Radiological studies of \textit{LMNB1}-related autosomal dominant leukodystrophy and Marinesco-Sjögren syndrome

JOHANNES FINNSSON
Dissertation presented at Uppsala University to be publicly examined in Grönwallsalen, Ingång 70, Akademiska Sjukhuset, Uppsala, Tuesday, 22 November 2016 at 13:00 for the degree of Doctor of Philosophy (Faculty of Medicine). The examination will be conducted in Swedish. Faculty examiner: Isabella Björkman-Burtscher (Diagnostisk radiologi, Lund).

Abstract

There are approximately 6000 to 8000 rare diseases, each with a prevalence of less than 1 / 10 000, but in aggregate affecting 6 to 8% of the population. It is important to evaluate disease development and progression to know the natural course of any disease. This information can be utilized in diagnostics and in assessing effects of therapeutic interventions as they become available. This thesis describes the natural clinical history and evolution of imaging findings of two rare diseases over approximately two decades.

Papers I, II and III present clinical, magnetic resonance imaging (MRI), magnetic resonance spectroscopy (MRS) and 18F-fluorodeoxyglucose positron emission tomography (FDG-PET) findings in LMBN1-related autosomal dominant leukodystrophy (ADLD). MRI was found to be very sensitive in finding pathology in patients with LMBN1-related ADLD, even before the onset of clinical symptoms. However, even patients with widespread MRI changes can have a relatively mild symptomatology and present only slight disturbances in metabolic examinations such as MRS and FDG-PET. This is compatible with relatively intact axons, even as myelin impairment is widespread.

Paper IV presents clinical and MRI findings in the brain and musculature in SIL1-positive Marinesco-Sjögren syndrome (MSS), and describes a new, mild phenotype of the disease with no intellectual disabilities and only slight motor disabilities. With a 19-year-long radiological follow-up, a slow progressive atrophic process in the cerebellum and brainstem could be demonstrated. MRI of the musculature shows early involvement of the quadriceps and gastrocnemii but not the tibialis anterior, progressing to widespread atrophy in the back and upper and lower limbs at the age of 20 years. In the mildest phenotype, the most severely affected muscles were the m gluteus maximus, m sartorius, m peroneus longus, and the lateral head of the m gastrocnemius.

Keywords: Leukoencephalopathies, hereditary central nervous system demyelinating diseases, autonomic dysfunction, adult-onset, neuromuscular disease, pediatric, neuro-ophtalmology, ataxia

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urn:nbn:se:uu:diva-303171 (http://urn.kb.se/resolve?urn=nbn:se:uu:diva-303171)
To Clara,

Daniel, Alba and Sofia
List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


III  **Finnsson, J.,** Lubberink, M., Fällmar, D., Savitcheva I., Melberg, A., Kumlien, E., Raininko, R. Glucose metabolism in the brain in $LMNB1$-related autosomal dominant leukodystrophy; a PET study. *Manuscript*

IV  **Finnsson, J.,** Kimber, E., Melberg, A., Raininko, R. Clinical and MRI evaluation of Marinesco-Sjögren syndrome with a 21-year-follow-up and a description of a mild form of the disease. *Manuscript*

* Denotes equal contribution

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They should avoid expressing the following sentiments: “Fewer doctors and more industrialists. The greatness of nations is not measured by what the former know, but rather by the number of scientific triumphs applied to commerce, industry, agriculture, medicine, and the military arts. We shall leave to the phlegmatic and lazy Teutons their subtle investigations of pure science and mad eagerness to pry into the remotest corners of life. Let us devote ourselves to extracting the practical essence of scientific knowledge, and then using it to improve the human condition. Spain needs machines for its trains and ships, practical advances for agriculture and industry, a rational health care system—in short, whatever contributes to the common good, the nation’s wealth, and the people’s well-being. May God deliver us from worthless scholars immersed in dubious speculation or dedicated to the conquest of the infinitesimal, which would be considered a frivolous if not ridiculous pastime if it weren’t so expensive.”

From Advice for a young investigator, by Santiago Ramón y Cajal (1897), translation by Swanson N and Swanson LW, 1999
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### Abbreviations

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<th>Description</th>
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<tr>
<td>ADC</td>
<td>apparent diffusion coefficient</td>
</tr>
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<td>ADLD</td>
<td>autosomal dominant leukodystrophy</td>
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<tr>
<td>ARCA</td>
<td>autosomal recessive cerebellar ataxia</td>
</tr>
<tr>
<td>CADASIL</td>
<td>cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy</td>
</tr>
<tr>
<td>CCFDN</td>
<td>congenital cataracts, facial dysmorphism and neuropathy</td>
</tr>
<tr>
<td>Cho</td>
<td>choline</td>
</tr>
<tr>
<td>CNS</td>
<td>central nervous system</td>
</tr>
<tr>
<td>Cr</td>
<td>creatine</td>
</tr>
<tr>
<td>CRLB</td>
<td>Cramér-Rao lower bound</td>
</tr>
<tr>
<td>CRV</td>
<td>cerebroretinal vasculopathy</td>
</tr>
<tr>
<td>CSF</td>
<td>cerebrospinal fluid</td>
</tr>
<tr>
<td>CT</td>
<td>computed tomography</td>
</tr>
<tr>
<td>DWI</td>
<td>Diffusion-weighted images</td>
</tr>
<tr>
<td>EDSS</td>
<td>Kurtzke Expanded Disability Status Scale</td>
</tr>
<tr>
<td>FDG</td>
<td>¹⁸F-fluorodeoxyglucose</td>
</tr>
<tr>
<td>FLAIR</td>
<td>fluid attenuation inversion recovery</td>
</tr>
<tr>
<td>FSE</td>
<td>fast spin-echo</td>
</tr>
<tr>
<td>FWHM</td>
<td>full width half maximum</td>
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<tr>
<td>GLD</td>
<td>globoid cell leukodystrophy</td>
</tr>
<tr>
<td>GRE</td>
<td>gradient-echo</td>
</tr>
<tr>
<td>HDLS</td>
<td>hereditary diffuse leukoencephalopathy with spheroids</td>
</tr>
<tr>
<td>MLD</td>
<td>metachromatic leukodystrophy</td>
</tr>
<tr>
<td>MRC</td>
<td>medical research council</td>
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<tr>
<td>MRI</td>
<td>magnetic resonance imaging</td>
</tr>
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<td>MRS</td>
<td>magnetic resonance spectroscopy</td>
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<tr>
<td>MS</td>
<td>multiple sclerosis</td>
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<tr>
<td>MSA</td>
<td>multiple system atrophy</td>
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<tr>
<td>MSS</td>
<td>Marinesco-Sjögren syndrome</td>
</tr>
<tr>
<td>NAA</td>
<td>N-acetyl aspartate</td>
</tr>
<tr>
<td>NIH</td>
<td>National Institutes of Health</td>
</tr>
<tr>
<td>OPCA</td>
<td>olivopontocerebellar atrophy</td>
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<tr>
<td>PCWH</td>
<td>peripheral demyelinating neuropathy, central dysmyelinating leukodystrophy, Waardenburg syndrome, and Hirschsprung disease</td>
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<tr>
<td>PET</td>
<td>positron emission tomography</td>
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<td>Abbreviation</td>
<td>Full Form</td>
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<td>--------------</td>
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<tr>
<td>PMD</td>
<td>Pelizaeus-Merzbacher disease</td>
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<td>POLD</td>
<td>pigmentary orthocromatic leukodystrophy</td>
</tr>
<tr>
<td>PRESS</td>
<td>point-resolved spectroscopy</td>
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<tr>
<td>ROI</td>
<td>region of interest</td>
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<tr>
<td>SD</td>
<td>standard deviation</td>
</tr>
<tr>
<td>SE</td>
<td>spin-echo</td>
</tr>
<tr>
<td>SI</td>
<td>signal intensity</td>
</tr>
<tr>
<td>SNR</td>
<td>signal to noise ratio</td>
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<tr>
<td>T1W</td>
<td>T1-weighted</td>
</tr>
<tr>
<td>T2W</td>
<td>T2-weighted</td>
</tr>
<tr>
<td>WES</td>
<td>whole-exome sequencing</td>
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<tr>
<td>WM</td>
<td>white matter</td>
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<tr>
<td>VWM</td>
<td>leukoencephalopathy with vanishing white matter</td>
</tr>
<tr>
<td>X-ALD</td>
<td>X-linked adrenoleukodystrophy</td>
</tr>
<tr>
<td>Z-score</td>
<td>standard score</td>
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</tbody>
</table>
Introduction

Rare diseases

The definition of a rare disease varies from country to country. According to the definition by the Swedish National Board of Health and Welfare a rare disease is a disease that affect less than 1 / 10 000 people and which leads to a disability (Socialstyrelsen, 2016). The NIH defines a rare disease purely based on prevalence, as a disease that affects fewer than 200 000 people in the United States, which approximates to 6 / 10 000 (GARD, 2016). The EU definition is “a life-threatening or chronically debilitating disease which is of such low prevalence (fewer than 5 / 10 000) that special combined efforts are needed to address them, so as to prevent significant morbidity, perinatal or early mortality, or a considerable reduction in an individual’s quality of life or socioeconomic potential.” (Montserrat Moliner et al., 2014).

It has been estimated that there are 6000 to 8000 rare diseases, together affecting between 6 and 8 % of the population (Bonkowsky, 2016). Most, though not all, rare diseases are genetic, often caused by a single gene defect. Although many debut in childhood, more than half debut in adulthood (orpha.net, 2012).

The reasons to study rare diseases are manifold. The primary is one of fairness, just because a disease is rare, it should not be ignored, and it is important to know the natural course of any disease to evaluate disease progression and assess effects of therapeutic interventions. Another reason is that defining rare diseases and finding diagnostic criteria for them can prevent unnecessary testing and insecurity in patients. Finally, knowledge of rare diseases can help elucidate the normal function of biological systems, especially as many of them are monogenetic and the dysfunction of a gene or protein helps define its likely normal function.

Leukodystrophies

Leukodystrophy is a compound of the Greek words leuko = white, dys = lack of, and trophy = growth. The word was introduced in 1928, for metachromatic leukodystrophy (Bielschowsky et al., 1928; Kevelam et al., 2016). A modern definition of leukodystrophy is a genetic and progressive disorder that primarily and directly affects CNS myelin (Love et al., 2015). However, some controversy exists regarding which diseases should be classified as leukodystrophies.
A consensus paper from 2015 presented a more detailed definition (Vanderveer et al., 2015):

“Leukodystrophies are heritable disorders affecting the white matter of the central nervous system with or without peripheral nervous system involvement. These disorders have in common glial cell or myelin sheath abnormalities. Where known, neuropathology is primarily characterized by the involvement of oligodendrocytes, astrocytes and other non-neuronal cell types, although in many disorders the mechanism of disease remains unknown, and in other cases is suspected to include significant axonal pathology.

In leukodystrophies, on magnetic resonance imaging (MRI), T2 hyperintensity in the affected white matter is present and T1 signal may be variable. Mildly hypo-, iso- or hyperintense T1 signal relative to the cortex may be consistent with a hypomyelinating leukodystrophy. Demyelinating leukodystrophy leads to significantly hypointense T1 signal.

Leukodystrophies do not include acquired CNS myelin disorders, such as multiple sclerosis and related acquired demyelinating processes, infectious and post-infectious white matter damage, toxic injuries and non-genetic vascular insults.

In addition, CNS diseases in which neuropathology shows primary involvement of neurons in cerebral cortex or other gray matter structures should not be characterized as leukodystrophies. Also, inborn errors of metabolism, in which the clinical manifestations of systemic illness, such as liver, muscle, or heart predominate, but in which brain MRI can detect significant abnormalities of white matter, should not be characterized as leukodystrophies.”

In the same paper a distinction was made between leukodystrophies and “genetic leukoencephalopathies”, representing disorders with significant, if not primary, white matter abnormalities, not meeting the criteria for inclusion as a leukodystrophy. Even more recently Kevelam et al. (2016) suggested that “leukodystrophy” should refer to all genetic diseases primarily affecting CNS white matter, not limited to progressive disorders or only disorders directly affecting myelin. The term “leukoencephalopathy” is more inclusive, representing any white matter disorder, genetic or acquired.

The majority of leukodystrophies debut in childhood and exhibit an autosomal recessive inheritance pattern (Kohlschütter et al., 2010), some are X-linked or sporadic. A minority exhibit an autosomal dominant inheritance pattern. Table 1 lists leukodystrophies with autosomal dominant inheritance where the genetic defect is known. Comprehensive tables, presenting mode of inheritance, associated genes and clinical findings in most leukodystrophies can also be found in the papers by Kohlschütter et al. (2010), Ahmed et al., (2014, reporting on adult-onset leukodystrophies) and Barkovich et al. (2016, reporting on hypomyelinating leukodystrophies).

Exact, and valid, epidemiological information about the total prevalence of leukodystrophies is difficult to find. In the United Kingdom the estimated lifetime risk per one million live births is 31 for childhood onset leukodystrophies, and 40 for childhood onset genetic encephalopathies (Stellitano et al., 2016). The most common childhood onset leukodystrophies in the UK are
MLD (representing 22% of cases), X-ALD (21% of cases) and Krabbe/GLD (16% of cases). In the study by Stellitano et al. (2016), 51% of diagnosed children with a progressive intellectual and neurological deterioration had a leukodystrophy or genetic leukoencephalopathy. It should be noted that 58% of the children with a progressive and neurological deterioration never received a specific diagnosis. This number is likely to decrease, given the increased access to genetic testing. The reported prevalence of adult onset leukodystrophies is rising, as more and more forms are described and defined. Ahmed et al. (2014) estimates their total prevalence to 300 / 1 000 000.

**LMNBI-related autosomal dominant leukodystrophy**

LMNBI-related ADLD is a lethal adult onset disease first described by Eldridge et al. in 1984 as a “hereditary adult-onset leukodystrophy simulating chronic progressive multiple sclerosis”. In their material, 20 out of 21 patients had been diagnosed with MS, before the availability of CT scanning, and without regard to family history. The report presented a pedigree of four generations and they concluded that the disease was inherited in an autosomal dominant fashion. The clinical picture was that of a slowly progressive multisystem neurologic disorder debutting in the 4th to 6th decade with patients coming to the attention of neurologists when they start losing fine motor skills. Before that, they debut with autonomic symptoms, involving the bladder or bowel and/or orthostatic hypotension. Late in the course the patients are bedridden, and they reported that death usually occurred 20 years after the appearance of overt symptoms. CT scanning, performed in 5 patients, revealed findings distinct from those found in MS with extensive, symmetric decrease in white-matter density, first in frontal lobes and cerebellum and later in parietal and occipital lobes.

A detailed description of the histopathological and MRI findings of the brain was published by Melberg et al. in 2006. In that material, subjects exhibited extensive T2 hyperintensities in cerebellar peduncles and the cerebral white matter, most prominent frontoparietally. In symptomatic subjects the whole lengths of the corticospinal tracts and the corpus callosum were affected. It was characteristic of the disease that there was a less-affected periventricular rim around the lateral ventricles. On histological examinations, myelin appeared rarefied and vacuolated, though there was only minimal reactive astrogliosis and no increase of lymphocytes or phagocytic cells. There was a relatively close match between grossly visible lesions in neuropathologic inspection and those revealed by MR imaging, while microscopically the disease extended beyond the MR lesions, especially in the cerebellum. The axons seemed well preserved and there was no significant pathology in the cerebral cortex, while in the cerebellum the number of Purkinje cells was red-
<table>
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<th>Disorder</th>
<th>Onset</th>
<th>Clinical characteristics</th>
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<tr>
<td>18q minus syndrome*</td>
<td>Highly variable. Mental retardation, short stature, hypotonia, hearing impairment, foot deformities.</td>
<td></td>
</tr>
<tr>
<td>Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL)**</td>
<td>Adult-hood</td>
<td>Recurrent ischemic stroke, cognitive decline, migraine with aura, mood disturbance, apathy.</td>
</tr>
<tr>
<td>Adult-onset leukoencephalopathy with axonal spheroids and pigmented glia (ALSP)***</td>
<td>Adult-hood</td>
<td>Progressive personality change, motor impairment, parkinsonism, seizures (rare).</td>
</tr>
<tr>
<td>Hypomyelinating leukodystrophy 6</td>
<td>Infant-hood</td>
<td>Developmental delay, extrapyramidal movement disorders, progressive spastic tetraplegia, ataxia.</td>
</tr>
<tr>
<td>LMNB1-related autosomal dominant leukodystrophy (ADLD)</td>
<td>Adult-hood</td>
<td>Debuts with autonomic disease, progressing with ataxia and symptoms from the corticospinal tracts.</td>
</tr>
<tr>
<td>Peripheral demyelinating neuropathy, central dysmyelinating leukodystrophy, Waardenburg syndrome, and Hirschsprung disease (PCWH)</td>
<td>Infant-hood</td>
<td>Developmental delay, nystagmus, myopia, hypotonia, deafness, aganglionosis, hypomelanotic skin patches.</td>
</tr>
<tr>
<td>Retinal vasculopathy with cerebral leukodystrophy (RVCL)</td>
<td>Adult-hood</td>
<td>Vision loss, seizures, hemiparesis, apraxia, dysarthria, memory loss.</td>
</tr>
</tbody>
</table>

* Categorized as a leukodystrophy in the paper by Vanderver et al. (2015), but not 100% consensus.
** Not categorized as a leukodystrophy in the consensus paper by Vanderver et al. (2015).
*** Includes hereditary diffuse leukoencephalopathy with spheroids (HDLS) and pigmentary orthochromatic leukodystrophy (POLD)
Table 1. (continued)

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<thead>
<tr>
<th>MRI</th>
<th>Gene</th>
<th>References</th>
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<tr>
<td>(1) Extensive cerebral white-matter abnormalities with frontal preponderance.</td>
<td></td>
<td>van der Knaap et al., 2001, 2005a, 2006</td>
</tr>
<tr>
<td>(2) Periventricular rim of decreased T2W signal and elevated T1W signal.</td>
<td></td>
<td>Wang et al., 2007</td>
</tr>
<tr>
<td>(3) SI changes in basal ganglia and thalami.</td>
<td></td>
<td>Farina et al., 2008</td>
</tr>
<tr>
<td>(4) Brainstem lesions.</td>
<td></td>
<td>Sawaishi, 2009</td>
</tr>
<tr>
<td>(5) Contrast enhancement of: Ventricular lining / periventricular rim / frontal white matter / optic chiasm / fornix / basal ganglia / thalamus / dentate nuclei / brain stem Sometimes predominant or isolated involvement of posterior fossa structures. Adult form: Brainstem and spinal cord atrophy and signal intensity changes, contrast enhancement in cerebrum or brainstem. Increased T2W signal in upper corticospinal tracts.</td>
<td>GFAP</td>
<td>Graff-Radford et al., 2014</td>
</tr>
<tr>
<td>Poor differentiation between grey and white matter on T2W images.</td>
<td></td>
<td>Linnankivi et al., 2006</td>
</tr>
<tr>
<td>WM lesions first affecting temporal poles and external capsules, the whole WM affected in end-stage disease.</td>
<td></td>
<td>Joutel et al., 1996</td>
</tr>
<tr>
<td>Initially focal, later confluent WM lesions particularly affecting frontal and parietal lobes.</td>
<td></td>
<td>Dichgans et al., 1998</td>
</tr>
<tr>
<td>Initially lesions along corticospinal tracts and cerebellar peduncles, almost all WM affected in end stage disease, sparing a periventricular ribbon.</td>
<td></td>
<td>Sundal et al., 2012a, b</td>
</tr>
<tr>
<td>Hypomyelination, cerebellar atrophy, absence or disappearance of the putamen.</td>
<td></td>
<td>Lynch et al., 2016</td>
</tr>
<tr>
<td>Tumour-like lesions which may resolve spontaneously or multiple small white matter lesions.</td>
<td></td>
<td>Simons et al, 2013</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Melberg et al., 2006</td>
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<td></td>
<td></td>
<td>Inoue et al., 2004</td>
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<tr>
<td></td>
<td></td>
<td>Elmaleh-Bergès et al., 2013</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mateen et al., 2010</td>
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</tbody>
</table>
uced and the number of Bergmann astroglial cells slightly increased (Eldridge et al., 1984; Schwankhaus et al., 1994; Coffeen et al., 2000; Melberg et al., 2006). The paper by Melberg et al. (2006) suggested the disease should be named “adult-onset autosomal dominant leukodystrophy with autonomic symptoms”.

The same year, 2006, duplications of LMNB1 was found to cause the disease (Padiath et al., 2006). LMNB1 is the gene encoding lamin B1. Nuclear lamins are the proteins composing the nuclear lamina, associated with the inner face of the nuclear envelope. Apart from providing support they seem to regulate DNA replication (Moir et al., 1994) and participate in chromatin organization (Shimi et al., 2008). Nuclear lamina also play a role in neuronal migration (Coffinier et al., 2010a, b; Young et al., 2014; Lee et al., 2014). Diseases caused by defects in nuclear lamins are called laminopathies (Burke et al., 2002; Worman et al., 2009). The group includes, but is not limited to, autosomal dominant Emery-Dreifuss muscular dystrophy (Bonne et al., 1999), dilated cardiomyopathy with conduction system disease (Fatkin et al., 1999), Charcot-Marie-Tooth disorder type 2 (De Sandre-Giovannoli et al., 2002), Dunnigan-type familial partial lipodystrophy (Cao et al., 2000) and Hutchinson-Gilford progeria syndrome, a pediatric disorder presenting as premature ageing (Eriksson et al., 2003; De Sandre-Giovannoli et al., 2003). Apart from duplications of the LMNB1 gene LMNB1-related ADLD can be caused by increased lamin B1 expression through enhancer adoption due to a genomic deletion (Giorgio et al., 2015). The increased levels of lamin B1 seem to cause age-dependent inhibition of lipid synthesis in oligodendrocytes, resulting in dysmyelination (Lin et al., 2009; Padiath et al., 2010; Rolyan et al., 2015).

Although the number of reported families with LMNB1-related ADLD has grown from 3 to numbering in the tens since the publication by Melberg et al. in 2006 (Padiath et al., 2006; Schuster et al., 2011; Meijer et al., 2008; Brussino et al., 2009; Dos Santos et al., 2012; Fogel et al., 2012; Molloy et al., 2012; Flanagan et al., 2013), no longitudinal study, presenting the evolution of clinical radiological findings in the disease has been published.
Autosomal recessive cerebellar ataxias

Autosomal recessive cerebellar ataxias are a heterogeneous and complex group of diseases. There are at least 20 different clinical forms of ARCA, caused by over 30 genes/loci (Mancuso et al., 2014; Hamza et al., 2015). Their combined prevalence is estimated to 22-70 / 1 000 000, with total as well as relative prevalence of the different forms varying between populations (Koht et al., 2007; Anheim et al., 2010; Coutinho et al., 2013). Friedrich ataxia is the most common ARCA in most populations. Ataxia telangiectasia is the most common cerebellar ataxia with onset before the age of 5 years. Fogel et al. (2012a) discusses the evaluation of children presenting with cerebellar ataxia. Diagnostic flowcharts for ARCAs in general can be found in the papers by Anheim et al. (2012) and Mancuso et al. (2014). The advent of massively parallel, next-generation sequencing has made genetic diagnosis and screening faster and more cost-effective in the last years (Németh et al., 2013).

If patients present with congenital or childhood onset cataracts in addition to cerebellar ataxia, the differential diagnosis is substantially narrowed. One disease that should be considered is Marinesco-Sjögren syndrome, described in more detail below. Another differential diagnosis is CCFDN syndrome, found in Bulgarian Romani (Tournev et al., 1999). Although MSS and CCFDN share the clinical characteristics of childhood cataracts, nystagmus, somatic and mental retardation, ataxia, skeletal deformities and hypogonadism, they differ in that CCFDN patients present mild facial dysmorphism, microcornea and demyelinating neuropathy (Lagier-Tourenne et al., 2002). CCFDN is caused by a mutation in the CTDP1 gene (Varon et al., 2003). Ataxia-microcephaly-cataract syndrome (Ziv et al., 1992) resembles MSS, but microcephaly is not part of MSS. Patients with cataract-ataxia-deafness-retardation syndrome (Begeer et al., 1991) present hearing loss. Cerebellar ataxia, cataract, deafness and dementia or psychosis / ITM2B related cerebral amyloid angiopathy (Strömgren et al., 1970; Vidal et al., 2000), presents autosomal dominant inheritance and symptom onset later in life than in MSS. Cataract, ataxia, short stature and mental retardation described by Guo et al. (2006) presents X-linked recessive inheritance. Schulz et al. (2007) reported on two siblings with the clinical picture of MSS but without marked cerebellar atrophy and without mutations in the SIL1 gene, the only gene found so far causing MSS.

Marinesco-Sjögren syndrome

Marinesco-Sjögren Syndrome is a rare (1 – 9 / 1 000 000) autosomal recessive disorder characterized by cerebellar ataxia, childhood cataracts, progressive myopathy and mild to severe mental retardation (Marinesco et al., 1931; Sjögren T, 1950). It has been described in Mendelian Inheritance in Man,
since the book’s first edition (McKusick, 1966). In 2005, two groups independently identified mutations in the \textit{SIL1} gene to be a cause for the disease (Anttonen \textit{et al.}, 2005; Senderek \textit{et al.}, 2005). \textit{SIL1} acts as a co-chaperone for BiP, a key regulator of endoplasmatic reticulum functions (Dudek \textit{et al.}, 2009). \textit{SIL1} depletion in mice has a variety of pathophysiological consequences, including alterations of the endoplasmatic reticulum/nuclear envelope, of mitochondria, of the cytoskeleton and of vesicular protein transport (Roos \textit{et al.}, 2015). Curiously, Lamin B1 is one of a number of proteins with altered expression in woozy-type mice, a spontaneous mutant serving as a model for MSS, and a link has been found between chaperone dysfunction and nuclear envelope pathology (Roos \textit{et al.}, 2014).

Neuropathology in MSS is non-specific with marked cerebellar atrophy, especially of the vermis and variable cortical atrophy. Histology shows an almost complete loss of nerve cells in the cerebellar cortex and severe nerve fiber loss in the white matter of the cerebellum. In the pons and medulla oblongata, there is severe gliosis and nerve cell loss in the pontine nuclei and inferior olives (Mahloujdi \textit{et al.}, 1972). Light microscopy of the muscle shows variation in the fiber size, with atrophic fibers and fatty replacement as well as vacuole formation (Herva \textit{et al.}, 1987; Superneau \textit{et al.}, 1987; Sewry \textit{et al.}, 1988; Komiyama \textit{et al.}, 1989; Suzuki \textit{et al.}, 1997). On electron microscopy, an electron-dense membranous structure surrounding the nuclei can be seen and is considered specific for the disease (Sewry \textit{et al.}, 1988; Goto \textit{et al.}, 1990; Sasaki \textit{et al.}, 1996).

Reported neuroradiological findings in MSS include cerebellar hypoplasia or atrophy (Georgy \textit{et al.}, 1998; Slavotinek \textit{et al.}, 2005; Fujitake \textit{et al.}, 2011), which is nonspecific (Poretti A \textit{et al.}, 2008) and nonobligatory in MSS (Reinhold \textit{et al.}, 2003). A small anterior pituitary gland and absence of the high signal intensity in the posterior pituitary gland have also been reported (McLaughlin \textit{et al.}, 1996; Reinhold \textit{et al.}, 2003), as well as a T2 hyperintense cerebellar cortex, best seen on the coronal FLAIR images (Harting \textit{et al.}, 2004).

One previous study documents CT findings in the muscles in adult patients with MSS (Mahjneh \textit{et al.}, 2006) and there is a case report of MRI findings in the lower extremities of a single adult patient with MSS (Fujitake \textit{et al.}, 2011). Thus far, no description of muscle CT or MRI findings in children affected by the disease has been published, even though muscle pathology usually is investigated as a part of the diagnostic work-up, and MRI can help by guiding the choice of the biopsy site.
Aims

General aim
The general aim of the thesis was to describe the natural clinical history and evolution of radiological findings in *LMNB1*-related autosomal dominant leukodystrophy and Marinesco-Sjögren syndrome.

Specific aims

**Paper I**
To describe the metabolic changes in the brain of patients affected by *LMNB1*-related ADLD, as demonstrated by $^1$H-MRS.

**Paper II**
To describe the natural clinical and radiological development of *LMNB1*-related ADLD based on a follow-up study over a two-decade period.

**Paper III**
To investigate glucose metabolism in the brain in *LMNB1*-related ADLD using $^{18}$F-fluorodeoxyglucose positron emission tomography (FDG-PET).

**Paper IV**
To present a long-term clinical description and MRI follow-up in the brain and muscles throughout childhood and young adulthood in patients affected by MSS and to describe a new mild phenotype of MSS.
Subjects and Methods

**LMNB1-related ADLD**

**Subjects**

Twenty-five subjects, from 2 nonrelated families segregating LMNB1-related ADLD were initially recruited and underwent clinical assessment and radiological studies. Once the genetic basis of the disease became known (Marklund et al., 2006; Padiath et al., 2006), 2 asymptomatic subjects without MRI pathology were found to be non-carriers of the LMNB1 duplication and were excluded. The LMNB1-duplications were of different sizes in the 2 families; 203,432bp in Family I and 189,731bp in Family II (Giorgio et al., 2013). The final material consisted of 23 subjects, 12 women and 11 men. All 23 subjects were included in the longitudinal study, presented in Paper II, 14 of the subjects were included in the MRS study, presented in Paper I and 8 of the subjects in the PET study, presented in Paper III.

Due to the differing sample sizes, the numbering of the patients varies between Paper I, II and III. Throughout this thesis however, the numbering is consistent for comparative purposes.

**Methods**

Subjects were studied assessing history, clinical neurological and physical examinations, and followed up by one and the same experienced neurologist. Blood pressure was recorded in the supine and standing upright position within 3 minutes. The Kurtzke Expanded Disability Status Scale was applied in retrospect based on medical records for clinical scoring of pyramidal, cerebellar, brain stem, sensory, bladder and bowel, visual, and mental functions (Kurtzke, 1983). Symptoms of orthostatic hypotension were included in the autonomic bowel and bladder functional systems score. The EDSS is commonly used to rate MS disability. Our rationale for applying the EDSS is that LMNB1-related ADLD, like MS, affects the white substance in the brain and spinal cord.

Clinical examinations were accompanied by radiological investigations. The brain was examined with CT in 5 subjects. Twenty-one subjects underwent brain MRI, 4 of whom had also undergone CT. In sagittal series, the upper spinal cord was visualized and appeared thin. Therefore, MRI of the
spinal cord was added in the study protocol and was performed in 14 subjects. MRS of the brain was performed in 14 subjects, twice in 3 of them. CT of the brain was performed 4 times during 6 years in one subject. MRI of the brain was repeated at least once in 13 subjects with a median follow-up time of 10 years (range, 0.5–17) and MRI of the spinal cord in 9 subjects with a median follow-up time of 5 years (range, 2–10). Age distribution of subjects at the time of examinations is displayed in Figure 1.

Figure 1. Age distribution of subjects at radiological examinations and neurological controls at our hospital. — = time span for clinical follow-up at our hospital. ● = MRI of the brain. ★ = MRS of the brain. ■ = PET of the brain. □ = MRI of the spine. △ = CT of the brain.
The symptomatology of the 14 subjects included in the MRS study ranged from asymptomatic to having severe autonomic and motor symptoms at the time of the first investigation (Table 2). For that study, two healthy controls per subject, matched for age (± 3 years) and sex were recruited for comparison. The control groups consisted of people working at the radiology department and their acquaintances.

Table 2. Clinical and MR imaging characteristics of the 14 subjects included in the MRS-study.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Age (yrs.)</th>
<th>Symptoms and signs</th>
<th>MRI changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>34–40</td>
<td>Asymptomatic</td>
<td>Mild-moderate</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>37–39</td>
<td>Asymptomatic</td>
<td>Extensive</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>48–58</td>
<td>Autonomic symptoms</td>
<td>Extensive</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>45–70</td>
<td>As in Group 3 + pyramidal signs and/or ataxia</td>
<td>Extensive</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>45</td>
<td>As in Group 4 + severe motor handicap</td>
<td>Extensive</td>
</tr>
</tbody>
</table>

In the PET study, presented in Paper III, data from 18 healthy controls, aged 58-69 years, median and mean 64 years, were used for comparison in a quantitative analysis.

**Computed tomography**

Brain CT was performed using a standard technique. Intravenous contrast medium was used in one examination.

**Magnetic resonance imaging**

Some subjects had undergone their first MR examination at other hospitals, causing some variation in the sequences. All examinations were performed with 1.5 tesla clinical MR systems. Brain MRI contained at least a sagittal T1-weighted and an axial T2-weighted SE sequence. Follow-up examinations were all performed at our department. Brain images were obtained using a standard imaging protocol, including T1-weighted sagittal and axial SE images, T2-weighted axial and coronal FSE images, and T2-weighted axial FLAIR images. Contrast medium was used in 5 examinations. DWIs were obtained in 10 subjects, 4 of them had a DWI follow-up of 4 to 7 years. The spinal cord was examined with sagittal T1-weighted SE and T2-weighted FSE sequences through the entire spinal cord. Axial T2-weighted images were obtained by using a three dimensional SE sequence at the levels of C2, C7, T5 to T6, and conus medullaris in 9 subjects. In 5 subjects, examined in other hospitals, transverse images were obtained with different T2-weighted SE or GRE sequences at varying levels.

All images were reviewed by an experienced neuroradiologist. Distribution of signal-intensity changes and substance loss as well as CSF spaces were graded, the effect of the patient’s age was eliminated by comparing the images to the copies progress between examinations were assessed and a five-grade
scale was devised (Figure 6). When sizes of intracranial CSF spaces were graded, the effect of the patients’ ages was eliminated by comparing the images to the copies of a standard image series used in a study of a neurologically healthy population (Salonen et al., 1997). Maximal width of the third ventricle was measured. Measurements of the brain stem were compared to those in a healthy population in different age groups (Raininko et al., 1994). Anteroposterior and transverse diameters as well as cross-sectional area of the spinal cord were measured at levels C2, T6, and the conus by two readers. These results were compared to the normal values (Krabbe et al., 1997).

**Magnetic resonance spectroscopy**

MRS examinations were performed using a single-voxel technique. The voxel was placed in the supraventricular white matter with the posterior end under the sensory-motor cortex because this was the area first affected (Melberg et al., 2006). The size and form of the voxel was individually adjusted to the subject’s anatomy in order to select a representative sample of white matter but to avoid or minimize partial volume effects with grey matter. Contamination by CSF was avoided. A typical voxel placement can be seen in Figure 2. The median size of the voxels was 14.6 ml (range 3.8–22.9 ml): in subjects 15.6 ml (range 3.8–22.9 ml) and in controls 13.1 ml (range 6–20.7 ml).

We used a PRESS sequence with 128, exceptionally 256, acquisitions, 1,024 points, and a spectral bandwidth of 1,000 Hz. A long repetition time (6,000 ms) and short echo time (22 ms) were used to reduce the quantification errors due to T1 and T2 relaxation effects. In four subjects, aged 35–61 years, MRS was also performed with a repetition time of 2,000 ms and an echo time of 136 ms to better demonstrate lactate. For quantification 16 unsuppressed water reference acquisitions were obtained. An unsuppressed water signal was used as an internal reference when metabolite concentrations were estimated with LCModel v 6.2-1G. We restricted the model to the range 0.2–4.0 ppm. The spectra were corrected for eddy currents. All spectra were manually assessed to exclude obvious non-randomness in the residuals or erroneous assignment of metabolites. All the analyses were made by one radiologist supported by one physicist.

NAA was evaluated as (NAA+ NAA-glutamate), Cr as total Cr (Cr + phosphocreatine), and Cho as total Cho (phosphocholine + glyceryl-phosphocholine). Glutamine and glutamate were evaluated as a glutamine–glutamate complex (Glx). Millimolar concentrations (mM, millimoles/liter substance) were measured using tissue water as a reference. Ratios were calculated using total Cr as a reference.
Figure 2. Typical MRS voxel placement from the anterior part of the parietal lobe continuing forward into the frontal lobe, encompassing T2-hyperintense changes

**Positron emission tomography**

Patients 12 and 15 were examined according to a standard clinical protocol, using a dynamic scan with start directly after intravenous injection of FDG and sampling of arterialized venous blood, as described in a previous publication (Engler et al., 2008). Patient 12 was examined with a Siemens ECAT EXACT HR+ scanner (CTI PET Systems Inc., Knoxville, TN, USA) and patient 15 with a GE 2048-15B Plus PET camera (General Electric Medical Systems, Uppsala, Sweden). Attenuation correction was performed using a 10-minute transmission scan with rotating 68Ge rod sources.

Patients 4-9 were examined with a Discovery ST (GE Healthcare, USA) PET/CT scanner after injection of 3 MBq/kg FDG. In patients 4, 6, 8 and 9, emission data acquisition started at the time of FDG injection, and the scan time was 45 minutes, with the following frame durations: 6 × 10 s; 3 × 20 s; 2 × 30 s; 2 × 1 min; 2 × 2.5 min; 7 × 5 min. A heat pad was used to arterialize venous blood, and blood sampling was performed at 15, 25, 35, 45, 60 and 90 s, and 2, 3, 5, 7, 10, 20, 30 and 45 min. In patients 5 and 7, the scanning started 20 minutes after FDG injection, and the scan time was 25 minutes with frame durations 5 × 5 min. Blood sampling was performed at 45 s and 1, 2, 3, 5, 10, 20, 45 and 60 min. Blood glucose levels were used to calculate absolute values for glucose metabolism in the brain. A low dose CT was performed in the same session for attenuation correction.

**Data analysis**

In patient 5, blood sampling failed and no quantitative data could be obtained. In patients 4, 6-9 and 12, quantitative glucose metabolism images were produced by a modified version of the method described by Patlak (Patlak et al.,...
The data were post-processed using the software PVElab (Quarantelli et al., 2004) and automatically divided into 46 ROIs using a probability based method (Svarer et al., 2005). Of these 46 ROIs, 44 were bilateral and from these an average value of the two sides was calculated. Data from the 3 ROIs of the brainstem were also combined, leaving 22 ROIs plus the values of the global glucose metabolism.

In patient 15, software post-processing could not be performed as the original data of the examination was no longer extant. Quantitative FDG-PET data had been analysed at the time of examination using Patlak analysis and manual delineation of cortical and subcortical ROIs as described in detail in a previous publication (Engler et al., 2008).

FDG-PET data from patients 4-9 and 12 were also analysed semiquantitatively with the software suite CortexID (GE Healthcare, Marlborough, MA, USA), using the average metabolism of the whole brain as a reference and comparing the findings to a dataset of 140 healthy controls. The original dataset of patient 15 was not extant and could not be analysed using the CortexID software.

**Statistical analysis**

To perform statistical calculations and draw graphs, the free software package R was used (R Core Team, 2013). In Paper I we used the paired t test to look for differences between subjects and controls and Pearson linear correlation to look for relationships between metabolite levels. In Paper II Bland-Altman plots were used to assess variability of repeated measurements and Pearson linear correlation was used to look for relationships between age and measurements obtained from the spinal cord. In Paper III Welch’s unequal variance t-tests were performed to find statistically significant differences in levels of FDG uptake.

The studies were approved by the local ethics committee and performed in accord with the ethical standards of the Declaration of Helsinki. Subjects gave informed consent before participating in the studies.
Marinesco-Sjögren syndrome

Subjects

Three patients with clinically and genetically confirmed MSS were included in the partially retrospective study presented in Paper IV. Two of them are identical twins (Patient 1 and 2) and compound heterozygotes for two mutations in the SIL1 gene (c.506_509dupAAGA and c.645 + 2T > C). Patient 3 is homozygote for the c.506_509dupAAGA mutation. The twins were first seen at our institution at the age of 16 months. They were followed at the department of pediatric neurology where one of the authors of Paper IV (EK) met them. All three patients were later followed by another of the authors (AM) at the neurology department of our hospital.

Methods

Patients were investigated with MRI of the brain and muscles. Five healthy controls, aged 5, 6, 8, 28, and 29 years, were recruited for comparison and investigated with MRI of the muscles. The types of MR examinations at each age are presented in Table 3. MR images were assessed by two radiologists visually, and a consensus report was created. To quantify the perceived pathologies and also to assess visually less affected muscles, SIs were measured at three levels in the representative muscles. The signal intensities were normalized by dividing the SI in a muscle ROI with that of nearby fat ROI. Thereafter, the mean of these three ratios in each muscle was calculated. As there were no significant differences between the ratios in the different muscles nor at different ages of the controls, a single mean and standard deviation of the muscle/fat SI ratios were calculated from the totality of the T1 respectively T2 measurements in the controls.

Table 3. MR examinations of the MSS patients and controls

<table>
<thead>
<tr>
<th>Subject</th>
<th>Pat 1 &amp; 2</th>
<th>Pat 1 &amp; 2</th>
<th>Con 5 yr</th>
<th>Con 6 yr</th>
<th>Con 8 yr</th>
<th>Con 20 yr</th>
<th>Con 27 yr</th>
<th>Con 28 yr</th>
<th>Con 29 yr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>16 mo</td>
<td>4 yr</td>
<td>5 yr</td>
<td>6 yr</td>
<td>8 yr</td>
<td>20 yr</td>
<td>27 yr</td>
<td>28 yr</td>
<td>29 yr</td>
</tr>
<tr>
<td>Brain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muscles</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower limbs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper limbs and trunk</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Pat = patient, Con = control, mo = months, yr = years
Results

LMNB1-related ADLD

Clinical findings

Figure 3 indicates asymptomatic subjects (n = 4), onset of symptoms, and EDSS scores at various ages, ages at which neurophysiological examinations were performed, and ages at death. Clinical characteristics of the subjects are summarized in Tables 4-6 and Figure 4A.

Table 4. Symptom onset of LMNB1-related ADLD

<table>
<thead>
<tr>
<th>First symptom</th>
<th>No. of subjects</th>
<th>Age at onset (yrs.) mean ± SD [Range]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autonomic</td>
<td>14</td>
<td>47 ± 5 [40–58]</td>
</tr>
<tr>
<td>Autonomic and gait problem</td>
<td>6</td>
<td>48 ± 5.5 [40–55]</td>
</tr>
<tr>
<td>Gait problem</td>
<td>2</td>
<td>50 ± 4 [47–53]</td>
</tr>
<tr>
<td>Total</td>
<td>22</td>
<td></td>
</tr>
</tbody>
</table>

ADLD = autosomal dominant leukodystrophy; SD = standard deviation

Autonomic symptoms

Autonomic symptoms were reported with onset between ages 40 and 58 years (median, 48). Subject 2, who had MRI pathology at age 34, had no symptoms at follow-up at age 43. He died after an accident at age 44. Subjects 1, 3, and 4, who were asymptomatic at the first evaluations, developed symptoms at ages 45 (constipation), 47 (bladder symptoms, erectile dysfunction, and constipation), and 47 years (bladder symptoms, erectile dysfunction, constipation, and gait problems), respectively. Their symptoms occurred 16, 13, and 9 years, respectively, after MR pathology was first documented. Ages of onset and types of the symptoms in the whole material are shown in Table 4. The type of symptoms at onset varied: Autonomic symptoms, usually bladder dysfunction, preceded other symptoms (n = 14); onset of autonomic symptoms and motor symptoms were simultaneous (n = 6); and in subjects 10 and 16, gait difficulties preceded autonomic symptoms by 3 and 1.5 years, respectively. In subject 10, gait problems first occurred during a long walk in the mountains and he needed support to walk. Autonomic symptoms included urinary urgency, incontinence, nocturia, and difficulty in emptying the bladder. Constipation was a common complaint from most study subjects and 4 men had erectile dysfunction as an early symptom. None of the patients reported
inability to sweat as an early feature. All 3 patients from Family II had an early onset of symptomatic orthostatic hypotension. In Family I, orthostatic hypotension was found early in the course (n = 9) or later during follow-up, in some cases after several years (n = 5). Dry skin was noted in 6 patients. Recurrent urinary tract infections were common.

<table>
<thead>
<tr>
<th>Subject</th>
<th>No.</th>
<th>Sex</th>
<th>Fam.</th>
<th>Age (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>I</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>I</td>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>I</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>I</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>I</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>II</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>I</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>I</td>
<td></td>
<td>6.5</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>II</td>
<td></td>
<td>7.5</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>I</td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>11</td>
<td>M</td>
<td>I</td>
<td></td>
<td>9</td>
</tr>
<tr>
<td>12</td>
<td>F</td>
<td>I</td>
<td></td>
<td>10</td>
</tr>
</tbody>
</table>

*Figure 3.* Evolution of symptoms and progress of disability. In addition to our own examinations, anamnestic data and information from the patient records in other hospitals have been used. That information was not always sufficient for EDSS scoring. Thin black lines = asymptomatic. Bold black lines = symptomatic. Numbers = EDSS scores. ▼ = neurophysiological examinations. + = deceased. M = male. F = female. Fam. = family. EDSS = Kurtzke Expanded Disability Status Scale.

**Motor symptoms**

In 14 patients, autonomic symptoms preceded motor symptoms by several months to years. Motor symptoms had a slow progressive course without acute exacerbations, with initial involvement of the legs, thereafter the arms, and,
finally, pseudobulbar palsy. Signs in the legs included spastic paraplegia that progressed slowly with weakness, brisk tendon reflexes, and extensor plantar signs. Maximum walking distance decreased with disease progression and patients used sticks, walkers, and, finally, a wheelchair. Mean age for EDSS 6 was 59 years and 61 years for EDSS 8. However, mean time lapse between EDSS 6 and 8 was 5 years (Table 5). As spasticity and weakness developed, the patients adopted a stooped posture, bending forward in the hips and semi-flexing the knees when standing and when supporting the stride, resulting in low back pain. Weak legs frequently resulted in falls. Weakness was also present in the arms, but to a lesser extent than in the legs. Patients eventually developed tetraparesis, flaccid paralysis of the legs with weak or abolished tendon reflexes (subjects 6 and 20), and pseudobulbar palsy, with difficulties in articulation and swallowing in the seventh and eighth decade, corresponding to EDSS 9.5. Gastrostomy was required for appropriate nutrition in 4 patients (subjects 15, 17, 19, and 22).

![Figure 4](image_url)

**Figure 4.** Evolution of EDSS scores in all 23 subjects (A) and of radiological grades in the 21 subjects examined with brain MRI (B). Multiple observations of individual subjects maintain the same shade of grey and are connected with lines. Some of the circles and segments of the lines may represent more than 1 subject. For MRI grading, see Figure 6. EDSS = Kurtzke Expanded Disability Status Scale; MRI = magnetic resonance imaging.
Disease duration
Disease duration from onset of symptoms to death (n = 11) was between 3 and 24 years (Table 5), with a median survival of 18 years. Four patients survived more than two decades. Eight patients died with EDSS 8.5. Median age at death of symptomatic subjects was 68 years. In 3 cases, cause of death was not directly leukodystrophy-related: myocardial infarction in subjects 11 and 23 and pulmonary embolism after an operation in subject 16 (EDSS 3.5–6.5).

Table 5. Disability and survival time in LMNB1-related ADLD

<table>
<thead>
<tr>
<th>EDSS score</th>
<th>No. of subjects</th>
<th>Age at reaching the score (yrs.) mean ± SD [range]</th>
<th>Time from symptom onset (yrs.) mean ± SD [range]</th>
<th>Time between the scores 6 and 8 (yrs.) mean ± SD [range]</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>16</td>
<td>59 ± 8 [45–74]</td>
<td>11 ± 5 [4–19]</td>
<td></td>
</tr>
<tr>
<td>Deatha</td>
<td>11</td>
<td>66 ± 6 [56–75]</td>
<td>17 ± 6 [3–24]</td>
<td></td>
</tr>
</tbody>
</table>

*One asymptomatic subject with an accidental death not included.
ADLD = autosomal dominant leukodystrophy; EDSS = Kurtzke Expanded Disability Status Scale; SD = standard deviation

Pseudoexacerbation
Patients reported heat intolerance and worsening of neurological symptoms during periods of infections or fever (Table 6). Exacerbations included impaired cognition, motor functions, and consciousness that resulted in repeated hospitalizations. Complications were reversible when patients recovered from infection and when body temperature normalized. Subject 5 developed hypothermia during a urinary tract infection and pneumonia. Body temperature was 29.5°C upon admission to the hospital, and he required assisted ventilation. The patient had 3 months of hospitalization, including a period of rehabilitation, and EDSS score changed from 6.5 before hypothermia to 8 afterward.
Table 6. Prevalence of some symptoms and signs in the 22 symptomatic subjects

<table>
<thead>
<tr>
<th>Symptoms and signs</th>
<th>No. of subjects (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autonomic</td>
<td></td>
</tr>
<tr>
<td>Bladder dysfunction and/or obstipation</td>
<td>22 (100)</td>
</tr>
<tr>
<td>Orthostatic hypotension</td>
<td>17 (77)</td>
</tr>
<tr>
<td>Erectile dysfunction as early symptom</td>
<td>4 (40 [of 10 men])</td>
</tr>
<tr>
<td>Pyramidal, including: Lower limbs, upper limbs and pseudobulbar</td>
<td>20 (91)</td>
</tr>
<tr>
<td>Ataxia, including: Spectrum of imbalance of gait, ataxia in upper limbs, truncal ataxia</td>
<td>20 (91)</td>
</tr>
<tr>
<td>Pseudoexacerbations</td>
<td>17 (77)</td>
</tr>
<tr>
<td>Tremor</td>
<td>10 (45)</td>
</tr>
<tr>
<td>Sensory deficits in lower limbs</td>
<td>7 (32)</td>
</tr>
<tr>
<td>Hypothermia</td>
<td>1 (5)</td>
</tr>
</tbody>
</table>

**Ataxia**

Ataxia (Table 6) was present in patients with pyramidal symptoms. Gait unsteadiness, and subsequently difficulty or inability to stand upright without support, with eyes open and closed was observed. Patients with pyramidal signs and impaired sensation in the legs, including decreased vibration sense, had an atactic heel-knee-shin test with eyes open and closed. There was commonly intention tremor in the arms and, in subsequent stages, dysmetria and dysdiadochokinesis. Only 2 patients had overt nystagmus. Bedside examination showed that smooth pursuit was interspersed by saccadic interruptions in several patients.

**Tremor**

Postural tremor of the arms was observed in 10 patients (patients 5, 6, 8, 9, 12, 13, 15, 16, 19, and 21) accompanied by neck tremor in 4. Patient 13 had tremor involving the jaw as well as having an effect on chewing and speech. Primidone abolished the tremor, but the patient did not tolerate the medication.

**Sensory impairment**

Sensory impairment started in the feet (n = 7; patients 6, 9, 12, 13, 19, 20, and 21) and was found in some of the subjects with long disease duration, weakness, spastic paraplegia, and EDSS 6. Subject 6 developed decreased sensation of all modalities that progressed with spinal segments reaching the mid-thoracic level at age 58. This patient had the most severe atrophy of the spinal cord and flaccid paralysis of the legs.

**Cognition**

Early in the course of the clinical disease, patients had normal or mild deficit on Kokmen’s short test of mental status (Kokmen et al., 1987). Detailed neuropsychological testing was not performed. Patients at an advanced disease stage and with pseudobulbar palsy usually had dementia or were unable to perform the test. The severely affected subjects had apraxia, difficulties in speaking, or anarthria (n = 8). Subjects 18 and 23, with EDSS 4 and 6, had a score level above dementia level at >70 years of age.
Neurophysiology

Neurophysiological results are presented in Table 7. Nerve conduction studies and electromyography performed in subjects with EDSS 0 to 7 did not indicate polyneuropathy. Somatosensory evoked potentials with stimulation of the median and tibial nerves showed signs compatible with myelopathy and diffuse involvement of the central nervous system (CNS) in some subjects (EDSS 3–7), but was normal in other subjects with EDSS 0 to 3.5. Magnetic cortical stimulation revealed conduction delay in the motor pathways in 3 subjects, but was normal in 1. Visual evoked potential, performed in 7 subjects, was normal. Sympathetic skin response measured in the hands and feet disclosed decreased sweat reaction (sympathetic) in the feet in 6 subjects and was normal in 9.

Table 7. Neurophysiological examinations

<table>
<thead>
<tr>
<th>Examination</th>
<th>No. of subjects</th>
<th>Result</th>
<th>EDSS score</th>
<th>Subject No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nerve conduction</td>
<td>16</td>
<td>No polyneuropathy</td>
<td>0–7</td>
<td>3–13, 17–19, 21, 23</td>
</tr>
<tr>
<td>Somatosensory evoked potential</td>
<td>16</td>
<td>Normal, N = 6</td>
<td>0–3.5</td>
<td>3, 4, 7, 8, 10, 17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Delayed conduction compatible with myelopathy and diffuse CNS involvement, N = 10</td>
<td>3–7</td>
<td>5, 6, 9, 11, 12, 16–19, 23</td>
</tr>
<tr>
<td>Magnetic cortical stimulation</td>
<td>4</td>
<td>Normal, N = 1</td>
<td>3.5</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Conduction delay in motor pathways, N = 3</td>
<td>3.5–8.0</td>
<td>5, 6, 13</td>
</tr>
<tr>
<td>Visual evoked potential</td>
<td>7</td>
<td>Normal</td>
<td>3–6</td>
<td>6–8, 12, 13, 16, 23</td>
</tr>
<tr>
<td>Sympathetic skin response</td>
<td>15</td>
<td>Normal, N = 9</td>
<td>0–5.5</td>
<td>3, 4, 7, 8, 10–13, 16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Decreased in feet, N = 6</td>
<td>3–5</td>
<td>5, 6, 9, 18, 19, 23</td>
</tr>
</tbody>
</table>

Other Findings

RR interval (parasympathetic) was tested in response to normal and deep breathing (n = 14; subjects 3–10, 12, 13, 18, 19, 21, and 23), during Valsalva maneuver (n = 7; subjects 7, 8, 12, 13, 18, 19, and 23), and during stand-up test (n = 8; subjects 7–10, 12, 13, 18, and 23). The stand-up test implied lying down for 10 minutes, followed by standing up for 1 minute, during which time the RR interval was recorded. Decreased RR interval variability was observed during stand-up test in 3 of 8 subjects (subjects 9, 10, and 13), during Valsalva maneuver in 2 of 7 (subjects 13 and 19), and during deep breathing test in 2 of 14 (subjects 4 and 19). These 14 subjects had autonomic symptoms, except for subjects 3 and 4, who were asymptomatic at the time of the RR interval test.
Radiological findings

**Computed tomography**

CT was pathologic in all 5 investigated subjects, 4 of whom were symptomatic. The principal CT findings were hypodense areas predominantly in the supraventricular cerebral white matter and in the middle cerebellar peduncles (*Figure 5*). On follow-up, the extent of the hypodensities increased and there was progressive loss of white matter.

![Figure 5. Example of computed tomography findings in a 60-year old patient with hypodense areas in the cerebellar peduncles (A) and cerebral white matter (B).](image)

**Magnetic resonance imaging**

MRI revealed pathology in all examinations of all subjects.

*Brain*

T2 signal intensity changes were more prominent than substance loss. Abnormal MRI findings preceded clinical symptoms and signs, with more than a decade in 3 cases. Progress could be observed in MRI in 22 of the 25 repeated examinations, usually, but not always, with a contemporaneous change in EDSS score. The shortest interval at which there was progress in signal intensity changes in the brain was 18 months. *Figure 6* exemplifies the evolution of signal intensity changes over time in the brains of 2 subjects. The course of imaging findings over time is summarized in *Figure 4B*. A certain order in the evolution of the signal intensity changes could be noted. Signal intensity changes started with small T2 hyperintensities under the motor cortex and extended downward through the pyramidal tracts, affecting the cerebral peduncles and the pyramids of the medulla oblongata (*Figure 7*) and also the cerebellar peduncles, even in subjects who still were asymptomatic (*Figure 6*). With increasing disease duration, T2 hyperintensities gradually became more widespread and confluent throughout the cerebral white matter, affecting the cerebral lobes usually in the order frontal–parietal–occipital–temporal. Most subjects over the age of 40 years had widespread signal intensity changes in
the white matter (grade 3). All subjects with motor symptoms had extensive areas of T2 hyperintensities in the cerebral white matter (grade 3).

**Figure 6.** Examples of the evolution and grading of signal intensity changes over time in the brains of 2 subjects in T2-weighted images (fluid attenuation inversion recovery sequences except for grade 3 in subject 6 which is a spin-echo sequence). Changes on brain magnetic resonance imaging and EDSS score are not directly comparable, partly because EDSS also reflects changes in the spinal cord.

Definitions of the radiological grades:
Grade 1: Small T2 hyperintensities under the motor cortex (top arrow) and extending down through the pyramidal tracts, affecting the cerebral peduncles (open arrow) and the pons (bottom arrow).
Grade 2: More widespread abnormalities in the cerebellar peduncles (arrow) and cerebral white matter, extending into the frontal and anterior parietal lobes. The
periventricular white matter is spared. Cerebrospinal fluid (CSF) spaces are minimally larger than in the first examination, but not abnormally wide.

Grade 3: Further progress of white matter changes in the cerebrum and the cerebellar peduncles. The changes extend along the occipital horns. Periventricular white matter (open arrow) is less affected than the surrounding pathological white matter. CSF spaces are slightly enlarged.

Grade 4: Further enlargement of cerebral white matter changes, which have extended into the temporal lobes. Periventricular less-affected rim marked with an open arrow. CSF spaces larger than in the examination in grade 3 and the lateral ventricles and the third ventricle are pathologically wide.

Grade 5: Further progress of white matter changes. The less-affected periventricular rim is still visible, but thinner. CSF spaces are markedly enlarged. EDSS = Kurtzke Expanded Disability Status Scale.

As described previously (Melberg et al., 2006), the periventricular white matter was spared or less affected. On T2-weighted SE images, signal intensity of periventricular white matter was consistently lower than that of more peripheral white matter (Figures 6 and 8-10). The same signal pattern was observed on T2-weighted FLAIR images in most cases (Figures 6 and 9). However, the T2 FLAIR signal intensity of the most severely affected white matter was suppressed in later stages in 12 patients (Figures 8 and 10), making the periventricular less-affected rims less well seen. This relative increase of the periventricular signal intensity on FLAIR images started in the slices above the ventricles. In 4 patients, periventricular signal intensities looked partially even higher than in more peripheral pathological white matter (Figure 10B).

Figure 7. High signal intensity in the pyramids in the medulla oblongata (arrow) is a typical finding, detectable when the entire length of the intracranial pyramidal tract is affected, noted already in grade 1. T2-weighted fluid attenuation inversion recovery (FLAIR) image of an asymptomatic 43-year-old man.
Apparent diffusion coefficient (ADC) values were increased or simulated normal values in affected areas of the cerebral white matter (Figures 9 and 10), but the abnormal area could sometimes look smaller than in morphological T2-weighted images. Pathological areas in cerebellar peduncles did not show ADC changes in 5 of 10 subjects. Periventricular ADC values were first lower than in more peripheral affected white matter (Figure 9C), but they increased during disease progress, resulting in a reverse ADC pattern (Figure 10C), at least partially, in 5 subjects. ADC values were never lower than in normal brain.

No pathological enhancement was noted after contrast medium administration. Starting in the fifth decade, there was progressive loss of white matter leading to widening of ventricles and peripheral CSF spaces. Cortical thickness was relatively well preserved.

Figure 8. Periventricular white matter (open arrows) is typically less hyperintense than more peripheral white matter on T2-weighted spin-echo images (A), but in this severely affected 65-year-old patient (subject 12, EDSS 8.5), this relationship is reversed on T2-weighted fluid attenuation inversion recovery images (B) in which the signal intensity is suppressed in the most severely affected areas (arrows). EDSS = Kurtzke Expanded Disability Status Scale.
Figure 9. Comparison of a T2-weighted spin-echo (SE) image (A), T2-weighted fluid attenuation inversion recovery (FLAIR) image (B), and an (ADC) map (C) in a 46-year-old woman (grade 3, EDSS 1). Slice position just above the lateral ventricles is shown in a coronal T2-weighted SE image (D). Dashed line represents the middle line of the 5-mm-thick slice. Area of pathological signal intensity is the same in the T2-weighted images both with SE and FLAIR sequences. The apparent diffusion coefficient map shows increased diffusion in the same area. Observe nonaffected rims around the lateral ventricles in all images (arrows). EDSS = Kurtzke Expanded Disability Status Scale.

Spinal cord

All measurements obtained from the spinal cord were significantly (>2 standard deviations) smaller than in the normal population (Krabbe et al., 1997). On follow-up examinations, only 3 subjects exhibited a decrease in cross-sectional area or diameter, defined as >8mm$^2$ or >1.5mm difference between two measurements. These cut-off points were estimated based on Bland-Altman plots of the variability between two readings. A 1.5-mm cut-off is also consistent with voxel size. We found a strong linear correlation between age and cross-sectional area at the level of the conus ($R^2 = 0.67$) and slight-to-moderate correlations between age and the other measurements obtained from the
Figure 10. Comparison of a T2-weighted spin-echo (SE) image (A), T2-weighted fluid attenuation inversion recovery (FLAIR) image (B), and an apparent diffusion coefficient (ADC) map (C) in a more advanced case: 51-year-old subject 5 (grade 4, EDSS 6.5). Slice position just above the lateral ventricles is shown in a coronal T2-weighted SE image (D). Dashed line represents the middle line of the 5-mm-thick slice. Total area of pathological signal intensity is the same on the T2-weighted images both with SE and FLAIR sequences, but in the areas with the highest signal intensity on the SE image, the signal has been suppressed on the FLAIR image (open arrows). A less-affected periventricular rim is observed on the SE image (white arrow), but on the FLAIR image that rim looks most hyperintense (white arrow). The same area shows high ADC values (white arrow), but in other pathological areas, diffusion is less pathological or even simulates normal: There is a dark line between the affected and normal brain (so called border effect). EDSS = Kurtzke Expanded Disability Status Scale.

spinal cord ($R^2 = 0.26–0.40$), except for the sagittal diameter at the level of the conus. All subjects, even asymptomatic ones, exhibited abnormal T2 signal in the white matter of the spinal cord. The typical finding, observed even in 1 asymptomatic subject (subject 4), was high white matter signal intensity in the entire spinal cord (Figure 11). Only in 2 subjects was the white matter not totally involved. One of them (subject 3) was asymptomatic when he was
examined at ages 34 and 40 years. In the first examination, only lateral parts of the white matter were hyperintense; however, in the second examination, the entire white matter emitted a high T2 signal intensity. The other subject (subject 16 with EDSS 3.5) only underwent one spinal MRI in which anterior and lateral white matter did not appear totally pathological.

*Figure 11.* T2-weighted spin-echo images of the spinal cord, sagittal (A) and transverse (B) slices of a 63-year-old female (EDSS 3). Pronounced spinal cord atrophy. Increased signal intensity in the whole white matter. In healthy subjects, signal intensity is lower in white matter than in grey matter. Pathological signal intensity in the pyramids in the medulla oblongata is also well observed (arrow). EDSS = Kurtzke Expanded Disability Status Scale.

**Magnetic resonance spectroscopy**

The spectrum of the subject in group 5 had an SNR of 3 and was excluded, together with its associated controls. This subject was also one of the three in the follow-up examinations. The remaining spectra had a high quality with a median FWHM of 0.046 for subjects as well as for controls (range 0.038–0.053 for subjects, 0.038–0.069 for controls) and a median SNR of 14 (range 5–29): 12 in subjects (range 5–26) and in controls 14 (range 6–29). Myo-inositol, total Cho, total Cr, and total NAA had a CRLB < 20 in all examinations, signifying that the “true” metabolite level with 95% certainty lies within ±20% of the estimated value. Glx had a CRLB < 20 in 9 subjects and 22 controls, but in the remaining 8 subjects, CRLB was 20–35.
Representative spectra from one control and one subject with extensive imaging changes are shown in Figure 12. Results from the group comparisons of subjects versus controls are presented in Figure 13. Metabolite concentrations of total Cr, total Cho, total NAA, and Glx using tissue water as a reference were significantly lower in the subjects with extensive imaging changes than in the controls. However, the 2 subjects with minimal or moderate changes in the voxels (MRI grade 1-2) exhibited the same metabolite levels as the controls (Figure 13A).

Figure 12. Example of spectra from one control (a) and one subject with extensive MRI changes (b). The thick line is the fitted spectrum and the thin line beneath is the baseline. Residuals (the total data minus the fit to the data) are shown in the upper part of the image.
Figure 13. Metabolite levels in the first MRS measured using internal water (A), and creatine (B) as a reference. The subjects presenting symptoms and having extensive changes in MR images have consistently approximately 30% lower metabolite levels when measured using water as a reference (A). There are no significant differences between subjects and controls when measured using creatine as a reference (B). Subjects are divided into groups as in Table 2: □ = group 1, ■ = group 2, ■ = group 3, ■ = group 4, ○ = control **P values of paired two-tailed t test, subjects with extensive MRI changes (groups 2–4) vs. average of their respective controls

We found strong linear correlations between all these metabolite levels in the subjects with a Pearson product–moment correlation coefficient ranging from 0.61 to 0.95. When total Cr was used as a reference, no significant difference could be detected between subjects and controls (Figure 13B). There were no high lipid peaks in any of the subjects and only a minority of subjects and controls had lipids and macromolecules with a CRLB <20. Therefore, lipid/macromolecule measurements are not shown in Figure 13. Lactate with CRLB < 20 was not detected in any of the subjects.

In the follow-up examinations, one of the two subjects who showed clinical progression was excluded from the analysis due to low SNR in the MRS. The other individual with clinical progression showed a decrease of 25–30% in the total NAA/total Cr ratio and a decrease of 60% in the myoinositol/total Cr ratio in 5 years.

Positron emission tomography

Absolute glucose metabolism analysed with PVElab in patients 4, 6-9 and 12

Figure 14 presents glucose metabolism in individual ROIs as well as globally in patients and controls. There was a 16% reduction in mean uptake in the cerebellum (p < 0.01), a 15% reduction in the brainstem (p < 0.05) and a 14% reduction in the grey matter globally (p < 0.05). No significant difference in mean uptake was found in any other region. Patient 12, the oldest patient to be investigated, had significantly (> 2 SD) lower glucose metabolism in 5 of 22 ROIs (cerebellum, orbitofrontal cortex, medial and inferior frontal gyrus,
middle and inferior temporal gyrus, posterior cingulate cortex and hypothalamus), and in the grey matter globally. Glucose metabolism in patient 6 (Figure 15), who was clinically most severely affected, with the most pronounced findings on MRI, was significantly lower in 9 ROIs (brainstem, cerebellum, thalami, putamina, caudate nuclei, insular cortex, medial and inferior frontal gyrus, anterior cingulate cortex and ventrolateral prefrontal cortex) as well as in the grey and white matter globally. Patient 9 had significantly lower metabolism in the caudate nuclei and global white matter compared to the controls.

![Figure 14. Glucose metabolism vs age by region of interest in patients 4, 6-9 and 12 (triangles) and healthy controls (dots) as analysed with PVElab.](image-url)
Quantitative analysis in patient 15, using a standard clinical protocol
Patient 15 had approximately 30% or 1.9 SD lower global glucose metabolism compared to normal values, with cerebellum showing the most marked reduction, approximately 45% or 2.2 SD lower than normal values.

Figure 15. FDG-PET images of the most severely affected patient (Patient 6, A-C) compared with a healthy control (D-F). Axial (A, D), coronal (B, E) and sagittal (C, F) images showing decreased glucose metabolism in the patient.
**Semiquantitative glucose metabolism of Patients 4-9 and 12 analysed with CortexID**

Regions of interest with statistically decreased or increased FDG uptake (z-scores < -2 or > 2, respectively) are presented in Table 8. In patients 4, 6, 9 and 12, an apparent increased metabolism was found in parts of the temporal lobes compared to the global metabolism. In patient 5 and 12, a decreased metabolism was seen in the cerebellum.

**Table 8. Regions with low and high glucose metabolism in the semiquantitative analysis with the CortexID software suite**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Areas with z-score &lt; -2</th>
<th>Areas with z-score &gt; 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>None</td>
<td>R precuneus [+2.25]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R parietal superior [+2.56]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R temporal lateral [+2.63]</td>
</tr>
<tr>
<td>5</td>
<td>Cerebellum [-3.59]</td>
<td>R prefrontal lateral [+2.68]</td>
</tr>
<tr>
<td></td>
<td>Pons [-3.29]</td>
<td>R parietal inferior [+2.17]</td>
</tr>
<tr>
<td>6</td>
<td>R prefrontal lateral [-3.01]</td>
<td>L occipital lateral [+2.52]</td>
</tr>
<tr>
<td></td>
<td>R anterior cingulate [-3.00]</td>
<td>L temporal lateral [+2.25]</td>
</tr>
<tr>
<td></td>
<td>L anterior cingulate [-3.48]</td>
<td>R temporal mesial [+2.72]</td>
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<td></td>
<td></td>
<td>L temporal mesial [+2.73]</td>
</tr>
<tr>
<td>7</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>8</td>
<td>L precuneus</td>
<td>None</td>
</tr>
<tr>
<td>9</td>
<td>L Anterior Cingulate [-2.01]</td>
<td>L Occipital Lateral [+2.05]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R Temporal Lateral [+3.95]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L Temporal Lateral [+2.21]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R Temporal Mesial [+2.36]</td>
</tr>
<tr>
<td>12</td>
<td>L Prefrontal Lateral [-2.44]</td>
<td>L Occipital Lateral [+2.44]</td>
</tr>
<tr>
<td></td>
<td>R Posterior Cingulate [-3.08]</td>
<td>L Temporal Mesial [+2.10]</td>
</tr>
<tr>
<td></td>
<td>R Parietal Inferior [-2.34]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cerebellum [-2.10]</td>
<td></td>
</tr>
</tbody>
</table>

z-score = standard score (number of standard deviations from the mean), L = left, R = right.
Marinesco-Sjögren syndrome

Clinical histories

Patients 1 and 2

The medical histories of the twins were very similar. They were born in week 34, weighing 1801 g and 2149 g. Strabismus and a delayed motor development was noticed at six months of age. The first MRI of the brain was performed at 16 months of age. At this age, their length and weight were -1 SD to -2 SD and the head circumference was -2.5 SD. They could sit without support, though somewhat unsteadily, and stand with support, with hyperextended knees. They could not get up to a sitting or standing position independently. They had convergent strabismus and mild vertical nystagmus. They presented with decreased muscle tone in the trunk and extremities and their pincer grip was clumsy. EMG showed myogenic changes both proximally and distally in the extremities. Muscle pathology from the tibialis anterior muscle showed myopathic changes with some necrotic fibers, compatible with myogenic myopathy. At 3 ½ years, they could walk with support and get up to a sitting position independently, but they could not get up from sitting to standing or back without help. Their walk was atactic. The second MRI including both brain and leg musculature was performed at the age of 4 years.

Biopsy from the vastus lateralis muscle was performed during the same anesthesia, and muscle pathology showed slight myogenic myopathy changes similar to the previous biopsy as well as some variation in the fiber diameters. A new finding was dispersed cytoplasmic inclusions, similar to what can be seen in inclusion body myositis. Also, a few rimmed vacuoles and fibers positive for fetal and neonatal myosin (MHCd and MHCn) were found. Electron microscopy revealed foci of cell debris, granular material, and membranous whorls. There was no fatty replacement, nor fibrotic change.

Both twins were operated upon for bilateral cataracts at 4 years of age. At 5 ½ years of age, they attended normal preschool. Their speech was somewhat slurred but was easily understood by friends and family. They were both wheelchair users. Both twins continued their schooling, attending mainstream school until 15 years of age, continuing to and completing upper secondary school. At the time of their last follow-up at the age of 22 years, both were approximately -2 SD in length and weight. They were wheelchair users and needed assistance transferring from the wheelchair to an examination table. They had convergent strabismus, nystagmus, and mild dysarthria. They could not lift up their heads when in supine position, and muscle strength in the arms and legs was generally decreased, being able to extend the knees when lying down but not when sitting up.
**Patient 3**

She was born at term after a normal pregnancy. She learned to walk at 2–2½ years of age and always had difficulties in running. She had surgery for bilateral cataracts at the age of 5 years and strabismus at the ages of 10 and 12 years. She never learned to swim or ride a bike and had difficulties climbing the stairs. She attended normal school and continued to university to get a bachelor’s degree. As she did not enter puberty, hormone replacement therapy was started at the age of 19 years. She was of normal height and weight. She expressed a hypersensitivity to sounds and visual stimuli. She exhibited gaze nystagmus, mild ataxia, especially in heel/shin testing, and some unsteadiness of gait as well as mild muscle weakness in the extremities (MRC 4).

A biopsy of the deltoid muscle was performed, and the histopathology showed muscle fiber caliber variation with both hypertrophic and atrophic fibers and an abundance of rimmed vacuoles on light microscopy. Electron microscopy showed dense perinuclear stranding, suggestive of Marinesco-Sjögren syndrome.

**Radiological findings**

**Magnetic resonance imaging of the brain**

MRI findings in the brain are exemplified in *Figures 16 and 17*. The findings in the twins were similar at each age. At 16 months of age, the vermis was small and cerebellar sulci were prominent with slightly small cerebellar hemispheres. The cerebellar cortex was hyperintense on T2-weighted images. The cerebellar peduncles and pons were slightly small, the latter measuring 15 and 14 mm in the anteroposterior direction (normal width 17–20 mm, Raininko R *et al.*, 1993). The fourth ventricle was wide, and there was an arachnoid cyst in the posterior fossa. In one of the twins, the frontal and interhemispheric subarachnoid space was slightly widened. The signal intensity pattern of the white matter and supratentorial gray matter was normal. At the age of four years, the image was very similar. The anteroposterior width of the pons had increased to 18 and 16 mm (normal width 18–22 mm). At 20 years of age, the cerebellar peduncles and medulla oblongata were slightly thinner than before. The anteroposterior width of the pons was 15 and 14 mm (normal width 20–25 mm). The general impression was that the cerebellum had not grown between the two investigations but rather decreased in size. Liquor spaces, including the cerebellar sulci and the fourth ventricle, were wider and the adenohypophysis was flat.
Figure 16. MRI images of the brain showing progressive cerebellar (skinny arrow) and pontine (fat arrow) atrophy in Patient 1, and atrophy in the superior cerebellum in Patient 3 (open arrow). T1-weighted sagittal images.

In Patient 3, examined at 27 years of age, the vermis and superior part of the cerebellar hemispheres were small with increased T2 SI in the cortex. The inferior cerebellum was essentially normal in volume and exhibited normal SIs. The anteroposterior diameter of the pons was normal (22 mm). No pathological findings were found supratentorially. This patient also had a flat adenohypophysis.

Figure 17. MRI images of the brain, showing T2 hyperintense cerebellar cortex in Patients 1 and 2 (arrows) and high signal intensity in the superior half of the cerebellar cortex in Patient 3 (open arrow). Axial (A, B) and coronal (C-F) T2-weighted images with SE (A-D) and FLAIR sequences (E, F). Note also the progression of cerebellar atrophy, best seen in C and D.

Magnetic resonance imaging of muscles

The muscle MRI findings are exemplified in Figures 18 and 19 and the measured T1 and T2 SI ratios are presented in Figure 20. At 4 years of age, the thigh musculature, especially the vastus medialis, vastus lateralis and adductor magnus were atrophic and presented increased muscle/fat SI ratios. By visual assessment, no pathological changes were seen in the calf musculature, though a slightly increased muscle/fat SI ratio was found in the m gastrocnemius and m soleus in the patients compared to the controls. There was no difference in the ratio in the m tibialis anterior.
Figure 18. MRI images of lower leg muscles of Patient 1 at the ages of 4 and 20 years and a young control, showing progressive atrophy and fatty infiltration. am = adductor magnus; bf = biceps femoris; edl = extensor digitorum longus; gcl = gastrocnemius, lateral head; gem = gastrocnemius, medial head; gr = gracilis; pl = peroneus (fibularis) longus; rf = rectus femoris; sa = sartorius; sm = semimembranosus; sol = soleus; ta = tibialis anterior; tp = tibialis posterior; vi = vastus intermedius; vl = vastus lateralis; vm = vastus medialis

At 20 years of age, the muscles of the thighs and calves were very atrophic with extensive areas of high T1 and T2 signal, which had markedly progressed compared to the previous examination. The hamstrings, m adductor magnus, m adductor longus, and the m tibialis anterior were somewhat less affected than the other muscles. This examination also included the upper limbs, where the musculature was generally atrophic with increased SI especially in the m triceps. There was also atrophy of and signal increase in the erector spinae and gluteal musculature. No increased signal was seen on the fat-suppressed T2-weighted images.

The musculature of Patient 3, investigated at 27 years of age, though severely affected, was not as pathological as in the twins. The m gluteus maximus was severely affected. In the thighs, there was some increase in the T1 and T2 muscle/fat SI ratios in most muscles, with the most affected muscles being the m gracilis, m biceps femoris, and m sartorius. The normalized T1 and T2 SI of the m vastus lateralis were also increased, to a level similar to that seen in the twins at 4 years of age. In the calves, the most severely affected muscles were the m peroneus longus and the lateral head of the m gastrocnemius. There was a relative sparing of the m tibialis anterior. In the upper limbs, the m triceps, biceps, and brachialis were smaller than usual. The muscle/fat
T1 and T2 SI ratio was slightly increased in the m triceps but normal in the m biceps and m deltoideus. No increased signal was seen on the fat-suppressed T2-weighted images.

Figure 19. MRI findings in musculature in adults showing extensive atrophy and fatty infiltration in Patient 1 and affection of the m peroneus longus and the lateral head of the gastrocnemius in Patient 3. al = adductor longus; am = adductor magnus; bb = biceps brachii; bf = biceps femoris; edl = extensor digitorum longus; gel = gastrocnemius, lateral head; gem = gastrocnemius, medial head; gm = gluteus maximus; gr = gracilis; pl = peroneus (fibularis) longus; rf = rectus femoris; sa = sartorius; sol = soleus; ta = tibialis anterior; tb = triceps brachii; tp = tibialis posterior; tfl = tensor fasciae latae; vl = vastus lateralis
Figure 20. Signal intensity ratios of the muscles in the patients. ■ = Patient 1 at 4 years of age, □ = Patient 1 at 20 years of age, ▲ = Patient 2 at 4 years of age, △ = Patient 2 at 20 years of age, ○ = Patient 3 at 27 years of age. The vertical lines represent the mean values, and the dashed lines the limits for ±2 standard deviations of the measurements in the controls.
Discussion

*LMNB1*-related ADLD

Paper II presents the first clinical and radiological long-term follow-up in subjects with an *LMNB1* gene duplication leading to *LMNB1*-related ADLD. The study comprised 23 individuals, which is extensive considering the rareness of the disease. Importantly, the recruitment of asymptomatic individuals preceding clinical onset made it possible to follow the entire course of the disease.

In line with previous reports, symptoms started in the fifth to sixth decade (Melberg *et al*., 2006; Schuster *et al*., 2011; Meijer *et al*., 2008), and in the great majority of patients, autonomic symptoms preceded or occurred together with gait problems and dyscoordination (Eldridge *et al*., 1984; Melberg *et al*., 2006; Coffeen *et al*., 2000; Schuster *et al*., 2011; Meijer *et al*., 2008; Brussino *et al*., 2009; Dos Santos *et al*., 2012; Fogel *et al*., 2012b; Giorgio *et al*., 2013). In the original family reported by Eldridge et al, onset was reported from the fourth to fifth decade (Eldridge *et al*., 1984). Two of our patients reported gait difficulties preceding bladder control problems. To our knowledge, there are only two reports of *LMNB1*-related ADLD that describe motor signs as the first symptoms: in a Serbian family (Potic *et al*., 2013) and in a Brazilian patient (Giorgio *et al*., 2013). In an Italian ADLD family, with increased expression of lamin B1 due to a genomic deletion leading to an enhancer adoption, pyramidal signs were the presenting feature (Brussino *et al*., 2010; Giorgio *et al*., 2015).

We observed that motor deficits progressed in the same order in both families. Initial symptoms and signs of the lower spinal segments were spastic paraplegia developing to tetraplegia and eventual pseudobulbar palsy in the seventh to eighth decade. Tremor, previously reported in *LMNB1*-related ADLD (Brown *et al*., 1987), was present in less than half of the patients and caused major complaint in 1 patient. We found no signs of polyneuropathy, confirming earlier reports (Eldridge *et al*., 1984; Schwankhaus *et al*., 1994; Melberg *et al*., 2006; Coffeen *et al*., 2000; Sundblom *et al*., 2009). A new observation in this follow-up study is that sensory impairment started in the distal part of the legs and ascended over time to the thoracic level, consistent with myelopathy, and development of flaccid paralysis of the lower limbs.

Pseudoexacerbation in association with exposure to heat, fever, and infections was a frequent complication in patients from both families. This is an
important aspect associated with this type of leukodystrophy, previously mentioned only in one German and one Swedish patient (Schuster et al., 2011). Interestingly, similar pseudoexacerbations are observed in MS, which shares several clinical features with LMNB1-related ADLD. Life-threatening hypothermia, previously reported in a Canadian patient having LMNB1-related ADLD (Meijer et al., 2008), occurred in one of our patients.

Detailed neuropsychological testing has not been performed in a larger series of patients with LMNB1-related ADLD. Below-average test results or cognitive deficits have been described in a 47-year-old German patient (Dos Santos et al., 2012) and a 55-year-old Canadian patient (Laforce et al., 2013), respectively. In one Italian family, mild cognitive impairment and dementia were reported (Brussino et al., 2009) while patients from another family presented impaired selective attention and progressive decline in abstract thinking abilities (Terlizzi et al., 2016). Eldridge’s original report concluded that intellectual acuity is often well maintained (Eldridge et al., 1984). In our study, patients did not complain of cognitive impairment or they only showed mild cognitive deficit early in the disease course. However, patients with advanced disease and pseudobulbar palsy usually had dementia.

Median survival time after onset of symptoms was 18 years, which is comparable to earlier reports (Eldridge et al., 1984; Meijer et al., 2008).

MRI revealed pathological findings in the brain and spinal cord in all subjects and a regular progression of brain changes, in both symptomatic and asymptomatic subjects. In the brain, subjects first present T2 hyperintensities in the white matter underlying the motor cortex. These were present in even our youngest subject (age 29), so we cannot say for certain at what age they can first be observed. T2 hyperintensities progress down through the pyramidal tracts, and these findings can precede symptoms of the disease by up to 16 years. The extent of the changes in some of the asymptomatic subjects is astonishing. Many patients with very large pathological areas manifested relatively mild symptoms. One explanation may be the lack of inflammation and preservation of neurons (Melberg et al., 2006). Increased ADC values in the white matter, consistent with increased diffusivity, and the drop in signal intensity in some T2-weighted FLAIR images are also in agreement with an increase in free water content. The spinal cord was thin and displayed pathological white matter signal intensity in all 14 imaged subjects. We only found radiological progress in 4 of 9 subjects examined twice, but progress may be difficult to demonstrate in a very thin spinal cord because of the limited spatial resolution of the MRI. In addition, the area is technically difficult and artifactual signal intensity changes may hamper morphological evaluation.

The involvement of the corticospinal tracts and cerebellum, observed radiologically and pathologically, explains the clinical pyramidal signs and ataxia (Melberg et al., 2006). The cause of autonomic symptoms and signs, such as urinary bladder dysfunction, has been suggested to be a manifestation of frontal lobe disease (Schwankhaus et al., 1994). A distal lesion of sympathetic
noradrenergic neurons has also been suggested (Brown et al., 1987), as well as isolated noradrenergic failure (Guaraldi et al., 2011; Terlizzi et al., 2016). Spinal cord involvement in LMNB1-related ADLD has been reported previously (Sundblom et al., 2009). The follow-up study presented in Paper II gives a strong indication that spinal cord involvement is clinically significant in this type of leukodystrophy and may also, at least partly, explain the autonomic dysfunction. Previous histopathological studies have verified involvement of the spinal cord in a patient already at 3 years after onset of symptoms (Sundblom et al., 2009) and more-severe changes in a patient with longer disease duration (Schwankhaus et al., 1994). In our study, clinical assessment, radiological findings, and neurophysiological examinations indicate myelopathy. The most severe case with the most advanced atrophy of the spinal cord had ascending sensory impairment over time, reaching the mid-thoracic level, clearly indicating clinical myelopathy. Signs from the legs, in this case, included flaccid paralysis and weak-to-abolished tendon reflexes, although there were no signs of spasticity. Early autonomic dysfunction, such as disturbed bladder and bowel control as well as erectile dysfunction, is likely, in part, a result of myelopathy. A similar conclusion has recently been presented in an autosomal recessive leukodystrophy, adult polyglucosan body disease, where bladder dysfunction is an initial symptom, likely correlating to spinal cord atrophy (Mochel et al., 2012).

In the MRS-study, presented in Paper I, we found low concentrations of all investigated metabolites in all subjects with extensive changes on MR imaging (11/13), when they were quantified using tissue water as a reference (Figure 13A). We also found linear correlations between all these metabolite concentrations. We conclude that the decreased quantities are mostly due to increased water content in the tissues, “diluting” all metabolites to a similar degree. When calculating the millimolar concentrations of metabolites, using an internal water reference, an assumption is made that the water content in the tissue is constant, but if this is not true, all millimolar metabolite levels of a specific subject will be “off” by an unknown scaling-factor, which varies from subject to subject. High water content is also in agreement with the high signal intensity the subjects exhibit in the white matter on T2W MR images, as exemplified in Figure 2. However, there can be also factors other than high water concentration increasing signal intensity of T2-weighted images. There was no obvious difference in metabolite levels comparing the subgroups of clinically affected subjects. In subjects with minimal imaging findings and no symptoms, the concentrations in millimolar did not differ from those in healthy controls. Using total Cr as a reference, no statistically significant difference can be seen between subjects and controls (Figure 13B).

Spectroscopic signs of active demyelination in the form of increased choline, lactate, or lipids were not found in our study. Our findings seem to be in agreement with the histopathological findings of vacuolated myelin (Melberg et al., 2006; Coffeen et al., 2000), where myelin was partly replaced with
fluid, although axons and glia appeared normal. The relative levels of the metabolites have not changed, suggesting that the relative distribution of the glial and neuronal cellular compartments is not altered.

One of the subjects investigated twice did show a slight decrease in total NAA/total Cr over time, suggesting that some neurodegeneration might have occurred, even if it was not seen in the group comparison between subjects and controls. This subject had a rather high, though not pathological, myo-inositol/total Cr ratio in the first investigation, suggestive of glial reaction, while the second value was more normal. However, as a group, the subjects showed no clear spectroscopic signs of gliosis nor apparent loss of neuronal function, as can be seen in Figure 13B.

In the FDG-PET study, presented in Paper III, we found a reduced mean glucose metabolism in the cerebellum, brainstem and global grey matter in patients with LMNB1-related ADLD. The findings in the cerebellum are consistent with the clinical cerebellar symptoms as well as the MRI finding of early and pronounced changes in the middle cerebellar peduncles. In addition, they are compatible with the earlier reported histopathological findings of a reduction of Purkinje cells and increase of Bergmann astroglial cells in the cerebellar cortex in this disease (Melberg et al., 2006).

In the patients analysed with a semiquantitative method, 4 of 7 had an increased glucose metabolism in the temporal lobes compared to global metabolism. However, since none of the patients showed an increased metabolism in the quantitative analysis, we interpret these findings as ‘pseudo-increases’ related to a globally reduced metabolism. This is of importance for interpreting semiquantitative values in relation to choice of reference region. The relatively higher temporal metabolism is in line with the evolution of MRI findings since the white matter of the temporal lobes is the last white matter area to be affected.

Previously, 10 studies or case reports describing FDG-PET findings in a total of 21 patients with leukodystrophies have been published (Volkow et al., 1987; Iinuma et al., 1989; Bakheet et al., 1999; Salmon et al., 1999; Sawaishi et al., 1999; Al-Essa et al., 2000; Johannsen et al., 2001; Renard et al., 2011; Kim et al., 2012; Salsano et al., 2014), 17 of the 21 patients had X-ALD. The methodology and reference regions or reference data used varies across the publications, and there are no obvious metabolic patterns. Four out of six publications on X-ALD reported a low FDG uptake in the temporal lobes, including the only multi-subject study (Salsano et al., 2014). Low uptake in the cerebellum is described in four reports, one on Krabbe’s disease, the infantile form of which typically affects the cerebellum (Al Essa et al., 2000; Abdelhalim et al., 2014), and three on X-ALD, including one on a patient with cerebello-brainstem dominant form of ALD (Kim et al., 2012).

Hypothesising on the cause of the abnormalities of the glucose metabolism seen in X-ALD, Salsano et al. (2014) suggested an afferent dysfunction, due
to white matter lesions affecting axons giving excitatory input. Their hypothesis was supported by the lack of cortical atrophy in affected locales on MRI. Postmortem studies of X-ALD have also not shown any atrophy (Schaumburg et al., 1975). Kim et al. found decreased FDG metabolism diffusely in the cortex, most prominently bifrontally and in the cerebellum in a patient with X-ALD (Kim et al., 2012). On MRI, T2 hyperintensities were seen along the pyramidal tracts and in the cerebellum. They also interpreted this mismatch in findings as a result of an altered connectivity in the axons connecting cortical cerebral and cerebellar areas, decreasing the feedback and feed-forward connections between the areas.

In histopathological studies of patients with LMNB1-related ADLD, the cerebral cortex has been normal (Coffeen et al., 2000; Melberg et al., 2006). Therefore, similar to the theory proposed by Salsano et al. (2014), we assume that the globally decreased glucose metabolism in the cerebral cortex is secondary to myelin vacuolation and loss, consistent with the extensive T2 hyperintensities seen on MRI, causing decreased functioning of the axons connecting different cortical areas and deep grey matter structures. However, given the level of atrophy presented by patients with LMNB1-related ADLD in advanced stages, exemplified in Figure 8, it is plausible that some neuronal loss occurs in the cortex, and the decreased global metabolism might be due to a combination of axonal dysfunction and neuronal loss.

Differential diagnosis

A few disorders may resemble the clinical and/or radiological findings observed in LMNB1-related ADLD. Patients with adult polyglucosan body disease and bladder dysfunction, spinal cord atrophy, and axonal neuropathy develop cerebral white matter lesions, but these are different from the changes observed in this study (Mochel et al., 2012). Another type of leukodystrophy that may present with autonomic dysfunction, ataxia, and pyramidal signs is autosomal dominant adult-onset Alexander disease. However, these patients usually have palatal myoclonus and focal enhancing MRI changes in the cerebellum, brain stem, and cervical cord (Schwankhaus et al., 1995; Martidis et al., 1999; Okamoto et al., 2002; Farina et al., 2008; Pareyson et al., 2008). A clinical presentation with autonomic symptoms including orthostatic hypotension may resemble MSA/Shy-Drager syndrome (Schatz, 1996; Tada et al., 2007; Jellinger, 2014). Furthermore, spastic paraplegia and autonomic dysfunction may suggest a spinal cord compression/lesion. In fact, MRI of the spinal cord was the first radiological investigation performed in 2 of our patients. T2 hyperintensity in the middle cerebellar peduncles is a relatively rare finding, and besides in LMNB1-related ADLD, it is characteristic in OPCA/MSA-C (Savoiardo et al., 1990) and in symptomatic elderly men with a fragile-X permutation (Brunberg et al., 2002). But the finding can be present in a number of diseases (Okamoto et al., 2003). OPCA is a neurodegenerative syndrome representing several distinct entities, characterized by prominent
cerebellar and extrapyramidal signs, dysarthria, and dysphagia (Berciano et al., 2006). Patients with fragile-X permutation present cognitive decline and ataxia from approximately 60 years of age and a family history consistent with X-linked inheritance. Further differential diagnosis between LMNB1-related ADLD and other leukodystrophies and leukencephalopathies has been presented in articles by Melberg et al. (2006) and Sundblom et al. (2009)

The MRI findings of the brain in LMNB1-related ADLD are highly specific. Patients exhibiting extensive T2 hyperintense areas in the lobar supratentorial white matter with spared or less severely affected periventricular white matter and T2 hyperintensities in the cerebellar peduncles and along the corticospinal tract can be suspected of having the disease, even without clinical information. Together with the clinical history, the imaging findings presented herein and well demonstrated on T2-weighted SE images can be considered as pathognomonic for LMNB1-related ADLD. Early MRI changes can be easier to detect on FLAIR images, but advanced cases are more difficult to interpret on them. Recently a paper was published describing a 4 members of a family with an autosomal dominant leukodystrophy presenting symptoms of migraine, mild cognitive decline and early onset dementia (Corlobé et al., 2015). MRI images presented in the publication are very similar to the findings we usually see in LMNB1-related ADLD. However, no gene defect was found in LMNB1, NOTCH3, GFAP or CSF1R, raising the possibility of this being a hitherto undescribed adult-onset leukodystrophy. The authors performed WES on the patients and one healthy relative, but given the small size of the family no single gene variant segregating with the disease could be found.

A number of leukodystrophies have been studied with MRS (Farina et al., 2000; Brockmann et al., 2003a, b; Janson et al., 2006; van der Voorn et al., 2006; Wang et al., 2007; Bizzi et al., 2008; Sohn et al., 2010). Sometimes results are presented as concentrations in millimolar measured against tissue water and sometimes as quotes against Cr. Bizzi et al. (2008) presented 70 patients classified into three groups: hypomyelinating diseases, usually exhibiting increased levels of creatine and myoinositol; demyelinating diseases with increased choline/creatine, decreased NAA/creatine ratios and presence of lactate; and diseases of white matter rarefaction. Van der Voorn et al. (2006) used a similar classification, reporting on 42 patients.

Comparing our findings of normal metabolite levels, using creatine as a reference, no other of the reported diseases has similar findings. NAA/Cr is most often reduced, signalling the loss of functioning neurons. Lactate is often present indicating an anaerobic metabolism. Adult-onset GLD can have normal total NAA/total Cr but shows an increase in total Cho/total Cr (Farina et al., 2000; Brockmann et al., 2003b; Wang et al., 2007), while in hypomyelinating diseases, such as PMD, creatine is increased (van der Voorn et al., 2006; Bizzi et al., 2008). Our findings of low metabolite levels quantified using tissue water as a reference is somewhat similar to VWM, however, in
VWM, total NAA is relatively more decreased than total Cr, giving a lower total NAA/total Cr ratio (van der Voorn et al., 2006). Patients with VWM also present a more severe clinical picture than patients with LMNB1-related ADLD. Taking all this together, our findings of relatively spared metabolite levels measured using creatine as a reference are consistent with the relatively slow clinical course in patients with LMNB1-related ADLD present, compared with most other leukodystrophies.

In the longitudinal study presented in Paper II, we found MRI to be the method that first reveals abnormalities in subjects affected by LMNB1-related ADLD displaying T2 hyperintensities in the brain and the spinal cord white matter long before onset of symptoms. These findings can thus be considered as early markers of the disease in subjects with the pertinent family history. Given that the radiological changes precede the clinical symptoms, they could, theoretically, be incidental in individuals imaged for other reasons. T2 hyperintensities along the corticospinal tracts are nonspecific, although not very common in the pyramids in other diseases. In LMNB1-related ADLD, they are accompanied by early and typical changes in the cerebellar peduncles. The first MRI changes in LMNB1-related ADLD restricted to the upper corticospinal tracts may be identical to those in adult-onset Krabbe disease (Wang et al., 2007). However, at that stage, patients with adult-onset Krabbe disease present with clinical symptoms such as paraparesis, in contrast to patients with LMNB1-related ADLD who likely would be asymptomatic.

Marinesco-Sjögren syndrome

In different materials, a SIL1 mutation has been found in approximately 50 to 66% of individuals with the clinical triad of cataracts, cerebellar atrophy, and myopathy (Senderek et al., 2005; Annesi et al., 2007; Anttonen et al., 2008; Eriguchi et al., 2008; Takahata et al., 2010; Krieger et al., 2013). Traditionally, intellectual disability has been considered as being a part of MSS (Marinesco et al., 1931; Sjögren T, 1950; Müller et al., 1962; Superneau et al., 1985), but one-third of the patients with a SIL1 mutation have normal intellectual capacity (Krieger et al., 2013). According to previous studies, no clear genotype-phenotype correlations can be seen in MSS due to SIL1 mutations, with subjects with the same genotype exhibiting a variation in the phenotypes (Anttonen, Lehesjoki, 2006). Whole-exome screening has found SIL1 mutations in patients presenting with cerebellar ataxia but without cataracts or muscular dystrophy (Noreau et al., 2015). Patient 3 has a genotype common in Finnish MSS patients, with published cases presenting varying degrees of psychomotor delay and moderate to severe ataxia (Anttonen et al., 2005; Ezgu et al., 2013). In a screening of healthy Finnish controls, Anttonen et al. found one carrier of the 506_509dupAAGA mutation among 96 individuals. We therefore consider the unusual, mild phenotype of Patient 3, a homozygote for
a 506_509dupAAGA mutation, to be an extension of the clinical spectrum of the disease. Patients 1 and 2 who had a more severe disease were compound heterozygotes for two distinct mutations in the \textit{SIL1} gene (c.506_509dupAAGA and c.645 + 2T > C).

The distinction between cerebellar hypoplasia and atrophy is sometimes unclear, and it can be impossible to determine from a single imaging study. Even with multiple studies, the follow-up time might not be sufficiently long to show any change due to the slow processes. As a case in point, the typical findings in MSS have sometimes been described as cerebellar hypoplasia (Tachi \textit{et al.}, 1991; McLaughlin \textit{et al.}, 1996; Georgy \textit{et al.}, 1998). Subjectively, no change was seen over a span of 7 years in a patient examined at the ages of 56 and 63 years, nor in a patient imaged at 15 months and 7 years (Georgy \textit{et al.}, 1998; Harting \textit{et al.}, 2004). In the present study, having a longer follow-up time of 19 years, we saw a progressive atrophy of the cerebellum together with a measurable decrease in the diameter of the pons.

Hyperintense cerebellar cortex in MSS patients was first reported in 2004 (Harting \textit{et al.}, 2004). It seems to be a relatively constant finding in the disease, although the cerebellar cortex appeared normal in a patient imaged before his first birthday (Al-Maawali \textit{et al.}, 2012). The finding is not mentioned in a publication from 2003 describing cerebellar atrophy in MSS but it can be difficult to discern on sagittal T2-weighted images, like the ones in that publication (Reinhold \textit{et al.}, 2003). However, the examination of the patients also included coronal T2 FLAIR images, where the hyperintense cortex is most easily visible. Therefore, it is possible that the finding is not obligatory or that the patients suffered from some phenotypically similar, but not genetically identical disease. It has been hypothesized that the increased signal is due to gliotic changes as histopathology has shown an increase of astroglial cells in the cerebellar cortex (Mahloudji \textit{et al.}, 1972; Poretti \textit{et al.}, 2015). The finding can also be seen, though sometimes inconsistently, in a number of other diseases including infantile neuroaxonal dystrophy (INAD) (Steinlin \textit{et al.}, 1998; Poretti \textit{et al.}, 2008), DOOR syndrome (Deafness, Onycho-Osteodystrophy and mental Retardation, Nomura \textit{et al.}, 2009), congenital disorders of glycosylation type 1a (Melberg \textit{et al.}, 2011; Feraco \textit{et al.}, 2012), Christianson syndrome (Bosemani, 2014), Complex I deficiency (Wolf \textit{et al.}, 2003; Kevelam \textit{et al.}, 2013), late-onset GM2 gangliosidosis, and spinocebellar ataxia type 5 and type 7 (Al-Maawali \textit{et al.}, 2012). In an article by Scaglia \textit{et al.} (2005), a T2 hyperintense cerebellar cortex can be seen on a figure depicting a patient with an unspecified, nonspecific mitochondrial encephalopathy, though the finding is not mentioned in the accompanying text. In the present study, the twins exhibited the common finding of generally increased T2 signal in the cerebellar cortex. The third, less affected patient, had high T2 signal only in the superior half of the cerebellar cortex with the inferior part appearing normal, both in signal intensity and volume. This specific pattern has not been
described before. The smaller extent of the pathological changes on MRI seems to correlate with the milder phenotype of the patient.

Paper IV is the first study presenting radiological findings in the muscles of children with MSS. Muscle MRI has been found to be a useful tool for diagnosing and evaluating disease progression in congenital myopathies and muscular dystrophies, with some diseases exhibiting specific patterns of muscular involvement (Mercuri et al., 2005; Quijano-Roy et al., 2012; Bönne-mann et al., 2014; Kana et al., 2014; North et al., 2014; Quijano-Roy, Carlier, 2014). Comprehensive tables can be found in the publication by Quijano-Roy et al. (2012). Mahjneh et al. (2006) describes the CT findings in the muscles of 10 MSS patients aged 20–51 years. Similar to our findings, the gluteal musculature was severely affected, as well as the thigh musculature. In agreement with our findings, the m biceps femoris was somewhat less affected. Also, the mm gastrocnemii, m soleus, and m peroneus were more affected than the m tibialis anterior and the m extensor digitorum longus. In their patients, the consistently most severely affected muscles were the gluteals, m sartorius, m gracilis, and the m rectus femoris. We also found the m sartorius to be the most severely affected muscle in adulthood; however, in childhood, the most severely affected muscles were the quadriceps. In Patient 3, four of the muscles we investigated had a muscle / fat SI ratio > 0.5 (Fig.3): m gluteus maximus, m sartorius, m peroneus longus, and the lateral head of the m gastrocnemius. As there was no increased signal in the fat-suppressed images, we interpret the increased T1 and T2 signal as being due to fatty infiltration and not edema or inflammation. This is consistent with histopathological findings of fatty replacement (Superneau et al., 1987; Komiyama et al., 1989; Sasaki et al., 1996). An advantage of looking at the pattern of muscle involvement in the less affected Patient 3 is that she was ambulatory and active to a normal degree. Therefore, any muscle affection is more likely to be due to the disease specific mechanisms rather than general disuse. Of course, given the large phenotypic variation, one could suspect that the more severely affected patients have more widespread myopathic changes per se. Lastly, looking at the early involvement of the gastrocnemii muscles and the differential involvement of the lateral head of the gastrocnemii in Patient 3, one could consider performing muscle biopsies from these instead of from the relatively spared tibialis anterior muscle.
Final remarks

On the naming of diseases

In these 4 papers, presenting studies on 2 different diseases, 3 different “naming schemes” are exemplified. Eponymous naming, as in “Marinesco-Sjögren syndrome” has the advantage of being reasonably compact and memorable. Of course it tells us nothing of the nature of the specific disease, especially if you know nothing about the persons after whom it is named. This, however makes it rather robust in the sense that the name can be kept unchanged as specific knowledge about a disease evolves.

“Adult-onset autosomal dominant leukodystrophy with autonomic symptoms”, though unwieldy, is more descriptive, in contrast with eponymous naming. This can be especially helpful in the naming of rare diseases, as the diagnosis can be made given information on symptomatology and inheritance. In fact, when the paper by Melberg et al. was published in 2006, there were published cases from 3 different families. This number has since steadily grown, and there are now approximately 20 known families world-wide. Descriptive naming of course requires that the clinical picture is specific enough, otherwise the name is likely to describe a variety of diseases with differing prognoses and pathophysiologies. Conversely, if the name becomes too long, there is a tendency to abbreviate it. Many papers reporting on this disease call it adult-onset ADLD, or even just ADLD. This shortened name becomes less descriptive and one might even argue ‘erroneous’ as there are a number of adult-onset autosomal dominant leukodystrophies with differing clinical pictures and genetic bases (see Table 1).

Adding the associated gene to the name of the disease as in LMNB1-related ADLD gives another level of specificity. With genetic sequence analysis rapidly becoming faster and more accessible, making the diagnosis can become exceedingly easy. This type of naming scheme is also useful in many rare diseases as they often are monogenetic. However, the connection between genotype and phenotype is not always direct or simple, and reducing the diagnosis and taxonomy of disease to finding genetic markers can possibly hide these complexities. Knowing the exact type of mutation (if several disease-causing mutations are known) can be more exact, but is not always sufficient to give an accurate prognosis. For example, as mentioned previously, patients sharing the genotype of Patient 3 in Paper IV (homozygosity for the c.506_509dupAAGA mutation in the SIL1-gene) have been found to have variable phenotypes ranging from the common MSS phenotype with intellectual disability to the very mild phenotype of Patient 3. Several factors may play a role in the phenotypical expression of a specific gene defect, including environmental factors, the complete genetic makeup of an individual and epigenetics (Zoghbi and Beaudet, 2016).
Radiology in the age of genetic diagnosis
Kevelam et al. (2016), present a recent overview of the history of leukodystrophy diagnosis and describes the usefulness of whole-exome sequencing in their differential diagnosis. They acknowledge that, since its advent in the 1980s, MRI has proven to be a very useful tool for the diagnosis of leukoencephalopathies. Mainly due to its high sensitivity to white matter abnormalities. As the imaging patterns of more and more diseases have been described, MRI has also been found to be a reasonably specific tool for making a differential diagnosis (van der Knaap et al., 1991; van der Knaap and Valk, 2005b). MRI has also been useful in classifying novel disorders, based on their distinct MRI patterns (Hanefeld et al., 1993; Schiffmann et al., 1994; van der Knaap et al., 1995; van der Knaap et al., 1999a, b; van der Knaap et al., 2003; Wolf et al., 2005; Timmons et al., 2006; Bernard et al., 2010; Steenweg et al., 2012b, c). Later, the causative genes for some of these diseases have been found, validating this MRI based taxonomy (Leegwater et al., 2001a, b; Zara et al., 2006; Scheper et al., 2007; Steenweg et al., 2012a).

This role of MRI may be waning. Gene-sequencing is hard to match for sensitivity and specificity in finding the differential diagnosis of monogenetic diseases and as it becomes cheaper and faster it becomes ever more cost-effective. Radiology, with its ‘multi-dimensionality’ does however have some advantages over gene testing. As exemplified in this thesis, the same gene defect can give rise to different clinical pictures mirrored in a variation of MRI findings which can add information when trying to give a prognosis. Imaging is still important when grouping patients before gene identification. Kevelam et al. (2016), reports a success rate of 80-90% for gene identification by WES in small, homogenous patient groups, compared to success rates of 42% for mixed leukodystrophy cases (Vanderver et al., 2016) and 16-53% for unselected patients (de Ligt et al., 2012; Srivastava et al., 2014; Taylor et al., 2014).

The multi-dimensionality of imaging also includes the time dimension. Of course, the traditional golden standard in nosology is pathology, but, as Berciano et al. (2006) remarks: “Pathological classification (…) is not particularly helpful to clinicians who, not unnaturally, prefer to make some sort of working diagnosis before the autopsy results are available.” And one is usually reluctant to perform serial brain biopsies in slowly progressive neurological diseases. Thus, imaging plays an important role in showing the evolution of diseases over time and, as a consequence, in the evaluation of the efficacy of therapies, as they become available.
Conclusions

Paper I
The absolute concentrations of all metabolites measured in millimolar were decreased in the lesion areas, which seems to be attributable to increased water content in the tissues, coherent with the MR imaging findings of hyperintense areas on T2-weighted images and histopathologically shown vacuolation in earlier reports. The relative quantities of the metabolites appeared preserved, suggesting a preservation of the relative distribution of the neuronal and glial cellular compartments, which is in accordance with the fact that patients with extensive MRI changes may have relatively mild clinical symptoms.

Paper II
*LMNB1*-related ADLD is a slowly progressive disease affecting both the brain and the spinal cord. Radiological findings in the brain and spinal cord may precede the clinical symptoms by more than a decade and subjects having radiological abnormalities develop a clinical disease. The early symptoms, including autonomic dysfunction and pyramidal signs, indicate myelopathy. Clinical symptoms of myelopathy with autonomic dysfunction in combination with characteristic radiological findings enable diagnosis of *LMNB1*-related ADLD.

Paper III
In patients with *LMNB1*-related ADLD, mean glucose metabolism is decreased in the cerebellum, brainstem and global grey matter. The cerebellar findings are consistent with clinical cerebellar symptoms, MRI findings and histopathology. The global reduction of glucose metabolism most likely depends on a combination of cortical afferent dysfunction and neuronal loss.

General conclusions regarding *LMNB1*-related ADLD
MRI imaging is sensitive in finding pathology in patients with *LMNB1*-related ADLD and shows changes already before onset of clinical symptoms. In more advanced disease, the changes are also very specific. However, even patients with widespread MRI changes can have relatively mild symptomatology and present only slight disturbances in metabolic examinations. This is compatible with relatively intact axons, even as myelin impairment is widespread.
Paper IV

The phenotypical spectrum of S/1L1-positive Marinesco-Sjögren syndrome includes mild forms with no intellectual disabilities and only very slight motor disabilities. With a 19-year-long radiological follow-up, a slow progressive atrophic process in the cerebellum and brainstem in MSS could be demonstrated. MRI of the musculature shows early involvement of the quadriceps and gastrocnemii but not the tibialis anterior, progressing to widespread atrophy in the back, upper and lower limbs at the age of 20 years. In the mildest phenotype, the most severely affected muscles were the gluteus maximus, sartorius, peroneus longus, and the lateral head of the gastrocnemius.
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