Hypomyelinating Leukodystrophies: Translational Research Progress and Prospects

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Hypomyelinating leukodystrophies represent a genetically heterogeneous but clinically overlapping group of heritable disorders. Current management approaches in the care of the patient with a hypomyelinating leukodystrophy include use of serial magnetic resonance imaging (MRI) to establish and monitor hypomyelination, molecular diagnostics to determine a specific etiology, and equally importantly, careful attention to neurologic complications over time. Emerging research in oligodendrocyte biology and neuroradiology with bedside applications may result in the possibility of clinical trials in the near term, yet there are significant gaps in knowledge in disease classification, characterization, and outcome measures in this group of disorders. Here we review the biological background of myelination, the clinical and genetic variability in hypomyelinating leukodystrophies, and the insights that can be obtained from current MRI techniques. In addition, we discuss ongoing research approaches to define potential outcome markers for future clinical trials.

ANN NEUROL 2014;76:5–19

The concept of hypomyelinating disorders was originated by Schiffmann, van der Knaap, and colleagues.[1–3] Among the inherited white matter (WM) disorders, hypomyelinating leukodystrophies (HLDs) are notable for abnormalities in myelin development rather than destruction. This class of disorders is distinguished by their characteristic appearance on magnetic resonance imaging (MRI), namely, lessening or absence of the T_2 hypointensity that typically signifies the presence of myelin, often without the significant lessening of T_1 hyperintensity seen in the other, nonhypomyelinating leukodystrophies. Other MRI features help to narrow the differential diagnosis and focus genetic and metabolic testing.[3]

We are entering a phase of clinical research for HLDs where identification of outcome measures of potential treatment benefit is crucial. In ultrarare diseases, clinical features, and natural history are often

View this article online at wileyonlinelibrary.com. DOI: 10.1002/ana.24194

Received Mar 4, 2014, and in revised form Jun 5, 2014. Accepted for publication Jun 5, 2014.

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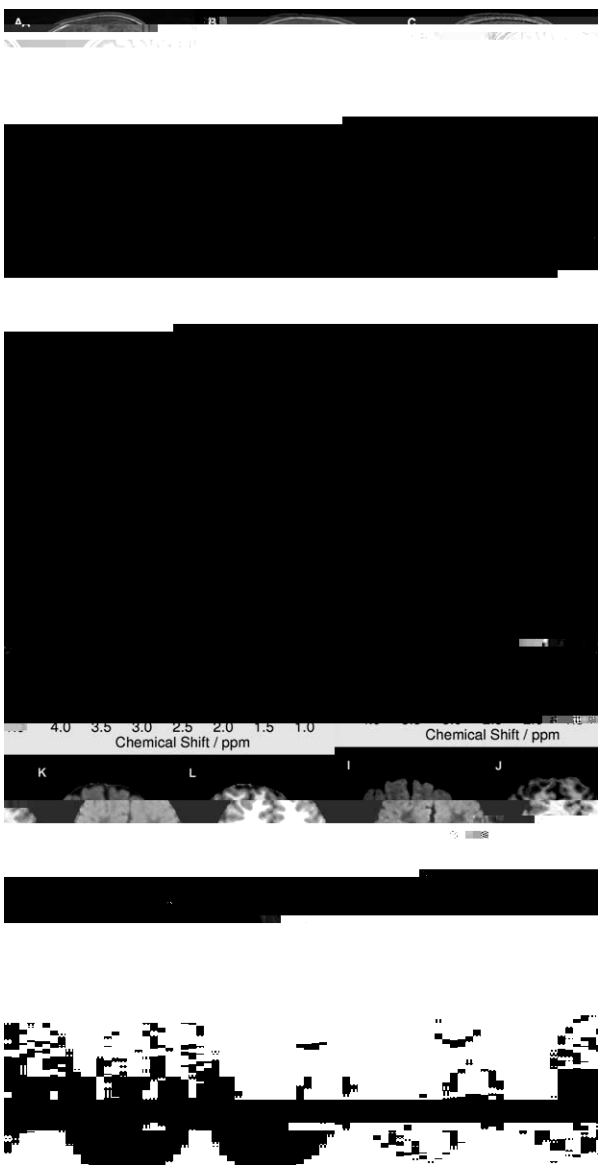
monocarboxylate transporter 1.[12,13] Finally, initiation of myelination and myelin maintenance is regulated by the availability of glycolytic and lipid substrates such as purines, glucose, and lactate.[6,14] OPCs are widespread in the normal adult CNS, where they contribute to myelin repair (eg, in multiple sclerosis[15]) and turnover of myelin within normal WM.[16]

The focus of this review will be the HLDs. As shown in Figure 1, early oligodendrocyte development comes under control by specific transcription factors that promote glial subtype specification of OPCs. Transcription factors Olig2, Sox10, and Nkx2.2 are essential for early stages of OPC development, whereas other transcriptional proteins, including myelin regulatory factor (MyRF), as well as chromatin remodeling and signaling pathways such as integrin and PI3 kinase, coordinate to promote later stages of oligodendrocyte differentiation and myelin remodeling.[17,18] No HLD-causing mutations have been identified in these pathways, perhaps because they are essential in many cell types. The sheath then formed is enriched in myelin-specific lipids and proteins including proteolipid protein 1 (PLP1). Mutations in *1* are known to be causative of Pelizaeus–Merzbacher disease (PMD), a classic example of an HLD. Different types of mutations in *1* may have different impacts on the oligodendrocyte lineage. The most common alteration is duplication of the entire gene, suggesting that gene dosage is essential. Severe missense mutations in *1* trigger the unfolded protein response and cell death of OPCs, preventing myelination, leading to the severe connatal form of the disease.[19] Milder missense mutations and null mutations are associated with milder forms. Although OPCs likely respond to the loss of myelin in HLDs, their intrinsic mutation likely renders them ineffective in repair.



Diagnosis and Management of HLDs

HLDs are characterized by a paucity of myelin development based on histochemistry and MRI criteria. MRI typically shows variable signal (ie, hyper-, hypo-, or isointense) on T_1-weighted imaging and mild hyperintensity on T_2-weighted imaging of the WM compared to gray matter (GM) signal (Fig 2A–F).[2] This is distinct from other leukodystrophies, in which more hypointense

TABLE 1. Hypomyelinating Leukodystrophies, Their Inheritance, and Their Respective Genetic Cause, When Known

Hypomyelinating Disorder	OMIM Number	Abbreviation	Inheritance	Gene
18q- syndrome	601808		Sporadic	*1* -
Cockayne syndrome	216400		AR	
Hypomyelination with atrophy of the basal ganglia and cerebellum	612438	H-ABC	Sporadic	***T*** *4*
Hypomyelination with congenital cataracts	610532	HCC	AR	*12*
Hypomyelination of early myelinated structures		HEMS	X-linked	
Hypomyelination with brainstem and spinal cord involvement and leg spasticity	615281	HBSL	AR	
Free sialic acid storage disease	604369		AR	*1 5*
Fucosidosis	230000		AR	*1*
Pelizaeus–Merzbacher disease	312920	PMD	X-linked	*1*
Pelizaeus-Merzbacher–like disease	608Aof the5n22.2(erzeas14144414g6.979n6s1(my.6(la60769)-5.9(1)-1807.der)-9369			

T_1-weighted and more severely hyperintense T_2-weighted WM imaging signals are seen, usually in a more geographic or localized distribution.

It is also important to differentiate HLDs from neuronal diseases with secondary hypomyelination, which carry an independent differential diagnosis, such as *1*-,[20,21] *1*-,[22] and *1*-related disorders.[23,24] Neuronal diseases with secondary hypomyelination have prominent GM symptoms, such as early onset epilepsy and severe intellectual disability. They commonly present with microcephaly and/or early and severe cerebral atrophy. It is also important to differentiate HLDs from delayed myelination. When a lack of myelin deposition is noticed on an MRI in a child younger than 2 years, a second MRI should be performed at least 6 months later to assess for significantly improved myelination, diagnostic of delayed myelination (in distinction, increase in myelination is not observed in HLDs).

HLDs are genetically and clinically diverse (Table 1), but have commonalities as a group. Most HLD patients present in the neonatal or infantile period with axial hypotonia, which evolves to spastic quadriparesis, and have or will develop nystagmus. Patients with PMD, Pelizaeus-Merzbacher–like disease caused by mutations in *2*,[25] and *10*-related disorders[26] have early onset of congenital nystagmus, whereas patients with hypomyelination, hypodontia, and hypogonadotropic hypogonadism (4H),[27,28] oculodentodigital dysplasia, and 18q- syndrome develop nystagmus later in the course of their disease or never. Cerebellar signs are often present and can be the predominant clinical manifestation, such as in 4H, a RNA polymerase III–related leukodystrophy. Extrapyramidal signs are not uncommon, especially dystonia, but typically occur later in the disease course, with the exception of hypomyelination with atrophy of the basal ganglia and cerebellum (H-ABC), where dystonia is often seen early in the disease. Cognitive function is relatively preserved in most patients but typically declines slowly or relatively late in the disease course. Another possible neurological manifestation of HLDs is the presence of a peripheral neuropathy, which can be seen inconsistently with -null syndrome (one of the

milder PMD forms), Cockayne syndrome, *10*-related disorders, and hypomyelination with congenital cataracts (HCC). A full description of these conditions and the reasons they are included within the HLDs is beyond the

4H, and should be managed with appropriate pharmacotherapy. Scoliosis and hip dislocations are frequent complications, and should be carefully prevented and treated in a timely manner. Swallowing difficulties are present early in severe forms, and in milder forms develop over time. Epilepsy is an infrequent complication of HLDs. If present, appropriate treatment with antiepileptic drugs should be initiated. Specific complications of certain entities among the HLDs include endocrine abnormalities (hypogonadotropic hypogonadism and, less frequently, growth hormone deficiency) in 4H. Endocrine monitoring should be done regularly; treatment decisions should be made on an individual basis. Management of the dental anomalies in 4H includes prosthetic treatment and early detection of cavities.

Myelin Assessment by MRI

In addition to conventional T_1- and T_2-weighted imaging, several advanced MRI techniques might be more appropriate to clinically detect myelin in the human brain. In the following sections, we discuss proton magnetic resonance spectroscopy (MRS), quantitative T_1 and T_2, magnetization transfer imaging (MTI), and diffusion tensor imaging (DTI).

Myelin Assessment by Proton MRS

Proton MRS allows separation of protons in different chemical environments based upon the effects of surrounding electron clouds upon the net strength of the magnetic field felt by the proton (chemical shift) and the influences of neighboring nuclei (spin–spin coupling). Used for decades in analytical chemistry, it has been applied to human diseases as a part of the MRI examination. Recently, MRS has been investigated as a tool in the assessment of metabolic disorders and specifically HLDs.[30–32] However, the spectrum of myelin itself is quite complex, essentially composed of overlapping spectra of the many functional groups that are part of the proteins and complex molecules that are components of myelin.[33] The peaks from most of the protons within these functional groups are split (into doublets, triplets, quadruplets, and more) by adjacent protons and/or have T_2 relaxation times too short to be apparent on in vivo proton spectra.[34] Therefore, the use of in vivo proton MRS in patients with disorders of myelin formation is mainly limited to assessment of the major peaks seen in the human brain: choline, creatine, myo-inositol, gluta-

signatures, allowing more direct assessment of myelin content. Conventionally, this has been accomplished through the acquisition of multiple[32–48] spin-echo T_2 decay data spanning a wide range of echo times (up to 320 milliseconds).[51] Assuming a slow exchange regime with respect to T_2,[52] a non-negative least squares approach is used to fit a semicontinuous log T_2 distribution to the sampled decay data.[51]

[RD]).[83] RD has been interpreted as an indicator of myelin density, based on increased values in mouse models of hypomyelination, the mouse,[84] the transgenic mouse,[85] and the fixed brains of Plp1-transgenic mice.[86] AD has been interpreted as a measure of axonal integrity; it is normal in mice with relatively intact axons, and decreased in mice with reduced axonal caliber.[84,85] The increased RD in poorly myelinated WM has been confirmed in the pup; of all investigated DTI parameters, the relative increase of RD was most prominent, whereas the increase of AD was much smaller, in accordance with paucity of myelin being the most apparent histopathological observation.[87] However, the actual quantification of myelin by means of these parameters has not yet been demonstrated.

It should be noted that the tensor model, although widely used, is limited by the assumption of a Gaussian

DTI changes in the region of the transplant showed increasing FA and decreasing RD, suggestive of engraftment and consistent with the possibility of myelin formation in those regions. Thus, this study encourages later (phase II/III) testing to prove efficacy of these approaches in HLDs.

However, the outcomes of such studies require expert opinion as to surrogate biomarkers and/or clinical measures that can be employed in the proof of efficacy. By definition, ultrarare disorders have no normal baseline and stereotyped natural history, but rather involve a spectrum of outcomes. To enhance the testing of potential clinical interventions for HLDs, surrogate biomarkers should be adopted as primary outcomes, with careful tracking of clinical outcomes as secondary measures. The following section establishes expert opinion on the current knowledge and research of possible endpoints for clinical trials in HLDs.

Exploring Surrogate Clinical Endpoints for HLDs

To define clinical endpoints for ultrarare disorders such as HLDs is challenging. Clinical presentation varies from one HLD to another, and complexity is also conferred by individual/developmental changes in the first years of life. For example, classic PMD patients usually have intellectual disability and a complex neurological syndrome consisting of spasticity, ataxia, and an extrapyramidal movement disorder, which is so severe that walking and even sitting without support are not possible. In contrast, patients with the other common HLD, 4H, usually learn to walk without support before their third year of life and have much better cognitive abilities, their main neurological sign being ataxia.[94,95] Even within a given HLD, the spectrum of severity is wide. For PMD, there is clear genotype–phenotype correlation[96,97]; for 4H and other HLDs, this relationship is less obvious. To make things even more complicated, the majority of patients with HLD slowly deteriorate after a period of clinical stability, probably due to axonal degeneration, mirrored in the global atrophy seen in longitudinal imaging (Fig 4).[98]

Observational studies for most HLDs are lacking. Specifically, there is urgent need to study the utility of standardized assessment tools such as the Gross Motor Function Classification System (GMFCS) and dystonia, dyskinesia, and ataxia scales, as well as the applicability of tests of cognitive function in a population with severe motor handicaps. Besides brainstem auditory responses, which are typically absent in PMD, there is no evidence that neurophysiologic or biochemical tools could be used as surrogate outcome markers in HLDs at this point, but this needs further investigations, especially in newly described HLDs.

FIGURE 4: Global atrophy as shown on longitudinal magnetic resonance imaging. (A, B) Axial T_2-weighted images of an 8-year-old hypomyelination, hypodontia, and hypogonadotropic hypogonadism patient with a homozygous missense mutation in POLR3A. (C, D) severe global atrophy is visible in the same patient at the age of 19 years, manifested both by an increased volume of cerebrospinal fluid spaces and by a decreased intracranial volume (thicker skull).

MRI modalities are currently the most promising surrogate biomarker for clinical trials of HLD, but any improvement seen in MRI surrogates needs correlation with measurable clinical improvement. Of note, MRI modalities that are used as a surrogate biomarker will have to take into consideration any physiologic differences between innate myelination and post-therapeutic remyelination. These may include such considerations as the maturity of myelin wrapping, the hydrophobicity of the myelin membranes, and the size of cytoplasmic channels after myelin wrapping (see Fig 3A). As discussed below, new approaches to quantify myelination may provide the best objective evaluation of effect of a therapeutic trial.

Exploring Basic and Translational Research to Develop Endpoints for HLDs

New Approaches to Augment Capability of MRI in Myelin Detection in Humans

The sections above have highlighted scientific opportunities for glial biology and MRI technology to come

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