Update on Leukodystrophies: A Historical Perspective and Adapted Definition

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Abstract

Leukodystrophies were defined in the 1980s as progressive genetic disorders primarily affecting myelin of the central nervous system. At that time, a limited number of such disorders and no associated gene defects were known. The majority of the leukodystrophy patients remained without a specific diagnosis. In the following two decades, magnetic resonance imaging pattern recognition revolutionized the field, allowing the definition of numerous novel leukodystrophies. Their genetic defects were usually identified through genetic linkage studies. This process required substantial numbers of cases and many rare disorders remained unclarified. As recently as 2010, 50% of the leukodystrophy patients remained unclassified. Since 2011, whole-exome sequencing has resulted in an exponential increase in numbers of known, distinct, genetically determined, ultrarare leukodystrophies. We performed a retrospective study concerning three historical cohorts of unclassified leukodystrophy patients and found that currently at least 80% of the patients can be molecularly classified. Based on the original definition of the leukodystrophies, numerous defects in proteins important in myelin structure, maintenance, and function were expected. By contrast, a high percentage of the newly identified gene defects affect the housekeeping process of mRNA translation, shedding new light on white matter pathobiology and requiring adaptation of the leukodystrophy definition.

Keywords
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► genetics
► myelin

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Introduction

Leukodystrophies have a history of just over a 100 years. For long, the field has been dominated by the problem of unclassified and unclassifiable cases. Over the years, multiple different techniques have been applied to address this problem depending on technological developments, with limited success. Since a few years, however, an exponential increase in numbers of ultrarare cases with novel genetic defects is seen. In this update, we address the proportion of classified leukodystrophies and discuss the impact of the insights gained from all newly detected genetic defects on what we define as a leukodystrophy.

Pathology

Research on brain white matter disorders started in the 1830s, when the first pathology descriptions appeared. In the subsequent 150 years, their diagnosis and classification remained almost exclusively based on pathology. “Multiple sclerosis” was first defined,1,2 after 60 years, followed by the definition of “diffuse sclerosis” to distinguish disorders with a diffuse abnormality of the brain white matter from those with multifocal abnormalities.3 The word “leukodystrophy” was introduced in 1928 for metachromatic leukodystrophy (MLD)4. “Leukodystrophy” comes from the Greek roots leuko = white, dys = lack of, and trophy = growth. Consensus on the definition of “leukodystrophies” as a disease category emerged in the 1980s. In two papers in this journal, they were defined as disorders primarily affecting myelin, either directly or through the involvement of oligodendrocytes, caused by a genetic defect, and clinically progressive.5,6 So, the initial definition of leukodystrophy was myelin focused. As of today, it is this definition that most physicians have in mind when using the word. “Leukoencephalopathy” is a more neutral term for any brain white matter disorder, genetic or acquired.

Magnetic Resonance Imaging

In the 1970s, the advent of computed tomography (CT) scanning provided the first opportunity to visualize leukoencephalopathies in vivo, but due to the low sensitivity and tissue differentiation, CT was unable to distinguish between most different disorders.7 In the early 1980s, the advent of magnetic resonance imaging (MRI) had a major impact on the leukencephalopathy field. It was immediately clear that MRI had a very high sensitivity for white matter abnormalities,5 but the specificity of these findings was initially considered as low.5,10

When we started to study leukoencephalopathies in the late 1980s, a limited number of disorders and no genes associated with a leukodystrophy were known. The diagnosis depended on metabolic investigations (metabolites in body fluids and enzyme activities) for most disorders11 and pathology findings for a few.12,13 MRI changed the diagnostic approach entirely. We noticed that patients with a diagnosis that was verifiable by laboratory testing presented with distinct patterns of MRI abnormalities, shared by patients with the same diagnosis, but different from the patterns observed in patients with other diagnoses.14 This observation prompted the development of MRI pattern recognition to enhance the specificity of MRI interpretation.15

For the diagnosis of leukoencephalopathies known at that time, MRI pattern recognition worked well.16 In the 90s, it became, however, clear that in substantial numbers of leukoencephalopathy cases no specific diagnosis could be established. We estimated that over 60% of the leukoencephalopathy cases remained unsolved.17 The heavy load of unclassified leukoencephalopathies impelled us and others to work on them and define novel disorders by their distinct MRI patterns.17 In this way, new disease entities as megalencephalic leukoencephalopathy with subcortical cysts (MLC),18 vanishing white matter (VWM),19–21 hypomyelination with atrophy of the basal ganglia and cerebellum (H-ABC),22 leukoencephalopathy with brainstem and spinal cord involvement and lactate elevation,23 hypomyelination, hypodontia, and hypogonadotropic hypogonadism (4H syndrome),24–26 leukoencephalopathy and thalamic and brainstem involvement and high lactate (LTBL),27 and hypomyelination of early myelinating structures (HEMS)28 were defined and criteria were drafted for MRI-based diagnoses.

Genetic Linkage

The first genes associated with known leukodystrophies were identified at the end of the 1980s, initially mainly by a candidate gene approach directed at the likely genetic defect. Examples are PLP1 for Pelizaeus–Merzbacher disease,29 ARSA for MLD,30 ASPA for Canavan disease,31 and GALC for Krabbe disease.32 Later, genes were identified by genetic linkage, for example, ABCD1 for X-linked adrenoleukodystrophy.33 Validation of the concept that novel leukodystrophies could be defined by their MRI pattern came in 2001, when the first genes mutated in MRI-defined disorders were identified: EIF2B1–5 for VWM34,35 and MLC1 for MLC.36 Over the following years, several genes mutated in new, MRI-defined disorders were identified.37–41 Linkage analysis using positional cloning to pinpoint the chromosomal location of the candidate gene and subsequent narrowing of the candidate region, followed by sequential analysis of candidate genes in the region by Sanger sequencing were the main techniques used. This approach required substantial numbers of patients at all stages. Several patients with the same MRI pattern were necessary to define the disease and multiple genetically informative families were needed for genetic linkage. For most patients with an unclassified or defined, but molecularly undetermined leukodystrophy, this technique did not succeed, mainly due to the rarity of the disorders or dominant de novo inheritance.

Despite the fact that the most prevalent novel leukodystrophies had been identified between 1990 and 2010 and their gene defect had been elucidated, it was found that 50% of leukodystrophy patients still remained without a specific diagnosis in 2010.42

Exome Sequencing

The advent of next-generation-sequencing technology in 2005 had a major impact on the leukodystrophy field. It
created a paradigm shift in the approach of gene discovery for rare Mendelian disorders.\(^{43}\) Massive parallel sequencing of the protein-coding part of the genome is referred to as whole-exome sequencing (WES).\(^{43}\) In 2011, the first genetic defect for a leukodystrophy without known molecular cause, “he-reditary diffuse leukoencephalopathy with spheroids (HDLS),” was identified using WES, which revealed dominant mutations in CSF1R.\(^{44}\) Soon thereafter WES revealed recessive mutations in EARS2 in patients with LTBL,\(^{45}\) and dominant de novo mutations in TUBB4A in the sporadic disease H-ABC.\(^{46}\) The number of novel genes associated with new or previously defined Mendelian disorders identified between 2011 and 2016 using WES has been enormous and illustrates the high potential of this technique for gene finding.\(^{47,48}\) In addition, WES has proven to greatly facilitate a molecular diagnosis for disorders associated with numerous different gene defects, such as hypomyelination and respiratory chain defects, in which sequential gene analysis is costly and time consuming.

Although extremely successful, one of the biggest challenges of WES still is an interpretation of the data. From which sequential gene analysis is costly and time consuming.\(^{8}\) We have had a success rate of 80 to 90% for gene identification by WES in small, homogeneous patient groups from our database of unclassified leukoencephalopathies, whereas several larger WES studies have reported success rates of 42% for mixed leukodystrophy cases\(^{57}\) and 16 to 53% for unselected patients.\(^{58-60}\) The most important advantage of WES in homogeneous patient groups is that the patients validate each other in that the conclusion on the pathogenicity of the identified variants does not only depend on knowledge of gene function or predicted effect of the variant, but also on whether variants in the same gene are observed in other patients.

### Percentage of Unsolved Cases

In 2010, 50% of the leukodystrophy cases were still unclassified.\(^{42}\) We suspected that with WES the percentage has decreased substantially. To confirm this, we conducted a retrospective study of three different historical cohorts, including a total of 430 patients with an unclassified leukoencephalopathy despite extensive available diagnostic workup.

The first and oldest cohort, cohort 1, was published in 1999 and contains 92 leukoencephalopathy patients from 82 families, including both inherited and acquired disorders, evaluated at the Kennedy Krieger Institute in Baltimore during 1990 to 1996.\(^{17}\) Follow-up of outcomes was executed in October 2015. A specific diagnosis had been established in 40 of the 82 families: 27 through DNA confirmation, 10 based on established MRI criteria (four MLC and six Alexander disease), and three based on pathology (Alexander disease and HDLS). For the latter 13 patients, the diagnosis could not be confirmed molecularly because of lack of available DNA. The percentage of almost 50% of the cases with a specific diagnosis is an underestimation. As expected for such an old cohort, many cases were lost to follow-up and most cases never had the genetic workup that would now be state-of-the-art. In addition, part of the patients may not have a genetic disease.\(^{17}\)

The second and third cohorts consist of cases from the MRI database of the Center for Childhood White Matter Disorders in Amsterdam that contains over 3,000 unclassified leukoencephalopathy cases. In 2011, cases from this database were entered on lists if they presumably had a leukodystrophy, but no molecular diagnosis, and had hypomyelination (cohort 2) or were suspected of a mitochondrial defect (cohort 3). Hypomyelination was defined as evidence of stable lack of myelin on two successive MRIs with an interval of at least 6 months and the second MRI after 1 year of age.\(^{61}\) A “suspected mitochondrial leukodystrophy” was determined by the MRI pattern, showing features as cystic lesions in the abnormal white matter, additional gray matter lesions, restricted diffusion, contrast enhancement, and elevated lactate on magnetic resonance spectroscopy of the brain.\(^{62,63}\) Outcomes were assessed in December 2015 and exclusively based on the presence or absence of a molecular diagnosis. Counting siblings as one, 181 cases were present in cohort 2 and 167 in cohort 3. For cohorts 2 and 3, 67 (37%) and 61 (37%) of the cases were lost to follow-up leaving 114 and 106 cases, respectively (\(\text{Table 1}\)). For these remaining cases, a molecular diagnosis was established in 60/114 (53%) for cohort 2, 69/106 (65%) for cohort 3, and 129/220 (59%) for the overall group (\(\text{Table 1}\)).

##### Table 1 Outcome for cohorts 2 and 3

<table>
<thead>
<tr>
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<th>Cohort 2 : Hypomyelination</th>
<th>Cohort 3: Suspected mitochondrial leukodystrophy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cases (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Informative cases</td>
<td>114 (100%)</td>
<td>106 (100%)</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>60/114 (53%)</td>
<td>69/106 (65%)</td>
</tr>
<tr>
<td>No diagnosis, WES unrevealing</td>
<td>4/114 (4%)</td>
<td>3/106 (3%)</td>
</tr>
<tr>
<td>No diagnosis, no WES performed</td>
<td>50/114 (43%)</td>
<td>34/106 (32%)</td>
</tr>
<tr>
<td>Lost to follow-up</td>
<td>67</td>
<td>61</td>
</tr>
<tr>
<td>Total cases</td>
<td>181</td>
<td>167</td>
</tr>
</tbody>
</table>

Abbreviation: WES, whole-exome sequencing.
It is important to realize that the patients included in the present retrospective study represent the 60% (cohort 1) or 50% (cohorts 2 and 3) patients in whom no diagnosis could be established 16 or 5 years before, respectively. A positive molecular diagnosis in these cases results in an estimated decrease of the unclassified group to approximately 20%. This is still an underestimation of the percentage of classifiable leukodystrophy cases. Most of the remaining unclassified leukodystrophy patients did not undergo WES (50/54 cases for cohort 2 and 34/37 cases for cohort 3). With WES applied in all patients, the percentage of unclassified cases would be even lower.

**Genetic Defects and Leukodystrophy Concepts**

Fig. 1 presents the reported diagnoses in the cohorts. An intriguing finding is that only three myelin- or oligodendrocyte-specific protein defects were found: PLP1 for HEMS, GJC2 for Pelizaeus–Merzbacher-like disease, and GJB1 for brain manifestations of X-linked Charcot–Marie–Tooth disease. Based on the original definition of the leukodystrophies, numerous defects in proteins important in the myelin build-up, maintenance, structure, and function would be expected, but instead a high percentage of the newly identified gene defects affect the housekeeping process of mRNA translation, both in the hypomyelination and in the suspected mitochondrial leukodystrophy groups (Fig. 1).

mRNA translation is a highly complex process and numerous proteins are involved, including proteins mediating activation, initiation, elongation or termination of mRNA translation; aminoacyl-tRNA synthetases; ribosomal proteins; as well as cofactors and modifying proteins. VWM was the first example of a leukodystrophy caused by a defect in mRNA translation. In cohorts 2 and 3 many patients had a defect in one of the mitochondrial or cytosolic aminoacyl-tRNA synthetases. These are housekeeping enzymes that attach amino acids to their cognate tRNA molecules as an essential step in protein synthesis. Furthermore, a large group of patients had mutations in genes encoding subunits of RNA polymerase POLR3 (POLR3A, POLR3B, and POLR1C). Strikingly, POLR3-related disorders prove to be among the most prevalent hypomyelinating leukodystrophies. In line with the housekeeping function in the mRNA translation of the affected proteins, patients never have two mutations that would cause complete function loss, because the absence of the housekeeping function would not be compatible with life. In fact, we suspect that the housekeeping function of the mutated proteins is still guaranteed and that it is not the hampered housekeeping function that causes the disease. We hypothesize that noncanonical functions of the affected proteins play a role in the pathogenesis of mRNA translation-related leukodystrophies.

For some disorders accepted as leukodystrophies, WES revealed defects in proteins specifically expressed in unexpected cell types. The CSF1R gene, mutated in HDLS, encodes a growth factor specific for microglia, macrophages, and monocytes, while pathology suggests a white matter axonopathy. TUBB4A, mutated in H-ABC, encodes a β-tubulin protein, a building block of microtubules. Microtubules are an essential component of the cytoskeleton and allow cell organelles and vesicles to move. In H-ABC, in which pathology points at the primary axonal damage, the defective microtubule system may hamper axonal transport, leading to axonal dysfunction and loss and secondary myelin deficit.

The findings of the last 5 years have major implications for how we understand leukodystrophies. The original definition was focused on myelin and required a progressive disease course. With the information on newly identified defects underlying leukodystrophies, there is no justification for a myelin focused definition of the leukodystrophies. In addition, molecular progress has made clear that there are also nonprogressive or even improving leukodystrophy variants. MLC, widely accepted as a leukodystrophy, was found to have an improving variant along with only transient abnormalities on MRI. Another example is LTB1, which is typically characterized by a single episode of deterioration early in life, followed by improvement. The timing and severity of the episode determine the outcome, which varies from no or almost no handicap to severe dysfunction or death. The conclusion based on these observations is that progression should not be regarded as a prerequisite for inclusion in the category of the leukodystrophies.

Definitions reflect the state of knowledge at the time and should be regularly updated to incorporate new insights. Recently the GLIA consortium suggested a modified leukodystrophy definition to include heritable disorders affecting

![Fig. 1](Image)

**Fig. 1** Overview of diagnoses established (A) Diagnoses in cohort 1. (B) Genes mutated in cohort 2, hypomyelination. (C) Genes mutated in cohort 3, suspected mitochondrial leukodystrophies.
the central nervous system white matter with abnormalities involving myelin or all macroglial cell types, both oligodendrocytes and astrocytes. The progressive disease course was no longer a criterion. Weighing all new information, we propose to take the next step and define leukodystrophies as all genetically determined disorders primarily affecting central nervous system white matter, irrespective of the structural white matter component involved, the molecular process affected and the disease course. Leukodystrophy definitions are, although necessarily imperfect, of fundamental importance, because they determine how we understand white matter physiology and pathophysiology and how we approach treatment. Not only do remyelination and glia restoration need to be achieved for leukodystrophies, but a complex tissue that contains many more components needs to be repaired.

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