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.

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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

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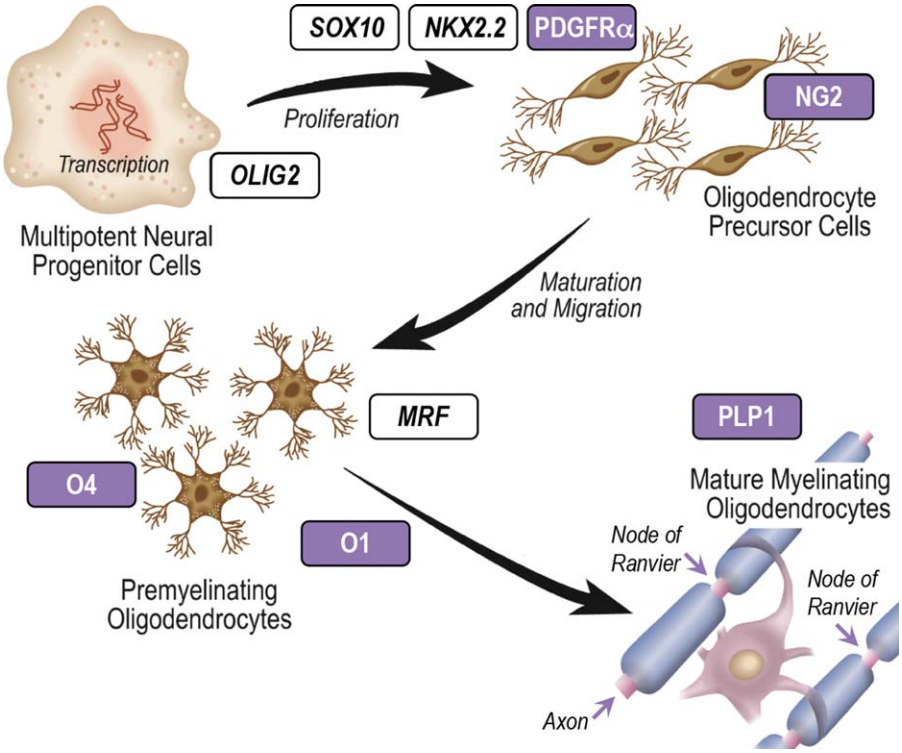

FIGURE 1: Overview of oligodendrocyte lineage development. Oligodendrocyte development commences in the midgestation mammalian embryo. Multipotent neural progenitor cells competent to produce oligodendrocytes express the transcription factor OLIG2, and cell intrinsic and environmental interactions with other transcription factors (eg, SOX10, NKX2.2) lead to specification of oligodendrocyte precursor cells (OPCs) marked by expression of platelet-derived growth factor receptor-α (PDGFRα) and neuron-glial antigen 2 (NG2). Such OPCs proliferate after specification, migrate throughout the central nervous system parenchyma, and differentiate under control of myelin regulatory factor (MRF) and other intrinsic programs. In white matter, premyelinating oligodendrocytes engage with axons, and it is thought that physical and activity-dependent cues trigger myelin wrapping, marked initially by transition from $O4^+$ to $O1^+$ stages, and eventually expression of proteolipid protein 1 (PLP1) and other mature markers. One oligodendrocyte can provide hundreds of myelin segments to adjacent axons. Myelination enables formation of nodes of Ranvier, which cluster sodium channels for saltatory conduction. Myelination is also thought to stabilize axonal membranes, which results in physical properties detectable by magnetic resonance imaging. Transcription factors are indicated by unfilled boxes and cellular markers by violet/filled boxes.

poorly known; therefore, clinical/neurological parameters alone are insufficient as endpoints for clinical efficacy studies. In this case, another possible acceptable clinical trial design includes the use of biomarkers as surrogate endpoints. The adoption of criteria for biomarkers of efficacy is an important feasibility step for the planning and execution of clinical studies because it provides an objective endpoint at a defined period of time after an intervention has been initiated. Proposing a standard measure of myelination based upon magnetic resonance (MR) metrics as a reliable biomarker could therefore greatly encourage clinical research.

With these challenges in mind, we organized a multidisciplinary group to address clinical and future research priorities for HLDs. The group makes consensus recommendations for MRI and neurological assessments in clinical care, endpoints for clinical research, and potential methods to detect myelin in the human brain with greater specificity and sensitivity.

Biological Basis of Myelination and HLDs

Oligodendrocytes are the myelinating cells of the central nervous system (CNS; Fig 1). Myelin enables rapid transmission of action potentials through saltatory conduction,[4,5] provides trophic support and protection for axons,[6–8] and allows for packing of greater axon densities during the evolution of brain complexity.[9] Almost half of the human brain is comprised of tracts of myelinated axons in WM, and it is therefore not surprising that leukodystrophies, caused by deficient deposition or destruction of myelin, have significant functional effects.

Myelination is an energy-expensive process, as developing oligodendrocyte precursor cells (OPCs) undergo as much as a 6,500-fold increase in membrane surface area as they differentiate into oligodendrocytes that provide up to ~100 myelin segments on multiple axons through extension of protoplasmic processes.[10,11] Mature oligodendrocytes also provide ongoing trophic support to axons, in part through functions of the

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 *PLP1* are known to be causative of Pelizaeus–Merzbacher disease (PMD), a classic example of an HLD. Different types of mutations in *PLP1* 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 *PLP1* 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

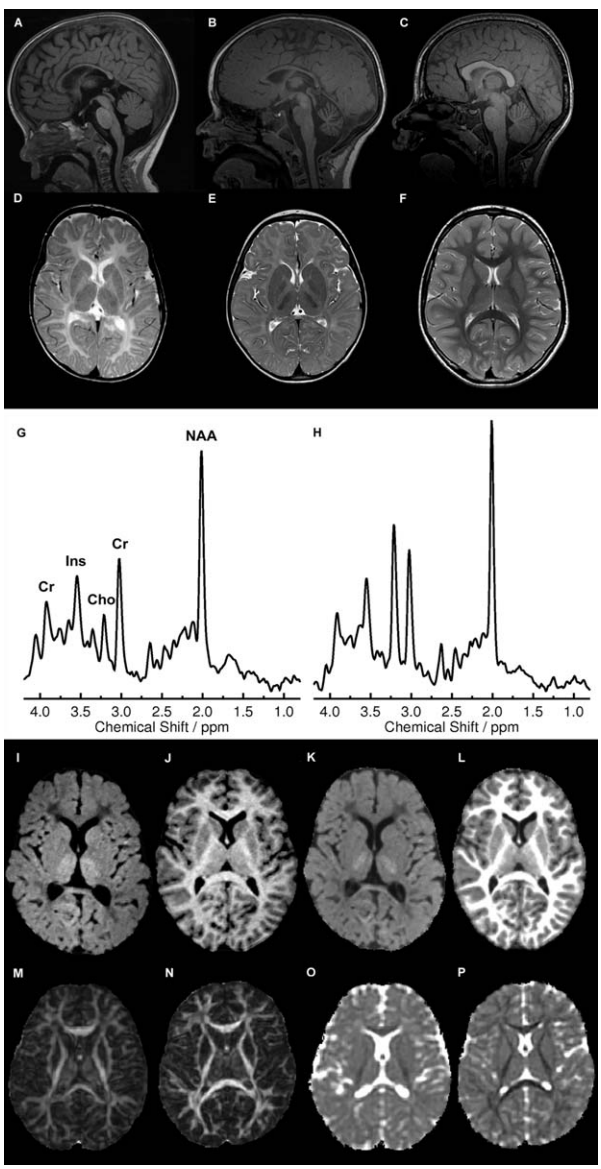

FIGURE 2: Magnetic resonance imaging (MRI) of hypomyelinating leukodystrophy patients in comparison to subjects with normal MRI. (A–C) Sagittal T_1-weighted images show hypointense signal of corpus callosum in (A) a 4-year-old Pelizaeus–Merzbacher disease (PMD) patient and (B) additional cerebellar atrophy in a 2-year-old hypomyelination, hypodontia, and hypogonadotropic hypogonadism (4H) patient, in comparison to (C) a 5-year-old control subject. (D–F) Axial T_2-weighted images show homogeneous T_2 hyperintensity of white matter in (D) a 4-year-old PMD patient and similar white matter hyperintensity in addition to hypointensity of the globi pallidi and lateral thalami in (E) a 2-year-old 4H patient in comparison to (F) a 5-year-old control subject. (G, H) Magnetic resonance spectrum of white matter in (G) a 17-year-old 4H patient shows reduced choline (Cho) in comparison to (H) a 5-year-old control subject. (I, J) Magnetization transfer (MT) ratio maps and (K, L) MT saturation maps show severe global reduction of MT values (manifested as decreased hyperintensity) in (I, K) a 6-year-old 4H patient compared to (J, L) a 6-year-old control subject. (M, N) Fractional anisotropy (FA) maps and (O, P) radial diffusivity (RD) maps show a reduction of FA (manifested as decreased white matter hyperintensity in M compared with N) and an increase of RD (manifested as less white matter hypointensity in O compared with P) in a 4-year-old 4H patient (M, O) in comparison to a 7-year-old control subject (N, P). The images of each pair I-J, K-L, M-N, and O-P are shown at the same intensity scale. Cr = creatine; Ins = inositol; NAA = N-acetylaspartate.

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

Hypomyelinating Disorder	OMIM Number	Abbreviation	Inheritance	Gene
18q- syndrome	601808		Sporadic	*18q-*
Cockayne syndrome	216400		AR	*ERCC6*, *ERCC8*
Hypomyelination with atrophy of the basal ganglia and cerebellum	612438	H-ABC	Sporadic	*TUBB4A*
Hypomyelination with congenital cataracts	610532	HCC	AR	*FAM126A*
Hypomyelination of early myelinated structures		HEMS	X-linked	*unknown*
Hypomyelination with brainstem and spinal cord involvement and leg spasticity	615281	HBSL	AR	*DARS*
Free sialic acid storage disease	604369		AR	*SLC17A5*
Fucosidosis	230000		AR	*FUCA1*
Pelizaeus–Merzbacher disease	312920	PMD	X-linked	*PLP1*
Pelizaeus-Merzbacher–like disease	608804	PMLD	AR	*GJC2*
Pol III-related leukodystrophies/4H	607694, 614381	4H	AR	*POLR3A*, *POLR3B*
Oculodentodigital dysplasia	164200	ODDD	AD, sporadic	*GJA1*
RARS-associated hypomyelination, *SOX10*-associated disorders	609136		AR, AD, sporadic	*RARS*, *SOX10*
Trichothiodystrophy with hypersensitivity to sunlight	601675		AR	*ERCC2, ERCC3, GTF2H5, MPLKIP*

4H = hypomyelination, hypodontia, and hypogonadotropic hypogonadism; AD = autosomal dominant; AR = autosomal recessive; OMIM = Online Mendelian Inheritance in Man database.

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 *AGC1*-,[20,21] *HSPD1*-,[22] and *AIMP1*-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 *GJC2*,[25] and *SOX10*-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 *PLP*-null syndrome (one of the

TABLE 2. MRI Characteristics and the Diagnoses They Suggest

MRI Characteristic	Suggests
Diffuse and homogeneous hypomyelination	PMD
Diffuse and homogeneous hypomyelination with hypomyelination of the brainstem, especially the pons	PMLD
Hypomyelination with areas of increased T_2 signal and decreased T_1 signal	HCC, 18q- syndrome, HBSL
Relative T_2 hypointensity of the dentate nuclei, optic radiation, globi pallidi, anterolateral nuclei of the thalami, corticospinal tract at the level of the PLIC	Pol III/4H
Hypomyelination of early myelinating structures	HEMS
Calcifications	Cockayne syndrome, AGS[a]
Thin corpus callosum	Pol III/4H, fucosidosis, Cockayne syndrome, sialic acid storage disease
Cerebellar atrophy	Pol III/4H, sialic acid storage disease, H-ABC, ODDD, Cockayne syndrome, fucosidosis, 18q- syndrome (hypoplasia)
Basal ganglia abnormalities	Atrophy (especially putamen), H-ABC; hyper T_1 and hypo T_2 of GP, fucosidosis; hypo T_2 of GP, Pol III/4H, ODDD
Prominent cerebral atrophy	Infantile sialic acid storage disease, AGS[a]

4H = hypomyelination, hypodontia, and hypogonadotropic hypogonadism; AGS = Aicardi–Goutières syndrome; GP = globus pallidus; H-ABC = hypomyelination with atrophy of the basal ganglia and the cerebellum; HBSL = hypomyelination with brainstem and spinal cord involvement and leg spasticity; HCC = hypomyelination with congenital cataracts; HEMS = hypomyelination of early myelinating structures; hyper = hyperintensity; hypo = hypointensity; MRI = magnetic resonance imaging; ODD-D = oculodentodigital dysplasia; PLIC = posterior limb of the internal capsule; PMD = Pelizaeus–Merzbacher disease; PMLD = PMD-like; Pol III = Pol III–related leukodystrophies.
[a]AGS may present with a hypomyelinating pattern on MRI.

milder PMD forms), Cockayne syndrome, *SOX10*-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 scope of this review and described elsewhere.[29]

Some HLDs can present with an adult onset hereditary spastic paraparesis phenotype. In addition to progressive spastic paraplegia, dysarthria, dysphagia, and later cognitive decline are frequent in adulthood. The late onset HLD phenotypes have much milder MRI WM abnormalities. In 4H, some affected patients present in late adolescence or early adulthood with hypomyelination on MRI that is not well correlated with the disease severity. A late presentation is often associated with milder clinical symptoms and slower deterioration. A spectrum with more severe infantile onset cases on one end and milder adolescent or adult onset variants at the other extreme will probably be part of most if not all HLDs.

In the diagnosis of a HLD, MRI pattern recognition is very useful (Table 2),[3] but non-neurological features, when present, can help the clinician in suspecting one disorder versus another (Table 3). For example, hypodontia and delayed puberty point to 4H, whereas the presence of cataracts suggests HCC. Overall, it is important to note that these systemic manifestations are inconstant and may not be present in all affected individuals.

Molecular diagnosis has become the mainstay in diagnosis of hypomyelinating conditions, and a targeted number of genes specific to hypomyelination should be tested. A definitive molecular diagnosis will aid the clinician with management, prognosis, and genetic counseling.

Management of patients with HLDs will largely depend on the specific diagnosis and individual severity of the disease. Typical complications in patients with the classic form of PMD include severe spasticity necessitating oral or intrathecal treatment with baclofen, chemodenervation, or selective dorsal rhizotomy. Dystonia is seen more frequently in certain HLDs such as H-ABC and

TABLE 3. Useful Non-Neurological Clinical Features to Orient the Diagnosis in Hypomyelinating Leukodystrophies

Clinical Characteristics	Suggests
Dysmorphic signs	18q- syndrome
Facial coarsening	Sialic acid storage disease, fucosidosis
Cataracts	HCC, Cockayne syndrome, Pol III/4H (rarely)
Myopia, typically progressive and severe	Pol III/4H
Hearing loss	Cockayne syndrome, *SOX-10*
Dental abnormalities	Pol III/4H[a]; Cockayne syndrome, propensity to cavities; ODDD, enamel hypoplasia
Skin abnormalities	Cockayne syndrome, hypersensitivity to sunlight; fucosidosis, angiokeratoma corporis diffusum
Hand and feet abnormalities	ODDD
Cardiac abnormalities	18q- syndrome; fucosidosis, cardiomegaly
Hepatosplenomegaly	Sialic acid storage disease, fucosidosis
Endocrine abnormalities	18q- syndrome, Pol III/4H (delayed or absent puberty)

[a]The dental abnormalities encountered in Pol III–related leukodystrophies are not universal and are highly variable: oligodontia, hypodontia, delayed teeth eruption, abnormal sequence of teeth eruption, and abnormal color or shape of sometimes 1 but typically several teeth.
4H = hypomyelination, hypodontia, and hypogonadotropic hypogonadism; HCC = hypomyelination with congenital cataracts; ODDD = oculodentodigital dysplasia; Pol III = Pol III–related leukodystrophies.

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, glutamate, glutamine, and N-acetylaspartate (NAA; see Fig 2G, H). None of these peaks has been shown to directly reflect the presence or quantity of myelin in the

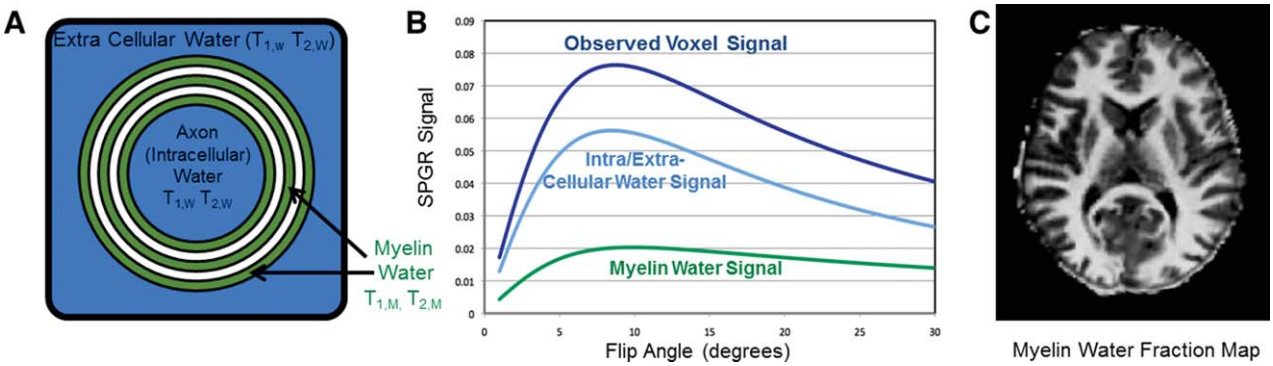

FIGURE 3: Background of myelin water fraction determination. (A) Because of more interactions with surrounding molecules and electrical/magnetic variations, myelin water has shorter relaxation times ($T_{1,m}$ and $T_{2,m}$) than intra-/extracellular water ($T_{1,w}$ and $T_{2,w}$). (B) The signal intensity per voxel is a combination of myelin water and intra-/extracellular water, as shown here for T_1-weighted spoiled gradient recalled (SPGR) as a function of flip angle. (C) Additional T_1/T_2-weighted sequences allow for the estimation of a myelin water fraction map.[62]

developing brain, although combinations of peak heights can be used to roughly monitor normal brain development.[35–37]

Some authors have studied proton MRS in HLDs and compared them with other leukodystrophies.[38] They found high creatine and myo-inositol levels and low choline levels compared to controls.[38] Takanashi et al have studied the use of proton MRS in 2 mouse models of HLDs, the *msd* mouse (a model of connatal PMD)[31] and the *shiverer* (*Shi*) mouse (deficient for myelin basic protein),[39] and found very different proton MRS patterns. NAA, which is normally metabolized to acetate and aspartate in oligodendrocytes,[40] is elevated in the classic form of PMD.[30,32] The most likely reason for this is that the PLP1 proteins in PMD patients are abnormally folded and accumulated in the endoplasmic reticulum, resulting in the activation of an unfolded protein response that finally leads to oligodendrocyte apoptotic death before normal myelination occurs, leading to higher NAA levels.[41] In contrast, NAA is low in *Shi* mice, presumably because the number of cortical neurons is decreased[39] for reasons that are not currently understood. Choline may be reduced in HLDs, as cultured oligodendrocytes have a higher concentration of choline than neurons, astrocytes, or oligodendrocyte precursors.[42] However, at this time, it does not seem that MRS will be useful by itself to quantify myelination.

Myelin Assessment by Quantitative T_1 and T_2

Much of the exquisite soft tissue contrast we have come to expect from MRI arises from differences in the intrinsic T_1 and T_2 relaxation properties. In their most basic description, both T_1 and T_2 relaxation processes result from molecular motion and interactions, which are influenced by the biophysical structure and biochemical environment.[43] In particular, characteristics such as density (ie, water content/mobility); macromolecule, protein, and lipid composition; and paramagnetic atom (eg, iron) concentrations alter the local tissue environment, and consequently affect tissue T_1 and T_2. In WM, for example, the phospholipid-rich myelin sheath and associated proteins, cholesterol, iron-containing oligodendrocytes and glial cells, and reduced free water content all result in decreased T_1 and T_2 relative to GM.[44] These relaxation time differences are readily apparent in the GM/WM contrast in conventional T_1- and T_2-weighted images of adult brain, and onset or alteration of this contrast has been used as a qualitative measure of myelin changes. For example, the emergence of GM/WM contrast in the developing brain, associated with drastic reductions in the T_1 and T_2 relaxation times, broadly parallels the histological timeline of myelination.[35] Similarly, a change of contrast driven by prolonged T_1 and T_2 in WM disorders, such as multiple sclerosis, can primarily reflect focal myelin loss.[45]

Relaxation properties are extremely sensitive to local microstructural and biochemical changes in WM, but they are nonspecific and can reflect developing myelination, differing concentrations of iron (ferritin),[46] water content changes associated with fiber density and diameter, or pathological processes such as edema and inflammation.[47] In an effort to improve myelin sensitivity and specificity, a multiple-component approach has been proposed.[48–50] Here, the T_1 and/or T_2 signal curves observed within an imaging voxel are assumed to be a composite mixture from multiple distinct microanatomical tissue environments that, through their unique microstructure and biochemistry, have differing T_1 and T_2 characteristics and thus distinct MRI signal signatures (Fig 3A, B). The aim of multiple component relaxation analysis is to decompose and quantify these individual

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] Results of this fitting have consistently revealed a bi- or trimodal T_2 profile, with a short T_2 peak ($T_2 < 30$ milliseconds), moderate T_2 peak ($60 < T_2 < 150$ milliseconds), and a long T_2 peak ($T_2 > 2$ seconds).[52] Through imaging–histology comparisons[53,54] and in vivo studies of demyelinating disorders,[55] these peaks have been ascribed to 3 layers: water trapped between the lipid myelin bilayers; the less restricted intra- and extracellular water; and cerebrospinal fluid, respectively.[51,52] The volume ratio of the short T_2 peak to the total area under the T_2 distribution, termed the *myelin water fraction* (MWF), has strong correlation with histological assessments of myelin content,[51,52] irrespective of inflammation or changes in water content.[56] Recently, similar multicomponent T1 relaxation has been demonstrated at ultrahigh field strengths.[57] Thus, MWF has the potential to be a better quantitative marker of myelin than measures derived from MTI or DTI.[58,59] However, MWF has not yet gained entrance into routine clinical MRI because of limited spatial coverage, time requirements, and frequent technical difficulties that result in stimulated echo artifacts.

Due to the lengthy acquisition times required, recent work has centered on the development of 3-dimensional multicomponent relaxation approaches, such as rapid spiral imaging,[60] or alternative non–spin echo based imaging methodologies (ie, GRASE[61] or mcDESPOT[62]). These newer methods have the potential to overcome some of the difficulties in integrating MWF into standard clinical protocols (see Fig 3C), but need further validation.[63] They have already provided preliminary new insight into brain development and myelination,[64] as well as myelin changes that reflect disease course and severity in other WM disorders.[65,66]

Myelin Assessment by MTI

MTI targets magnetization exchange between immobile macromolecular protons (bound pool), such as those bound in proteins and lipid bilayers of myelin, and free water protons (free pool). Magnetization transfer (MT) is evoked by selectively saturating the bound pool with an off-resonance radiofrequency pulse. This saturation is then transferred to the free pool and observed as an attenuation of the MR signal.[67] In WM the MT effect is overproportionately determined by MWF,[68] suggesting that MT characteristics mainly relate to myelin.

Quantitative MT methods adopt complex modeling of the 2 pools, allowing mapping of multiple parameters with the bound pool fraction f being most relevant.[69,70] The bound pool is about 2 to 3× larger in WM (11%) than in GM (4%), small in blood (2%), and absent in cerebrospinal fluid.[71] In demyelination, strong correlations of f and magnetization transfer ratio (MTR) with histologically quantified myelin density have been detected in animal models[72,73] and in human postmortem multiple sclerosis studies.[74,75] However, bovine brain imaging and imaging–histology correlations have suggested that nonmyelin WM, containing macromolecules such as axonal membranes and glial cells, also modulate MT measures.[75–77] Moreover, general WM pathological changes in demyelinating diseases have been shown to influence and complicate MT measures of myelin.[74,76,78]

The utility of quantitative MT (qMT) parameters has benefited greatly from studies in hypomyelinating conditions such as those in *shaking* (*Sh*) pups, a canine model with a *PLP* gene mutation,[79] with f being most discriminative. Because axons are preserved in *Sh* pups, the results indicate a high correlation to normal myelination. Similar results have been obtained with qMT in hypomyelinating *Shi* mice.[80,81]

Not surprisingly, MTR has been found significantly decreased in childhood HLDs.[38] MT saturation maps in various HLDs in children revealed an overall decrease with distinct spatial distributions of higher MT saturation values, reflecting lack of myelin, and characteristic regions containing more myelin in accordance with histopathological findings (see Fig 2I–L).[82] The sensitivity to changes in myelination has rendered MT saturation a suitable parameter to monitor treatment effects, as demonstrated in cerebral folate transporter α deficiency with hypomyelination. In preliminary studies, folinic acid treatment may improve/normalize MT saturation values (Dreha-Kulaczewski, unpublished observations).

Myelin Assessment by DTI

DTI can be used to assess the magnitude and direction of water motion in a volume of tissue. If small volumes are imaged at high resolution, this technique gives insight into the microstructure of WM. Parallel myelinated axons give rise to a high fractional anisotropy (FA), indicating the direction of overall motion of water molecules in the sampled voxel. In WM, this direction is determined by the largest eigenvalue of the diffusion tensor, representing diffusivity parallel to the axons (axial diffusivity [AD]), and by the 2 lower eigenvalues, representing diffusivity perpendicular to the axons (radial diffusivity

[RD]).[83] RD has been interpreted as an indicator of myelin density, based on increased values in mouse models of hypomyelination, the *Shi* mouse,[84] the transgenic *hcv1-tk* 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 *Shi* mice with relatively intact axons, and decreased in *hcv1-tk* mice with reduced axonal caliber.[84,85] The increased RD in poorly myelinated WM has been confirmed in the *Sh* 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 distribution of water diffusion, and DTI parameters are sensitive to partial volume averaging, because the size of the voxels being assessed is much larger than that of the crossing fiber tracts.[88] To overcome these limitations, high b-value diffusion imaging was additionally applied in the abovementioned *Shi* pup study.[87] Results showed that the zero-displacement probability P_0, which is obtained from high b-value diffusion imaging and which describes the degree of water restriction in tissue, was similarly sensitive to the paucity of myelin as the DTI parameter RD, which is more widely available on clinical scanners.

Human DTI studies comparing FA, AD, and RD parameters indicate RD to be the most sensitive in distinguishing HLD patients from controls (see Fig 2M–P). A case study of a family with a unique mutation of PMD has described increases in the lowest eigenvalues,[89] and a preliminary study in a larger group of patients with several HLDs also identified RD as the most relevant DTI parameter (Steenweg et al, unpublished).

Although DTI parameters are indicative of myelin content, they are sensitive to changes in myelination, and thus may serve to detect treatment effects. With the *hsv-tk1* mouse model, which has a phenotype with a transient, reversible hypomyelination,[85] strongly elevated RD was observed at 2 weeks of age, which normalized in the following weeks with myelination. Another study performed high-field ex vivo DTI to follow transplantation of immunodeficient *Shi* mice with human neural stem cells (NSCs).[90] The study did not determine AD and RD separately, but an increase of FA was observed in those areas that also showed myelination on histopathology. A recent human phase I study evaluated safety and evidence of myelin formation after transplantation of human CNS stem cells in 4 subjects with an early onset severe form of PMD.[91] DTI showed an increase of FA and decrease of RD consistent with myelination, in the region of transplantation compared to control white matter regions remote to the transplant sites.

Currently, these techniques all have roles in assessing hypomyelination. MRS shows specific findings in some disorders, but none that is applicable to the entire group of HLDs; it does not allow myelin quantification. MTI is quantitative, and its results are clearly associated with myelin presence, but it is not specific for the binding of water to myelin and therefore will never be precisely quantitative for myelin. DTI is very sensitive to changes in water motion associated with myelination, but the changes in water motion are not specifically caused by myelin; therefore, although very characteristic diffusion changes are caused by myelination, observation of those changes cannot be absolutely interpreted as being the result of myelin, nor can they be used to quantify myelin. Diffusion techniques are likely to improve substantially with technical improvements. MWF, although currently still limited by lack of spatial coverage, time requirements, and frequent technical difficulties that result in artifacts, shows promise for quantitative myelin analysis with technical improvements.

Emerging Therapies in HLDs

Despite current limitations of MRS, DTI, and MTI in quantifying myelin, these MRI techniques, or a combination thereof, will become particularly important when therapies become available for different HLDs. Because OPCs are defective in HLDs, and the following considerations, cell-based therapies have emerged as candidates for therapeutic research. In preclinical studies, biological properties of OPCs suitable for transplantation include self-renewal and migration.[92] Second, NSCs and OPCs can engraft successfully in mouse models of hypomyelination, conferring functional properties of myelin, such as an enhanced conduction velocity to host axons.[90] A final attribute making HLD a focus of clinical studies is that MRI might comprise a noninvasive method for identifying functional engraftment of myelinating cells in the grossly hypomyelinated background of the host/patient brain.

For these reasons, a 2007 National Multiple Sclerosis Society workshop proposed PMD as a proof of concept disorder for cell-based therapies to restore myelin.[93] These considerations promoted a phase I clinical study of allogeneic NSC transplantation in 4 patients with connatal PMD who were followed clinically and with frequent MRI.[91] These studies showed that the procedure, the immunosuppression, and the transplanted cells themselves were safe 1 year after the transplant. Moreover,

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.

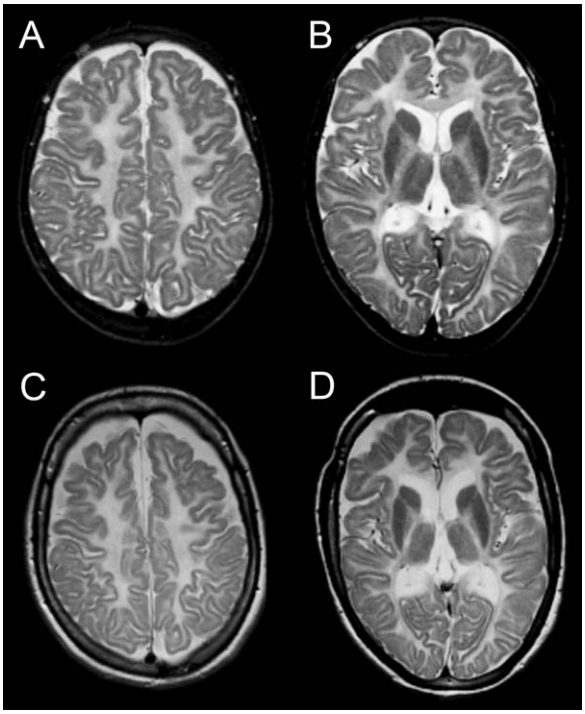

FIGURE 4: Global atrophy as shown on longitudinal magnetic resonance imaging. (A, B) Axial T_2-weighted images of an 8-year-old hypomyelination, hypodontia, and hypogonadotropic hypogonadism patient with a homozygous missense mutation in *POLR3A*. (C, D) severe global atrophy is visible in the same patient 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

together in characterization of myelin disorders. As described above, although MRI techniques do not directly quantify myelin, semiempirical or empirical proton MR models may have promise. For example, several small animal transgenic mouse models exist with hypomyelination and hypermyelination that could be used to "train" MRI methods for greater fidelity. After imaging, the tissue sample or animal would be analyzed for myelin quantity (the "myelin score") by histology, electron microscopy, and myelin G-ratios (the ratio of axon to myelin circumference).[99] A weighted combination of MR parameters can be selected that best explain the experimentally determined myelin quantities, a process that needs iterative validation. It would be essential to also study animals or samples with inflammatory infiltrates to ensure that a differentiation could be made based on specific parameters (for example, FA, MWF, and MT would be expected to be increased and RD to be decreased with myelination as compared to inflammation). Others have developed myelin-specific gadolinium-complexed MR contrast agents that appear to bind myelin with high specificity and may become clinically useful if they can be modified to cross the blood–brain barrier after intravenous infusion (current compounds require intraventricular infusion).[100] If the compounds bind to myelin in a consistent manner, quantification would potentially be obtainable by relaxometry alone. The goal of these studies would be to refine MRI power to identify myelin quantity in vivo.

Several centers have adopted the use of positron emission tomography in clinical studies of multiple sclerosis patients. These studies have used ^{11}C-labeled N-methyl-4,4′-diaminostilbene[101] to demonstrate accumulation of the compound in myelinating structures in developing animal models, disappearance of the tracer after autoimmune demyelination, and reappearance after remyelination.[102,103] This compound readily crosses the blood–brain barrier and appears to be highly specific for myelinated regions.[101] One disadvantage is exposure of the brain to (relatively low levels of) radiation.

Finally, in vitro systems of myelination are helping to define processes that regulate myelin initiation and wrapping.[104] Dual mode imaging incorporating light and/or fluorescent microscopy with simultaneous MRI is envisaged as a way that basic glial biology and MR science might come together to enhance our capabilities for sensitive and specific detection of myelin by MRI.

Patient-Specific Models and Potential Therapies for HLDs

The past decade has seen a revolution in stem cell biology that has resulted in new and promising techniques for investigation of human disease and patient-specific modeling. Embryonic stem cells (ESCs) are defined by their capacity for self-renewal and totipotency, and mouse and human ESCs can be differentiated into oligodendrocytelike precursor cells.[100,105,106]

Findings in the past decade have shattered the dogma that differentiation is a one-way process; it is possible to convert fibroblasts back into pluripotent cells.[107,108] Such induced pluripotent stem cells (iPSCs) can be converted into OPC-like progenitors for studies of myelin biology in vitro[100] and rescue of the *Shi* mouse in vivo.[109] Direct conversion of fibroblasts into OPC-like cells, bypassing the iPSC stage, has recently been demonstrated in rodents.[110,111] Thus, many avenues are available for direct production of OPC-like progenitor cells derived from patient material for in vitro and in vivo studies to gain new insights into the nature of pathogenic mutations and therapeutic approaches in HLDs.

New technologies for site-specific mutations using TALEN[112] and CRISPR[113,114] technologies make it feasible to create targeted patient-specific mutations to study effects on oligodendrocyte biology, such as engineering patient-specific *PLP1* mutations into ESCs to elucidate their impact on oligodendrocyte maturation, survival, and myelination. Another exciting opportunity may be the reversal of patient-specific mutations through gene correction. Future cell-based therapies for HLDs might proceed from production of iPSCs, gene correction, production of OPCs, and eventual autologous transplantation. However, use of such applications in the brain is likely years away because of safety concerns (eg, potential for oncogenic transformation).

Conclusion

We anticipate that new clinical trials (pharmacological and cell-based therapies) will be forthcoming in the next several decades for HLDs. In ultrarare disorders, clinical trials require biomarker surrogates of efficacy and might be eligible for US Food and Drug Administration Fast Track status. MRI is commonly used in diagnosis of all myelin disorders including HLDs, and is currently the most promising biomarker. We believe it is of utmost importance to prepare for these clinical trials with natural history studies and collection of clinical MRIs. We therefore recommend careful and systematic collection of clinical data via consortiums such as GLIA (Global Leukodystrophy Initiative) and LeukoNet, as well as standardized minimal clinical MRI sequences, including at least DTI, MTI, and combined T_1 and T_2 relaxometry.

Due to the variable clinical presentation of the HLDs, neurological parameters, which can be used as

secondary endpoints, are more difficult to define. Reliable parameters include GMFCS and the appropriate rating scales for spasticity, ataxia, and dystonia. Visually evoked potentials or brainstem auditory evoked potentials might prove to be other objective and easy to use parameters, although they have yet to be validated.

Regarding primary endpoints to be collected in clinical trials, we recommend the following MRI techniques: conventional imaging, MTI (for MTR and MT saturation), DTI (especially for RD and FA), combined T_1 and T_2 relaxometry (for MWF), and proton MRS. Despite the promise of MRI, current individual techniques are not optimized for specific detection of myelin. We propose a multidisciplinary effort to enhance MRI sensitivity using new experimental genetic and humanized animal systems.

Systematic collection of clinical, electrophysiological, and MRI data collection in HLDs in natural history studies will allow us to determine which findings correlate best with MRI characteristics and could therefore represent strong secondary endpoints for future therapeutic trials.

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Authorship

P.J.W.P., A.V., G.B., and N.I.W and AJB. wrote the article and created figures. S.F.D.-K., P.J.W.P., and S.C.L.D. provided expertise regarding MRI modalities. E.B., A.K., W.R., and C.f.-C. provided expertise in myelin biology. A.V., G.B., N.I.W., and W.K. provided expertise regarding the clinical outcomes and classification of hypomyelinating conditions. W.K., D.R., and A.J.B. provided overall revision and coordination of the manuscript. P.J.W.P., A.V., G.B., and N.I.W. share the role of first author for this article. W.K., D.R., and A.J.B. share the role of senior author for this article.

Potential Conflicts of Interest

G.B.: speaking fees, Actelion Pharmaceuticals, Shire, Genzyme; board membership, Actelion Pharmaceuticals, Shire; NPC registry, Actelion Pharmaceuticals. A.K.: personal fees, BioMarin Pharmaceutical.

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