IV

Update on leukodystrophies
Chapter 11

Update on leukodystrophies: origin, evolution and two revolutions


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SUMMARY

When we initiated our studies of leukodystrophies in the nineteen eighties, a limited number of disorders were known and no associated gene defects. We estimated then that over 60% of the cases were unclassified. In the following two decades MRI pattern recognition revolutionized the field, allowing definition of numerous novel leukodystrophies. Their genetic defects were usually identified by genetic linkage studies. This process required substantial numbers of cases and many rare disorders remained unclassified. As recently as 2010, 50% of the leukodystrophy patients remained unclassified. Soon after, whole-exome sequencing caused another revolution with an exponential increase in numbers of distinct, genetically determined, ultra-rare leukodystrophies. Results from our retrospective studies concerning three historical cohorts of unclassified leukodystrophy patients indicate that currently at least 80% of the patients can now be molecularly classified. Based on the original definition of 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. With this new information, a myelin focused definition of leukodystrophies is outdated; we propose a further adaption of the definition.
PATHOLOGY ERA

Research on brain white matter disorders started in the eighteen thirties, when the first pathology descriptions of brain disorders appeared. In the subsequent 150 years, diagnosis and classification of brain white matter disorders 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 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 emerged in the nineteen eighties. They were defined as disorders primarily affecting myelin, either directly or through 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.

MRI ERA

In the nineteen seventies, the advent of 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 nineteen eighties, the advent of MRI had a major impact on studies of leukoencephalopathies. It was immediately clear that MRI had a very high sensitivity for white matter abnormalities,8 but the specificity of these findings was considered as low.9,10

When we started to study leukoencephalopathies in the late nineteen eighties, 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 and caused a revolution in the white matter field. 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. In the early nineties 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. The heavy load of unclassified leukoencephalopathies impelled us to work on them and define novel disorders by their distinct MRI patterns. In this way, new disease entities as megalencephalic leukoencephalopathy with subcortical cysts (MLC), vanishing white matter (VWM), hypomyelination and atrophy of the basal ganglia and cerebellum (H-ABC), leukoencephalopathy with brainstem and spinal cord involvement and lactate elevation (LBSL), hypomyelination, hypodontia and hypogonadotropic hypogonadism (4H syndrome), leukoencephalopathy and thalamus and brainstem involvement and high lactate (LTBL), and hypomyelination of early myelinating structures (HEMS) were defined and criteria were drafted for MRI-based diagnoses.

**GENETIC LINKAGE ERA**

The first genes associated with known leukodystrophies were identified at the end of the nineteen eighties, initially mainly by a candidate gene approach directed at the likely genetic defect. Examples are \textit{PLP1} for Pelizaeus-Merzbacher disease, \textit{ARSA} for MLD, \textit{ASPA} for Canavan disease, and \textit{GALC} for Krabbe disease. Later, genes were identified by genetic linkage, e.g. \textit{ABCD1} for X-linked adrenoleukodystrophy. 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: \textit{EIF2B1-5} for VWM and \textit{MLC1} for MLC. Soon many other genes mutated in newly MRI-defined disorders were identified. 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 \textit{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.
EXOME SEQUENCING ERA

The advent of next-generation-sequencing technology in 2005 caused a second revolution in 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, ‘hereditary 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 Additionally, 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 is interpretation of the data. From the approximately 20,000-25,000 variants identified in each individual exome, a single candidate gene has to be selected. WES approaches using small groups of patients with presumably the same MRI-defined novel leukodystrophy are quite successful in gene identification.45,46,49-56 and appears much more powerful than performing WES in large, unselected patient groups. We have had a success rate of 80-90% for gene identification by WES in small, homogeneous patient groups from the Amsterdam database of unclassified leukoencephalopathy cases, whereas several larger WES studies have reported success rates of 42% for mixed leukodystrophy cases 57 and 16-53% 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 pathogenicity of 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 remained 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 work-up.
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 1990-1996. Follow-up for 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 work-up that would now be state-of-the-art. Additionally, a part of the patients may not have a genetic disease.

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 3000 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 six months and the second MRI after one year of age. 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.

Outcomes were assessed in December 2015 and exclusively based on presence or absence of a molecular diagnosis. Counting siblings as one, a total of 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 (Table 1). For these remaining cases, a molecular diagnosis was established in 60/114 (53%) for cohort 2 and 69/106 (65%) for cohort 3 (Table 1), and 129/220 (59%) for the overall group.

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 17 or 5 years ago, 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.
Table 1. Outcome for cohort 2 and cohort 3

<table>
<thead>
<tr>
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<th>Cohort 2 – hypomyelination</th>
<th>Cohort 3 – suspected mitochondrial leukodystrophy</th>
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</thead>
<tbody>
<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>
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GENETIC DEFECTS AND LEUKODYSTROPHY CONCEPTS

Figure 1 presents the reported genetic defects in cohorts 2 and 3. 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 leukodystrophies, numerous defects in proteins important in 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 (Figure 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 our cohorts 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 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.

It is intriguing that several defects in mRNA translation result in distinctly different leukodystrophies, whereas other, similar defects in mRNA translation genes are associated with non-neurological phenotypes. Each disease has its own cell type- and tissue-specificity. In line with the housekeeping function of the affected proteins,
Figure 1. Overview of genes identified.
A. Genes mutated in cohort 2, hypomyelination. B. Genes mutated in cohort 3, suspected mitochondrial leukodystrophies. Each color represents a different gene. This is true for both for the hypomyelination and suspected mitochondrial leukodystrophy groups.
patients never have two mutations that would cause complete function loss, because 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. VWM is an excellent example. Although a decreased eIF2B guanine exchange factor activity is observed in VWM patient-derived lymphoblasts and fibroblasts, no effect on protein synthesis rate is noted in these cells.\[^{69,70}\] There is no correlation between residual eIF2B activity in patient cells and severity of the VWM phenotype.\[^{71}\] The clinical phenotype of VWM includes no signs of overall compromised protein synthesis.\[^{72}\] Another example is the striking similarity in selectively affected brain structures in ‘hypomyelination with brain stem and spinal cord involvement and leg spasticity’ (HBSL) and LBSL, despite the fact that the involved aspartyl tRNA synthetases have different subcellular localization, cytoplasmic versus mitochondrial, where they are involved in unrelated mRNA translation processes.\[^{38,51}\] This observation suggests that a shared, alternative function of these enzymes is responsible for the overlapping phenotype. Most likely other, non-canonical functions of the affected proteins play a role in the pathogenesis of mRNA translation-related leukodystrophies.\[^{73,74}\]

A striking finding is that many new leukodystrophies are caused by mitochondrial defects. Formerly, defects in energy metabolism were expected to mainly affect neurons, with predominant involvement of the cerebral cortex (e.g. MELAS), basal ganglia, thalami and brain stem nuclei (e.g. Leigh syndrome).\[^{75,76}\] The identification of novel gene defects affecting proteins that are part of the respiratory chain for pure white matter disorders\[^{52,53}\] or combined white and gray matter disorders\[^{49}\] shed new light on the effect of respiratory chain dysfunction in the brain.

For some disorders accepted as leukodystrophies WES revealed defects in proteins specifically expressed in unexpected cell types. The \textit{CSF1R} gene, mutated in HDLS, encodes a growth factor specific for microglia, macrophages and monocytes,\[^{77}\] while pathology suggests a white matter axonopathy.\[^{13}\] \textit{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 primary axonal damage, the defective microtubule system may hamper axonal transport, leading to axonal dysfunction and loss and secondary myelin deficit.\[^{78}\] The findings of the last five 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
leukodystrophies. Additionally, molecular progress has made clear that there are also non-progressive 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 LTBL, 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 leukodystrophies.

Definitions reflect the state of knowledge at the time and should be regularly updated to reflect new insights. Recently the GLIA consortium suggested a modified leukodystrophy definition to include heritable disorders affecting the central nervous system white matter with abnormalities involving myelin or all macroglial cell types, both oligodendrocytes and astrocytes. 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, but a complex tissue that contains many more components needs to be repaired.
REFERENCES
