Diagnosis, prognosis, and treatment of leukodystrophies

Marjo S van der Knaap, Raphael Schiffmann, Fanny Mochel, Nicole I Wolf

Lancet Neurol 2019; 18: 962–72

Published Online
July 12, 2019
http://dx.doi.org/10.1016/S1474-4422(19)30143-7

Department of Pediatric Neurology, Emma Children's Hospital, Amsterdam University Medical Centre, Amsterdam, Netherlands (M S van der Knaap MD, N I Wolf MD); **Amsterdam Neuroscience, Amsterdam, Netherlands** (M S van der Knaap, N I Wolf); **Department of Functional Genomics, Center for Neurogenomics and Cognitive Research, Vrije Universitiet Amstardam, Amsterdam, Netherlands** (M S van der Knaap); **Institute of Metabolic Disease, Baylor Scott and White Research Institute, Dallas, TX, USA** (R Schiffmann MD); **Institut du Cerveau et de la Moelle Épinière (Sorbonne Université, Université Pierre et Marie Curie, Institut National de la Santé et de la Recherche Médicale, and Centre National de la Recherche Scientifique), Paris, France** (F Mochel MD); **and Department of Genetics and Reference Center for Adult Neurometabolic diseases, Pitié-Salpêtrière University Hospital, Assistance Publique des Hôpitaux de Paris, Paris, France** (F Mochel)

Correspondence to:
Prof Marjo S van der Knaap, Department of Pediatric Neurology, University Medical Centre, 1081 HV, Amsterdam, Netherlands
ms.vanderknaap@vumc.nl

Leukodystrophies comprise a large group of rare genetic disorders primarily affecting CNS white matter. Historically, the diagnostic process was slow and patient prognosis regarded as poor because curative treatment was only available for very few leukodystrophies in early stages of the disease. Whole-exome sequencing has both greatly increased the number of known leukodystrophies and improved diagnosis. Whether MRI keeps its central place in diagnosis and what the role is of whole-exome sequencing are relevant questions for neurologists. Improved diagnosis has revealed the phenotypic variability of leukodystrophies, requiring adaptation of prognostication. Technological advance in molecular techniques and improved insight into the pathophysiology of individual leukodystrophies have led to therapeutic developments, including drug design and gene therapy. Despite this progress, therapies are only beneficial early in the disease course, emphasising the need for a speedy diagnosis and for research on regenerative approaches to repair the damage already present.

Introduction

Leukodystrophies constitute a large, highly heterogeneous group of rare genetic disorders, characterised by selective and primary involvement of the CNS white matter.[1,2] Such disorders can manifest in people of all ages. Various underlying gene defects are known, each defining a specific leukodystrophy.[1,2] Advances in molecular techniques have had a fundamental effect on the diagnosis, understanding, and treatment of leukodystrophies.[3–6] Diagnosis of leukodystrophy used to be time-consuming and cumbersome, but whole-exome sequencing (WES; mostly used in clinical settings) and whole-genome sequencing (WGS; currently mostly used for research) now allow rapid identification of the underlying gene defect. WES and WGS have led to the identification of the molecular basis of many leukodystrophies, solving the persistent problem of a high proportion of leukodystrophy cases without molecular diagnosis, increasing the number of diagnosable disorders,[3,4] and adding knoweldge about clinical variability and prognosis.[5] Therapy for leukodystrophies has lagged, but prospects are improving.[6] Gene-editing techniques are rapidly advancing, facilitating in-vivo and in-vitro gene correction, necessary for gene therapy. Other treatment options include drugs that modulate disease pathways, antisense oligonucleotide therapy, and therapy based on stem cells.[6]

The almost overwhelming increase in the number of known leukodystrophies necessitates an updated categorisation system to facilitate diagnosis. WES availability requires reconsideration of the traditional step-by-step approaches to achieve definitive diagnoses. The role of MRI as a primary diagnostic tool needs be also reconsidered, and perhaps redefined. For the leukodystrophies with improving therapeutic prospects, a speedy diagnosis is particularly important, as such therapies are only relevant in early disease stages, when the brain is not yet irreparably damaged. New knowledge on wide clinical variation and benign disease variants requires modification of the traditional view on leukodystrophies as disorders with an invariably poor prognosis.[7] New insights into the molecular and cellular bases of the disorders have changed the concept of leukodystrophies and have led to recognition that all structural white-matter components (including myelin, oligodendrocytes, astrocytes, microglia, axons, and blood vessels) can be primary disease targets.[2,8] This new view affects the understanding of disease mechanisms and directs therapy development. This Review provides an update on these advances in diagnosis, prognosis, and treatment of leukodystrophies.

Definition and categorisation

The definition of leukodystrophy has evolved over time, first focused only on myelin and oligodendrocytes, and subsequently including astrocytes.[1] The latest definition includes all genetically determined disorders with selective and primary involvement of CNS white matter, irrespective of the structural white-matter component involved and molecular process affected.[2,8] By contrast, leukoencephalopathies comprise all primary CNS white-matter disorders, both genetic and acquired.[2] This Review specifically addresses the leukodystrophies. Some leukodystrophies are traditionally called leukoencephalopathies; although for clarity, we will refer to them as leukodystrophies. However, the outdated myelin-focused concept of leukodystrophies persists, and the new definition requires further implementation in clinical practice. For example, a review published in 2018 only recognised demyelinating and hypomyelinating categories,[9] hampering understanding of, for instance, leukodystrophies with a primary astrocytic or microglial defect.[10] Importantly, the myelin-focused concept of leukodystrophies directs all therapy developments towards remyelination, which is not a useful approach for leukodystrophies in which the primary problem does not concern myelin.

For the diagnostic work-up, a leukodystrophy categorising system is helpful, providing a framework, inside which the numerous individual disorders can be recognised, differentiated between, and understood. The most appropriate categorising principle might differ depending on purpose or target group. A proposed system for pathologists was based on a cellular pathology approach, taking into account the contribution of various white-matter cell types and structures driving white-matter disease.[2] Although this system is helpful for pathologists,

this perspective is less suitable for neurologists, for whom understanding the types of metabolic and molecular causes of leukodystrophies, and distinguishing categories that facilitate the diagnostic process is more helpful. In a framework for neurologists, we have subdivided leukodystrophies into categories, which are typically diagnosed in the same specialised laboratory (appendix pp 1–3): lysosomal disorders, peroxisomal disorders, mitochondrial respiratory chain disorders, defects in amino acid and organic acid metabolism, and DNA repair disorders. Genetic vasculopathies,[11,12] translation defects,[3] and defects in ion and water homoeostasis[13] are newly defined categories. Newly detected disorders can be easily placed within this framework.

This proposed categorisation should enhance insight into pathophysiology by revealing the types of defects that CNS white matter is vulnerable to. This framework will be adapted whenever new insights are gained. Importantly, since all disease categorisations represent simplifications of complex biology and pathophysiology,[2] the categorisation of some defects is difficult, as they would fit multiple categories (eg, mitochondrial translation versus mitochondrial defect). Similarly, mutations in the same gene can differentially affect white and also grey matter structures, depending on the specific mutation.[14]

Diagnosis

Whole-exome sequencing

The introduction of WES has had a major effect on the diagnosis of rare diseases, including leukodystrophies. Many previously undetermined cases have been given a specific diagnosis and numerous new leukodystrophy genetic defects have been identified. The percentage of leukodystrophy cases without specific diagnosis has decreased from about 50% in 2010 to 20–30% in 2016,[3,4] and this percentage is still decreasing. WES is cheaper[15] and delivers a much faster diagnosis (which takes a few months) than the traditional step-by-step diagnostic approach (which can take years).[16,17] With the major diagnostic success, high cost effectiveness, and easy applicability of WES, the traditional diagnostic approach used in clinical practice needs to be reconsidered. Additionally, available and emerging therapies—only applicable in early disease stages—put pressure on the diagnostic process, requiring speed.

For these reasons, it might be suggested that the use of MRI in guiding the diagnostic process should be replaced by WES. However, WES has its own limitations. WES is a labour-intensive and, in itself, a costly technique, and its turnaround time varies from a few weeks to several months depending on local facilities. Some genetic variants, such as copy number variations, variants in non-coding parts of the gene, and variants in non-coding RNAs, are not detected by WES.[18] For instance, leukodystrophy with calcifications and cysts, which is caused by mutations in *SNORD118*, a non-coding RNA, is not detected by WES, but it is easily diagnosed by MRI.[19] The recessive variant of hypomyelination with atrophy of the basal ganglia and cerebellum (H-ABC) that is caused by a deletion in the promoter region of *UFM1* is not detected by WES, while the MRI abnormalities are pathognomonic.[20] Sometimes WES reveals variants of unclear significance and diagnosis thus requires confirmatory evidence from other sources.[21] Sometimes WES findings are negative, because the patient does not have a genetic disease. Sometimes WES findings are negative and clinical and MRI recognition of the disease and targeted genetic testing are still necessary. WES-based gene-panel analysis is faster than open WES, but it does share its drawbacks. How the limitations of WES should be weighed against the obvious advantages and which place WES should have in the diagnostic process to maximise contribution and avoid pitfalls are important questions.

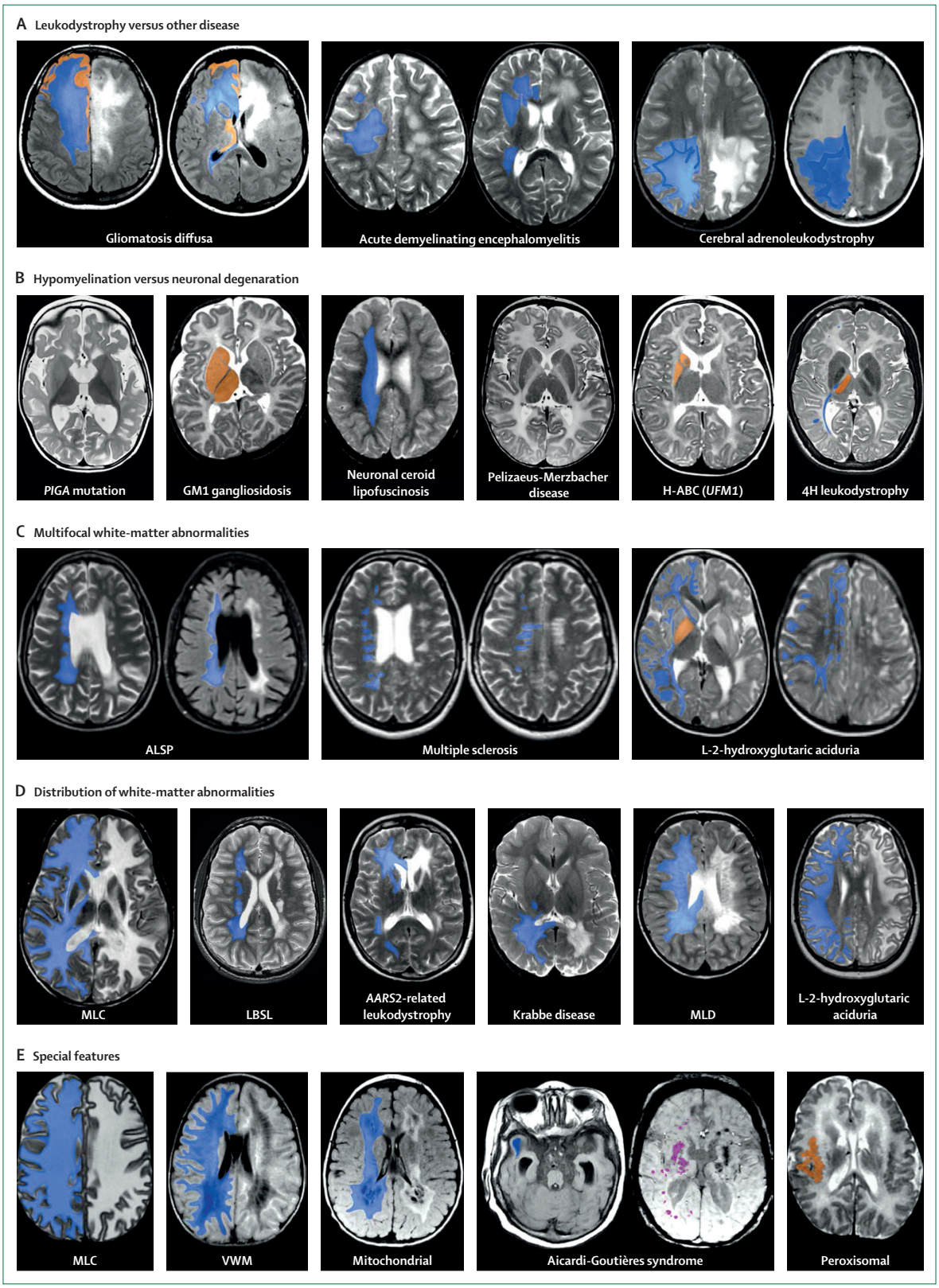
See Online for appendix

MRI

The clinical presentation and neurological findings in patients with leukodystrophy are often non-specific. With its extreme sensitivity for brain white-matter abnormalities, MRI is still typically the first technique used to diagnose a CNS white-matter disease, and all diagnostic algorithms proposed in the past few years are still MRI-centred.[9,11,12,22–25] Considering that an MRI of the patient is available and considering the limitations of WES as a stand-alone test, we agree that the diagnostic process should start with in-depth MRI pattern recognition,[26] which aids in the crucial distinction between genetic and acquired disease and might allow a specific diagnosis or short differential diagnosis containing only a few items (figure 1; appendix pp 10–17).

However, MRI also has limitations and can be inconclusive, especially in adults. For example, in adults with multifocal and asymmetrical white-matter lesions, adult-onset leukodystrophy with axonal spheroids and pigmented glia (ALSP) constitutes an important differential diagnosis to multiple sclerosis (figure 1C).[27,28] The distinction by MRI between genetic vascular leukodystrophies and acquired white-matter abnormalities related to vascular risk factors like hypertension might be impossible.[11] Unusual MRI presentations pose diagnostic dilemmas. For example, an MRI of cerebral adrenoleukodystrophy (also known as ALD) with asymmetrical abnormalities might suggest acquired disease.[29] Additionally, MRI pattern recognition is an evolving tool, and new information on disease variation should be continuously incorporated into this tool as it becomes available.

With the use of new pulse sequences and higher field strengths, the potential of MRI regarding the diagnosis of leukodystrophies is further increasing.[30–32] New MRI techniques specifically assess white-matter microstructural integrity. The measurement of the intramyelin water fraction is a promising technique to produce markers for myelin content and intactness,[31] while neurite orientation dispersion and density imaging allows an estimation of microstructural complexity of dendrites and


A Leukodystrophy versus other disease
Gliomatosis diffusa
Acute demyelinating encephalomyelitis
Cerebral adrenoleukodystrophy
B Hypomyelination versus neuronal degenaration
PIGA mutation
GM1 gangliosidosis
Neuronal ceroid lipofuscinosis
Pelizaeus-Merzbacher disease
H-ABC (UFM1)
4H leukodystrophy
C Multifocal white-matter abnormalities
ALSP
Multiple sclerosis
L-2-hydroxyglutaric aciduria
D Distribution of white-matter abnormalities
MLC
LBSL
AARS2-related leukodystrophy
Krabbe disease
MLD
L-2-hydroxyglutaric aciduria
E Special features
MLC
VWM
Mitochondrial
Aicardi-Goutières syndrome
Peroxisomal

axons within the white matter.[32] The capacity of these new MRI techniques to distinguish between different leukodystrophies or to provide better insight into leukodystrophy pathology has not been assessed yet.

Proposed diagnostic algorithm

Weighing up the available information, we propose a diagnostic algorithm (figure 2), in which we combine the excellent diagnostic capabilities of MRI and the contribution of clinical diagnostic features (if present) with the powerful diagnostic options of WES or WGS to achieve a molecular diagnosis in the highest percentage of patients of all ages as fast as possible. MRI is central in answering the initial crucial question as to whether the patient has a leukodystrophy (figure 1; appendix pp 10–11). If so, analysis of clinical details might also be valuable for the diagnosis, especially if systems other than the CNS are involved (appendix p 12).[7,9,11,22–25] If the combination of MRI and clinical features suggests one or a few specific disorders, targeted metabolic and genetic analyses are the fastest way to a confirmed diagnosis. If negative, the diagnosis of leukodystrophy and the differential diagnoses should be reconsidered. Importantly, in some cases, MRI is pathognomonic for a specific disorder and this diagnosis should not be discarded after initial negative test results.[19,20] If the MRI pattern and clinical picture are less specific or the differentiation from acquired leukoencephalopathies is challenging, a leukodystrophy expert should be consulted. The expert might recognise the disease and go for targeted testing. If the expert does not recognise the disease, non-selective screening should be started. Screening using next-generation sequencing with analysis of a white-matter gene panel or open WES or WGS analysis is, if available, the most fruitful approach with the highest diagnostic yield, fastest result, and lowest cost compared with all other testing approaches.[4,15,17] However, if next-generation sequencing techniques are not available, not accessible, or have a long turnaround time, one screening round of metabolic biomarker assessment (figure 2) can be applied, with relatively fast results compared with WES or WGS (which is the fastest) and the traditional step-wise approach (which is the slowest).[9] Importantly, sequential metabolite and gene analysis should be avoided, because the approach is expensive and takes many months or years.[17] If all tests are unrevealing, the patient should be referred to a leukodystrophy expert centre for diagnosis;[33] if no diagnosis is made, participation in studies to identify new genes associated with leukodystrophies should be considered.

Analysis of WES and WGS data will improve as more information on disease-causing and benign variants becomes available. WGS, in part, addresses the problems of WES discussed here.[34] When WES, and particularly WGS become less expensive and more widely available worldwide, the algorithm will need to be adapted again. We envision a time when assessment of metabolites in body fluids will be abandoned as a first-line screening technique, but will remain important in the evaluation of pathogenicity of variants and in monitoring therapy.

In contrast to the diagnostic algorithm outlined here, newborn screening aims at identification of patients before disease onset to install treatment before or at the beginning of disease manifestations. The technique is available for a growing number of genetic disorders, including leukodystrophies, but the number of disorders screened for differs per country or even region within a country due to local decisions based on ethical considerations and financial means.[35,36] With this approach, means to confirm presence of the disease and predict onset and severity are essential for making decisions regarding therapy.[18]

Prognosis

Leukodystrophies can present at any age. Age of onset typically correlates inversely with disease severity and

Figure 1: **MRI patterns of leukodystrophies**

(A) Non-selective involvement of grey and white matter in a patient who does not have a leukodystrophy or leukoencephalopathy, but was diagnosed with gliomatosis diffusa, confirmed at autopsy. Multifocal white matter abnormalities with an asymmetrical distribution are more common in acquired diseases, illustrated here by inflammatory demyelination in acute demyelinating encephalomyelitis, while confluent and symmetrical abnormalities are more common in leukodystrophies, illustrated here by cerebral adrenoleukodystrophy. (B) Primary hypomyelination versus secondary hypomyelination in neuronal degenerative disorders. Neuronal degenerative disorders commonly have earlier onset and more severe atrophy than leukodystrophies, as illustrated by a patient with *PIGA* mutations versus a patient with Pelizaeus-Merzbacher disease. Infantile-onset neuronal degenerative disorders are characterised by abnormal myelination, as shown by a patient with infantile GM1 gangliosidosis and a patient with *PIGA* mutations; later-onset neuronal degenerative disorders are often characterised by a wide, ill-defined periventricular zone of mild signal abnormality, as illustrated by the case of juvenile neuronal ceroid lipofuscinosis. In infantile GM1 gangliosidosis, abnormalities are also present in the basal ganglia and thalami. Special features facilitate a diagnosis in hypomyelinating disorders, like low T2 signal in the lateral thalamus in 4H leukodystrophy and absence of the putamen in H-ABC. The abnormal signal in the lateral part of the head of the caudate nucleus is pathognomonic for H-ABC caused by recessive *UFM1* mutations. (C) Multifocal cerebral white matter abnormalities are more common in acquired disorders, but can also occur in specific leukodystrophies, hampering the differential diagnosis. Here these abnormalities are shown in ALSP, multiple sclerosis, and L-2-hydroxyglutaric aciduria. The latter case also displays signal abnormalities in the basal nuclei, facilitating the diagnosis. (D) Diagnostic value of the analysis of the distribution of white matter abnormalities, with diffuse involvement in MLC versus involvement of periventricular and deep cerebral white matter in LBSL, anterior predominance in *AARS2*-related leukodystrophy versus posterior predominance in juvenile Krabbe disease, and periventricular and deep predominance in MLD versus subcortical predominance in L-2-hydroxyglutaric aciduria. (E) Diagnostic value of special features. Swelling of the abnormal white matter is a feature of MLC. Diffuse white matter rarefaction, as evident on FLAIR, is a feature of VWM. The presence of well delineated cysts in the abnormal white matter is a feature of mitochondrial leukodystrophies. Anterior temporal cysts and calcium deposits, as observed on susceptibility-weighted images, are features of Aicardi-Goutières syndrome. Perisylvian polymicrogyria is often a feature of severe variant of peroxisome biogenesis defects. White matter structures are marked in blue, grey matter structures in orange, and calcium deposits in purple.
4H leukodystrophy=hypomyelination, hypodontia, and hypogonadotropic hypogonadism. ALSP=adult-onset leukodystrophy with axonal spheroids and pigmented glia. FLAIR=fluid-attenuated inversion recovery.
H-ABC=hypomyelination with atrophy of the basal ganglia and cerebellum.
LBSL=leukodystrophy with brain stem and spinal cord involvement and lactate elevation. MLC=megalencephalic leukodystrophy with subcortical cysts.
MLD=metachromatic leukodystrophy. VWM=vanishing white matter.

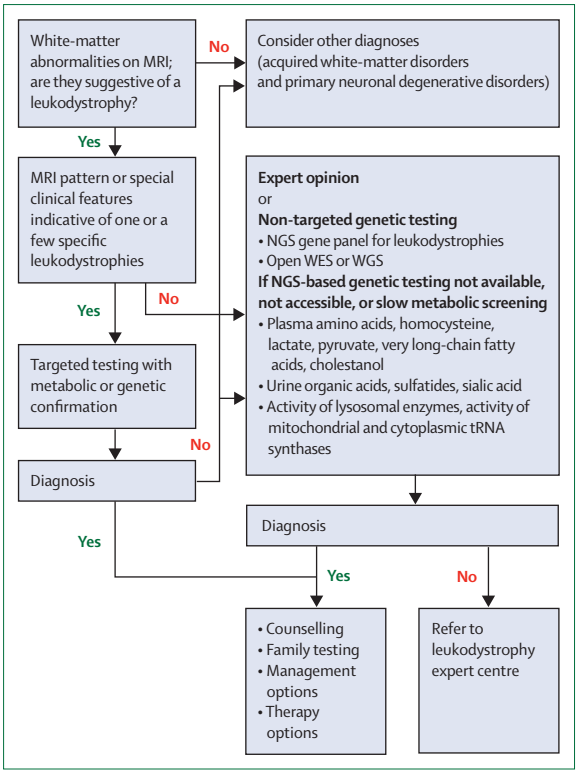


Figure 2: **Proposed diagnostic algorithm**
The authors' view on the up-to-date testing framework for leukodystrophies. Next-generation sequencing has an important role and should be considered early, as its diagnostic yield is high, speeding up the diagnostic process. NGS=next-generation sequencing. WES=whole-exome sequencing. WGS=whole-genome sequencing.

rate of progression.[37,38] Leukodystrophies have a reputation of being relentlessly progressive and fatal.[7] Although this concept is true for many leukodystrophies, more variable and more benign disease phenotypes with long episodes of stability, permanent improvement, or complete recovery are now apparent.[9,12] For example, megalencephalic leukodystrophy with subcortical cysts (MLC) invariably has its onset with macrocephaly in the first year of life, but the subsequent disease course is of variable severity: some patients die in adolescence, but others display hardly any disease signs as adults.[39] Interestingly, an MLC variant with dominant inheritance and transient white-matter disease is also part of the phenotypic range.[39]

Counterintuitively, leukodystrophies with similar genetic defects are not necessarily associated with a similar prognosis. Even though defects in mitochondrial tRNA synthetases all affect the same process of translation of 13 genes of the mitochondrial genome into respiratory chain proteins,[40] only some defects are associated with leukodystrophies, and these have different disease courses. Leukodystrophy with brain stem and spinal cord involvement and lactate elevation (LBSL), caused by *DARS2* mutations, most often has a childhood or adolescent onset and slow disease course without a life-limiting effect, while early-infantile onset disease can be rapidly progressive and fatal.[41] Leukodystrophy with thalamus and brain stem involvement and lactate elevation (also called LTBL), caused by mutations in *EARS2*, is associated with a single period of neurological decline in the antenatal or infantile period, followed by improvement and then stabilisation; a second episode of clinical deterioration has not been reported.[42] MRI shows improvement of most early brain abnormalities, and only structures that were permanently damaged remain visibly abnormal.[42] Timing and severity of the episode determine the clinical outcome, varying from death to complete recovery.[42,43] Disease phenotype can be extremely discordant between affected siblings, and some people have *EARS2* mutations with no or minimal disease.[42] Consequently, biallelic *EARS2* mutations might be picked up by WES in patients with another disease, causing diagnostic confusion.[44] In *AARS2*-related ovarioleukodystrophy, onset is relatively late, in adolescence or adulthood, but deterioration is often rapid, causing death within a few years after onset.[45,46] So, for unclear reasons, defects in three neighbouring enzymes involved in the translation of 13 genes of the mitochondrial genome into respiratory chain proteins cause leukodystrophies, but with major differences in MRI presentation and disease course.

The group of hypomyelinating disorders comprises disorders of myelin development, suggesting an early onset and subsequent deterioration. However, scarcity of myelin might occur without clinical manifestations for years up to decades.[47] An example is oligosymptomatic hypomyelination, hypodontia, and hypogonadotropic hypogonadism (4H leukodystrophy) in adults.[48] Another example is *TMEM106B*-associated hypomyelination, which presents in infancy, but only mild clinical features are present in teenagers and adults, contrasting with the severe disability observed, for instance, in individuals with Pelizaeus-Merzbacher disease, which manifests at the same early age with the same clinical signs and MRI changes.[49]

The prognoses of diseases with episodic deterioration are difficult to predict and are not always poor. Mitochondrial leukodystrophies often have an onset in infancy, but can present at any age.[50] The disease course is typically characterised by episodes of neurological decline, provoked by febrile infections or other stressors, followed by partial recovery. Patients might die or survive for decades with highly variable disease signs or even no disability.[50] The course of vanishing white matter (VWM), also called childhood ataxia with CNS hypomyelination, is similar and similarly variable.[37] The episodic deterioration is often related to sensitivity of some leukodystrophies to external factors, which co-determine disease course. In VWM, deteriorations are provoked by activators of the integrated stress response;[51] in microglia-related leukodystrophies, such as adrenoleukodystrophy, by activators of immune cascades;[52] and in mitochondrial disorders by high energy demand during febrile infections.[50] In such disorders,

environmental factors and genetic make-up, which for instance determines susceptibility to infections and febrile response, have a strong effect on the disease course and, more so with increasing disease duration.[37]

Thus, a general statement on the prognosis of leukodystrophies cannot be made; statements regarding prognosis have to be ascertained for each leukodystrophy separately. Several factors can be considered when addressing the wish of the patient and family for a clear prognosis (panel 1). Large and long-term natural history studies combining genetic, clinical, imaging, and environmental, information (such as infections, trauma, or food habits) will help identify the contribution of each and to improve prediction. These studies will also clarify the true variation in disease phenotype and prognosis. In analogy to adrenoleukodystrophy,[56] we foresee that milder and later-onset leukodystrophy variants than are now known will be identified more frequently, since these variants are currently underdiagnosed.

Management and treatment

Currently, many interventions are available to manage disease manifestations, improve the quality of life of patients,[57] reduce the use of medical resources, and reduce expenses.[58] Some clinical manifestations are disease-specific, while others are general (panel 2). Anticipation and monitoring of manifestations allow initiation of treatment (eg, hydrocortisone replacement for adrenal failure in adrenoleukodystrophy[59] and cholecystectomy for gallbladder involvement in metachromatic leukodystrophy [MLD])[61] before manifestations substantially impair quality of life. Spasticity is a central problem in leukodystrophies, which is usually successfully managed by oral drugs, intramuscular botulinum toxins, intrathecal baclofen,[53] or selective dorsal rhizotomy.[62] Infections constitute a major contribution to the disease burden and clinical outcome. They are a potentially modifiable factor, for instance by vaccinations and prophylactic antibiotic treatment to prevent respiratory tract infections.[58,63] Given the rarity of the disorders and heterogeneity of patient groups, evidence-based data on these approaches do not exist, and is unlikely to be available in the future.

Curative therapy development for leukodystrophies has been lagging more than management. Considering the complexity of these diseases and the fact that pathomechanisms might not only differ for individual leukodystrophies, but also for different mutations in the same gene,[14,21] this delay is not surprising. Fortunately, with technological developments, this prospect is reversing.[6,60,64] For designing clinical trials, in-depth knowledge on natural history in large patient cohorts[65] (often hampered by the ultra rarity of the disease) and identification of suitable biomarkers (often MRI-derived)[30–32] are essential.

Established treatments with proven efficacy are haematopoietic stem-cell transplantation (HSCT) and ex-vivo gene therapy. The feasibility and efficacy of other treatment modalities remain to be proven.

Panel 1: **Prognostic factors**

Specific disease
Some disorders are predictable in their course (eg, metachromatic leukodystrophy [MLD], leukodystrophy with brain stem and spinal cord involvement and lactate elevation [LBSL], and megalencephalic leukodystrophy with subcortical cysts [MLC]),[39,41,53] whereas others are highly unpredictable (eg, vanishing white matter [VWM]).[37]

Age at presentation
In general, early onset is associated with more rapid decline;[37] however, exceptions exist (eg, MLC).[39]

Disease severity at presentation
A severe and chronically progressive disease predicts a poor prognosis (eg, VWM).[37] A subacute severe encephalopathy is more unpredictable (eg, mitochondrial leukodystrophies).[50]

Specific mutations
A clear genotype–phenotype correlation is present in some disorders (eg, VWM or Pelizaeus-Merzbacher disease),[37,54] but not in others (eg, MLC).[39]

MRI
In general, more extensive damage is associated with more severe disease course.[47,48] For the episodic disorders, what damage is irreparable might not be immediately clear.[50,55] For some disorders, the white-matter abnormalities are extensive or diffuse, yet disease severity is variable (eg, MLC or hypomyelination).[39,49]

Haematopoietic stem-cell transplantation

For decades, a low-phenylalanine diet for phenylketonuria, chenodeoxycholic acid for cerebrotendinous xanthomatosis, and HSCT for early stages (presymptomatic or early symptomatic) of cerebral adrenoleukodystrophy, juvenile and adult MLD, and juvenile or adult Krabbe disease were the only efficacious therapies available.[6,64–66] HSCT in later disease stages and in patients with early symptomatic late-infantile MLD or Krabbe disease are associated with poor outcome.[67–69] Unfortunately, the outcome of timely HSCT is not uniformly favourable either. In patients with Krabbe disease, diagnosed by prenatal testing or newborn screening, early HSCT does not cure the disease, but only modifies its course.[70] ALSP is caused by mutations in *CSF1R*, which encodes the macrophage colony-stimulating factor 1 receptor (CSF-1-R). CSF-1 is a cytokine that controls the production, differentiation, and function of macrophages and microglia. As HSCT provides microglia derived from bone marrow, ALSP is an excellent candidate for this therapy. Stabilisation of the disease after HSCT has been reported,[71] but overall experience is scarce and criteria for timing of the intervention in the disorder are still inadequate. As most leukodystrophies are currently without therapeutic options, HSCT is sometimes done out of desperation, without rationale, and outside of a formal trial setting.[72] Patients and families are confronted with high costs, and considerable morbidity and mortality, without any benefit;[70] thus, use outside of current indications is strongly discouraged.

Panel 2: **Management of patients with leukodystrophies**

Disease specific

- Hydrocortisone supplementation for Addison syndrome in adrenoleukodystrophy or adrenomyeloneuropathy[57,59,60]
- Cholecystectomy for gallbladder dysfunction, polyps, and to prevent cancer in metachromatic leukodystrophy (MLD)[61]
- Avoid head trauma and ensure prompt treatment of fever and infections to avoid triggering deterioration in vanishing white matter (VWM)[37]
- Growth hormone substitution if deficient; hormone supplementation to induce puberty and prevent osteoporosis in hypomyelination, hypodontia, and hypogonadotropic hypogonadism (4H leukodystrophy)[57]
- Treatment of ovarian failure in VWM or *AARS2*-related leukodystrophy[60]
- Monitoring and treatment of immune-mediated disease features in Aicardi-Goutières syndrome[60]
- Avoid head trauma and ensure prompt treatment of status epilepticus in megalencephalic leukodystrophy with subcortical cysts (MLC)[39]

General

- Treatment of spasticity with spasmolytic drugs, botulinum toxin, intrathecal baclofen, or selective dorsal rhizotomy[53,57,62]
- Maintenance of sufficient nutritional state (eg, percutaneous gastrostomy)[57]
- Prevention (eg, with prophylactic antibiotics) and treatment of infections[37,57,58,63]
- Treatment of bladder and bowel dysfunction[57]
- Monitoring and treatment of orthopaedic problems such as hip dislocation and scoliosis[57]
- Ensuring adequate calcium and vitamin D supplementation[57]
- Treatment of neuropathic pain (eg, with amitriptyline or gabapentin)[57]
- Treatment of irritability and disturbed sleep (eg, with melatonin or alimemazine)[57]
- Treatment of sialorrhea (eg, with anticholinergic drugs or botulinum toxin injections)[57]
- Treatment of epilepsy[57]
- Ensuring adequate communication[57]

Gene therapy

Ex-vivo gene therapy allows transplantation of genetically engineered autologous haematopoietic cells in patients with cerebral adrenoleukodystrophy or MLD.[73,74] This new strategy is a solution for patients without matched donors and allows further genetic modification. For example, in patients with MLD, enhanced arylsulfatase A activity is achieved by gene modification strategies and, for the first time, good functional outcomes are reported for patients with the late-infantile disease.[73] The procedure eliminates risks related to incompatibility between graft and host, and even stabilises peripheral neuropathy, which does not respond well to HSCT.[73] The therapeutic window, however, does not differ from that of donor-derived HSCT—for a good outcome, treatment has to be performed early.[73] Careful monitoring is required to indicate whether the overexpression of arylsulfatase A becomes problematic over time.

Gene therapy directly targeting the brain is becoming a realistic option. Treatment with a viral vector carrying the *ASPA* gene in 13 patients with Canavan disease proved to be safe and resulted in some clinical and MRI improvement.[75] However, a small trial evaluating intracerebral gene therapy in patients with MLD was terminated because of continuing patient deterioration.[76] Why this approach was unsuccessful is unclear; gene delivery alone might not be sufficient and might have to be combined with immunomodulation or silencing of the mutant gene.[76,77]

The CRISPR (clustered regularly interspaced palindromic repeats)-Cas (CRISPR-associated protein) approach is a promising method for precise gene editing,[78] able to remove pathogenic variants, but also to silence genes. This tool has not yet been tested in animal models for leukodystrophies. Off-target effects are still a matter of serious concern.[78] Safety issues need to be addressed before application in the clinic.

Antisense nucleotides

Antisense nucleotides aim to target mutations with a dominant negative effect, silencing the mutant gene. A single intrathecal injection of this treatment in a mouse model of Alexander disease led to long-lasting improvement (until the mice were sacrificed at 4 months).[79] Other leukodystrophies with dominant inheritance, such as H-ABC caused by *TUBB4* mutations[14] are also candidates for this approach. Antisense nucleotides can also be used to correct aberrant splicing. This has been shown for a PLP-related disorder, hypomyelination of early myelinating structures (HEMS) in an animal model.[80] Dose, timing, and intervals of antisense nucleotide administration still have to be tested in clinical trials. The long-term consequences of gene silencing also need to be closely monitored.

Targeted drug treatment

Drugs for individual leukodystrophies are being identified in diverse ways. Drug screening can rapidly assess the effects of thousands of compounds on cell or zebrafish disease models.[81,82] Also, advancing insights into disease mechanisms facilitate identification of drug targets. For example, Janus kinase inhibition was used to block type I interferon signalling in a case of Aicardi-Goutières syndrome, showing clinical improvement with regain of previously lost skills and improvement of elevated inflammatory markers.[83] Also in Aicardi-Goutières syndrome, reverse transcriptase inhibitors are being investigated to decrease nucleotide load (NCT03304717/RTIAGS).[84] Guanabenz is being investigated for integrated stress response inhibition in VWM. The drug has been shown to be effective in ameliorating white-matter pathology in an animal model of VWM and a trial in a small group of patients with VWM is currently in preparation (NTR7482).[85] Finally, a PPAR-γ agonist, MIN-102 is being used to target multiple intracellular pathways (ADVANCE trial [NCT03231878] ongoing in adrenomyeloneuropathy). Substrate reduction therapy is an attractive approach to decrease the accumulation of storage material in MLD and Krabbe disease. Although effective in animal models, it has not yet been applied to patients.[86]

Enzyme-replacement therapy

For leukodystrophies, enzyme-replacement therapy has so far not proven effective, as intravenous formulations are not able to cross the blood–brain barrier.[87] Intraventricular enzyme administration might solve this problem and a phase 1/2 trial (NCT01510028) for patients with MLD has been done with possible disease-stabilising effects for the highest dose used.[88] Given the availability of proven therapies for patients with presymptomatic MLD and the disadvantages of frequent intrathecal enzyme administration, candidates for enzyme-replacement therapy trials will be patients who are already symptomatic, which might cause underestimation of efficacy. Still, as intrathecal enzyme-replacement therapy was also shown to be effective in an animal model of MLD,[89] a phase 2 trial (NCT03771898) with frequent intrathecal enzyme administration is just starting.[90]

Stem-cell-based therapies

Based on successful outcomes in leukodystrophy mouse models,[91,92] stem-cell-based therapy holds promise for patients with leukodystrophies. To our knowledge, the only published trial on patients with Pelizaeus-Merzbacher disease confirmed safety, but the procedure did not find a clinically significant effect in those patients.[93] The approach has been shown to be effective in an animal model of VWM.[92]

The most suitable type of stem cell (eg, pluripotent glial precursors, mesenchymal stem cells, or more differentiated cell types) and administration route (eg, intracerebral injections at multiple sites, or intrathecal or intranasal administration) remain to be identified and might differ between leukodystrophies.[93–95]

General considerations on therapy

For patients with cerebral adrenoleukodystrophy, Krabbe disease, MLD, or ALSP, HSCT is only effective when done before or soon after clinical disease onset.[66–69,71–74] This timing will also be true for other therapies currently under development. Thus, patients need to be identified early. Newborn screening allows treatment before clinical onset. In-depth knowledge of pathogenicity of gene variants is essential to predict presence, onset, and severity of the disease to make therapy decisions.[18]

So far, leukodystrophy therapy strategies have been aimed at white-matter pathology. When successful, patients live longer and other disease manifestations might become apparent, sometimes unexpectedly. An example is that HSCT in patients with cerebral adrenoleukodystrophy does not prevent later spinal cord disease caused by a long-tract axonopathy.[96] Another example is gallbladder carcinoma, occurring after an otherwise successful HSCT in patients with MLD.[61] Although VWM mainly causes neurological signs, the disorder is fundamentally a multiorgan disease.[37] One concern is that cure of white-matter pathology could be followed by disease of other organs.

Future treatments will most likely be multimodal. The Twitcher mouse model for Krabbe disease reacts much better to a combination of substrate reduction therapy, intraventricular enzyme-replacement therapy, and HSCT than to any of the three treatments alone.[86] Microglia abnormality might have an important role in several leukodystrophies, as shown in patients with Pelizaeus-Merzbacher disease, adrenoleukodystrophy, Krabbe disease, MLD, and ALSP,[97–99] and might become an additional therapeutic target, as already explored with success in the Twitcher mouse.[97]

Pathophysiological mechanisms

Increasing insights into the variety of gene defects underlying leukodystrophies has also greatly improved the understanding of the pathological mechanisms. Leukodystrophies are not only caused by myelin defects (either scarcity of myelin deposition [eg, in Pelizaeus-Merzbacher disease] or myelin loss [eg, in MLD]), but also by defects affecting astrocytes (eg, in Alexander disease and MLC), microglia (eg, ALSP), and small blood vessels.[2,8,10,11] At the molecular level, as opposed to the structural level, a substantial number of leukodystrophies are caused by respiratory chain defects.[3,50] Similarly, many leukodystrophies are caused by defects that directly or indirectly affect mRNA translation, together constituting a large leukodystrophy category comprising VWM, 4H leukodystrophy, and tRNA synthase defects.[3,100] Evidently, the brain white matter is vulnerable to defects affecting the translation and protein synthesis process. With the current state of knowledge, understanding why defects affecting translation so often lead to a leukodystrophy, why these leukodystrophies are dissimilar and have individual distinct features, and why other translation defects spare the brain white matter, is challenging.[101] Importantly, a defect in one molecular process could trigger a cascade in another, seemingly unrelated, pathway. An example of this mechanism occurs in an animal model of Pelizaeus-Merzbacher disease, where variants in the gene *PLP1*, encoding a major myelin protein, also result in enhanced inflammation.[99]

Conclusions and future directions

Substantial progress has occurred in many aspects of leukodystrophies, in particular regarding diagnosis, number of diseases known, insight into variability of clinical disease and prognosis, and options for therapy and management. The cumulative change is immense, demanding review and discussion of the implications for the clinical approach of leukodystrophies. The number of disorders and gene defects known has steeply increased, and with the introduction of next-generation sequencing, the tools for diagnosis have drastically improved. Nowadays, most patients with leukodystrophy can be accurately and timely diagnosed with molecular confirmation.[3,4] The increased knowledge of phenotypic

Search strategy and selection criteria

We searched PubMed for articles published in English, German, and French between Jan 1, 2013, and March 1, 2019, using the terms "leukodystrophy", "leukoencephalopathy", and assorted combinations of the following terms: "MRI", "mutation", "genetic", "pathophysiology", "management", "metabolic", "biomarker", "diagnosis", "treatment", "therapy", and "transplantation". We reviewed reference lists within original research and review articles for additional references. We finalised the reference list on the basis of originality and relevance to the scope of this Review.

variation of leukodystrophies facilitates a balanced discussion of prognosis with patients and families. Disease management options have improved, and early diagnosis facilitates proactive monitoring, management of disease manifestations, and prevention of complications. New knowledge of underlying gene defects and insights into leukodystrophy pathomechanisms are helping reveal which genes, molecular pathways, and cell types are therapy targets,[10] facilitating the design of new treatment strategies. Trials are being set up to test new therapies, for example to investigate drugs targeting disease-related pathways in patients with VWM (NTR7482) and antisense nucleotides in patients with Alexander disease. The efficacy of the new approaches still has to be proven, and the road to a cure for all patients with leukodystrophy is still long. Not only are strategies needed to halt the disease, but strategies aiming at repair of damaged brain tissue, be it exogenous repair or enhancing endogenous repair, also need urgent attention. In this respect, stem-cell transplantation or interventions enhancing recruitment of endogenous stem cells are promising.[91,92,102]

Establishing specialised leukodystrophy centres will facilitate studies on disease mechanisms and therapeutic trials. Each of these centres needs to focus on one or a few leukodystrophies to excel, and needs to collaborate in a network to make the benefits of progress available to patients worldwide.

Contributors

NIW designed, organised, and did the literature review. MSvdK contributed to the literature review. MSvdK, RS, FM, and NIW contributed to the design of the paper. MSvdK wrote the first draft of the paper. NIW and MSvdK made the figures. MSvdK, RS, FM, and NIW wrote the subsequent drafts together, reviewed and critiqued the report, and agreed on the final version of text, figures, and references. All authors gave final approval for publication.

Declaration of interests

MSvdK reports a patent on the therapeutic effects of guanabenz treatment in vanishing white matter pending to Stichting Vrije Universiteit Medisch Centrum (Amsterdam, Netherlands). The other authors declare no competing interests.

Acknowledgments

We thank Dr Susan Blaser, paediatric neuroradiologist at the Hospital for Sick Children in Toronto for critical reading of the manuscript.

References

1 Vanderver A, Prust M, Tonduti D, et al. Case definition and classification of leukodystrophies and leukoencephalopathies. *Mol Genet Metab* 2015; **114:** 494–500.
2 van der Knaap MS, Bugiani M. Leukodystrophies: a proposed classification system based on pathological changes and pathogenetic mechanisms. *Acta Neuropathol* 2017; **143:** 329–30.
3 Kevelam S, Steenweg M, Srivastava S, et al. Update on leukodystrophies: a historical perspective and adapted definition. *Neuropediatrics* 2016; **47:** 349–54.
4 Vanderver A, Simons C, Helman G, et al. Whole exome sequencing in patients with white matter abnormalities. *Ann Neurol* 2016; **79:** 1031–37.
5 Montaut S, Tranchant C, Drouot N, et al. Assessment of a targeted gene panel for identification of genes associated with movement disorders. *JAMA Neurol* 2018; **75:** 1234–45.
6 Gordon-Lipkin E, Fatemi A. Current therapeutic approaches in leukodystrophies: a review. *J Child Neurol* 2018; **33:** 861–68.
7 Waldman AT. Leukodystrophies. *Continuum (Minneap Minn)* 2018; **24:** 130–49.
8 Bugiani M, van der Knaap MS. Childhood white matter disorders: much more than just diseases of myelin. *Acta Neuropathol* 2017; **143:** 351–82.
9 Köhler W, Curiel J, Vanderver A. Adulthood leukodystrophies. *Nat Rev Neurol* 2018; **14:** 94–105.
10 van der Knaap MS, Bugiani M. Leukodystrophies—much more than just diseases of myelin. *Nat Rev Neurol* 2018; **14:** 747–48.
11 Ayrignac X, Carra-Dalliere C, Champfleur N de, et al. Adult-onset genetic leukoencephalopathies: a MRI pattern-based approach in a comprehensive study of 154 patients. *Brain* 2014; **138:** 284–92.
12 Lynch DS, Rodrigues Brandão de Paiva A, Zhang WJ, et al. Clinical and genetic characterization of leukoencephalopathies in adults. *Brain* 2017; **140:** 1204–11.
13 Min R, van der Knaap MS. Genetic defects disrupting glial ion and water homeostasis in the brain. *Brain Pathol* 2018; **28:** 372–87.
14 Curiel J, Rodríguez Bey G, Takanohashi A, et al. *TUBB4A* mutations result in specific neuronal and oligodendrocytic defects that closely match clinically distinct phenotypes. *Hum Mol Genet* 2017; **26:** 4506–18.
15 Richards J, Korgenski EK, Srivastava R, Bonkowsky JL. Costs of the diagnostic odyssey in children with inherited leukodystrophies. *Neurology* 2015; **85:** 1167–70.
16 Tan TY, Dillon OJ, Stark Z, et al. Diagnostic impact and cost-effectiveness of WES for ambulant children with suspected monogenic conditions. *JAMA Pediatr* 2017; **171:** 855–62.
17 Lionel AC, Costain G, Monfared N, et al. Improved diagnostic yield compared with targeted gene sequencing panels suggests a role for whole-genome sequencing as a first-tier genetic test. *Genet Med* 2018; **20:** 435–43.
18 Best S, Wou K, Vora N, et al. Promises, pitfalls and practicalities of prenatal whole exome sequencing. *Prenat Diagn* 2018; **38:** 10–19.
19 Jenkinson EM, Rodero MP, Kasher PR, et al. Mutations in *SNORD118* cause the cerebral microangiopathy leukoencephalopathy with calcifications and cysts. *Nat Genet* 2016; **48:** 1185–92.
20 Hamilton E, Bertini E, Kalaydjieva L, et al. *UFM1* founder mutation in the Roma population causes recessive variant of H-ABC. *Neurology* 2017; **89:** 1921–28.
21 Miyake N, Wolf NI, Cayami FK, et al. X-linked hypomyelination with spondylometaphyseal dysplasia (H-SMD) associated with mutations in *AIFM1*. *Neurogenetics* 2017; **18:** 185–94.
22 Parikh S, Bernard G, Leventer R, et al. A clinical approach to the diagnosis of patients with leukodystrophies and genetic leukoencephalopathies. *Mol Genet Metab* 2015; **114:** 501–15.
23 Ahmed RM, Murphy E, Davagnanam I, et al. Practical approach to diagnosing adult onset leukodystrophies. *J Neurol Neurosurg Psychiatry* 2014; **85:** 770–81.
24 Ashrafi MR, Tavasoli AR. Childhood leukodystrophies: a literature review of updates on new definitions, classification, diagnostic approach and management. *Brain Dev* 2017; **39:** 369–85.
25 Lynch DS, Wade C, Paiva ARB, et al. Practical approach to the diagnosis of adult-onset leukodystrophies: an updated guide in the genomic era. *J Neurol Neurosurg Psychiatry* 2019; **90:** 543–54.

26 Schiffmann R, van der Knaap MS. An MRI-based approach to the diagnosis of white matter disorders. *Neurology* 2009; **72:** 750–59.

27 Granberg T, Hashim F, Andersen O, Sundal C, Karrenbauer VD. Hereditary diffuse leukoencephalopathy with spheroids—a volumetric and radiological comparison with multiple sclerosis patients and healthy controls. *Eur J Neurol* 2016; **23:** 817–22.

28 Codjia P, Ayrignac X, Mochel F, et al. Adult-onset leukoencephalopathy with axonal spheroids and pigmented glia: an MRI study of 16 French cases. *Am J Neuroradiol AJNR* 2018; **39:** 1657–61.

29 Ulate-Campos A, Petanas-Argemi J, Rebollo-Polo M, et al. X-linked adrenoleukodystrophy with an atypical radiological pattern. *Rev Neurol* 2018; **66:** 237–40.

30 Inglese M, Fleysher L, Oesingmann N, Petracca M. Clinical applications of ultra-high field magnetic resonance imaging in multiple sclerosis. *Expert Rev Neurother* 2018; **18:** 221–30.

31 Mackay AL, Laule C. Magnetic resonance of myelin water: an in vivo marker for myelin. *Brain Plast* 2016; **2:** 71–91.

32 De Santis S, Bastiani M, Droby A, et al. Characterizing microstructural tissue properties in multiple sclerosis with diffusion MRI at 7 T and 3 T: the impact of the experimental Design. *Neuroscience* 2019; **403:** 17–26.

33 Foley AR, Donkervoort S, Bönnemann CG. Next-generation sequencing still needs our generation's clinicians. *Neurol Genet* 2015; **1:** e13.

34 Alfares A, Aloraini T, Subaie LA, et al. Whole-genome sequencing offers additional but limited clinical utility compared with reanalysis of whole-exome sequencing. *Genet Med* 2018; **20:** 1328–33.

35 Martínez-Morillo E, Prieto García B, Álvarez Menéndez FV. Challenges for worldwide harmonization of newborn screening programs. *Clin Chem* 2016; **62:** 689–98.

36 Rajabi F. Updates in newborn screening. *Pediatr Ann* 2018; **47:** e187–190

37 Hamilton EMC, van der Lei HDW, Vermeulen G, et al. The natural history of vanishing white matter. *Ann Neurol* 2018; **84:** 274–88.

38 Barañano KW. Leukodystrophies. *Semin Neurol* 2016; **36:** 362–66.

39 Hamilton EMC, Tekturk P, Cialdella F, et al. Megalencephalic leukoencephalopathy with subcortical cysts: characterization of disease variants. *Neurology* 2018; **90:** e1395–403.

40 Sissler M, González-Serrano LE, Westhof E. Recent advances in mitochondrial aminoacyl-tRNA synthetases and disease. *Trends Mol Med* 2017; **23:** 693–708.

41 van Berge L, Hamilton EM, Linnankivi T, et al. Leukoencephalopathy with brainstem and spinal cord involvement and lactate elevation: clinical and genetic characterization and target for therapy. *Brain* 2014; **137:** 1019–29.

42 Oliveira R, Sommerville EW, Thompson K, et al. Lethal neonatal LTBL associated with biallelic *EARS2* variants: case report and review of the reported neuroradiological features. *JIMD Rep* 2017; **33:** 61–68.

43 Güngör O, Özkaya AK, Şahin Y, Güngör G, Dilber C, Aydın K. A compound heterozygous *EARS2* mutation associated with mild leukoencephalopathy with thalamus and brainstem involvement and high lactate (LTBL). *Brain Dev* 2016; **38:** 857–61.

44 McNeill N, Nasca A, Reyes A, et al. Functionally pathogenic EARS2 variants in vitro may not manifest a phenotype in vivo. *Neurol Genet* 2017; **3:** e162.

45 Dallabona C, Diodato D, Kevelam SH, et al. Novel (ovario) leukodystrophy related to AARS2 mutations. *Neurology* 2014; **82:** 2063–71.

46 Lynch DS, Zhang WJ, Lakshmanan R, et al. Analysis of mutations in *AARS2* in a series of *CSF1R*-negative patients with adult-onset leukoencephalopathy with axonal spheroids and pigmented glia. *JAMA Neurol* 2016; **73:** 1433–39.

47 Steenweg ME, Wolf NI, van Wieringen WN, et al. Quantitative MRI in hypomyelinating disorders: correlation with motor handicap. *Neurology* 2016; **87:** 752–58.

48 Wolf NI, Vanderver A, van Spaendonk RM, et al. Clinical spectrum of 4H leukodystrophy caused by *POLR3A* and *POLR3B* mutations. *Neurology* 2014; **83:** 1898–905.

49 Simons C, Dyment D, Bent SJ, et al. A recurrent de novo mutation in *TMEM106B* causes hypomyelinating leukodystrophy. *Brain* 2017; **140:** 3105–11.

50 Morató L, Bertini E, Verrigni D, et al. Mitochondrial dysfunction in central nervous system white matter disorders. *Glia* 2014; **62:** 1878–94.

51 Chen N, Jiang YW, Hao HJ, et al. Different eukaryotic initiation factor 2Bε mutations lead to various degrees of intolerance to the stress of endoplasmic reticulum in oligodendrocytes. *Chin Med J* (Engl) 2015; **128:** 1772–77.

52 Gong Y, Sasidharan N, Laheji F, et al. Microglial dysfunction as a key pathological change in adrenomyeloneuropathy. *Ann Neurol* 2017; **82:** 813–27.

53 Van der Veldt N, Van Rappard DF, Van de Pol LA, et al. Intrathecal baclofen in metachromatic leukodystrophy. *Dev Med Child Neurol* 201; **61:** 232–35.

54 Nevin Z, Factor D, Karl R, et al. Modeling the mutational and phenotypic landscapes of Pelizaeus-Merzbacher disease with human iPSC-derived oligodendrocytes. *Am J Hum Genet* 2017; **100:** 617–34.

55 Kevelam SH, Rodenburg RJ, Wolf NI, et al. *NUBPL* mutations in patients with complex I deficiency and a distinct MRI pattern. *Brain* 2013; **136:** 1534–43.

56 Wiesinger C1, Eichler FS2, Berger J1. The genetic landscape of X-linked adrenoleukodystrophy: inheritance, mutations, modifier genes, and diagnosis. *Appl Clin Genet* 2015; **8:** 109–21.

57 Adang LA, Sherbini O, Ball L, et al. Revised consensus statement on the preventive and symptomatic care of patients with leukodystrophies. *Mol Genet Metab* 2017; **122:** 18–32.

58 Nelson C, Mundorff MB, Korgenski EK, et al. Determinants of health care use in a population-based leukodystrophy cohort. *J Pediatr* 2013; **162:** 624–28.

59 Huffnagel IC, Laheji FK, Aziz-Bose R, et al. The natural history of adrenal insufficiency in X-linked adrenoleukodystrophy: an international collaboration. *J Clin Endocrinol Metab* 2019; **104:** 118–26.

60 Helman G, Van Haren K, Bonkowsky JL, et al. Disease specific therapies in leukodystrophies and leukoencephalopathies. *Mol Genet Metab* 2015; **114:** 527–36.

61 van Rappard DF, Bugiani M, Boelens JJ, et al. Gallbladder and the risk of polyps and carcinoma in metachromatic leukodystrophy. *Neurology* 2016; **87:** 103–11.

62 Gump WC, Mutchnick IS, Moriarty TM. Selective dorsal rhizotomy for spasticity not associated with cerebral palsy: reconsideration of surgical inclusion criteria. *Neurosurg Focus* 2013; **35:** E6.

63 Anderson H, Wilkes J, Korgenski E, et al. Preventable infections in children with leukodystrophy. *Ann Clin Transl Neurol* 2014; **1:** 370–74.

64 Helman G, Van Haren K, Escolar ML, Vanderver A. Emerging treatments for pediatric leukodystrophies. *Pediatr Clin North Am* 2015; **62:** 649–66.

65 Augustine EF, Adams HR, Mink JW. Clinical trials in rare disease: challenges and opportunities. *J Child Neurol* 2013; **28:** 1142–50.

66 Laule C, Vavasour IM, Shahinfard E, et al. Hematopoietic stem cell transplantation in late-onset Krabbe disease. *Neuroimaging* 2018; **28:** 252–55.

67 van den Broek BTA, Page K, Paviglianiti A, et al. Early and late outcomes after cord blood transplantation for pediatric patients with inherited leukodystrophies. *Blood Adv* 2018; **2:** 49–60.

68 Groeschel S, Kühl JS, Bley AE, et al. Long-term outcome of allogeneic hematopoietic stem cell transplantation in patients with juvenile metachromatic leukodystrophy compared with nontransplanted control patients. *JAMA Neurol* 2016; **73:** 1133–40.

69 Allewelt H, Taskindoust M, Troy J, et al. Long-term functional outcomes after hematopoietic stem cell transplant for early infantile Krabbe disease. *Biol Blood Marrow Transplant* 2018; **24**: 2233–38.

70 Wright MD, Poe MD, DeRenzo A, et al. Developmental outcomes of cord blood transplantation for Krabbe disease: a 15-year study. *Neurology* 2017; **89:** 1365–72.

71 Eichler FS, Li J, Guo Y, et al. *CSF1R* mosaicism in a family with hereditary diffuse leukoencephalopathy with spheroids. *Brain* 2016; **139:** 1666–72.

72 Bonkowsky J, Wilkes J, Shyr D. Scope and burden of non-standard of care hematopoietic stem cell transplantation in pediatric leukodystrophy patients. *J Child Neurol* 2018; **33:** 882–87.

73 Sessa M, Lorioli L, Fumagalli F, et al. Lentiviral haemopoietic stem-cell gene therapy in early-onset metachromatic leukodystrophy: an ad-hoc analysis of a non-randomised, open-label, phase 1/2 trial. *Lancet* 2016; **388:** 476–87.

74 Eichler F, Duncan C, Musolino PL, et al. Hematopoietic stem-cell gene therapy for cerebral adrenoleukodystrophy. *N Engl J Med* 2017; **377:** 1630–38.

75 Leone P, Shera D, McPhee SW, et al. Long-term follow-up after gene therapy for Canavan disease. *Sci Transl Med* 2012; **4:** 165ra163.

76 Sevin C, Roujeau T, Cartier N, et al. Intracerebral gene therapy in children with metachromatic leukodystrophy: results of a phase I/II trial. *Mol Genet Metab* 2018; **123:** S129.

77 Kyriakoudi S, Sargiannidou I, Kagiava A, et al. Golgi-retained Cx32 mutants interfere with gene addition therapy for CMT1X. *Hum Mol Genet* 2017; **26:** 1622–33.

78 Salsman J, Dellaire G. Precision genome editing in the CRISPR era. *Biochem Cell Biol* 2017; **95:** 187–201.

79 Hagemann TL, Powers B, Mazur C, et al. Antisense suppression of glial fibrillary acidic protein as a treatment for Alexander disease. *Ann Neurol* 2018; **83:** 27–39.

80 Tantzer S, Sperle K, Kenaley K, et al. Morpholino antisense oligomers as a potential therapeutic option for the correction of alternative splicing in PMD, SPG2, and HEMS. *Mol Ther Nucleic Acids* 2018; **12:** 420–32.

81 Jang DS, Ye W, Guimei T, et al. Cell-based high-throughput screening identifies galactocerebrosidase enhancers as potential small-molecule therapies for Krabbe's disease. *J Neurosci Res* 2016; **94:** 1231–45.

82 MacRae CA, Peterson RT. Zebrafish as tools for drug discovery. *Nat Rev Drug Discov* 2015; **14:** 721–31.

83 Kothur K, Bandodkar S, Chu S, et al. An open-label trial of JAK 1/2 blockade in progressive IFIH1-associated neuroinflammation. *Neurology* 2018; **90:** 289–91.

84 Rice GI, Meyzer C, Bouazza N, et al. Reverse-transcriptase inhibitors in the Aicardi–Goutières Syndrome. *N Eng J Med* 2018; **379:** 2275–77.

85 Dooves S, Bugiani M, Wisse LE, et al. Bergmann glia translocation: a new disease marker for vanishing white matter identifies therapeutic effects of Guanabenz treatment. *Neuropathol Appl Neurobiol* 2018; **44:** 391–403.

86 Hawkins-Salsbury JA, Shea L, Jiang X, et al. Mechanism-based combination treatment dramatically increases therapeutic efficacy in murine globoid cell leukodystrophy. *J Neurosci* 2015; **35:** 6495–505.

87 Solomon M, Muro S. Lysosomal enzyme replacement therapies: historical development, clinical outcomes, and future perspectives. *Adv Drug Deliv Rev* 2017; **118:** 109–34.

88 Sevin C, Dali C, Giugliani R, et al. Intrathecal delivery of recombinant human arylsulfatase A in children with late-infantile metachromatic leukodystrophy: an update following extended treatment. *Mol Gen Met* 2017; **120:** S121 (abstr).

89 Matthes F, Stroobants S, Gerlach D, et al. Efficacy of enzyme replacement therapy in an aggravated mouse model of metachromatic leukodystrophy declines with age. *Hum Mol Gen* 2012; **21:** 2599–609.

90 Hironaka K, Yamazaki Y, Hirai Y, et al. Enzyme replacement in the CSF to treat metachromatic leukodystrophy in mouse model using single intracerebroventricular injection of self-complementary AAV1 vector. *Sci Rep* 2015; **5:** 13104

91 Wang S, Bates J, Li X, et al. Human iPSC-derived oligodendrocyte progenitor cells can myelinate and rescue a mouse model of congenital hypomyelination. *Cell Stem Cell* 2013; **12:** 252–64.

92 Dooves S, Leferink PS, Krabbenborg S, et al. Cell replacement therapy improves pathological hallmarks in a mouse model of leukodystrophy vanishing white matter. *Stem Cell Rep* 2019; **12:** 441–50.

93 Osorio MJ, Rowitch DH, Tesar P, et al. Concise review: stem cell-based treatment of Pelizaeus-Merzbacher disease. *Stem Cells* 2017; **35:** 311–15.

94 Barberini DJ, Aleman M, Aristizabal F, et al. Safety and tracking of intrathecal allogeneic mesenchymal stem cell transplantation in healthy and diseased horses. *Stem Cell Res Ther* 2018; **9:** 96.

95 Donega V, Nijboer CH, Tilborg G van, Dijkhuizen RM, Kavelaars A, Heijnen CJ. Intranasally administered mesenchymal stem cells promote a regenerative niche for repair of neonatal ischemic brain injury. *Exp Neurol* 2014; **261:** 53–64.

96 van Geel BMM, Poll-The BT, Verrips A, et al. Hematopoietic cell transplantation does not prevent myelopathy in X-linked adrenoleukodystrophy: a retrospective study. *J Inherit Metab Dis* 2015; **38:** 359–61.

97 Scruggs B, Zhang X, Bowles A, et al. Multipotent stromal cells alleviate inflammation, neuropathology, and symptoms associated with globoid cell leukodystrophy in the Twitcher mouse. *Stem Cells* 2013; **31:** 1523–34.

98 Sasaki A. Microglia and brain macrophages: an update. *Neuropathology* 2017; **37:** 452–64.

99 Groh J, Friedman HC, Orel N, et al. Pathogenic inflammation in the CNS of mice carrying human *PLP1* mutations. *Hum Mol Genet* 2016; **25:** 4686–702.

100 Dorboz I, Dumay-Odelot H, Boussaid K, et al. Mutation in *POLR3K* causes hypomyelinating leukodystrophy and abnormal ribosomal RNA regulation. *Neurol Genet* 2018; **4:** e289.

101 Meyer-Schuman R, Antonellis A. Emerging mechanisms of aminoacyl-tRNA synthetase mutations in recessive and dominant human disease. *Hum Mol Genet* 2017; **26:** R114–27.

102 Xing Y, Röth P, Stratton J, et al. Adult neural precursor cells from the subventricular zone contribute significantly to oligodendrocyte regeneration and remyelination. *J Neurosci* 2014; **34:** 14128–46.