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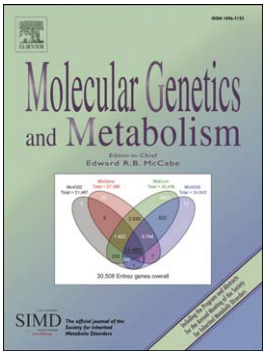

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A clinical approach to the diagnosis of patients with leukodystrophies and genetic leukoencephelopathies

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Abstract

Leukodystrophies (LD) and genetic leukoencephalopathies (gLE) are disorders that result in white matter abnormalities in the central nervous system (CNS). Magnetic resonance (MR) imaging (MRI) has dramatically improved and systematized the diagnosis of LDs and gLEs, and in combination with specific clinical features, such as Addison's disease in Adrenoleukodystrophy or hypodontia in Pol-III related or 4H leukodystrophy, can often resolve a case with a minimum of testing. The diagnostic odyssey for the majority LD and gLE patients, however, remains extensive – many patients will wait nearly a decade for a definitive diagnosis and at least half will remain unresolved. The combination of MRI, careful clinical evaluation and next generation genetic sequencing holds promise for both expediting the diagnostic process and dramatically reducing the number of unresolved cases. Here we present a workflow detailing the Global Leukodystrophy Initiative (GLIA) consensus recommendations for an approach to clinical diagnosis, including salient clinical features suggesting a specific diagnosis, neuroimaging features and molecular genetic testing. We also discuss recommendations on the use of broad-spectrum next-generation sequencing in instances of ambiguous MRI or clinical findings. We conclude with a proposal for systematic trials of genome-wide agnostic testing as a first line diagnostic in LDs and gLEs given the increasing number of genes associated with these disorders.

***Key words**:* Leukodystrophy, Glia, Myelin,

Abbreviations: LD – Leukodystrophies; gLE – Genetic leukoencephalopathy; MR – Magnetic resonance; MRI – Magnetic resonance imaging; GLIA – Global Leukodystrophy Initiative; CNS – Central nervous system; SIMD – Society for Inherited Metabolic Disorders; VWM – Vanishing white matter disease; X-ALD – X-linked Adrenoleukodystrophy; AMN – Adrenomyeloneuropathy; 4H syndrome **-** Hypomyelination, hypodontia and hypogonadotropic hypogonadism syndrome; AGS - Aicardi-Goutières Syndrome; HCC - hypomyelination with congenital cataracts; CTX - Cerebrotendinous xanthomatosis; PMD - Pelizaeus Merzbacher disease; PMLD - Pelizaeus Merzbacher like-disease; SLS -Sjögren-Larsson syndrome; CRMCC - Cerebroretinal microangiopathy with calcifications and cysts; Pol III – Polymerase III; CMV - congenital cytomegalovirus; Tay syndrome – trichothiodystrophy; MLD – Metachromatic Leukodystrophy; FLAIR - fluid-attenuated inversion-recovery; MRS –Magnetic resonance spectroscopy; CT – Computed tomography; NAA – N-acetyl aspartate; ADEM – Acute disseminated encephalomyelitis; NGS - Next-generation sequencing ; WES – Whole exome sequencing; WGS – Whole genome sequencing; P – Pathogenic; LP – Likely pathogenic; CSF - cerebrospinal fluid;

Contents:

1. Introduction and needs assessment

Leukodystrophies (LD) are genetic disorders affecting the white matter of the central nervous system (CNS) with or without peripheral nervous system involvement [1, 2]. There are over thirty conditions typically categorized as primary LD and a number of other heritable conditions (genetic leukoencephalopathies- abbreviated here as gLE) that affect the white matter of the brain [2]. Primary LDs are those heritable conditions of the white matter that primarily effect glial cells, while gLE are disorders with either primary neuronal, vascular or systemic involvement, in which the white matter changes are felt to be secondary [2]. Depending on the etiology of the LD or gLE, inheritance can be by any known mechanism: autosomal recessive, (*de novo*) dominant, X-linked, mitochondrially-encoded etc. These disorders include neonatal and adult presentations as well as the full spectrum through childhood and adolescence. Although individual features may vary, LDs and gLEs all share white matter abnormalities on imaging or pathology of the CNS, and most have motor deficits that often dominate the clinical picture, especially in younger individuals.

Due to challenges in diagnosis, the true prevalence and incidence of all LDs is not yet established. Estimates of their combined incidence range widely, from 1 in 50,000 to 1 in 7,663 [3, 4]. The early recognition of LDs can be challenging as they present insidiously, heterogeneously and are often not considered until neuroimaging shows abnormalities. Even then, they often remain undiagnosed or misdiagnosed, in part due to limited knowledge about their etiology. While advances in neuroimaging pattern recognition have improved diagnostic yield, curative treatments are currently limited and a definitive diagnosis is crucial for appropriate symptom management, prognostic and genetic counseling.

With the increasing number of LDs and gLEs a clinician must recognize, a simplified and standardized approach to facilitate identification of these diseases by child neurologists and geneticists is needed. The Global Leukodystrophy Initiative (GLIA) assessed clinicians' comfort in diagnosing LDs and gLEs and found that, despite the fact that these clinicians are members of the Society for Inherited Metabolic Disorders (SIMD) or Child Neurology Society, only a minority felt comfortable with the neuroimaging patterns and diagnostic approaches for LD and gLE patients (Table 1).

With the aim of providing clinicians with a simplified approach for diagnosing LDs and gLEs, here we (i) review the clinical presentations of various LDs and gLEs and highlight “red-flag” sine qua non neurologic symptoms along with extra-neurologic features, (ii) review established diagnostic algorithms for MRI pattern recognition, and (iii) present a decision tree workflow for molecular testing with specific attention to rapid diagnosis of treatable disorders and implementation of diagnostic genetic testing.

2. When to suspect a leukodystrophy or genetic leukoencephalopathy

The LDs and gLEs are a large group of relatively heterogeneous disorders [2]. Patients typically present with the onset of neurologic symptoms, though some may show worsening of long-standing, and typically milder, symptoms. It should be noted that some LDs and gLEs have a slow and progressive course, and can be mistaken for static encephalopathies unless a longitudinal view of the disease is taken (e.g. some hypomyelinating conditions), and a minority show slow improvement over time (e.g. LD caused by *HEPACAM* and *EARS2* mutations). The vast majority, however, present with gradual or abrupt deterioration of CNS function.

Most LDs and gLEs present with motor symptoms. This is in contrast with primary neuronal disorders, which usually present with cognitive decline and seizures - although there is often overlap between these symptom groups. Specific LDs and gLEs may have a typical age of onset which generally described as infantile (first year), late infantile (1-5 years), juvenile (5-12 years) or adolescence and adulthood. More typically, however, there is a spectrum of disease presentation across all age groups, whose presenting symptoms and specific signs change accordingly. Thus, the clinical features described below are broadly representative of the most typical presentations of the described disorders.

Often patients present to the neurologist with concern for a LD or gLE based on abnormal neuroimaging. However, if this is not the case, there are several clinical features or “red flags” that should alert the clinician to the possibility of a LD or gLE. These are highlighted below in italics. Establishing a differential diagnosis in patients with a suspected LD or gLE will begin by identifying these clinical features, assessing neurologic and systemic symptoms, and then performing appropriate diagnostic

investigations (i.e. genetic testing). Because patients with gLEs are not considered classic LDs but are often evaluated in clinics for patients with presumed inherited white matter conditions, these disorders are also detailed below [2].

3. Neurologic Features

LDs and gLEs have significant heterogeneity in disease course and in extraneurologic manifestations, as well as MRI patterns. Neurologic features may be more homogenous, though the degree to which certain features are present may vary with the age of presentation and the specific LD or gLE (Table 2).

LDs and gLEs will almost always affect the motor system. Patients may present to the clinician with concerns of delayed acquisition of motor milestones, stagnation of motor development or frank regression in motor skills. In an infant or young child, delayed motor development is more common in the hypomyelinating disorders, whilst motor regression is more common in the LDs with myelin destruction. In an older child the first symptom may be frequent falls or a clumsy gait, and in an adolescent or young adult, deterioration in functional skills such as sporting activities. Occasionally there is acute deterioration in motor skills in the context of an intercurrent illness or minor head injury; the latter can be seen in Vanishing White Matter disease (VWM), but can also be seen in a number of other LDs or inborn errors of metabolism.

The type of motor abnormality is often informative (Table 2). Patients may have early involvement of the corticospinal tracts resulting in a central pattern of weakness, upper motor neuron signs such as spastic quadraparesis or spastic paraparesis. In some cases, deep gray nuclei involvement occurs and patients may present with dystonia, chorea or a mixed movement disorder. Tremor may be present but is often non-specific and may be multifactorial in etiology.

Selected LDs and gLEs lead to prominent loss of cerebellar volume and may present with slowly progressive ataxia, in some cases as an isolated clinical finding. In many LDs and gLEs however, ataxia is part of a broader constellation of neurologic findings, which include prominent spasticity. For other LDs

and gLEs, a peripheral sensory neuropathy leading to altered proprioception and imbalance may contribute to alterations in gait, leading to a mixed cerebellar and sensory ataxia.

Additional neurologic features, such as autonomic dysfunction, alterations in head circumference, seizures or neurobehavioral abnormalities including extreme early infantile irritability, deterioration in school performance, new-onset hyperactivity or a change in personality can further help refine diagnostic considerations (Table 2).

4. Extra-Neurologic Findings

In addition to the neurologic findings associated with LDs and gLEs, a variety of extra-neural features can be helpful in suspecting a specific diagnosis (Table 3).

4.1 Adrenal insufficiency (Addison disease) is characteristic of only two LDs: X-linked adrenoleukodystrophy (X-ALD) / adrenomyeloneuropathy (AMN) and peroxisome biogenesis disorders. It usually presents with cutaneous hyperpigmentation, hyponatremia and more rarely hypoglycemia. Patients may show a prolonged recovery from general anesthesia as the first indication of adrenal insufficiency.

4.2 Other endocrine disturbances may be detected in patients with 4H leukodystrophy (Hypomyelination, hypodontia and hypogonadotropic hypogonadism syndrome), including hypogonadotropic hypogonadism (if the patient is of age to be pubertal) as well as less frequently growth factor deficiency and hypothyroidism. Hypothyroidism can also be seen in Aicardi-Goutières Syndrome (AGS). Post-natal growth failure may be seen in Cockayne syndrome but treatment with growth hormone is relatively contraindicated in this disorder. A significant proportion of patients with 4H leukodystrophy present with short stature and there have been a few reports of growth hormone deficiency.

4.3 Ophthalmologic abnormalities are noted in a number of the LDs and gLEs and findings can be quite

useful in restricting the differential diagnosis. Congenital *cataracts* are typical in hypomyelination with congenital cataracts (HCC), neonatal onset VWM, and some patients with peroxisome biogenesis disorders, whereas onset of cataracts later in childhood suggests cerebrotendinous xanthomatosis (CTX). Cataracts can be seen rarely in 4H leukodystrophy. *Retinitis pigmentosa* (and associated night blindness) develops in adolescent and adult patients with Refsum disease and is seen in some infants with peroxisome biogenesis disorders. *Retinal cherry red spots* are frequently seen in GM1- and GM2-gangliosidosis and sialidosis. *Optic atrophy* is a common feature of LDs and gLEs, most notable in Canavan disease, VWM, most hypomyelinating conditions and some mitochondrial disorders. *Nystagmus* is characteristic of Pelizaeus-Merzbacher disease (PMD), Canavan disease and a significant number of hypomyelinating LDs. Indeed, patients with PMD, Pelizaeus-Merzbacher like disease (PMLD) and SOX10-related disorders have early onset or congenital nystagmus whereas patients with 4H leukodystrophy, Oculodentodigital dysplasia, 18q- syndrome may have nystagmus at a later age. The presence of *retinal glistening white dots* with a perifoveal distribution in a patient with ichthyosis is pathognomonic for Sjögren-Larsson syndrome (SLS). *Retinal vascular defects* are seen in cerebroretinal micorangiopathy with calcification and cysts (CRMCC).

4.4 Cortical visual impairment can also be a late feature of many LDs and gLEs as the white matter disease progresses to involve the cortical visual tracts.

4.5 Hypodontia and oligodontia, as well as other dental abnormalities such as delayed teeth eruption, are characteristic of Pol III-related or 4H leukodystrophy [5]. Dental abnormalities with enamel dysplasia are a feature of oculodentogidigital dysplasia [6]. In peroxisome biogenesis disorders, teeth abnormalities include enamel defects of the secondary teeth. In Cockayne syndrome, the typical dental abnormality is the propensity for cavities, which is present in the majority of patients. Enamel hypoplasia, oligodontia, hypodontia and abnormal shape have also been described [7].

4.6 Dysmorphic facial features in an infant with hypotonia and/or seizures should suggest a peroxisome biogenesis disorder such as Zellweger syndrome. These infants often have dolichocephaly with wide anterior fontanelle. Other disorders with dysmorphic features associated with white matter abnormalities (often multifocal) include chromosomal abnormalities, Cohen syndrome, Costello syndrome and others. The development of coarsening facial features is typically seen in the lysosomal storage diseases with white matter involvement such as multiple sulfatase deficiency, mucopolysaccharidoses and sialic acid storage disease.

4.7 *Tendinous xanthomas*, particularly prominent in the Achilles tendon, along with white matter disease are specific signs of CTX.

4.8 *Skeletal radiographic abnormalities* are seen in several LDs and gLEs. Chondrodysplasia punctata is an early feature of the peroxisome biogenesis disorders. Development of radiographic features of dysostosis multiplex in a patient with white matter disease suggests multiple sulfatase deficiency or sialidosis.

4.9 Hearing impairment may be seen as a non-specific association of many LDs and gLEs involving the auditory nerves, but it is rarely the presenting symptom. Sensorineural deafness is typical of peroxisome biogenesis disorders and may also be seen in *SOX10* associated LD. It may be detected as early as the newborn period through routine newborn hearing screening programs, or later in infancy and childhood. An important diagnosis to consider in case of sensorineural deafness is congenital cytomegalovirus (CMV) infection, since this condition may also present with significant multifocal white matter abnormalities and myelin deficits. *RNASET2* deficiency, is an autosomal recessive disorder that mimic congenital CMV and therefore should also be considered in case of sensorineural hearing deficit. Later onset deafness in adults occurs in Refsum disease. More commonly, the hearing impairment in LDs and gLEs is central in origin.

4.10 Hepatosplenomegaly is a feature of certain lysosomal storage diseases with white matter involvement including multiple sulfatase deficiency, galactosialidosis and sialic acid disorders. Isolated hepatomegaly with or without hepatic dysfunction is often present in peroxisome biogenesis disorders. Hepatic dysfunction may also be seen in the congenital period or more rarely in infancy in AGS. gLEs caused by mitochondrial dysfunction may have associated hepatic abnormalities.

4.11 Cutaneous abnormalities are associated with several LDs and gLEs. *Angiokeratomas* are seen in galactosialidosis. *Ichthyosis* is a distinctive feature that is present at birth in SLS and later in childhood in multiple sulfatase deficiency or adults with Refsum disease. Ichthyosis with sparse, brittle hair is an unusual clinical finding in trichothiodystrophy (Tay syndrome) and is also associated with cutaneous photosensitivity. Cutaneous photosensitivity is also seen in Cockayne syndrome. *Hyperpigmentation* is a sign of adrenal insufficiency in children and adults with X-ALD/AMN.

4.12 *Ovarian dysgenesis* and dysfunction characterizes a distinctive variant of VWM (ovarioleukodystrophy). Ovarian dysfunction has also been recently recognized in leukoencephalopathy associated with the t-RNA synthetase deficiency caused by *AARS2* mutations. The primary ovarian failure seen in these disorders should not be confused with the hypogonadotropic hypogonadism seen in 4H leukodystrophy.

4.13 *Gastrointestinal symptoms*, often chronic, with diarrhea are often seen in CTX and in some patients with mitochondrial disorders, particularly mitochondrial neurogastrointestinal encephalopathy. Metachromatic Leukodystrophy (MLD) may be accompanied by severe, poorly understood feeding intolerance and gallbladder disease that may require cholecystectomy. Rare patients with AGS may have a condition mimicking inflammatory bowel disease. Many patients with LDs and gLEs have gastrointestinal reflux and chronic constipation which may cause significant morbidity.

5. Diagnostic Recommendations

The clinical evaluation of patients with LDs and gLEs should take into account all available clinical information including age of onset, family history, neurologic symptoms and the presence of characteristic extra-neurologic features as described above. Tables 2 and 3 can be used as a template for an initial assessment of a patient suspected of having any of the canonical LDs. Similarly detailed phenotypic descriptions of the gLEs are beyond the scope of this manuscript. Any initial evaluation that indicates strong concordance of a patient's presentation with that of a LD or gLE should trigger the following three actions, as described in detail below, which can occur in series or in parallel: (1) immediate assessment of the likelihood of a genetic etiology and if the putative LD or gLEs is treatable, (2) a detailed neuroimaging series, (3) molecular genetic testing (summarized in brief in Figure 1).

5.1 Neuroimaging. Brain MRI is the foundational investigation in a patient with a suspected LD or gLE (Figure 1, *box 1*) [8-16]. The imaging findings should be interpreted in the context of the clinical and family history and the examination findings, but may often be diagnostic even before these elements are known. Following MRI interpretation, it may be possible to determine a differential diagnosis for a given white matter disorder using a "pattern recognition" approach (Figure 1) [1, 8]. Correct interpretation of clinical and imaging findings will often allow the clinician to order tailored investigations rather than subjecting the patient to a battery of unnecessary and expensive testing. Sagittal T_1, Axial T_1, T_2-weighted and fluid-attenuated inversion-recovery (FLAIR) sequences should be obtained at a minimum. Other sequences may also be required such as administration of contrast (for disorders with an inflammatory component such as cerebral X-ALD), susceptibility weighting (for disorders with calcifications such as AGS), MR spectroscopy (MRS)(for mitochondrial disorders or Canavan disease to investigate abnormalities in lactate or N-acetyl aspartate (NAA) respectively), and diffusion-weighting (useful in disorders such as *AARS2*-related leukoencephalopathy). MRI is superior to CT, although CT may still be helpful to detect calcifications, in particular if newer MRI techniques helpful for detecting

calcifications were not performed. A spine MRI should be obtained on at least one occasion in all patients with an undiagnosed LD or gLE to assess for spinal cord involvement.

A single brain MRI, especially when it is performed in the first year of life, is not sufficient to distinguish between delayed myelination, hypomyelination and the early stages of a LD/gLE. Therefore, serial MRI scans are often required, usually with a minimum of 6-12 months interval between studies. Ideally, at least one scan should be obtained after the age of two years.

According to published MRI-based diagnosis algorithms [1, 8], three major MRI characteristics help to discriminate between the different types of LD and gLE. The first discriminator is the presence or absence of *hypomyelination* (Figure 1a) and in this regard assessment of the T_1 sequences are of particular importance, as T_1 shortening occurs before T_2 shortening as myelination progresses. Hypomyelination is defined as an unchanged pattern of deficient myelination on two MRI scans at least six months apart in a child older than one year [1, 8]. Hypomyelinating disorders can be further divided into those with and without involvement of the cerebellum and basal ganglia, and with and without global atrophy.

If the pattern is not one of hypomyelination, then the second discriminator is whether the white matter abnormalities are *confluent or isolated and multifocal* [1, 8] (Figure 1b). Multifocal changes often imply an acquired disorder such as infection or vasculopathy or a structural chromosomal disorder, whilst bilateral, symmetric confluent changes usually imply a gLE or LD. If the white matter abnormalities are confluent, then the third discriminator is the *predominant localization of the abnormalities* (Figure 1b). The most common patterns are frontal (e.g. Alexander disease(AxD)), parieto-occipital (e.g. X-ALD), periventricular (e.g. MLD), subcortical (e.g. Canavan disease), diffuse cerebral (e.g. VWM) or posterior fossa (e.g. peroxisomal disorders) predominant. Assessment of structures such as the cortex, basal ganglia, cerebellum, thalami and the descending white matter tracts is also important for further discrimination. Additional imaging features such as contrast enhancement, presence of calcifications, or macrocephaly can also help refine the diagnosis [1, 8]. Other MRI techniques, such as diffusion tensor imaging, spectroscopy and various multivariate analysis techniques of MRI data may be sensitive

indicators of involvement of certain white matter tracts or myelination but principally remain research tools. It should be noted that even with high quality imaging, expert imaging interpretation and a complete battery of clinical investigations at least 30-40% of LDs and gLEs, and 50% of hypomyelinating, cases remain unresolved.

5.2 Special attention to disease etiology and amenability to treatment. Once an abnormal MRI is observed it is of primary importance to resolve etiology of the patient's white matter disorder (Figure 1, *box 2*). Acquired and genetic white matter disorders share many imaging and phenotypic features, and in some cases are easily confused (and therefore may require expert evaluation – Figure 1, *box 4*). Failure to correctly identify the source of the patient's disorder quickly can have negative consequences. For example, in the case of patients with acquired white matter disorders it can lead to unnecessary interventions and diagnostic testing, and may result in failure to identify treatable entities (Figure 1, *boxes 5 & 9*). For example, acute disseminated encephalomyelitis (ADEM) can be controlled with high doses of corticosteroids and white matter abnormalities that result from B12 deficiency can be reversed with vitamin supplementation.

In the assessment of a patient with a suspected LD or gLE, we recommend explicit and rapid evaluation for those disorders with established therapeutic interventions (Figure 1, *boxes 3 -7* and Table 4). These include X--ALD, Krabbe disease, and MLD, which are all rapidly diagnosable biochemically, and some patients may benefit from bone marrow transplantation in the early stages of the disease {Helman, 2014 #97}. It also includes CTX which is arguably the most easily treatable LD and responds to both chenodeoxycholic acid and inhibitors of HMG-CoA reductase [17]. Although beyond the scope of the discussion here, we also note that a variety of gLEs with significant associated white matter involvement are also treatable and include the amino acidemias (e.g. Maple Syrup Urine Disease, Phenylketonuria), organic acidurias (Methylmalonic, isovaleric and propionic acidemias, etc.), Niemann-Pick type C, biotinidase deficiency, and Wilson's disease. To ensure that these disorders are not missed during work-up we strongly recommend *a minimum testing battery* in *all suspected LD or gLE* cases consistent with

these disorders that assesses very long chain fatty acids, lysosomal enzymes (including galactocerebrosidase activity, arylsulfatase A activity, and cholestanol) and a re-evaluation of newborn screening test results as well as possible indications for other treatable conditions.

5.3 Biochemical and molecular genetic testing. Following MRI pattern analysis, the standard diagnostic approach to LDs and gLEs consists of serial biochemical and single gene testing. In some cases, particularly for LDs and gLEs with clearly defined MRI patterns, this is an effective and timely approach (Figure 1, *boxes 3,6,7,10)*. Indeed, biochemical testing is essential for reliably diagnosing many of the clearly defined LDs and gLEs. Measurements of lysosomal enzymes for MLD, Krabbe disease, multiple sulfatase deficiency, and GM1/GM2 gangliosidosis are widely available. In some cases, enzymatic studies must be supported by biochemical measurements showing substrate accumulation. For example, determination of urinary sulfatides and glycosaminoglycans provides additional evidence for the diagnosis of metachromatic leukodystrophy or multiple sulfatase deficiency, respectively. Additionally, plasma very long-chain fatty acids measurement is a sensitive screening test for ALD, peroxisome biogenesis disorders and peroxisomal ß-oxidation defects. Urine organic acids analysis will detect biochemical abnormalities of L-2-hydroxyglutaric aciduria and Canavan disease, and may reveal Krebs cycle intermediates suggestive of mitochondrial diseases. Lastly, plasma cholestanol levels are typically elevated in CTX, which is one of the most easily treated LDs and gLEs (see above and Table 4). Single gene tests are also available for these disorders, and can provide additional or initial validation of the suspected diagnosis. When successful, these biochemical and genetic investigations can take as little as several weeks to complete.

For patients for whom there is no definitive MRI pattern, however, and therefore no definitive biochemical or single gene test, the diagnostic process may take nearly a decade [18] and will leave as many as half of individuals without a specific diagnosis [9]. High-throughput sequencing technologies, particularly gene panel-based approaches and whole exome sequencing, have now been used to identify the causal mutations underlying a wide variety of illnesses [19, 20]; and recent proof-of-principle studies

have indicated that partnering MRI pattern analysis and next-generation sequencing may lead to higher diagnostic yield and more timely diagnosis [21].

For those patients who have an abnormal but ambiguous MRI, and whose condition is clearly genetic, we advise broad spectrum next-generation sequencing (NGS) genetic testing using either gene panels, whole exome sequencing (WES) (which queries the entire coding sequence of the human genome), or whole genome sequencing (WGS) (Figure 1, *box 8*). The number of genes associated with LDs and gLEs continues to increase (a detailed list can be found in Table 5), and the phenotypic spectrum of disorders with secondary white-matter involvement continues to broaden, and it is therefore arguable that in many cases WES or WGS may therefore be the best near-term testing option.

Variants detected by NGS should be analyzed and categorized according to ACMG standards [22]. We recommend prioritizing known pathogenic (P) or likely pathogenic (LP) variants in disease genes that are known to have primary or secondary white matter involvement (e.g. gLEs), which can confirmed by an orthogonal approach (Figure 1, *box 11*). We note that genetic diagnosis requires mindful return of information to patients and their families with appropriate genetic counseling.

A proportion of patients will not achieve a specific diagnosis using NGS approaches. It is likely that these cases will represent instances in which the pathogenic variant resides in a gene that has not yet been causally associated with a human disease. In those circumstances we recommend that patients are given the option to participate in ongoing research programs, which aggregate patients with undiagnosed diseases with the aim of identifying new disease genes (Figure 1, *box 13*). These efforts have proven highly successful [23, 24]. We recommend the recruitment of the patients' mother and father to the study whenever possible, as sequencing of small family pedigree enables rapid identification of both compound heterozygous and de novo mutations. It should be taken into consideration that even with the ideal research conditions, causal variants are not always found, especially if the variant is located in a region of a gene that is not covered or not well covered, or if the type of variant is not easily detected by current technology (e.g. deletion, complex rearrangement).

5.4 Other diagnostic testing considerations. In cases where genetic testing results and other clinical investigations are ambiguous, we recommend consideration of additional supplementary investigations as detailed in Table 6. A lumbar puncture for analysis of cerebrospinal fluid (CSF) can be useful for evaluating a small number of LDs and gLEs. For example, CSF protein elevation is a hallmark of active demyelination. CSF leukocytosis, elevated interferon-α and neopterin suggest AGS. CSF NAA is elevated in Canavan disease, but urine organic acids testing is an equally effective diagnostic tool. In many cases, characterization of the neurologic disease using electrophysiologic tests, such as brainstem auditory-evoked potentials, sensory-evoked potentials and visual-evoked potentials can be useful. Nerve conduction studies and electromyography can also be useful in identifying peripheral nerve involvement (e.g. in AMN, MLD, Krabbe) or myopathy with or without a neuropathy (e.g. in mitochondrial diseases) or metachromatic leukodystrophy.

6. Conclusions and future directions

Leukodystrophies (LD), while primarily affecting the CNS, have a varied range of presentations with symptoms beginning at any age. Genetic Leukoencephalopathies (gLE) with white matter involvement and additional systemic or gray matter features, further add complexity to the diagnosis of these patients. Recognition of a few *sine qua non* “red flag” symptoms allows the clinician to astutely consider the LDs and gLEs in the patient’s differential diagnosis. Identifying other associated symptoms can help narrow the list of conditions one needs to test for. While MRI currently remains the mainstay of diagnosis in LDs and gLEs, in cases where the MRI pattern does not fit a specific entity, expanded genetic testing using NGS technologies is being used more commonly to confirm, or ab initio derive, the diagnosis. It is likely that future advances in genomic applications will demonstrate expanding utility to the early implementation of NGS testing, but this still requires trials to establish its clinical utility as a primary diagnostic strategy. With advancing research, specific therapies to treat patients in the earliest stages of their disease are now available for some disorders, with the future hope for therapeutic options in additional disorders. Thus, a renewed focus on rapid recognition and diagnosis of LDs is important to

afford patients an opportunity for early treatment and care.

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SP, GB, RL, AV, MP, MSVDK, NM, AP, JLS, JVH, and WBR contributed building consensus within the GLIA consortium on a clinical approach to the leukodystrophies. SP, GB, RL, WBR, AV, GB and RJT wrote this manuscript and AV, RL, MP, MSVDK, GH, WR and RJT provided critical review of the text.

9. Conflict of Interest:

During the course of the drafting of this manuscript RJT became an employee of Illumina, Inc. MCP: Editorial: Journal of Child Neurology, Child Neurology Open (Editor-in-Chief), Journal of Inherited Metabolic Disease (Editor). Otherwise authors report no conflict of interest.

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12. Tables

Table 1: Clinicians' Comfort Levels in the Diagnosis of Leukodystrophies

Respondents by specialty	
Biochemical Geneticists	43% (79)
Pediatric Neurologists	34% (62)
Clinical Geneticists	14% (26)
Other	9% (16)
Total	183
Comfort Levels	
Very confident of providing a diagnosis [5 on a scale of 0-5]	16%
Moderately confident of providing a diagnosis [3 or 4 on a scale of 0-5]	36%
Very confident in providing a differential diagnosis of a leukodystrophies	15%
Moderately confident in providing a differential diagnosis of a leukodystrophies	36%
Access to Resources	
Access to a regional leukodystrophy expert	76%
Cited a need for phone-based expert consult service	69%
Reported inadequate training in leukodystrophies	57%

Table 2. Major neurologic signs and symptoms in the leukodystrophies- Note, if nothing is noted, these are not commonly seen features, though in end stage disease almost all disorders can feature the described symptoms. Disorders that are not canonical leukodystrophies (i.e. genetic leukoencephalopathies) are not included in this table.

Disorder	Macrocephaly	Microcephaly	Cognitive involvement (with or without autistic features)	Psychiatric Symptoms	Irritability	Hypotonia, severe	Upper motor signs (e.g. spasticity)	Movement Disorder: tremor, dystonia or chorea	Isolated spastic Paraparesis	Ataxia	Peripheral neuropathy	Autonomic Dysfunction	Seizures, early in disease course	Other
Pol-III related disorders (4H leukodystrophy)			+/-				+	+	+/-	+				Nystagmus
18q minus syndrome			+/-				+							
X-Linked Adrenoleukodystrophy (X-ALD)				+			+		+					Adult onset cases with predominant myelopathy
Adult onset leukodystrophy with neuroaxonal spheroids and pigmented glia (including hereditary diffuse leukoencephalopathy with spheroids, HDLS, and Pigmentary type of orthochromatic leukodystrophy with pigmented glia, POLD)				+			+	+						Frontotemporal dementia
Aicardi-Goutières Syndrome (AGS)		+	+		+		+		+					
Alexander Disease (AxD)	+			+						+		+	+	Bulbar dysfunction in adult onset cases Nystagmus is prominent
Adult onset autosomal dominant leukodystrophy (ADLD)							+				+	+		Mixed lower and upper motor neuron disease
Canavan Disease	+		+		+	+	+	+					+	Nystagmus is prominent Patients may have sensorineural hearing loss

Cerebrotendinous Xanthomatosis (CTX)			+	+			+			+	+		+	
Chloride Ion Channel 2(ClC-2) related leukoencephalopathy with intramyelinic oedema				+			+							Severe headache in some cases
eIF2B related disorder (Vanishing WM Disease of CACH)							+			+				Acute neurologic deteriorations in the context of stressors Nystagmus is prominent
Fucosidosis			+				+	+						
Globoid Cell Leukodystrophy (Krabbe)			+	+	+		+		+	+	+		+	
Hypomyelination with atrophy of the basal ganglia and cerebellum (H-ABC)							+	+	+	+				
Hypomyelination with Brainstem and Spinal Cord involvement and Leg Spasticity (HBSL)*							+		+	+				May be steroid responsive
Hypomyelination with congenital cataract (HCC)							+			+	+			
Leukoencephalopathy with brainstem and spinal cord involvement and lactate elevation (LBSL)							+		+	+				
Leukoencephalopathy with thalamus and brainstem involvement and high lactate (LTBL)			+/-				+			+			+	May improve over time
Megalencephalic Leukoencephalopathy with subcortical cysts	+						+			+			+	May improve over time
Metachromatic Leukodystrophy and its biochemical variants			+/-	+		+	+			+	+		+	
Oculodentodigital dysplasia			+				+			+			+	Sensorineural hearing loss can be seen
Pelizaeus Merzbacher disease (PMD) *							+	+	+	+	+^			Nystagmus is prominent Respiratory stridor is a

														common feature in the connatal form. Neuropathy in PLP null syndrome.
Pelizaeus Merzbacher like-disease (PMLD) *										+	+			Nystagmus is prominent
Peroxisomal Biogenesis disorders (including Zelleweger, neonatal Adrenoleukodystrophy and Infantile Refsum)			+			+	+				+		+	
Polyglucosan Body Disease (PGBD)											+	+		
RNAse T2 deficient leukoencephalopathy		+	+/-				+							Deafness can be observed
Sialic acid storage disorders (Salla disease, Infantile Sialic Acid Storage Disease and Intermediate form)			+			+	+			+			+	
Single enzyme deficiencies of peroxisomal fatty acid beta oxidation (including only D-Bifunctional Protein Deficiency; SCPx deficiency ; Peroxisomal acyl-CoA-Oxidase Deficiency)			+			+	+							
Sjögren-Larsson syndrome*			+				+		+				+/-	
***SOX10*-associated PCWH: peripheral demyelinating neuropathy, central dysmyelinating leukodystrophy, Waardenburg syndrome, and Hirschsprung disease**							+			+				Deafness can be observed

Table 3. Extra-neurologic signs and symptoms in the leukodystrophies. Systemic involvement is more commonly seen in the genetic leukoencephalopathies, but disorders that are not canonical leukodystrophies are not included in this table

	Endocrine				Facial		Skin		Ocular					Gastro-intestinal			Musculo-skeletal			Genito-urinary	
	Adrenal insufficiency	Hypothyroidism	Growth Hormone def.	Failure to Thrive	Dysmorphism	Dental abnormalities	Ichthyosis	Hyperpigmentation	Cataracts	Cheery Red Spot	Glaucoma	Optic Atrophy	Retinitis Pigmentosa	Diarrhea	Gall Bladder Disease	Hepatic involvement	Joint abnormalities	Bony abnormalities	Myopathy	Ovarian Failure	Other
Pol-III related disorders (4H leukodystrophy)	+	+/−	+	+		+			+/−			+/−						+/−			Hypogonadotropic hypogonadism
18q minus syndrome					+																Genital abnormalities, congenital heart disease, immune manifestions and skin abnormalities may also be present
X linked Adrenoleuko-dystrophy*	+							+													
Aicardi-Goutières Syndrome (AGS)		+	+								+					+	+				Rare patients may have a cardiomyopathy Retinal vascular abnormalities can be seen in certain patients with autosomal dominant TREX1

																			mutations (RVCL)
Canavan disease*											+								
Cerebro-tendinous Xanthomatosis (CTX)		±							+		+		+				±	±	Atherosclerosis Tendon xanthomas Neonatal cholestatic jaundice
eIF2B related disorder (Vanishing WM Disease or CACH)									±									+	Connatal onset may have ovarian dysgenesis Only connatal cases have cataracts
Fucosidosis															+				Facial coarsening ad cardiomegaly are also present
Hypo-myelination with congenital cataracts									+										
Metachromatic Leukodystrophy and its biochemical variants*														+					
Oculo-dentodigital dysplasia					+	+				±	±					+			Bony abnormalities involving the digits are seen, as well as cleft lip and palate
Peroxisomal Biogenesis disorders (including Zelleweger, neonatal Adrenoleukodystrophy and Infantile Refsum)	+			+	+		+		+		+	+	+		+		+		Renal involvement, such as cysts, may be seen

Sialic acid storage disorders (Salla disease, Infantile Sialic Acid Storage Disease and Intermediate form)					+		+			+						+	+	+			Facial coarsening
Sjögren-Larsson syndrome*				+			+														Retinal "glistening" white dots can be seen in macular region

^^ The following disorders classified as leukodystrophies do not have prominent extra-neurologic features and as such are not listed on this table. These include: Adult onset leukodystrophy with neuroaxonal spheroids and pigmented glia caused by mutations in *CSF1R* glia, POLD, AxD, ADLD, ClC-2 related leukoencephalopathy with intramyelinic oedema, Krabbe, H-ABC, HBSL, HCC, LBSL, LTBL, Megalencephalic Leukodystrophy with subcortical cysts, Pelizaeus Merzbacher disease (PMD), Pelizaeus Merzbacher like-disease (PMLD),
PGBD, RNAse T2 deficient leukoencephalopathy, Single enzyme deficiencies of peroxisomal fatty acid beta oxidation (including only D-Bifunctional Protein Deficiency; SCPx deficiency ; Peroxisomal acyl-CoA-Oxidase Deficiency).

Table 4: Treatable leukodystrophies.*

Disease	Screening test	Treatment
Adrenoleukodystrophy (cerebral)	VLCFA	Bone marrow transplantation in early stages of the disease.
Cerebrotendinous Xanthomatosis	Cholestanol	Chenodeoxycholic acid; inhibitors of HMG-CoA reductase.
Krabbe	Galactocerebrosidase activity assay	Bone marrow transplantation in pre-symptomatic and early symptomatic patients, though the benefit of this is still undergoing testing.
Metachromatic leukodystrophy	Arylsulfatase A activity assay	Bone marrow transplantation in pre-symptomatic and early symptomatic patients though the benefit of this is still undergoing testing.

***Note, this does not include the many genetic leukoencephalopathies, including but not limited to amino acidemias (MSUD, PKU, etc.), organic acidurias (MMA, IVA, PA, etc.), Niemann-Pick type C, biotinidase deficiency, Wilson's disease, etc.*

Table 5: Minimum recommended gene list for broad spectrum genetic testing for single-nucleotide variants associated with leukodystrophies and genetic leukoencephalopathies.

Disease Name(s)	OMIM	Gene(s)
Hypomyelinating Leukodystrophies		
Pol-III related disorders (4H leukodystrophy)	607694	***POLR3A*, *POLR3B***
Hypomyelinating leukodystrophy Dystonia, type4 HABC	612438	***TUBB4A***
Hypomyelination and congenital cataract (HCC)	610532	***FAM126A***
Pelizaeus-Merzbacher disease (PMD)	312080	***PLP1***
Hypomyelinating leukodystrophy 2 (HLD2) Pelizaeus-Merzbacher-like disease 1 (PMLD1)	608804	***GJC2***
SOX10-associated PCWH: peripheral demyelinating neuropathy, central dysmyelinating leukodystrophy, Waardenburg syndrome, and Hirschsprung disease	609136	***SOX10***
Other Leukodystrophies		
X-linked Adrenoleukodystrophy (X-ALD)	300100	***ABCD1***
Adult-onset leukodystrophy with neuroaxonal spheroids and pigmented glia (including Hereditary diffuse leukoencephalopathy with spheroids (HDLS) and Pigmentary orthochromatic leukodystrophy (POLD))	221820	***CSF1R***
Aicardi-Goutières Syndrome (AGS)	615010 610333 610181 610329 612952 225750	***ADAR1*,** ***RNASEH2A*,** ***RNASEH2B*,** ***RNASEH2C*,** ***SAMHD1*,** ***TREX1***
Alexander disease (AxD)	203450	***GFAP***
Adult onset autosomal dominant leukodystrophy (ADLD)	169500	***LMNB1***
Canavan disease	271900	***ASPA***
Cerebrotendinosus Xanthomatosis (CTX)	213700	***CYP27A1***
Chloride Ion Channel 2(ClC-2) related leukoencephalopathy with intramyelinic oedema	615651	***CLCN2***
eIF2B related disorder (Vanishing WM Disease or CACH)	603896 603896 603896 603896 603896	***EIF2B1*,** ***EIF2B2*,** ***EIF2B3*,** ***EIF2B4*,** ***EIF2B5***
Fucosidosis	230000	***FUCA1***
Globoid cell leukodystrophy (Krabbe disease)	245200 611722	***GALC*,** ***PSAP***
Hypomyelination wit Brainstem and Spinal Cord Involvement and Leg Spasticity (HBSL)	615281	***DARS***
Leukoencephalopathy with brain stem and spinal cord involvement and lactate elevation (LBSL)	611105	***DARS2***

Leukoencephalopathy with thalamus and brainstem involvement and high lactate (LTBL)	NA	***EARS2***
Multiple sulfatase deficiency	272200	***SUMF1***
Megalencephalic leukoencephalopathy with subcortical cysts (MLC)	604004 613925	***MLC1*****,** ***HEPACAM***
Metachromatic Leukodystrophy (MLD)	607574 249900	***ARSA*****,** ***PSAP***
Oculodentodigital dysplasia (ODDD)	257850	***GJA1***
Polyglucosan Body Disease (PGBD)	263570	***GBE1***
RNAse T2 deficient leukoencephalopathy	612951	***RNASET2***
Sialic acid storage disease	269920	***SLC17A5***
Sjögren Larsson syndrome	270200	***ALDH3A2***
Progressive leukoencephalopathy with ovarian failure	615889	***AARS2***
Other Leukodystrophies: Peroxisomal biogenesis disorders		
Peroxisome biogenesis disorder 1A,B	214100, 601539	**PEX1**
Peroxisome biogenesis disorder 5A,B	614866, 614867	**PEX2**
Peroxisome biogenesis disorder 10A	614882	***PEX3***
Peroxisome biogenesis disorder 4A,B	614862, 614863	***PEX6***
Peroxisome biogenesis disorder 6A,B	614870, 614871	***PEX10***
Peroxisome biogenesis disorder 3A,B	614859, 266510	***PEX12***
Peroxisome biogenesis disorder 3A,B	614872, 614873	***PEX26***
Other Leukodystrophies: Peroxisomal single enzyme beta oxidation deficiencies		
D-bifunctional protein deficiency	261515	***HSD17B4***
Sterol carrier protein 2 deficiency Leukoencephalopathy with dystonia and motor neuropathy	613724	***SCP2***
Peroxisomal acyl-coA-oxidase deficiency	264470	***ACOX1***
Genetic Leukoencephalopathies		
L2-Hydroxyglutaric aciduria	600721 236792 615182	***D2HGDH*** ***L2HGDH*** ***SLC25A1***
Cockayne syndrome; UV-sensitive syndrome	609412	***ERCC6,*** ***ERCC8***
Deafness, dystonia, and cerebral hypomyelination	300475	**BCAP31**
Trichothiodystrophy, nonphotosensitive	234050	***MPLKIP***
Trichotiodystrophy with hypersensitivity to sunlight	601675	***ERCC2*****,** ***ERCC3*****,** ***GTF2H5*****,**
Hypomyelinating leukodystrophy 3	260600	***AIMP1***
Hypomyelinating leukodystrophy 4 Mitochondrial HSP60 chaperonopathy	612233	***HSP60***
Global cerebral hypomyelination	603667	***SLC25A12***

GM1-gangliosidosis	230500	***GLB1***
GM2-gangliosidosis Tay-Sachs disease	272800	***HEXA***
Infantile neuronal ceroid lipofuscinosis	256730	***PPT1***
Mucolipidosis IV	252650	**MCLON1**
Nasu-Hakola disease Dementia with bone cysts	221770	**TYROBP**
Phosphoglycerate dehydrogenase deficiency	601815	***PHGDH***
Phosphoserine aminotransferase deficiency	610992	***PSAT1***
Allan-Herndon-Dudley syndrome Monocarboxylate transporter 8 deficiency (MCT8)	300523	***SLC16A2***
Band-like intracranial calcification with simplified gyration and polymicrogyria	251290	***OCLN***
Cerebral arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL)	125310	***NOTCH3***
Cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL)	600142	***HTRA1***
Hereditary angiopathy with neuropathy, aneurysms and muscle cramps	611773	***COL4A1***
Wilson disease	277900	***ATP7B***
Menkes disease	300011	***ATP7A***
Leukodystrophy, hypomyelinating, 4 Spastic Paraplegia 13	612233 605280	***HSPD1***
Fabry disease	301500	***GLA***
Familial hemophagocytic lymphohistiocytosis 5	613101	***STXBP2***
Familial hemophagocytic lymphohistiocytosis 3	608898	***UNC13D***
Familial hemophagocytic lymphohistiocytosis 4	603552	***STX11***
Familial hemophagocytic lymphohistiocytosis 2	603553	***PRF1***
Lowe syndrome	309000	***OCRL***
Niemann-Pick type C2	601015	***NPC2***
Niemann-Pick type C1	257220	***NPC1***
Genetic Leukoencephalopathies: Mitochondrial Disorders		
Mitochondrial complex I disorders	252010	***NDUFS1, NDUFS4, NDUFS7, NDUFS8, NDUFV1, NDUFS2, NDUFAF1***
Mitochondrial complex II disorders	252011	***SDHA, SDHB, SDHAF1,***
Mitochondrial complex III disorders	124000	**BCS1L**
Mitochondrial complex IV disorders	256000 603644 604377 220110 256000 220110	***SURF1, SCO1, SCO2, COX10, COX15, TACO1***
Mitochondrial complex V disorders	220110	***ATPAF2***

Glutaric Acidemia IIC	231680	***ETFDH***
Coenzyme Q10 deficiency, primary, 4	612016	***ADCK3***
Combined oxidative phosphorylation deficiency 1	609060	***GFM1***
Combined oxidative phosphorylation deficiency 2	610498	**MRPS16**
Combined oxidative phosphorylation deficiency 4	610678	***TUFM***
Mitochondrial DNA depletion syndrome 4A, 4B and recessive ataxia syndrome	203700 613662 607459	***POLG***
Progressive external ophthalmoplegia with mitochondrial DNA deletions	610131	***POLG2***
Mitochondrial DNA depletion syndrome 1	603041	***TYMP***
Mitochondrial DNA depletion syndrome 5	612073	***SUCLA2***
Mitochondrial DNA depletion syndrome 7	271245	**C10ORF2**
Mitochondrial DNA depletion syndrome 8A	604712	**RRM2B**
Mitochondrial DNA depletion syndrome 12	615418	**SLC25A4**
Coenzyme Q10 deficiency, primary, 1	607426	**COQ2**
Coenzyme Q10 deficiency, primary, 5	614654	***COQ9***
Mitochondrial DNA depletion syndrome 3	251880	**DGUOK**
Genetic Leukoencephalopathies: Hereditary Spastic Paraplegias		
Spastic paraplegia 4 (SPG4)	182601	***SPAST***
Spastic paraplegia 5 (SPG5)	270800	***CYP7B1***
Spastic paraplegia 7 (SPG7)	607259	***SPG7***
Spastic paraplegia 11 (SPG11)	604360	***SPG11***
Spastic paraplegia 15 (SPG15)	270700	***ZFYVE26***
Spastic paraplegia 20 (SPG20)	275900	***SPG20***
Spastic paraplegia 21 (SPG21)	248900	***ACP33***
Spastic paraplegia 35 (SPG35)	612319	***FA2H***
Spastic paraplegia 56 (SPG56)	615030	***CYP2U1***

** 18q minus syndrome recommendations

Table 6. Clinical and Laboratory tests that aid in the diagnosis of LDs and gLEs

Clinical/Laboratory Test*	Diagnostic Target
Brain and spinal MRI (± gadolinium, ± MRS)	Establish white matter disease; ± evidence of leaky blood brain barrier and metabolite accumulation (mitochondrial disorders, Canavan, Sjögren Larsson Syndrome, peroxisomal biogenesis disorders)
Ophthalmologic exam	Document ophthalmologic signs in several leukodystrophies
Head CT	Assess for calcifications
Plasma very long-chain fatty acids	X-ALD/AMN and peroxisomal biogenesis disorders
Lysosomal enzymes (leukocytes)	Metachromatic Leukodystrophy, Krabbe, Multiple sulfatase deficiency, Galactosialidosis, Sialidosis
Blood Lactate, pyruvate, amino acids	Mitochondrial disorders
Lumbar Puncture (cell count, protein, ± CSF neopterin, ± interferon-alpha)	Non-specific marker of demyelination; ± pleocytosis and AGS markers
Urine sulfatides	MLD, Multiple sulfatase deficiency
Urine organic acids	L-2-hydroxyglutarate; NAA for Canavan disease; Krebs cycle intermediates (mitochondrial disorders)
Neurophysiologic studies (BAER, EMG/NCV, VEP, SSEP)	Characterize involvement of cranial and peripheral nerves, optic tracts and spinal tracts
Genetic analyses	As indicated for each LD or gLE

* Additional tests may be indicated for patients with certain distinctive clinical presentations or extra-neurologic features suggestive of one or more specific leukodystrophies.

13. **Figure Legends**

Figure 1. Recommended molecular diagnostic workflow. Note emphasis on identification of treatable disorders to enable rapid changes in care as appropriate. For a complete description of this figure please see the main text. Abbreviations: P = pathogenic; LP = likely pathogenic.

Figure 2. MRI pattern recognition in the LD and gLE (reprinted with permission from Genereviews). Three major MRI characteristics help to discriminate between the different types of LD and gLE. The first discriminator is the presence or absence of *hypomyelination* (Figure 2a). Within this subset, the presence of improvement of myelination or atrophy directs the clinician towards a series of gLEs. Within the true hypomyelinating LDs, the presence of basal ganglia and cerebellar involvement further helps refine the diagnosis. If the pattern is not one of hypomyelination, then the second discriminator is whether the white matter abnormalities are *confluent or isolated and multifocal* (Figure 2b). If the white matter abnormalities are confluent, then the third discriminator is the *predominant localization of the abnormalities* (Figure 2b).

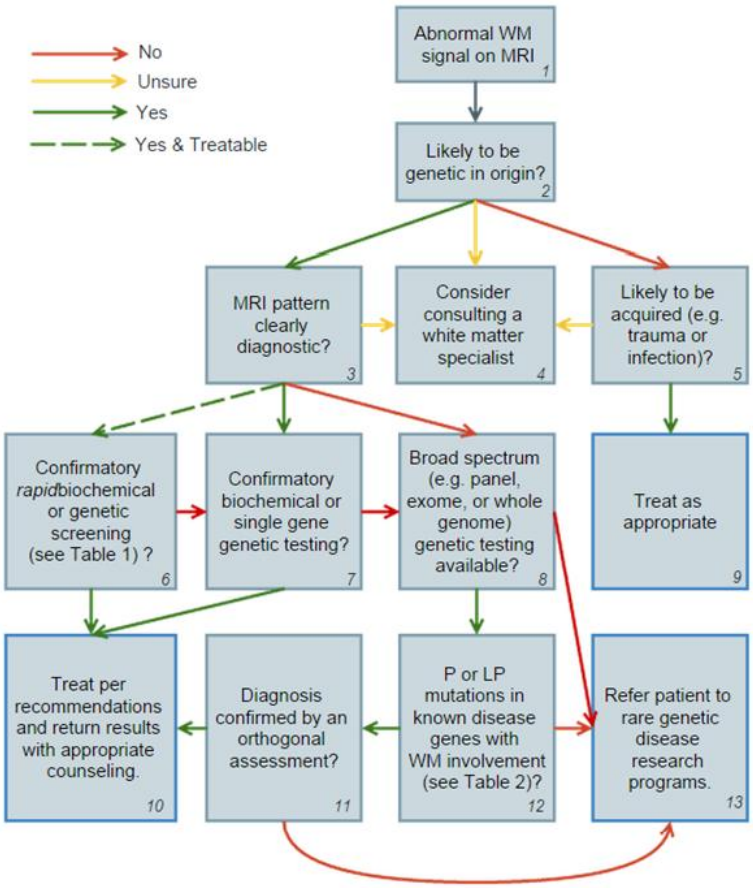


Figure 1

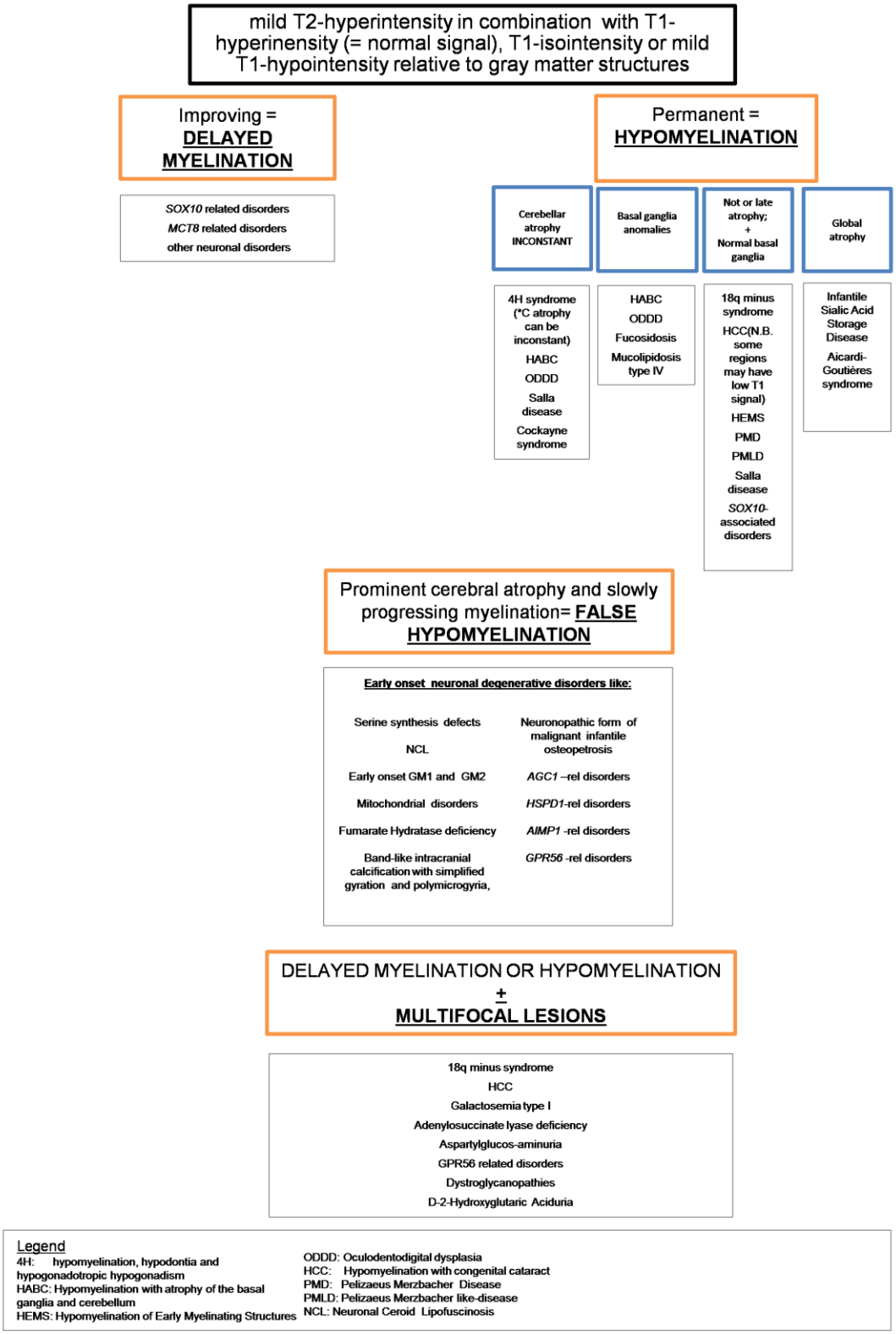
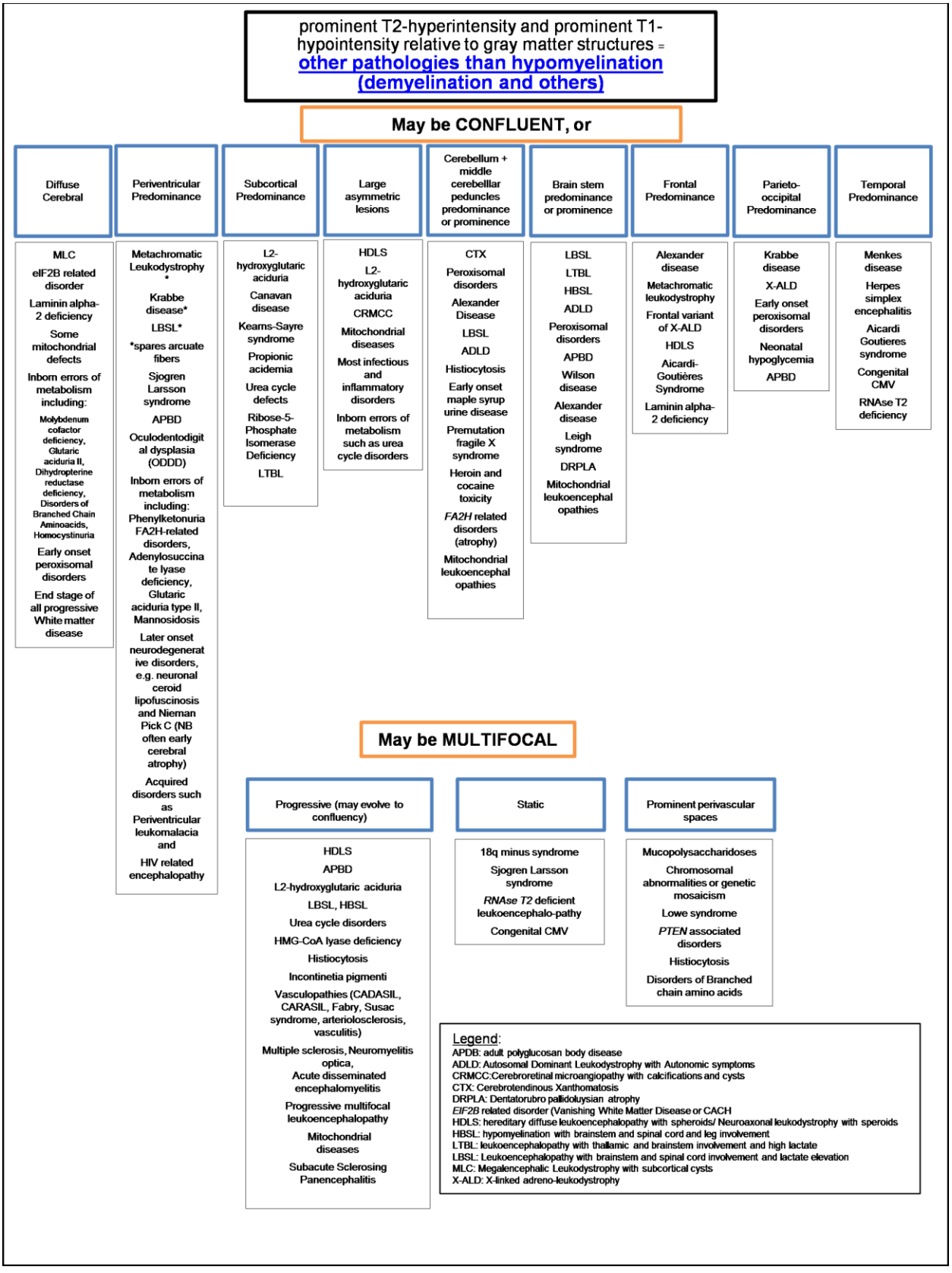


Figure 2



Highlights

If accepted, we propose the following highlights:

- Leukodystrophies are genetic disorders affecting the white matter of the central nervous system with or without peripheral nervous system involvement
- Although individual features may vary, leukodystrophies and genetic leukoencephalopathies all share white matter abnormalities on imaging or pathology of the CNS, and most have motor deficits that often dominate the clinical picture, especially in pediatric patients
- Brain MRI is the foundational investigation in a patient with a suspected leukodystrophy or genetic leukoencephalopathy
- The number of disorders with established therapies is small in number and as such these disorders require prompt recognition and downstream testing
- The partnering of MRI pattern analysis and next-generation sequencing may lead to higher diagnostic yield and more timely diagnosis