results, MRS revealed normal NAA/Cr and Cho/Cr as well as slightly increased Ins/Cr metabolite ratios. Our findings extend these reports because the determination of absolute metabolite concentrations allows for an unambiguous and specific assignment of the observed alterations to individual metabolites.

Macrocephaly in some patients and swelling of diffuse T2 hyperintensities in MDC1A point to edema of white matter. Reductions of the concentrations of most major metabolites, as demonstrated in this study, are consistent with white matter edema, if the normal concentrations of Ins are attributed to relative astrocytosis. This interpretation is in agreement with the hypothesis that laminin $\alpha 2$ deficiency results in leakage of fluids across the blood–brain barrier. Interestingly, the white matter alterations are not seen in compacted white matter such as the internal capsule or the corpus callosum. This observation is consistent with an edema rather than with a primary myelinopathy. Also the mild clinical signs of white matter dysfunction argue against a marked loss of vital neuroaxonal tissue.

We are not able to estimate the extent to which impaired myelin membrane formation due to laminin $\alpha 2$ deficiency may account for leukoencephalopathy in MDC1A. Dominant hypomyelination, however, is asso-

ciated with a distinct MRS pattern different from that reported here, i.e. increased concentrations of tNAA, tCr, and Ins together with a reduction of Cho as demonstrated in Pelizaeus–Merzbacher disease [25].

Our finding of largely normal spectra in cortical and subcortical grey matter is in line with the predominance of alterations in white matter well known from MRI studies of MDC1A. This difference is not explained by the fluid leakage hypothesis, as laminin $\alpha 2$ is expressed in the basal lamina of all cerebral blood vessels [18]. On the other hand, the relevance of laminin $\alpha 2$ for CNS myelination has been demonstrated in laminin $\alpha 2$ deficient mice, where defects in myelin lamellar compaction and sheath thickness have been observed [21]. We speculate that laminin $\alpha 2$ deficiency might lead to a locus minoris resistentiae in oligodendrocytes that facilitates leakage of water predominantly in white matter.

Clinical as well as MRI and MRS features of CNS alteration in laminin $\alpha 2$ deficiency show considerable overlap with those of megalencephalic leukoencephalopathy with subcortical cysts (MLC) [26,27]. Both conditions share in common early macrocephaly, extensive diffuse T2 hyperintensities of cerebral white matter that appears swollen, relatively mild signs of cerebral dysfunction, and a metabolic pattern comprising reduced tNAA, tCr, and Cho together with normal Ins. The MRS profile of white matter in MLC is in good agreement with the histopathological changes known to occur in this condition [28]. Histopathology revealed a cavitating spongiform leukoencephalopathy with vacuoles in the outermost lamellae of myelin sheaths. Subcortical white matter was altered by intense fibrillary astrogliosis, myelinated axons were well preserved [29]. Canavan disease (CD) is a further condition characterized by macrocephaly and swollen white matter with marked T2 hyperintensity. MRS in CD, however, shows highly elevated tNAA due to a deficiency of aspartoacylase, and an osmotic–hydrostatic mechanism resulting from the continuous synthesis and efflux of NAA has been proposed to be responsible for the CD phenotype [30].

In summary, the present results corroborate previous views and provide additional insights in the metabolic changes occurring in laminin $\alpha 2$ deficiency in human brain. We emphasize the need for histopathological investigations of white matter alterations in MDC1A for a better understanding of the detailed pathophysiology.

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