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ORIGINAL ARTICLE

Metachromatic leukodystrophy: natural course of cerebral MRI changes in relation to clinical course

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Abstract
Objective Metachromatic Leukodystrophy (MLD) is a rare disorder leading to demyelination and neurological impairment. A natural history study within the German leukodystrophy network analyzed MRI changes with respect to the clinical course.
Methods 113 MR images of 68 patients (33 late-infantile, 35 juvenile) were studied cross-sectionally and longitudinally. MRI and motor deterioration were assessed using standardized scoring systems.
Results The temporal and spatial patterns of MR severity scores differed between the late-infantile and juvenile form. Although early (involving central white matter, corpus callosum) and late signs (involving pons, cerebellum, cerebral atrophy) were similar, high MRI scores (mean 18, SD 1.2, $p<0.001$) were evident in the juvenile form already at the onset of first symptoms and even in presymptomatic patients. The progression rate of the MRI score was clearly higher and more uniform in the late-infantile (on average 8 per year, $p<0.0001$) than in the juvenile patients (on average 0.4 per year, $p<0.08$). In late-infantile patients, MRI changes correlated highly with motor deterioration (rho=0.73, $p<0.001$), this was less remarkable in the juvenile form (rho=0.50, $p<0.01$). Severe motor dysfunction was associated with U-fiber involvement and cerebellar changes ($p<0.05$).
Conclusions MRI showed a typical spatial pattern, which evolved gradually and uniformly during disease progression in late-infantile MLD. In juvenile MLD MRI changes were already observed at disease onset and temporal patterns were more variable. As therapeutic options for MLD are evolving, these findings are not only important for patient counseling but also for the evaluation of therapeutic interventions.

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Introduction

Metachromatic leukodystrophy (MLD) is an autosomal recessive lysosomal storage disorder caused by a deficiency of the catabolic enzyme arylsulfatase A (ASA) resulting in accumulation of metachromatic lipid material, 3-O sulfogalactosylceramide (sulfatide) (Gieselmann 2008). This leads to demyelination in the central and peripheral nervous system and consecutively to progressive neurological deterioration. According to the age of onset, a late-infantile, a juvenile and an adult form have been distinguished (Gieselmann and Krägeloh-Mann 2010). More than 100 mutations causing MLD have been described (von Figura et al. 2001).

MLD is a rare disorder occurring with an estimated incidence of around 1 per 100,000 live births (Poorthuis et al. 1999; Heim et al. 1997). Therefore, little systematic data exist on MRI changes during disease course. However, as therapeutic options evolve such as stem cell transplantation, enzyme replacement and gene therapy (Gieselmann and Krägeloh-Mann 2010; Biffi et al. 2008b), it seems essential to increase our knowledge about the natural course of this disease.

Typical MRI changes in MLD have been described in the literature (van der Knaap and Valk 2005; Faerber et al. 1999; Zafeiriou et al. 1999; Kim et al. 1997; Demaerel et al. 1991). Due to the relatively small number of patients (largest MRI report described 7 cases (Kim et al. 1997)) and lack of any quantitative tool a systematic assessment has not been possible until recently, when a scoring system of MRI changes in MLD has been proposed (Eichler et al. 2009). Here, we applied this MR scoring system in a large cohort of MLD patients mostly recruited within the German leukodystrophy network (Leukonet) in order to systematically describe the MR changes during disease course for the late-infantile and juvenile type. A validated scoring system for the description of gross motor function deterioration in MLD (Kehrer et al. 2011b) allowed to explore whether the MRI changes correlate with gross motor deterioration, the most prominent clinical symptom in MLD of late-infantile and juvenile onset.

Methods

Patients and data collection

113 MR images of 68 patients (37 female) were collected between 2006 and 2010 nationwide within the German Leukodystrophy network LEUKONET (99 MRIs of 55 patients) and from baseline data of a phase I/II therapeutic trial in Denmark (14 MRIs of 13 patients). Patients had either the late-infantile (n=33; onset before 2.5 years of age) or the juvenile (n=35; onset between 2.5 and 16 years of age) form of MLD. Diagnosis was confirmed by enzyme deficiency of ASA (all patients), accompanied by an increase of urine sulfatide excretion (available in 57), and/or a pathogenic mutation in the MLD gene (available in 52) and typical clinical symptoms together with leukodystrophic MRI-alterations (all patients). Multiple sulfatase deficiency was excluded in cases without known genotype by normal total arylsulfatase activity and/or absence of facial dysmorphism, skeletal deformities, ichthyosis or abnormal mucopolysaccharidurie. Disease onset was defined as the time when first neurological symptoms occurred and/or decline of motor, cognitive or behavioral function as recalled by parents or their primary physicians. Age at onset was between 6 to 30 months (mean 16.6 mo, SD 6.4 mo) in late-infantile patients and between 3 to 13.5 years of age (mean age 6.4 yrs, SD 3.2 yrs) in juvenile patients. Nine patients had MRI scans before the onset of any symptoms. Their disease was diagnosed because siblings were affected (in one case incidentally). Seventeen patients had follow-up scans, seven with the late-infantile and ten with the juvenile form (supplementary material, Fig. 1). None of the patients underwent any kind of therapeutic interventions prior to their brain scan. Peripheral neuropathy, a common symptom in MLD, was not analyzed for this study. The study was approved by the Ethical Committee of the University of Tübingen, Germany (401/2005) and Copenhagen, Denmark, respectively. Informed written consent was given by the parents. All data were verified and monitored by a third party (Center for Pediatric Clinical Studies (CPCS-III), Tübingen, Germany).

MRI analysis

T2-weighted images were acquired on different clinical MR scanners using T2-weighted sequences implemented in clinical routine examinations, usually with high spatial in-plane resolution and high slice thickness. Six expert raters with experience in MRI assessment of leukodystrophies visually assessed independently T2-weighted axial images using the MR Severity score as described recently (supplementary material, Table 1) (Eichler et al. 2009). Raters were blinded to patient identity and clinical information. Inter-rater reliability was tested in a subset of these images (n=8) by six raters using the intraclass correlation coefficient (ICC) (Shrout and Fleiss 1979). One of these raters (S.G.) rated all MR scans twice in order to obtain intra-rater reliability, also assessed with the ICC.

MR severity scores in relation to disease duration

MR severity scores were analyzed in relation to patients' age and disease duration. The latter is defined as the time from onset of symptoms. Due to the fact that longitudinal data were

available only in part of the patients, a cross-sectional approach with only the most recent MRI was used. A linear least-squares regression analysis for both the late-infantile and juvenile form of disease was performed in order to estimate the mean change of MRI over time and its 95% confidence intervals as a function of time from first symptoms. In addition, for children who were scanned more than once (late-infantile: n=7 children with two scans, juvenile: n=10 children with 2 to 11 scans) intercept and mean slope were calculated for both forms. This was done in order to investigate whether the trend observed by cross-sectional linear regression could be replicated in longitudinal observations.

Spatial and temporal pattern of MRI changes in specific structures

MR subscores (supplementary material, Table 1) were analyzed with respect to early and late changes. For this purpose, disease duration was divided into three groups: early (disease duration up to 10 months), middle (11–20 months for late-infantile, 11–100 months for juvenile), and late (more than 20 months for late-infantile, more than 100 months for juvenile); the different definitions accounted for the slower deterioration of juvenile patients (Kehrer et al. 2011a). Mean subscores were calculated. All subscores were adjusted to a range between 0 and 1 in order to make their means comparable.

MR severity scores in relation to gross motor deterioration

Gross motor function was assessed using the Gross Motor Function Classification system for MLD (GMFC-MLD) (Kehrer et al. 2011b). Referring to the age reference limit when children normally learn to walk (90th percentile), the GMFC-MLD can be applied to children from 18 months of age onwards. It consists of seven different levels as shown in the supplementary material, Table 2. Data on gross motor function were collected prospectively and retrospectively. Data source consisted of hospital records, clinical examinations and standardized patient questionnaires. Furthermore, families were periodically interviewed by telephone.

The correlation of the MR severity score and its subscores with the motor score (GMFC-MLD) was analyzed statistically using Spearman's rank correlation coefficient (rho). Comparing MR scores of patients with and without motor dysfunction was done using the Wilcoxon-Test.

The study was considered a hypothesis generating study to look for trends that describe the late-infantile and juvenile form and may distinguish between them. Therefore, no adjustment for multiple testing was done. All p-values were regarded as being descriptive.

Results

Patients' MR scores increased both with age (supplementary material, Fig. 1), disease duration (Fig. 1) and level of GMFC-MLD (Fig. 3). This increase, reflecting deterioration of MRI findings, was different between the late-infantile and juvenile form. In the late-infantile patients, MR scores deteriorated within a relatively small age range, namely between around 2 and 3.5 years of age (supplementary material, Fig. 1). In the juvenile patients, deterioration was more variable, as was age of disease onset (see 'Methods').

Analyses of inter-rater reliability (ICC 0.95, 95% confidence interval 0.86-0.99) and intra-rater reliability (ICC=0.95, 95% confidence interval 0.94-0.96) yielded good agreement, except for basal ganglia and thalamus where inter-rater reliability was below average (ICC 0.72, 95% confidence interval 0.10-0.95).

MR severity scores in relation to disease duration

Severity of MRI changes during disease course was described relating the MR severity score to the time from first symptoms (Fig. 1). The regression lines were based on the cross-sectional data only. For the MR severity score of the late-infantile form the best estimate of the intercept was 4 ($p=0.06$) and of the slope 8 ($p<0.0001$). For the juvenile form the best estimate of the intercept was 17.7 ($p<0.0001$) and of the slope 0.4 ($p<0.08$). Both the slopes (= rate of progression of MR score per year) and the intercepts (= MR score at onset of symptoms) of the regression lines were clearly different between the juvenile and late-infantile form in the linear regression analysis ($p<0.0001$). The mean

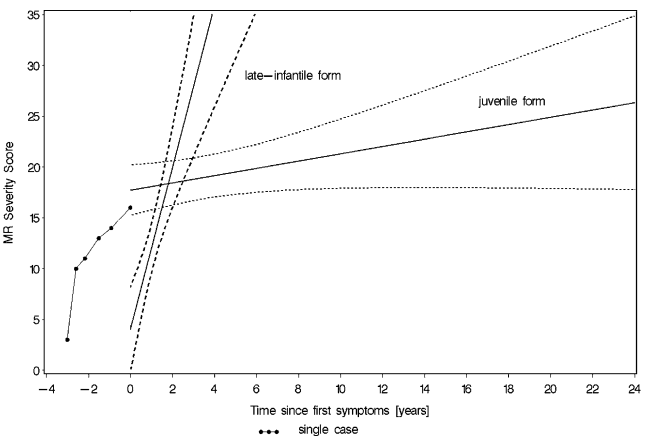

Fig. 1 MR Severity score (median and 95% confidence interval) of patients with late-infantile and juvenile MLD in relation to time from first symptoms (most recent scan, cross-sectional evaluation). A single presymptomatic juvenile case illustrates the evolution of MRI changes until first symptoms. She was followed-up for 3 years (negative values on x-axes for time before first symptoms)

intercepts and slopes for the longitudinal data were not identical with those from the cross-sectional data but showed the same trends with high slope / low intercept for the late-infantile group and low slope / high intercept for the juvenile patients (not shown). In addition, we were able to follow-up one juvenile patient during a presymptomatic situation until onset of first symptoms (illustrated in Fig. 1). This longitudinal observation further supports the evolution of MRI changes already before first symptoms.

Spatial and temporal pattern of MRI changes in specific structures

Means of MR subscores were analyzed according to the three disease stages, as defined above (supplementary material, Fig. 2). MRIs of patients in the early and late stage illustrating these findings are shown in Fig. 2. For the juvenile form, more structures already showed high scores (i.e. involvement) in the early phase of the disease and changes were more variable, but the sequence of early vs. late MRI changes was similar (supplementary material, Fig. 2).

- Early changes in the late-infantile form occurred in the parieto-occipital central WM, followed by frontal central WM changes, changes in the commissural fibres of the corpus callosum (first splenium, then genu) and changes in the periventricular WM in the same order (first parieto-occipital, then frontal and then temporal). In the juvenile form central and periventricular WM were affected in the early phase, together with the commissural fibres of the corpus callosum similar to the late-infantile form. The juvenile patients, however, revealed a more frontally pronounced pattern, i.e. the frontal WM and the genu were involved earlier and more severely compared to the late-infantile patients. A frontal predominance for the juvenile form was more pronounced in patients with a higher age of onset.

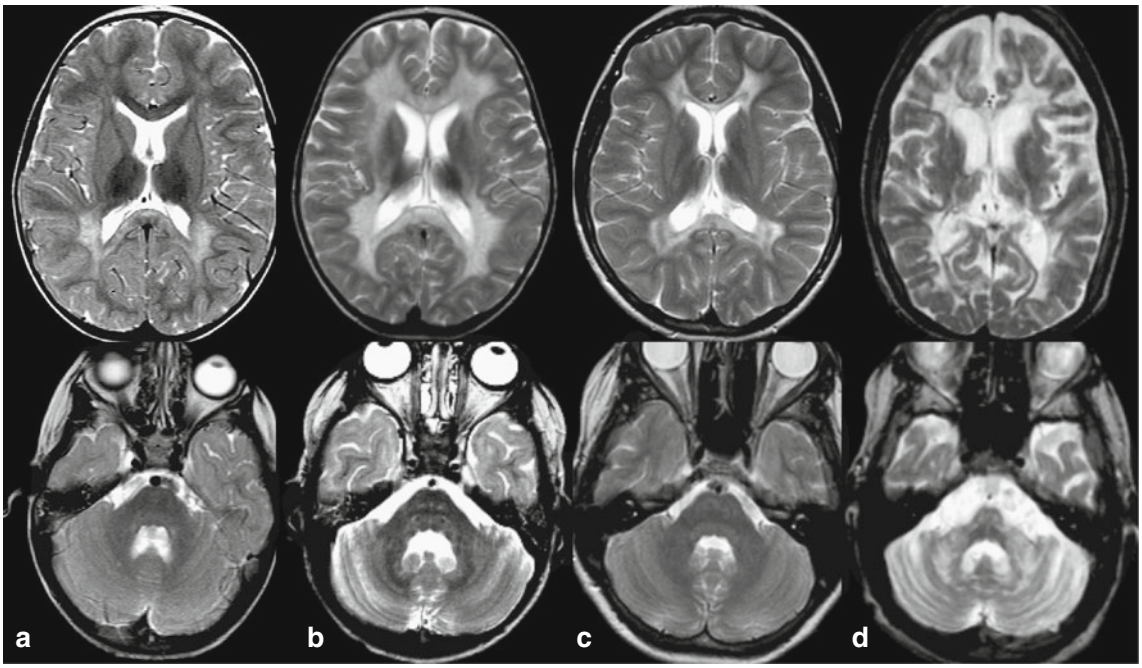

Fig. 2 Example T2-weighted axial MR images of patients with late-infantile MLD in the early (a) and late (b) phase and with juvenile MLD in the early (c) and late (d) phase of the disease. **a**) 2 years 4 months old girl, who had presented with first symptoms one month prior to the MR scan. MRI shows the typical pattern of severe parieto-occipital WM hyperintensity of periventricular and central WM, sparing subcortical WM (already myelinated). In the frontal WM only central parts are slightly involved. Both genu and splenium show signal hyperintensities, with the splenium more severely affected. Internal capsule is not yet involved, neither are there signs of cerebral atrophy or cerebellar involvement yet. **b**) 3 year-old girl, 23 months after first symptoms. Note the severe centrifugal spread of WM signal hyperintensities, involving partly subcortical areas and the corpus callosum. In addition, there is widening of inner ventricles as well as hyperintensity of the posterior limb of the internal capsule. Furthermore, cerebellar WM and projection fibers in the brain stem are affected. **c**) 12-year old girl, MRI done at the very onset of symptoms. Periventricular and central WM areas show signal hyperintensities including the commissural WM. Posterior limb of internal capsule is already involved. There are no cerebellar changes or cerebral atrophy. Subcortical WM (U-fibers) is also spared. **d**) 19 year-old patient who had presented with first symptoms at the age of 6 years. At this late stage of the disease severe cerebral and cerebellar atrophy and cerebellar WM signal hyperintensities are seen, in addition to affected projection fibers and reduced volume of supratentorial WM, which is diffusely hyperintense

- In a middle period during disease progression, the demyelination of the lobar and commissural WM advanced diffusely towards subcortical WM (U-fibers), for the juvenile with a more frontal predominance (frontal WM and genu) and for the late-infantile form with a more parieto-occipital (and splenium) predominance. Projection fibres in the internal capsule and pons were already affected at this stage (more pronounced in the juvenile form, where projection fibers were already affected in a few patients in the early stage).
- In a third and late stage of the disease, cerebral atrophy evolved, first with enlargement of inner ventricles, later with dilated outer CSF spaces, the latter was especially characteristic for the juvenile form (see Fig. 2d). From visual inspection it appeared that this atrophy was mainly related to shrinking WM volume, only later during the disease slightly smaller cortical bands could also be observed. In addition, cerebellar atrophy and demyelination of the cerebellar WM were typically observed at this late stage.

MR severity scores in relation to gross motor deterioration

In the late-infantile form the total MR Severity score was highly correlated with the level of GMFC-MLD (rho=0.73, $p<0.001$), this was less remarkable for the juvenile form (rho=0.50, $p<0.01$) (Fig. 3). Correlation coefficients were found to be, in general, higher in the late-infantile form compared with the juvenile form (supplementary material, Table 3). GMFC-MLD and cerebellar changes were positively correlated in both forms ($p<0.05$), whereas cerebral atrophy scores did not correlate with motor scores ($p>0.1$). Cerebellar WM involvement only occurred in patients in GMFC-MLD level 5 or 6, both for the late-infantile and juvenile form.

In patients with the juvenile form, distribution of motor scores was very inhomogeneous (Fig. 3). Thus, the correlation coefficient might not be informative. We therefore statistically compared the MR score of juvenile patients with (GMFC-MLD level 1–6) to those without motor symptoms (GMFC-MLD level 0) and found that both the total MR Severity score as well as the lobar periventricular and central WM subscores were, on average, higher in patients with motor symptoms ($p<0.01$). When comparing juvenile patients with mild motor deficit (GMFC-MLD level 1, i.e. motor problems, but able to walk without support) to patients with severe deficit (GMFC-MLD level 2–6, patients who had lost the ability to walk), scores for subcortical WM regions (U-fibers) were remarkably higher ($p<0.05$). Furthermore, cerebellar WM changes were associated with the severe motor deficit ($p<0.05$). Interestingly, projection fibres (pons and posterior limb of internal capsule) and cerebral atrophy were not associated with motor symptoms (GMFC-MLD level 1–6) or severe motor deficit (GMFC-MLD level 2–6) in the juvenile patients.

Discussion

The present study describes the natural course of late-infantile and juvenile MLD as depicted by cerebral MRI in the largest cohort reported so far. Standardized scores were applied in order to quantify MRI abnormalities and the deterioration of motor function. This allowed the description

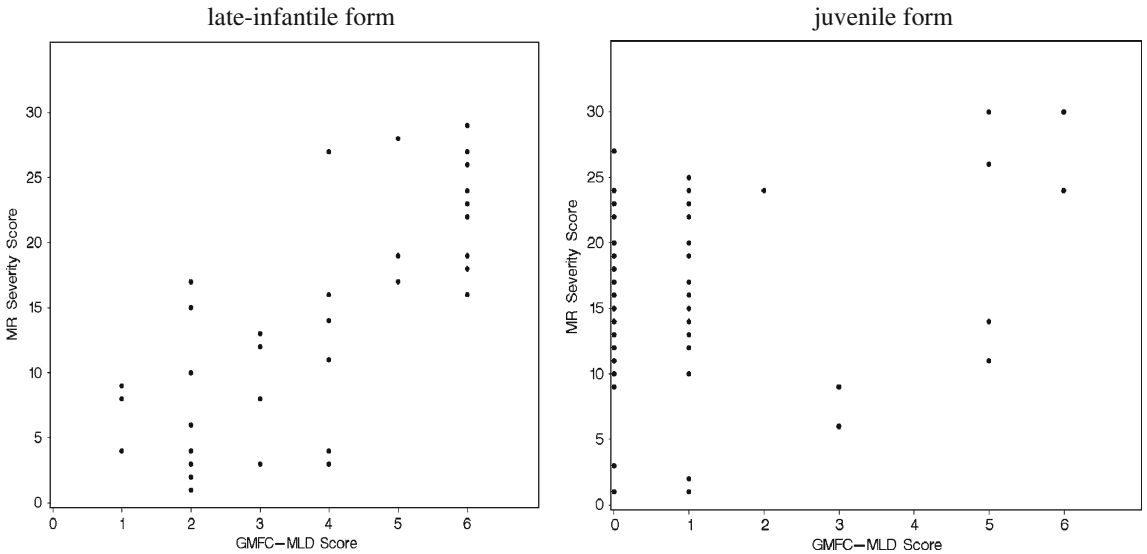

Fig. 3 MR Severity score in relation to the score for motor deterioration (GMFC-MLD), cross-sectional data only (most recent MRIs)

of a specific and progressive pattern of MRI changes in relation to the clinical course as well as certain distinct differences between the late-infantile and juvenile form. In the literature the MRI pattern in MLD has been described as diffuse WM signal abnormalities in periventricular and central regions, sparing the subcortical WM (U-fibers) early in the disease (van der Knaap and Valk 2005; Faerber et al. 1999; Zafeiriou et al. 1999; Kim et al. 1997; Demaerel et al. 1991). But no standardized tools were available and only few patients were studied systematically. We applied a recently developed scoring system (Eichler et al. 2009) to explore the spatial and temporal pattern of MR changes during natural disease course.

MRI changes in the late-infantile form were very homogeneous throughout the patient sample. The evolution of MRI abnormalities was not only uniform in its topographical distribution, but deterioration occurred also during a limited range of age, i.e. between 2 and 3.5 years. A typical spatial and temporal pattern could be identified with characteristic early and late MRI changes (Fig. 2). It seems noteworthy that WM changes started in the parieto-occipital lobes together with the splenium of the corpus callosum. In parallel to the cerebral changes, motor function deteriorated. This correlation was especially strong for lobar WM, but was also found for projection fibres and cerebellar changes. Only cerebral atrophy, a sign characterizing the late stage, did not correlate with motor deterioration.

In contrast to the late-infantile form, MRI in juvenile patients was clearly abnormal already before or at onset of first clinical symptoms. Although the spatial pattern of affected brain structures was similar, the variability of MRI abnormalities was higher. The WM affection showed a frontal predominance, which is considered typical for MLD (Schiffmann and van der Knaap 2009; Costello et al. 2009). Our systematic analyses confirmed observations that it seems to be an early sign only in juvenile MLD (van der Knaap and Valk 2005), in contrast to the late-infantile form with parieto-occipital predominance (van der Knaap and Valk 2005; Kim et al. 1997). The more variable course of MRI changes in juvenile patients probably reflects their clinical course, which is slower and more variable than in the late-infantile form (Kehrer et al. 2011b). Cognitive or behavioral symptoms may precede motor signs and may account for the weaker association of MR scores with motor function. Still, in juvenile patients with motor signs, MR scores were on average higher in periventricular and central WM regions than in patients not yet presenting with motor dysfunction. And changes in subcortical WM and cerebellum were associated with more severe motor dysfunction, i.e. patients unable to walk. Interestingly, cerebral atrophy, although a late sign of the disease, was again not associated with motor deterioration. We could, however, not analyze involvement of specific motor areas within the WM such as the central region in relation to motor function as the MR score does not allow this topographical distinction.

First symptoms may sometimes not be easy to identify (Biffi et al. 2008a), in retrospect ignored or overestimated, which may lead to artificial variability. This can, however, not sufficiently account for the MRI abnormalities at or even before onset of clinical disease. Obviously, rather widespread MR signal changes of the white matter can occur without causing symptoms in the juvenile patients. This raises the question of the nature of these changes. The histopathological brain findings in MLD are reported from autopsy cases for the late-infantile (Black and Cumings 1961; Jervis 1960), but also for the juvenile form (Haberland et al. 1973). The main feature was severe demyelination of the cerebral WM, so that MLD was initially even thought of as a primary disorder of oligodendroglia (Russel Brain and Greenfield 1950). Other important findings were astrocytal and microglial proliferation and sulfatide accumulation. Recently, in vivo MRI, postmortem MRI and histopathological investigation have been conducted in a patient with MLD and gave further evidence that the WM signal hyperintensity seen on T2-weighted images constitutes demyelination (van der Voorn et al. 2005). On the other hand, in an arylsulfatase A knock-out mouse model there was no histological finding of demyelination in the presence of T2-signal hyperintensity on MRI (Gieselmann et al. 2003). As all findings in the human situation come from late stages of the disease the histopathological nature behind early MRI changes remains unclear.

The MR severity score allowed a reliable quantification of brain involvement in this study. It has the advantage of being a standardized descriptive tool for a rare disease, similar to the MRI score in adrenoleukodystrophy (Loes et al. 1994). It can be applied to scans of different centers, MR scanners and sequences as long as axial T2-weighted images are available. This seems important in a rare disease where data collection has to be done in different settings and harmonization of methodology is not always possible.

The basal ganglia and thalamus were not further analyzed in this study because of the normal age-related and sequence-dependent signal variability (van der Knaap and Valk 2005). It proved to be difficult to reliably define abnormalities in these structures. However, the hypointensity in the ventrolateral part of the thalamus described earlier (Kim et al. 1997; Demaerel et al. 1991) was also seen in the present study (Fig. 2a).

The subjective aspect of a visual rating scale could be controlled for partly by intra-rater and inter-rater reliability tests, which both yielded good agreement. A limitation of this MR scoring system is the rather coarse categories of lobar white matter changes. Subtle changes within a defined area can not be described. Therefore, when quantifying MRI changes, a more sensitive and also objective tool could be a morphometric analysis of white matter changes

or atrophy. Beyond the descriptive analysis of white matter change MR spectroscopy and diffusion-weighted analyses might yield more insight into the pathophysiology of the disease.

It is essential to understand the natural course of MRI changes in relation to clinical findings not only in order to learn more about the disease process and the prognostic relevance of certain MRI changes, but also in order to have a background for the evaluation of new therapeutic strategies including decisions when to engage in therapeutic options. While enzyme replacement and gene therapy are currently evaluated in first clinical studies, stem cell transplantation (SCT) is already done in juvenile patients with MLD by several paediatric transplantation centres on the basis of an individual treatment trial as no other treatment options are available. Although no studies with larger numbers of treated patients against the natural disease course are available, it seems intuitive and is supported by case-reports and our own experience that the outcome after SCT is better the earlier (during the individual disease course) the treatment is given, as also reported in X-linked adrenoleukodystrophy (Mahmood et al. 2007). However, the decision when is “early enough” or “too late” is difficult. The results from our study indicate that the presence of MRI abnormalities, which may be present already before first symptoms in juvenile patients, are probably not a contraindication for doing SCT. Furthermore, the total MRI score may not be sensitive enough to define a threshold for intervention, as it is already high at onset and progresses slowly during disease in juvenile patients. Subscores identifying early and late MRI signs as given here may be more helpful to define the disease stage of the individual patient. In addition to the MRI score, clinical findings may help the decision when to engage into therapy. We recently described the motor deterioration of juvenile MLD in a large cohort and found that a relatively stable first stage characterized by mild motor abnormalities preceded a second phase of rapid motor deterioration (as rapid as in the late-infantile form) once independent walking was lost (Kehrer et al. 2011a). This indicates that SCT should be done long enough before the rapid regression occurs to allow stem cells to migrate to the brain, differentiate to microglia and start enzyme production, which is considered to be the treatment mechanism of SCT in MLD. However, clear answers to these questions will only be possible with systematic data on the disease course after SCT in comparison to the natural course of the disease.

The first step towards this goal is the description of the natural course with standardized tools in a larger cohort of unselected patients such as the MRI data in relation to disease course and motor deterioration as presented here.

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Conflict of interest disclosures

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