Chapter 1
Introduction

Myelin, myelination and myelin disorders:
from development to pathology to repair
**Genetic white matter disorders: definition, clinical features and diagnosis**

Genetic white matter disorders are inherited encephalopathies characterized by selective involvement of the white matter of the central nervous system. Most are progressive. The first report of a presumably familial white matter disorder dates back to passage between 18\textsuperscript{th} and 19\textsuperscript{th} century, when Pelizaeus and Merzbacher separately described the familial occurrence of a chronic progressive ‘diffuse sclerosis’ (as opposed to the already recognized ‘multiple sclerosis’), an encephalopathy with lack of myelin and sclerotic hardening of the cerebral white matter (Pelizaeus, 1899; Merzbacher, 1910). In 1913 Schilder reported an adult patient with ‘encephalitis periaxialis diffusa’, the sibling of whom presumably had the same disease (Schilder, 1913). Subsequent descriptions of similar cases prompted the coin of the term “leukodystrophy” (*leuko*, white and *dystrophy*, lack of growth, thus degeneration) (Bielschowsky & Henneberg, 1928). Hence, in the original concept of the word, leukodystrophies are hereditary, progressive diseases characterized by white matter degeneration. Disorders such as metachromatic leukodystrophy (MLD) and globoid cell leukodystrophy (GLD, also called Krabbe disease) owe their names to the staining reactions or unusual cell morphology seen on histopathological specimens. Analysis of stored substrates within white matter subsequently helped to unravel the underlying enzymatic defect in several instances. However, many genetic diseases that affect the white matter remain pathologically and pathogenetically poorly characterized with an unknown biochemical or molecular basis. Because many of these disorders demonstrate a failure in myelination (hypomyelination) rather than a loss of previously deposited myelin (demyelination), the term leukodystrophy *sensu stricto* has been considered too narrow, and recently juxtaposed by the broader term “leukoencephalopathy”.

This latter term would also cover those hereditary white matter disorders in which myelin is normally deposited and no myelin degeneration occurs, and those that do not progress and may even improve over time. Noteworthy, many also designate genetic and acquired defects causing secondary myelin degeneration as leukoencephalopathies (Kaye, 2001; Kohlschütter & Eichler, 2011). The choice for one term or the other and the opinion on which disorders are included under these headings are strongly coloured by convention, and the two words are employed by many interchangeably.

A scientific definition should reflect the knowledge of its time. An important question is how we should define genetic white matter disorders at this time. The term leukodystrophy in its intentional meaning is not applicable to all genetic white matter disorders, because many are not progressive or characterized by loss of
myelin. This term has survived because of its popularity, but has lost its precision in the light of the current knowledge. Many use the term leukencephalopathy to define all disorders that affect exclusively or predominantly the white matter of the brain. This choice is linguistically correct, but ignores the need to have terms to distinguish genetic from acquired disorders, and progressive from static conditions. Some destine the word leukodystrophy specifically to inherited white matter disorders. This choice may also be considered infelicitous, because it causes further confusion. The term would define both degenerative disorders, as in its original and still widely perceived meaning, and static conditions such as white matter disorders due to chromosomal abnormalities. Obviously, a consensus redefinition of these terms is urgently needed.

Genetic white matter disorders affect patients of all ages, from the prenatal period to senescence (Osterman et al., 2012). Incidence has been estimated to broadly range from 1:5,000 to 2:100,000 live births (Heim et al., 1997; Bonkowsky et al., 2010), but depending on definitions it is higher approaching 1:1000-2000 live births. As advances in imaging and molecular diagnostic techniques have enormously broadened the spectrum of these disorders, these numbers probably still represent an underestimation.

The clinical manifestations of white matter disorders are relatively heterogeneous and also depend on the age at onset (table 1). An insidious onset of symptom may complicate the diagnosis in the early disease stages, requiring a high index of suspicion and careful probing of the family history. Especially in infancy and early childhood, it can be difficult to distinguish whether a condition is static or truly progressive. With the exception of early infantile-onset cases, patients may exhibit normal early development before losing skills as the white matter disease evolves. A subtle cognitive decline may be the earliest sign preceding the loss of previously acquired motor skills. However, the onset may be or seem abrupt and triggered by traumas or infections, as in vanishing white matter (VWM), Alexander disease (AxD) and sometimes X-linked adrenoleukodystrophy (X-ALD). The bilateral and symmetric involvement of the cerebral white matter manifests typically as a spastic quadripareisis with cerebellar ataxia. More focal clinical signs may also appear, including dysphagia, dysarthria, extrapyramidal movement disorders, optic atrophy or seizures. Cognitive functions may remain relatively spared until late in the disease course. Although there is significant overlap in symptomatology, certain clinical signs can be clues to particular conditions. Peripheral neuropathy often coexists in Krabbe disease, MLD, Waardenburg-Hirschsprung’s syndrome and, in some instances, Pelizaeus-Merzbacher disease (PMD) and Pelizaeus-Merzbacher-
like diseases, and mitochondrial and peroxisomal disorders. Megalencephaly is characteristic for megalencephalic leukoencephalopathy with subcortical cysts (MLC), Canavan disease and AxD, and some peroxisomal disorders and organic acidurias. Characteristic accompanying non-neurological findings such as bone, tooth or skin anomalies or adrenal insufficiency, are also valuable diagnostic clues. Notably, as stated above, not all genetic white matter disorders are progressive. For example, children with the mild form of leukoencephalopathy with thalamus and brainstem involvement and high lactate (LTBL) or with MLC due to autosomal dominant GLIALCAM mutations improve clinically and radiologically over time.

The clinical manifestations in adults are broadly similar to those in children, reflecting the dysfunction of cerebral and/or spinal cord white matter (table 1) (Ahmed et al., 2014). Typically, adult patients present with progressive cognitive or neuropsychiatric difficulties, often associated with progressive lower limb spasticity or pseudobulbar signs. Cognitive dysfunction is generally characterized by impaired attention and memory, psychomotor slowing, defective visuospatial and executive skills, and personality changes typical of subcortical dementia. Notably, cortical symptoms such as aphasia, neglect, or apraxia are usually lacking. As in children, the clinical presentation may be precipitated by trauma, infection, or endogenous or exogenous toxins. Patients who present acutely are commonly misdiagnosed, since a genetic disorder is often not considered in adults. Additionally, the clinical picture may be confounded by concurrent substance abuse, psychiatric disease, underlying systemic conditions or incorrect preceding diagnostic labels such as vascular leukoaraiosis or multiple sclerosis. In the late stages, adult patients as well as children may progress into a state of akinetic mutism, stupor, coma, and ultimately death.

Recognition of leukodystrophies has been revolutionized by magnetic resonance imaging (MRI) complemented by ancillary advanced imaging technologies. The high sensitivity of MRI allows distinction of hypomyelinating leukodystrophies from other pathologies and recognition of myelination delay at follow up. Additionally, MRI may reveal disease-specific patterns of signal abnormalities and characteristic features that strongly address the clinical diagnosis (van der Knaap et al., 1991 & 1999; Schiffmann & van der Knaap, 2009; Steenweg et al., 2010; van der Voorn et al., 2006) (fig. 1). Examples are the contrast enhancement in X-ALD and AxD, or the presence of cysts in MLC and RNASET2-deficient leukoencephalopathy. Calcifications suggest Aicardi-Goutières syndrome (AGS) or RNASET2-deficient leukoencephalopathy, while massive rarefaction of the cerebral white matter is virtually pathognomonic of VWM. Indeed, the validity of MRI pattern recognition in
defining white matter disorders is confirmed beyond doubt by the increasing number of novel entities identified using this approach (van der Knaap et al., 1999).

The diagnostic work-up of genetic white matter disorders is further aided by neurophysiological testing and functional investigation of organs other than the nervous system. When revealing symmetric involvement of long spinal tracts and peripheral nerves, evoked potentials and nerve conduction velocities may be helpful in differentiating leukodystrophies from acquired demyelinating disorders. Absence of multimodal evoked potentials central responses addresses towards hypomyelination syndromes and, in particular, PMD. Thorough ophthalmological evaluation may reveal a cataract, as in cerebrotendinous xanthomatosis, some
hypomyelination syndromes (hypomyelination with congenital cataract), and congenital variants of VWM. Abnormal endocrine findings may suggest X-ALD (adrenal cortical insufficiency), PolR-III defects (hypogonadotropic hypogonadism), AARS2-related leukoencephalopathy or VWM (premature ovarian failure).

The definite diagnosis of a genetic white matter disorder is achieved through specific laboratory and genetic tests (table 1). An omnicomprehensive laboratory protocol does not exist. Age at onset, pattern of neurological involvement and MRI findings dictate the diagnostic work-up and allow for a successful clinical diagnosis (Kohlschütter & Eichler, 2011). However, despite the advances in MRI diagnostics and explosion of gene discovery over the last decade, a definite etiological diagnosis is still not reached in up to 20% of the patients (Kohlschutter et al., 2010; Perlman & Mar, 2012).
<table>
<thead>
<tr>
<th>Disease</th>
<th>Mutated genes and inheritance</th>
<th>Distinctive clinical features</th>
<th>Distinctive MRI findings</th>
<th>Diagnostic tests</th>
<th>Pathophysiology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult-onset autosomal dominant leukodystrophy</td>
<td>LMNB1 (AD)</td>
<td>Cerebellar, pyramidal, and autonomic symptoms</td>
<td>Cerebral WM and middle cerebellar peduncles involvement</td>
<td>MG</td>
<td>Inhibited transcription of myelin genes, including PLP1; altered Oct-1 nuclear localisation (oxidative stress)</td>
</tr>
<tr>
<td>Aicardi-Goutières syndrome</td>
<td>TREX1 (AR, AD de novo), RNASEH2A-C, SAMHD1, ADAR and IFIH1 (AR)</td>
<td>Neonatal form: microcephaly, spasticity, dystonia, marked developmental delay and regression; later-onset variants with milder phenotype</td>
<td>Extensive calcifications, cerebral hypoplasia, white matter signal abnormalities</td>
<td>Pleiocytosis (CSF), α-interferon (CSF), MG</td>
<td>Dysfunctional DNA repair</td>
</tr>
<tr>
<td>Alexander disease</td>
<td>GFAP (AD, most often de novo)</td>
<td>Megalencephaly, psychomotor regression, ataxia and seizures; adults: bulbar symptoms</td>
<td>Diffuse white matter abnormalities with anterior predominance; in adults mainly brain stem abnormalities</td>
<td>MG</td>
<td>Toxic aggregates of GFAP; defective proteasome degradation/auto-phagy; glutamate-mediated excitotoxicity</td>
</tr>
<tr>
<td>Allan–Herndon–Dudley syndrome</td>
<td>SLC16A2/MCT8 (XR)</td>
<td>Severe retardation in males</td>
<td>Severely delayed myelination</td>
<td>Thyroid function, MG</td>
<td>Decreased T3 uptake in neurons and glia</td>
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<tr>
<td>Adult-onset leukoencephalopathy with neuroaxonal spheroids and pigmented glia</td>
<td>CSF1R (AD)</td>
<td>Behavioral problems and dementia</td>
<td>Cerebral and callosal atrophy, frontal white matter involvement, no enhancement, possible lacunar- or stroke-like lesions</td>
<td>MG</td>
<td>Poorly understood</td>
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<td>Canavan disease</td>
<td>ASPA (AR)</td>
<td>Megalencephaly, hypotonia, psychomotor regression</td>
<td>Diffuse subcortical signal abnormalities; involvement of thalami and globi pallidi; increased NAA (MRS)</td>
<td>N-acetylaspartate (urine), MG</td>
<td>Poorly understood, possibly osmoregulation defect</td>
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<tr>
<td>Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy</td>
<td>NOTCH3 (AD)</td>
<td>Strokes, migraines</td>
<td>Vascular pattern, often involvement anterior temporal lobes</td>
<td>MG</td>
<td>Dysregulated Notch signalling leading to progressive vascular smooth muscle cell loss</td>
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<td>Cerebrotendinous xanthomatosis</td>
<td>CYP27A1 (AR)</td>
<td>Xanthomas, cataracts</td>
<td>Cerebellar white matter lesions, calcifications</td>
<td>Cholesterol (plasma), MG</td>
<td>Disrupted cholesterol homeostasis, cholestanol-induced apoptosis</td>
</tr>
<tr>
<td>Cockayne syndrome</td>
<td>ERCC6 and ERCC8 (AR)</td>
<td>Dwarfism, typical facies, peripheral neuropathy, cataract, pigmentary retinopathy, sensorineural hearing loss, lipoatrophy photosensitivity,</td>
<td>Hypomyelination, putaminal calcifications, vermian atrophy</td>
<td>DNA repair test (fibroblasts), MG</td>
<td>Impaired transcription-coupled subpathway of nucleotide excision DNA repair; altered redox balance; dysfunctional mitochondria</td>
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<tr>
<td>Cystic leukoencephalopathy without megalencephaly</td>
<td>RNASET2 (AR)</td>
<td>Non-progressive course</td>
<td>Anterior temporal cysts, calcifications, congenital CMV-like pattern</td>
<td>MG</td>
<td>Ribosomal RNA degradation defect with lysosomal storage; myelin pathology poorly understood</td>
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<td>Folate receptor defect</td>
<td>FOLR1 (AR)</td>
<td>Movement disorders, epilepsy; treatable</td>
<td>Hypomyelination</td>
<td>Folates (CSF), MG</td>
<td>Altered protein and lipid myelin composition</td>
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<tr>
<td>Fucosidosis</td>
<td>FUCA1 (AR)</td>
<td>Coarse facial features, dysostosis multiplex</td>
<td>Hypomyelination</td>
<td>α-L-fucosidase activity (leukocytes), MG</td>
<td>Accumulation of fucose-containing glycolipids and oligosaccharides; developmentally-regulated oligodendrocyte loss; selective Purkinje cell apoptotic loss</td>
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<tr>
<td>Giant axonal neuropathy type I</td>
<td>GAN (AR)</td>
<td>Curly hair, peripheral neuropathy</td>
<td>Features reminiscent of Alexander disease</td>
<td>MG</td>
<td>Impaired intermediate filament protein degradation, myelin pathology poorly understood</td>
</tr>
<tr>
<td>Globoid cell leukodystrophy</td>
<td>GALC (AR), PSAP (AR)</td>
<td>Developmental regression, spasticity, opisthotonus; late onset milder</td>
<td>Posterior peri-ventricular; streaky tigroid appearance; optic nerve swelling; involvement pyramidal tracts only (adults)</td>
<td>Galactocerebrosidase activity (leukocytes), MG</td>
<td>Psychosine cytotoxicity to oligodendroglia</td>
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<td>Glycine leukoencephalopathy and variants</td>
<td><em>AMT, GLDC, GCSH, LIAS, GLRX5</em> and <em>BOLA3</em> (AR)</td>
<td>Variable phenotype, from life-threatening neonatal to transient or late-onset forms</td>
<td>White matter vacuolation</td>
<td>Glycine (plasma versus CSF), MG</td>
<td>Bioenergetic and oxidative stress (increased lipid peroxidation, NMDAR-mediated glutathione reduction); myelin pathology poorly understood</td>
</tr>
<tr>
<td>Hypomyelination with atrophy of the basal ganglia and cerebellum</td>
<td><em>TUBB4A</em> (mostly de novo; rarely affected sibs of somatic mosaic parent)</td>
<td>Movement disorders, additionally encephalopathy of variable severity</td>
<td>Hypomyelination, characteristic anatomic pattern, vermian and putaminal atrophy</td>
<td>MG</td>
<td>Poorly understood</td>
</tr>
<tr>
<td>Hypomyelination and congenital cataract</td>
<td><em>FAM126A</em> (AR)</td>
<td>Cataracts and possibly peripheral neuropathy</td>
<td>Hypomyelination</td>
<td>MG</td>
<td>Poorly understood</td>
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<tr>
<td>Leukoencephalopathy with brainstem and spinal cord involvement and high lactate</td>
<td><em>DARS2</em> (AR)</td>
<td>In childhood to early adulthood cerebellar ataxia and spasticity; usually no cognitive impairment</td>
<td>Brainstem and spinal cord involvement; elevated lactate (MRS)</td>
<td>MG</td>
<td>Reduced catalytic activity, protein expression or dimerization; myelin pathology poorly understood</td>
</tr>
<tr>
<td>Cerebroretinal microangiopathy with calcifications and cysts (Labrune's disease)</td>
<td><em>CTC1, NDP</em> (AR)</td>
<td>Retinopathy</td>
<td>White matter cysts, calcifications</td>
<td>MG</td>
<td>Telomere DNA replication defect, myelin pathology poorly understood</td>
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<td>Leukoencephalopathy with metaphyseal chondrodysplasia</td>
<td>XR</td>
<td>Optic atrophy; broad knees and wrists, no contractures</td>
<td>Hypomyelination</td>
<td>Bone x-ray</td>
<td>Oligopenia</td>
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<td>Leukoencephalopathy with polycystic osteodysplasia (Nasu–Hakola disease)</td>
<td>TYROBP/DAP12 (AR), TREM2 (AR)</td>
<td>Pathologic fractures, rapidly progressing presenile dementia</td>
<td>Frontally accentuated atrophy, basal nuclei calcifications</td>
<td>Bone x-ray; MG</td>
<td>Aberrant regulation of microglial phagocytosis, oligodendropathy</td>
</tr>
<tr>
<td>Leukoencephalopathy with thalamus and brainstem involvement and high lactate</td>
<td>EARS2 (AR)</td>
<td>Later improvement</td>
<td>Thalamic and mesencephalic involvement, aplasia splenium corpus callosum; elevated lactate (MRS)</td>
<td>MG</td>
<td>Poorly understood</td>
</tr>
<tr>
<td>Megalencephalic leukoencephalopathy with subcortical cysts</td>
<td>MCL1 (AR) or GLIALCAM (AD, AR)</td>
<td>Megalencephaly, slowly progressive ataxia and spasticity, seizures; later improvement for the AD variant</td>
<td>Subcortical cysts in temporal poles and frontoparietal regions</td>
<td>MG</td>
<td>Ion-and-water homeostasis defect</td>
</tr>
<tr>
<td>Metachromatic leukodystrophy</td>
<td>ARSA (AR), PSAP (AR), SUMF1 (AR)</td>
<td>Behavioral changes, pyramidal signs, ataxia, peripheral neuropathy</td>
<td>Diffuse white matter abnormalities; streaky tigroid appearance; sparing of U-fibers</td>
<td>Arylsulfatase A activity (leukocytes), sulfatides (urine), MG</td>
<td>Accumulation of sulfatides within lipid membranes; cytotoxicity to oligodendroglia</td>
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<tr>
<td>Mitochondrial disorders</td>
<td>Nuclear and mitochondrial DNA genes</td>
<td>Large heterogeneous group of disorders, frequently multi-organ symptoms</td>
<td>Diffuse white matter involvement, possibly cavitations or cysts; elevated lactate (MRS)</td>
<td>Lactate (plasma, urine, CSF), respiratory chain activity (muscle, fibroblasts), MG</td>
<td>oxidative stress, ATP reduction, Ca(^{2+}) overload</td>
</tr>
<tr>
<td>Oculodentodigital dysplasia</td>
<td>GJA1 (AD, AR)</td>
<td>Typical craniofacial and skeletal anomalies, ocular anomalies, heart arrhythmias</td>
<td>Hypomyelination</td>
<td>MG</td>
<td>Abnormal posttranslational processing and localisation; diminished cell-cell coupling (heart and eye); myelin pathology poorly understood</td>
</tr>
<tr>
<td>Organic acidurias</td>
<td>Different genes</td>
<td>Large heterogeneous group of disorders, macrocephaly possible</td>
<td>White matter cystic degeneration, oedema</td>
<td>Urinary organic acids, MG</td>
<td>“Toxic” effect on oligodendrocytes and myelin</td>
</tr>
<tr>
<td>Ovarioleukodystrophy</td>
<td>AARS2</td>
<td>Frontal lobe dysfunction, pyramidal tract involvement; ovarian failure</td>
<td>Involvement of left-right connections and descending tracts; cerebellar atrophy</td>
<td>MG</td>
<td>Poorly understood</td>
</tr>
<tr>
<td>Peripheral neuropathy, central hypomyelination, Waardenburg and Hirschsprung syndrome</td>
<td>SOX10 (AD, affected sibs of germinal mosaic parents)</td>
<td>Sensorineural hearing loss, pigmentary abnormalities, Hirschsprung disease, neurologic symptoms</td>
<td>Hypomyelination</td>
<td>MG</td>
<td>Dysregulated developmental myelination</td>
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<tr>
<td>Pelizaeus–Merzbacher disease</td>
<td>PLP1 (XR)</td>
<td>Infantile onset in majority; nystagmus, impaired vision, ataxia, seizures</td>
<td>Symmetric confluent white matter signal abnormalities</td>
<td>MG</td>
<td>Poorly formed myelin; unfolded protein response</td>
</tr>
<tr>
<td>Pelizaeus–Merzbacher-like disease type I</td>
<td>GJA12/GJC2/Cx47 (AR)</td>
<td>Indistinguishable from PMD</td>
<td>Symmetric confluent abnormalities</td>
<td>MG</td>
<td>Poorly understood</td>
</tr>
<tr>
<td>Pelizaeus–Merzbacher-like disease type II</td>
<td>AIMP1 (AR)</td>
<td>Congenital to early infantile onset, early microcephaly, progressive course, kyphoscoliosis</td>
<td>Hypomyelination, atrophy; N-acetylaspartate (MRS)</td>
<td>MG</td>
<td>Poorly understood</td>
</tr>
<tr>
<td>Pol III-related leukodystrophies</td>
<td>POLR3A, POLR3B (AR)</td>
<td>Tooth anomalies, retarded puberty</td>
<td>Hypomyelination</td>
<td>MG</td>
<td>Oligodendrocyte loss</td>
</tr>
<tr>
<td>Sialic acid storage disease</td>
<td>SLC17A5 (AR)</td>
<td>Clinically intermediate between severe neonatal storage disease and the more benign Salla disease</td>
<td>Hypomyelination</td>
<td>Free sialic acid (urine, CSF), MG</td>
<td>Altered vesicular transport of N-acetyl-aspartylglutamate, reduced aspartate and glutamate transport into synaptic vesicles; myelin pathology poorly understood</td>
</tr>
<tr>
<td>Trichothiodystrophy</td>
<td>ERCC2/XPD, ERCC3/XPB</td>
<td>Skin, hair and nail abnormalities, photosensitivity</td>
<td>Hypomyelination</td>
<td>MG</td>
<td>Deregulation of thyroid hormone target genes</td>
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</table>
### Table 1 (cont). Typical clinical, MRI, diagnostic and pathophysiological features of a selection of genetic white matter disorders

<table>
<thead>
<tr>
<th>Disease</th>
<th>Mutated genes and inheritance</th>
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<tr>
<td>Vanishing white matter</td>
<td><em>EIF2B1</em>-5 (AR)</td>
<td>Rapid deterioration following minor head trauma and febrile illness; premature ovarian failure</td>
<td>Progressive white matter cystic rarefaction and cavitation</td>
<td>MG</td>
<td>Abnormal unfolded protein response</td>
</tr>
<tr>
<td>X-linked Adrenoleukodystrophy</td>
<td><em>ABCD1</em> (XR)</td>
<td>Cerebral: behavioral changes, motor regression, acute progression. Myeloneuropathy: chronic progressive spastic paraparesis Adrenal: failure</td>
<td>Cerebral: predominantly posterior periventricular, contrast enhancement. Myeloneuropathy: corticospinal tract involvement</td>
<td>Very long chain fatty acids (plasma); MG</td>
<td>Peroxisomal metabolic dysfunction; inflammation, lipoxidative stress, deficient ALDP function in microglia</td>
</tr>
<tr>
<td>Zellweger syndrome and Zellweger-like disorders</td>
<td>Different <em>PEX</em> genes (AR), <em>ACOX1</em> (AR)</td>
<td>Typical facies, visual and hearing loss, liver disease</td>
<td>Diffuse or restricted white matter involvement, polymicrogyria</td>
<td>Very long chain fatty acids and other peroxisomal metabolites (plasma); MG</td>
<td>Peroxisomal metabolic dysfunction, mitochondrial dysfunction, oxidative stress, protein misfolding, aberrant cell signalling, inflammation</td>
</tr>
</tbody>
</table>

AD, autosomal dominant; AR, autosomal recessive; XR, X-linked recessive; MG: molecular genetic analysis; CSF: cerebrospinal fluid; GFAP: glial fibrillary acidic protein; MRS: magnetic resonance spectroscopy; CMV: cytomegalovirus; NMDAR: N-methyl-D-aspartate receptor; ATP: adenosine triphosphate; ALDP: adrenoleukodystrophy protein (ABCD1).
Organization and structure of the brain white matter

The white matter of the brain, so called because of its gleaming blanching aspect on macroscopic examination, is composed of myelinated axons, glia cells (myelinating oligodendrocytes and oligodendrocyte progenitor cells [OPCs], NG2-glia, astrocytes and microglia) and blood vessels. The main component of white matter is the myelin sheath. Primordial myelin sheaths appeared in protostomes, lower bilateralia with centralized nervous system. Across evolution, the myelin sheath has evolved into an extended and modified plasma membrane wrapped around the axons that originates from and is part of a specific glia cell type, the oligodendrocyte (Geren & Raskind, 1953). Myelin is organized as multiple, spiral compacted layers (fig. 2). The non-compacted adaxonal (innermost) myelin layer is connected to the cell body through cytoplasmic channels containing cytoskeleton, vesicles and outposts of endoplasmic reticulum (ER) and Golgi apparatus that are necessary for myelin maintenance and turnover (Perkins et al., 2008). Each oligodendrocyte can myelinate as many as 40 or more separate axons, but provides myelin for only a segment of any given axon. The periodic interruptions where axons are left uncovered are the nodes of Ranvier, highly specialized posts of ions interchange and critical to the proper functioning of both axons and myelin (Quarles et al., 2006; Arancibia-Carcamo & Attwell, 2014).

Myelin

Myelin acts as a high resistance electrical insulator that facilitates conduction (Waxman & Bangalore, 2004). In myelinated axons, the excitable axonal membrane is exposed to the extracellular space only at the nodes of Ranvier, where the process of myelination ensures clustering of voltage-gated sodium channels. Because of the high resistance of the myelin sheath, membrane excitement at a node of Ranvier results in outward flow of the locally generated circuit and depolarization of the axonal membrane at the next node. The low capacitance of the myelin sheath implies that little energy is needed to depolarize the remaining internodal membrane, resulting in an increased speed of circuit spreading. This mode of impulse propagation is known as saltatory conduction. In myelinated fibres conduction velocity is proportional to the axonal diameter. If axons of projecting pathways were not myelinated, the human spinal cord would need to be as large as a big tree trunk and the optic nerves 0.75 meter in diameter.
in order to maintain equivalent conduction velocities. Therefore, myelin has the evolutionary advantage of facilitating conduction while preserving space and energy (Waxman & Bangalore, 2004; Nave & Werner, 2014).

Myelin plays a role in modulating the caliber and supporting the long-term structural integrity and viability of the axon it enwraps (Nave, 2010a & 2010b; Nave & Werner, 2014). Radial growth of axons is dependent on both myelination and organization and composition of the axonal cytoskeleton (mediated by the local accumulation and phosphorylation of neurofilaments), which is itself influenced by myelin cues (Brady et al., 1999; Rao et al., 2003, Sanchez et al., 2000). Axons in the brain of shiverer (Brady et al., 1999) and jimpy mice (Robain, 1977; Rosenfeld & Freidrich, 1983) respectively lacking the major CNS structural protein myelin basic protein (MBP) and mutant in the proteolipoprotein (PLP), are thinner than wild-type axons. Notably, some myelin constituents are not per se essential for

Figure 2. Myelin sheath ultrastructure. Transverse section of a myelinated axon from the optic nerve of an adult rat. The course of the flattened oligodendrocytic process beginning at the outer tongue (T) and ending at the internal mesaxon (Mi) can be traced. The major dens line (D) alternates with the paler intraperiod line (I). Note that where adjacent sheaths come together an intraperiod line is formed between them, so that an uninterrupted line is formed between them. The axon contains microtubules and neurofilaments. Reproduced from Peters & Vaughn, 1970.
myelination, but are required for axonal integrity. Mice lacking 2':3'-cyclic nucleotide 3'-phosphodisterase (CNP), another major protein concentrated in specific regions of the myelin sheaths associated with the oligodendrocytic cytoplasm, myelinate normally but later exhibit axonal swelling and neurodegeneration (Lappe-Siefke et al., 2003; Edgar et al., 2009). In Plp1-null mice, oligodendrocytes assemble compact myelin that has only ultrastructural abnormalities and allows a normal motor development. However, secondary focal blockage of axonal transport ensues leading to axonal swellings and Wallerian degeneration of the distal axons (Griffiths et al., 1998; Rosenbluth et al., 2006).

It has been suggested that myelin also provides essential trophic support to axons (Nave, 2010a & 2010b; Hirrlinger & Nave, 2014). This hypothesis stems from different observations, including the similarity between axonal pathology in Plp1-null mice and mitochondrial disorders (Ferreirinha et al., 2004; Tarrade et al., 2006), the higher risk of degeneration of thinner compared to thicker axons (thinner axons are ensheathed by thinner myelin) (Griffiths et al., 1998; Edgar et al., 2009), the presence of ATPases along the entire internodal membrane (where the majority of axonal mitochondria are located, but the myelin sheath creates a diffusion barrier to free metabolic exchange) (McGrail et al., 1991; Young et al., 2008), and the apparent contradiction of relatively short half-lived glycolytic enzymes being conveyed by slow anterograd axonal transport (Brady & Lasek, 1981; Oblinger et al., 1988; Yuan et al., 1999). On these bases, it has been speculated that myelinating glia support energy generation in axons by delivering glycolysis products (lactate, pyruvate and its derivatives) for mitochondria in long fiber tracts (Nave, 2010b). Indeed, monocarboxylate transporters for glycolytic breakdown products exist on oligodendrocytes and axons, and neuronal activity can be maintained by lactate as only energy source. Supporting this model, in conditional Cox10 mutant mice, in which mitochondrial respiration is selectively inactivated in myelinating cells, oligodendrocytes survive by aerobic glycolysis and release lactate to the axonal compartment (Funfschilling et al., 2012). Oligodendrocyte-derived lactate is then rapidly metabolized within white matter tracts in vivo. These findings suggest metabolic coupling as a novel physiologic aspect of axon-glia interaction. In this perspective, reduced oligodendroglial trophic support could promote neurodegeneration in many conditions, including different genetic white matter disorders (Hirrlinger & Nave, 2014).

Oligodendrocytes
Developmental myelination, the process by which myelin is first deposited around axons, starts in the human spinal cord at 12 weeks gestation, and in telencephalon around the 14th week. Myelin can be detected in the basal nuclei starting from 20 weeks, in the pre- and postcentral gyrus and optic radiation at 35 weeks, and in the acoustic radiation at 40 weeks. It then proceeds in a caudal-to-rostral and dorsal-to-ventral fashion in the brain white matter during the first two years of life (fig. 3), continuing through the fourth decade in the intra-cortical connections (Yakovlev & Lecours, 1967; van der Knaap & Valk, 2005; Lebel et al., 2008). There is, however, increased evidence that myelination occurs throughout the entire life, either to replace lost oligodendrocytes and myelin or to myelinate previously unmyelinated axons (Bartzokis et al., 2012).

The generation of myelin involves a tightly regulated and finely tuned pathway of OPC specification, proliferation, migration and differentiation culminating, given the appropriate environmental cues, in myelination of appropriate receptive axons (fig. 4). This process is regulated by the interplay of intrinsic and extrinsic cues, these latter impacting on the development of the oligodendrocyte lineage by signalling through intracellular pathways on transcription factors (Emery, 2010; Mitew et al., 2014). Many of these regulatory mechanisms shape different transitions in oligodendrocyte development, and are also reactivated in the post-natal brain for repair purposes (Fancy et al., 2011). Of note, the vast knowledge on oligodendrocyte development and myelination derives from studies of transgenic mice either (constitutively or conditionally) lacking or overexpressing given oligodendrocyte-, myelin- or myelination-related molecules, raising the issue of inter-species differences (Bradl & Lassmann, 2010). The many more oligodendrocytes needed to myelinate a huge amount of axons in the large white matter of the human brain and the much longer and complex developmental process support the idea that human oligodendrocytes may be generated for a longer time period and possibly from more progenitor sources than in the far smaller rodent brain. However, several lines of evidence indicate that a similar molecular machinery operates in humans as in rodents (Jakovcevski et al., 2009).

During development, OPCs appear in the forebrain in sequential waves. At early stages, they predominantly arise from ventral regions in the neural tube under the influence of extracellular signalling molecules as sonic hedgehog (SHH) (Orentas et al., 1999). The initial specification of the oligodendrocytic lineage is reliant on the basic helix-loop-helix (bHLH) transcription factor Olig2, the expression of which is induced (via Notch-1/Smootherened signalling) by SHH (Pringle et al., 1996; Lu et al., 2000). However, at later developmental stages ventrally-derived cells are
Figure 3. Development of myelin in the human brain. Parasagittal sections stained using the Weil method show development of myelin in the human brain at 28 (A) and 40 weeks gestational age (B), at 4 months (C) and at 2 years (D). Reproduced from Flechsig, 1920.

largely replaced by dorsally-derived OPCs (Vallstedt et al., 2005; Kessaris et al., 2006), which specify under the influence of the Wnt/β-catenin and bone morphogenic protein (BMP) pathways (Kasai et al., 2005; See & Grinspan, 2009). These OPCs give rise to the majority of adult oligodendrocytes in the mouse forebrain. Notably, SHH-independent pathways also participate to dorsal oligodendrocyte specification. The fibroblast growth factor (FGF)-2, for example, increases Olig2 expression and OPC specification via (MAPK, ERK1/ERK2-mediated) suppression of SMAD signalling, the main effector of dorsal BMP blockade of oligodendrocytes (Chandran et al., 2003; Bilican et al., 2008; Furusho et al., 2011). Other transcription factors at play during this phase contribute either to refine the dorso-ventral domains of the neural tube (NKX2.2, PAX6 [Vallstedt et al., 2005]) or to support oligodendrocyte specification (Ascl1/Mash1 [Parras et al., 2007; Sugimori et al., 2008; Nakatani et al., 2013], SOX10 [Wang et al., 2014]).
The spinal cord witnesses a similar sequence of molecular events, but OPC generation remains heavily skewed towards ventral origins.

Regulation of OPC proliferation and migration ensures that adequate OPC numbers reach the final site of myelination. OPC proliferation occurs not only during development, but also in the adult CNS and upon myelin loss, when developmental pathways are at least in part recapitulated (Kang et al., 2010; Clarke et al., 2012). Most extracellular signals promoting OPC proliferation also promote survival and inhibit differentiation (Wang et al., 2007; Cui et al., 2010). The most potent mitogen regulating total OPC numbers is platelet derived growth factor (PDGF)-A, signalling through the OPC receptor PDGFRα (Noble et al., 1988; Richardson et al., 1988; Pringle et al., 1992). Other OPC mitogens are FGF-2 and the insulin growth factor (IGF)-1 that operate alone or synergically to promote OPC proliferation (Chandran et al., 2003; Furusho et al., 2011; Baron et al., 2000; Zeger et al., 2007; Frederick et al., 2007). Extracellular effectors regulating OPC migration include motogenic factors (stimulating OPC motility), adhesion and contact molecules (present in the extracellular matrix [ECM] and on cells here contained), and long-distance chemotactic cues. The main motogenic molecules are PDGF-AA and FGF-2 (Baron et al., 2000). The effects of these molecules may be locally modified by cues present in the ECM, both proteins (laminin, fibronectin, merosin, tenascin-C, anosmin-1) and cell adhesion molecules (polysialylated neuronal cell adhesion molecule [PSA-NCAM], ephrins, αvβ1 integrins, claudin-11/oligodendrocyte specific protein, neural/glial antigen 2 [NG2], and N-cadherin) (de Castro et al., 2013; Mitew et al., 2014). Chemoattractants and repellents guide longer distance OPC migration. Among these, PDGF-AA, FGF-2 and the secreted chemokine (C-X-C motif) ligand 12 (CXCL12) attract OPCs, while astrocyte-secreted CXCL1 and semaphorins repel them (Noble et al., 1988; Bribián et al., 2006; Dziembowska et al., 2005; Tsai et al., 2002; Spassky et al., 2002; Taniguchi et al., 2009; Armendáriz et al., 2012). Importantly, the effects of chemotactic cues vary depending on the CNS region, the developmental stage and the specific receptors expressed by different OPC populations. Notably, axonal signals also regulate OPC proliferation and migration. Neuregulin-1 (NRG-1), for example, acts as (PI3K/Akt-mediated) proliferation signal as well as a (MAPK-driven) differentiation cue in presence of laminin-2α, and has temporally different effects on OPC migration, chemo-attracting OPC during development but being unnecessary for post-natal OPC migration (Canoll et al., 1999; Fernandez et al., 2000; Flores et al., 2000; Colognato et al., 2002 & 2004; Ortega et al., 2012).
Once they have reached their final destination, OPCs can terminally differentiate into post-mitotic pre-myelinating oligodendrocytes. This is a key point in the myelination process. In mice, OPC terminal differentiation and myelination are almost co-occurring events, with pre-myelinating cells rapidly progressing to myelination or undergoing apoptosis (Barres et al., 1992; Trapp et al., 1997). By contrast, the developing human brain seems to harbour pre-myelinating oligodendrocytes for longer periods, before these cells finally start to myelinate (Back et al., 2002). Greater complexity of the human brain, including its larger size and longer development, and the existence of unique regions and functions, presumably account for the need of a greater potential and more complex regulation of oligodendrocyte differentiation and myelination.

The current model of oligodendrocyte terminal differentiation has been heavily influenced by the simple observation that, in absence of mitogens and serum, cultured OPCs spontaneously exit the cell cycle and differentiate in myelinating oligodendrocytes. This has suggested that much of the regulation of oligodendrocyte differentiation and myelination may be at the level of inhibiting this “default” pathway, with relief of inhibition leading to oligodendrocyte differentiation and myelination (Emery, 2010). Observations in vivo, however, show that the balance between OPC proliferation and differentiation is a tightly regulated process ensuring that oligodendrocyte lineage progression takes place in an orderly sequence and preventing differentiated patterns of gene expression from being induced prematurely or in the wrong cells (Hughes et al., 2013). Indeed, many of the factors participating in this process are inhibitory. Among these are the axonal signals Leucin-rich repeat and immunoglobulin domain-containing-1 (LINGO-1), Jagged-1 and Delta-1. These two latter signal via Notch-1 in OPCs and, through activation of the Hes1 and Hes5 transcription factors, hamper OPC differentiation by inhibiting pro-differentiation factors such as SOX10 (Jepson et al., 2012; Wang et al., 1998; Kondo & Raff, 2000; Liu et al., 2006). Interestingly, in presence of contactin/F3, Notch-1 signalling promotes rather than inhibits OPC differentiation (Hu et al., 2003). BMPs and the oligodendrocyte-specific G-protein coupled receptor 17 (GRP17) also act as negative regulators of oligodendrocyte differentiation and myelination. Their signalling is mediated by the bHLH molecules ID2 and ID4 that directly bind Olig1 and Olig2 inhibiting their function (Cheng et al., 2007; Chen et al., 2009; Samanta & Kessler, 2004). Promoters of differentiation include insulin growth factor-1 (IGF-1) and the triiodothyronine/thyroid hormone 3 (T3), the importance in vivo of which is supported by the hypomyelination phenotype of patients with congenital hypothyroidism (Carson et al., 1993;
**SPECIFICATION**

**DIFFERENTIATION, MATURATION & MYELINATION**

**OPC**  
(PDGFRα, NG2)

- Olig2, Sox10
- Shh/Gli

**pre-myelinating oligodendrocyte**  
(PLP, DM20, APC)

- Nkx2.2
- Jagged1

**myelinating oligodendrocyte**  
(MBP, MOG)

- YY1, MRF, HDAC

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**neurotransmitters**

**astrocyte**

- GFAP, DAPI

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**ATP adenosine**

**WHITE MATTER**

**CORTEX**

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**neuron**

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**expression**

- Nkx6.1
- Sox9
- Sox10, Ascl1, Zfp191
- Nkx2.2
- Nkx2.2, Nkx6.2
- Olig1 nuclear, Tcf712, Tcf4, active WNT
- Olig1 cytoplasmic, MRF, YY1

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**miR-219, miR-338**

**Hes1, Hes5, Id2, Id4, Sox5, Sox6**
Signalling via the Wnt/β-catenin pathway has emerged as a key regulator of oligodendrocyte development, and exemplifies how the targets of signalling pathways may change depending on the differentiation stage and cellular context. Canonical Wnt signalling leads to nuclear localisation of β-catenin, which recruits the transcription factor Tcf7l2/Tcf4 and inhibits terminal differentiation (Shimuzu et al., 2005; Fancy et al., 2009; Fu et al., 2012). Mice with elevated Wnt/β-catenin signalling in the oligodendrocyte lineage show blocked oligodendrocyte differentiation and hypomyelination (Fancy et al., 2009). Repression of Wnt signalling is then necessary for oligodendrocyte differentiation. Decrease in β-catenin levels allows for binding of Tcf7l2 to the chromatin-remodelling proteins histone deacetylase complexes 1 and 2 (HDAC1/2, see below) and to Olig2 (Ye et al., 2009). In agreement with a role of Tcf7l2 in chromatin remodelling during differentiation, Tcf7l2 expression is tightly regulated and occurs only in post-mitotic non-dividing pre-myelinating oligodendrocytes given that nuclear levels of β-catenin are low (Fu et al., 2012). Tcf7l2 would therefore act as a molecular switch promoting or inhibiting oligodendrocyte differentiation depending on the available binding partners.

The identification of oligodendrocytic transcription factors required for terminal differentiation has been greatly aided by knockout animal models. These have revealed that Olig2, the main oligodendrocyte lineage marker, has stage-specific functions in oligodendrocyte development and that its target genes differ according to the differentiation stage. Olig2 is a key player in OPC specification (see above) and is also necessary for terminal differentiation of postnatal OPCs, but has no effects on myelination at later differentiation stages (Mei et al., 2013).

In OPCs, Olig2 induces chromatin remodelling by directing the histone acetylating

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**Figure 4. Regulation of oligodendrocyte development.** After specification, proliferating (circular green arrow) oligodendrocyte progenitor cells (OPCs) undergo sequential steps of differentiation and maturation toward immature pre-myelinating and then myelinating cells. Markers identifying the maturation stage are specified. The process is highly controlled and regulated by a program composed by several intrinsic and extrinsic factors. Boxes represent oligodendrocyte-lineage transitions that are dependent on specific transcription factors (based on mouse knockout experiments). Coloured gradient bars represent predicted temporal expression patterns. The contribution of astrocytic and axonal signals is indicated. Abbreviations are listed on page 7 and found in the text.
molecule BRG1 to target genes critical for terminal differentiation (including SOX10) and myelination (including the myelin regulatory factor [Myrf]), therefore inducing their expression (Yu et al., 2013). The transcription factor Ascl1/Mash1 also supports oligodendrocyte terminal differentiation via promoting the expression of Nkx2.2 (Sugimori et al., 2008). Notably, in both Mash1 and Nkx2.2 knockout mice the total numbers of post-mitotic oligodendrocytes are reduced (Parras et al., 2007; Qi et al., 2001). Nkx2.2 must then be downregulated concurrent with the onset of myelination.

In addition to genetically programmed extracellular ligands, there is evidence that OPC terminal differentiation is also driven by the level of axonal activity. Release of adenosine by active axons activates purinergic receptors on OPCs and promotes differentiation. Axonal release of ATP stimulates adjacent astrocytes to secrete the pro-myelination cytokine leukaemia inhibitory factor (LIF), which in turn acts on OPCs counteracting the inhibitory effects of BMPs (Ishibashi et al., 2006).

The final stage of oligodendrocyte development is myelination. Myelination occurs in a very short time window in the lifetime of the individual oligodendrocyte, during which myelin sheaths are formed and the number of sheaths is determined (Watkins et al., 2008; Czopka et al., 2013). For this to take place, intrinsic and extrinsic regulators interact dynamically to control the balance between differentiation and myelination in a spatiotemporally specific manner, preventing transcriptional repressors of myelin genes from remaining activated after positive activators of myelin gene transcription are brought into play.

The simplest mechanism for determining whether an individual axon is myelinated would be the expression of inhibitory or permissive cues for myelination on the surface of the axon itself. This mechanism would also have the important benefit of allowing control of myelin at the subcellular level, explaining how individual axons are selected for myelination. Intriguingly, most axonal ligands influencing myelination are inhibitory, preventing myelination initiation or excessive myelination. Among these, Lingo1, PS-NCAM, and Jagged-1 inhibit oligodendrocyte process extension and initial contact with the axon (Decker et al., 2000; Fewou et al., 2007; Wang et al., 1998; Hu et al., 2003; Watkins et al., 2008; Mi et al., 2005). Conversely, axonal signals promoting myelination include laminin-α2 (binding oligodendrocyte β1-integrin receptors), netrin-1 (binding the DCC receptor) and axonal L1 ligand (binding oligodendroglial contactin). These pro-myelination signals converge in activating the non-receptor tyrosine kinase Fyn
eventually resulting in reorganisation of the cytoskeletal actin and microtubules, extension of oligodendrocyte processes and increased branch formation (Morisette & Carbonetto, 1995; Colognato et al., 2002; Hu et al., 2009; Liang et al., 2004; Bauer et al., 2009; Laursen et al., 2009; Rajasekharan et al., 2009). Fyn-mediated morphological remodelling occurs partly via activation of Rho GTPases by the non-receptor tyrosine kinase FAK (focal adhesion kinase) (Chun et al., 2003; Bacon et al., 2007; Kim et al., 2008; O’Meara et al., 2013). Interestingly, FAK regulates oligodendrocytic morphology in an opposite fashion depending on the composition of the ECM, inhibiting process expansion in the presence of fibronectin and promoting process maturation in the presence of laminin-α2 (Lafrenaye & Fuss, 2010). Thus, FAK appears to be a key regulator of ECM-cytoskeletal responses.

Axonal signals are also required to establish adequate myelin thickness. Amongst these are laminin-α2/β1 integrin and NRG-1/ErbB (Lee et al., 2006; Barros et al., 2009; Taveggia et al., 2007; Brinkmann et al., 2008). Notably, NRG-1 expression is influenced by neuronal activity (Ziskin et al., 2007; Liu et al., 2011), confirming this as a major promoter of OPC proliferation, oligodendrogenesis, and myelin remodelling (Gibson et al., 2014). Other pathways downstream of signals regulating myelin thickness are the Raf/MEK/ERK pathway, targeted by FGF-2 and brain-derived neurotrophic factor (BDNF), and the PI3K/Akt/mTOR (mammalian target of rapamycin) pathway, the constitutive activation of which results in hypermyelination (Furusho et al., 2012; Xiao et al., 2010 & 2012; Ishii et al., 2013; Wong et al., 2013; Flores et al., 2008; Narayanan et al., 2009).

Numerous transcription factors have been identified that are involved in regulating myelination. Of note, some are also expressed at earlier differentiation stages, suggesting that their role in promoting differentiation and activity at myelin gene promoters must be subject to regulation of additional cues differentially expressed between OPCs and myelinating cells. Olig1, for example, is expressed throughout oligodendrocyte differentiation, but becomes necessary only at the onset of myelination when it interacts with SOX10 to promote the transcription of MBP (Xin et al., 2005; Li et al., 2007). Interestingly, oligodendrocyte maturation coincides with nucleo-cytoplasmic translocation of Olig1, which is a prerequisite to membrane expansion and maturation (Niu et al., 2012). Nucleo-cytoplasmic translocation is a known, although not fully explored mechanism of transcription factor inactivation. The mechanisms by which Olig1 promotes membrane maturation when excluded from the nucleus are still obscure, but may involve sequestration of inhibitory factors away from the nucleus or recruitment of other promoting factors in or to the cytoplasm. Besides acting at myelin gene promoters,
SOX10 directly interacts and induces the pro-myelination factor Myrf. Myrf is present only in post-mitotic oligodendrocytes during terminal differentiation, when it mediates the transition from pre-myelinating to myelin forming cells. Conditional inactivation of Myrf in oligodendrocytes causes differentiation to stall at an early pre-myelinating stage (Emery et al., 2009). Inducible conditional ablation of Myrf in mature oligodendrocytes causes demyelination even in adult animals, suggesting a fundamental role of Myrf also in myelin maintenance (Koenning et al., 2012). In agreement with this, Myrf binding to DNA is enriched close to genes for myelin components and enzymes and cytoskeletal proteins involved in the generation of the myelin sheath (Cahoy et al., 2008; Bujalka et al., 2013).

As the window of time available for the onset of myelination is very narrow (Czopka et al., 2013), oligodendrocytes possess a complex machinery to synthesize, sort, and traffic enormous amounts of membrane components in short time and in a coordinated manner (Quarles et al., 2006; Simons & Trotter, 2007). To ensure the proper lipid-rich composition of the myelin sheath, specific lipid-lipid and lipid-protein interactions must ensue. The myelin sheath has a unique lipid composition, as it is enriched in cholesterol, galactosylceramide, sulfatide and ethanolamine plasmalogens. The importance of maintaining the optimal ratio of individual lipid classes is illustrated by the adaptive plasticity of the process in genetically manipulated mice. Most lipids are synthesized in the ER, possibly preassembled early in the secretory pathway, and are transported to the membrane via vesicular and non-vesicular mechanisms (Simons et al., 2000; Taylor et al., 2002; Krämer et al., 1997; White & Kramer-Albers, 2014). The major protein (PLP)/DM20 associates with lipids and is transported to myelin by vesicular transport through the biosynthetic pathway (Bradl & Lassmann, 2007; Simons et al., 2000). MBP, which is crucial for the proper assembly of myelin, is targeted by transport of its mRNA in the form of granules assembled in the cell body, transported along microtubules in the oligodendroglial processes, and then localized to the myelin membrane where it ensures myelin compaction (Ainger et al., 1997; Barbarese et al., 1999; Aggarwal et al., 2013; Müller et al., 2013). This alternative mechanism of local synthesis could have been developed to ensure that MBP exerts its adhesive action only at the appropriate site. In vitro studies suggest that both delivery systems may be at least in part under the control of neuronal signals (Trajkovic et al., 2006; Bradl & Lassmann, 2007).

Recent studies using in vivo time-lapse imaging of zebrafish embryos and quantitative analysis of electron micrographs from optic nerve serial sections have shown that radial and longitudinal expansion of the future myelin sheath occur
Quite simultaneously (Snaidero et al., 2014). According to this model, the innermost layer of the sheath is laterally expanding and squeezes in between the preceding layer and the axon, an event called wrapping. Myelin compaction would then progress from the abaxonal layers towards the adaxonal inner tongue, allowing the growing sheath to expand underneath the previous layer (Snaidero et al., 2014; Nave & Werner, 2014).

Epigenetic regulation, including chromatin remodelling, DNA methylation and non-coding RNAs, has recently been recognized as a fundamental player contributing to oligodendrocyte development and myelination (Yu et al., 2010; Liu & Casaccia, 2010). One of the best characterized chromatin remodelling is nucleosomal histone modification by acetylation on lysine residues, which is dynamically mediated by histone acetyltransferases (HATs) and HDACs. HATs add acetyl groups and favour a transcriptionally competent chromatin structure, while HDACs remove acetyl groups resulting in chromatin compaction. Recent work, however, shows that HDACs can also participate in transcriptional activation of certain myelin genes (Liu et al., 2009). During oligodendrocyte development, HDAC activity is essential for OPC differentiation and myelin gene expression with temporally-specific effects: the activity is critical for OPC differentiation, but is apparently not required for myelin gene expression after myelination onset (Shen et al., 2005; Humphrey et al., 2008; Swiss et al., 2011). Actually, HDACs likely promote oligodendrocyte differentiation by inhibiting the expression of pathways and genes that otherwise block differentiation, including the Wnt/β-catenin and Notch signalling pathways (see above) (Shakéd et al., 2008; Kim & Lassar, 2003; Ye et al., 2009; Kao et al., 1998; Yamaguchi et al., 2005). In line with this, HDAC mutants develop severe myelination deficits due to a block of oligodendrocyte differentiation (Jacob et al., 2011).

MicroRNAs are short non-coding RNAs processed from endogenous genomic loci that target mRNA species with complementary sequence to repress their translation and stability (Bartel, 2009). MicroRNAs function at multiple stages during oligodendrocyte development to direct and finely tune differentiation processes (Svaren, 2014; He et al., 2014). MicroRNAs expression is required for cell cycle exit and terminal differentiation. A possible negative regulator of proliferation is miR34a, which targets cyclin D1 and Notch1 (Bremer et al., 2010; Viader et al., 2011). MiR219 and miR338 target genes (Hes5, SOX6, PDGFRα) that act to maintain OPCs in a proliferative undifferentiated state (Zhao et al., 2010; Dugas et
MiR219 may additionally target transcription factors potentially involved in neurogenesis (Dugas et al., 2010; Zhao et al., 2010). This suggests a dual role of microRNAs in regulating oligodendrocyte lineage progression and preventing expression of neuronal or other cell lineage genes. Finally, expression of microRNAs is required on a continual basis in mature oligodendrocyte for proper maintenance of myelin. MiR23 targets the nuclear envelope protein lamin b1 (Lin & Fu, 2009). \( \text{LMNB1} \) gene duplications cause adult-onset autosomal dominant leukodystrophy, which is associated with myelin loss and repressed production of MBP, PLP and myelin oligodendrocyte glycoprotein (MOG) (Lin et al., 2014). MiR219 regulates lipid metabolism by targeting a fatty acid elongase that synthesizes saturated and polyunsaturated very long chain fatty acids incorporated into the myelin sheath (Shin et al., 2009).

NG2 glia

NG2 glia are the most recent member of the club of glial cells, identified by their expression of the chondroitin sulphate proteoglycan 4 (CSPG4) (Levine & Nishiyama, 1996). In the developing brain, NG2 glia are a highly proliferating population giving rise to OPCs and protoplasmic astrocytes in the gray matter, whereas their lineage appears restricted to OPCs in the white matter (Zhu et al., 2008 & 2011). In the adult brain, NG2 glia constitute the virtual totality of proliferating cells outside the neurogenic niches and are maintained in homeostatic balance under normal conditions (Hughes et al., 2013). They co-express PDGFR\( \alpha \) and Olig2 (like OPCs during development) and generate oligodendrocytes and further NG2 glia (Dimou et al., 2008; Ge et al., 2009; Simon et al., 2011; Nishiyama et al., 2009; Geha et al., 2010). They maintain however their regional intrinsic differences, with white matter NG2 glia having a much higher propensity to generate mature oligodendrocytes than their gray matter counterparts (Dimou et al., 2008; Kang et al., 2010; Vigano et al., 2013). The existence in the adult brain of NG2 glia not involved in oligodendrocyte generation has raised the hypothesis that this glial cell type has other functions beyond being OPCs. NG2 glia differ from other glia cell types by receiving synapses from neurons with clear ultrastructural specializations (Behrendt et al., 2013; de Biase et al., 2010; Sakry et al., 2011), a feature for which they are also referred to as “synantocytes”. Different observations suggest that this synaptic communication regulates NG2 glia proliferation and/or differentiation. In mice, sensory deprivation during development leads to NG2 glia proliferation, whereas physical activity in adult animals induces their cell cycle exit.
and differentiation into mature oligodendrocytes (Dimou & Götz, 2012; Mangin et al., 2012; Simon et al., 2011). In line with this hypothesis, differentiation of NG2 glia coincides with loss of their synaptic input (Mangin & Gallo, 2011). Within this scenario, NG2 glia may contribute to activity-dependent regulation of myelination and of myelin remodelling in the adult brain. The discovery of proliferating NG2 glia also in the human brain (Geha et al., 2010) suggests that these events take place in our species as well.

**Astrocytes**

Astrocytes are the predominant cell population in the CNS. Once considered simple scaffolding elements, astrocytes are now recognized as an extremely heterogeneous cell type essential for CNS development and maintenance of CNS homeostasis (Freeman, 2010; Zhang & Barres, 2010; Sofroniew & Vinters, 2010; Verkhratsky & Parpura, 2010). During development, astrocytes progenitors promote synaptogenesis and neurite outgrowth and are involved in synapse stabilization and elimination (Molofsky et al., 2012; Barres, 2008). Postnatally, astrocytes preserve the integrity of the blood-brain and blood-cerebrospinal fluid barriers, control the extracellular ionic milieu, provide metabolic support to neurons, facilitate perivascular flow of cerebrospinal fluid, ensure proper synaptic transmission and plasticity, and are involved in cerebral blood flow regulation (Verkhratsky et al., 2012; Schummers et al, 2008; Sofroniew & Vinters, 2010; Verkhratsky & Parpura, 2010; Barres, 2008; Iliff & Nedergaard, 2013). Astrocytes also participate in regulating developmental myelination and myelin maintenance in the adult brain (Barnett & Linington, 2013; Lanciotti et al., 2013). The role of astrocytes as modulators of oligodendrocyte proliferation and differentiation postulated over 20 years ago based on experimental studies (Noble et al., 1988; Richardson et al., 1988) was definitively confirmed by the identification of human leukoencephalopathies linked to mutations in astrocyte-specific gene products such as the intermediate filament glial fibrillary acidic protein (GFAP, AxD; Brenner et al., 2001) and MLC1 (MLC; Leegwater et al., 2001).

*In vitro and in vivo* studies have shown that astrocytes are a major source of many regulatory signals that influence OPC survival, oligodendrocyte differentiation/maturation and myelination (IGF-1, LIF, BDNF, Lingo-1, BMPs, CXCL10, CXCL12, CXCL1, PDGF, FGF-2) (Barnett & Linington, 2013). Astrocytes secrete ECM components (tenascin C, hyaluronan, fibronectin, laminin and members of the metalloproteinase family) and are implicated in ECM remodelling,
which may affect OPC proliferation/differentiation and myelination (Barnett & Linington, 2013; Harlow & Macklin, 2014). Simplified culture systems show that astrocytes also couple neuronal activity with myelinogenesis via ATP-, LIF-dependent mechanisms and possibly promote rapid myelin wrapping by material contribution of packaged lipids (Ishibashi et al., 2006; Sorensen et al., 2008; Watkins et al., 2008).

Astrocytes contribute to myelin maintenance by orchestrating the control of ion-water homeostasis at the nodes of Ranvier and preventing intramyelinic edema (Benfenati & Ferroni, 2010; Rash, 2010). When action potentials are transmitted through the white matter, depolarization of myelinated axons is associated with influx of sodium at the nodes of Ranvier and compensatory efflux of potassium at the paranodal regions covered by myelin. These fluctuations of ions are accompanied by osmotically driven shifts in water that require immediate compensation to allow further impulse transmission and prevent cellular swelling. Excessive osmotic water and potassium are siphoned away across the paranodal myelin into astrocytes via gap junctions. Long distance disposal of water and ions occurs via dispersion through the panglial syncytium, a network of astrocytes, oligodendrocytes and ependymal cells also interconnected by gap junctions. The crucial role of astrocytes in maintaining myelin integrity by potassium siphoning and gap junction communication is shown by white matter vacuolization and myelin degeneration in mice lacking gap junctions that form heterotopic interactions between oligodendrocytes and astrocytes (Tress et al., 2011 & 2012; Lutz et al., 2009; Kleopa et al., 2010).

The essential roles of astrocytes in maintaining CNS physiology have challenged the neurocentric view of brain functions. It has been suggested that failure of glial function results in development of many (if not all) neurological diseases (Verkhratsky & Parpura, 2010; Verkhratsky et al., 2012 & 2014). Given the important astrocytic contribution to the regulation of myelin deposition and maintenance, it is highly likely that astrocytes also participate in the pathogenesis of genetic white matter disorders other than AxD and MLC.

**Axons**

Axons and the ensheathing glia interact bidirectionally and throughout life. This interaction is essential for both partners. Axonal degeneration leads to loss of myelin and lack of myelin leads to axonal degeneration (Ellison et al., 2004; Bjartmar et al., 1999).
Although oligodendrocytes differentiate well in the absence of axons and can wrap even chemically fixed axons or nanofibers in vitro (Rosenberg et al., 2008; Lee et al., 2012), axonal signals participate in regulating oligodendroglial lineage progression and myelination in vivo (Zuchero & Barres, 2010). As illustrated above, axonal NRG-1 influences OPC proliferation, migration and differentiation (Canoll et al., 1999; Fernandez et al., 2000; Flores et al., 2000; Colognato et al., 2002 & 2004; Ortega et al., 2012), while LINGO-1, Jagged-1 and Delta-1 prevent differentiated patterns of gene expression from being induced prematurely in OPCs (Mi et al., 2005; Jepson et al., 2012; Wang et al., 1998). Axonal ligands like PSNCAM, laminin-α2 and netrin-1 control myelination initiation and mediate the influence on myelination of ECM components (Decker et al., 2000; Fewou et al., 2007; Morisette & Carbonetto, 1995; Rajasekharan et al., 2009). Neurons regulate membrane trafficking in oligodendrocytes and coordinate the transport of myelin components like PLP to the plasma membrane, thereby ensuring delivery at the appropriate developmental time (Trajkovic et al., 2006; Kippert et al., 2007). Axonal signals as laminin-α2/β1 integrin and NRG-1/ErbB are also required to establish adequate myelin thickness (Lee et al., 2006; Barros et al., 2009; Taverggia et al., 2007; Brinkmann et al., 2008).

In addition, neuronal activity may influence myelination (Demerens et al., 1996). Blockade of activity in the developing rat optic nerve by tetrodotoxin or axotomy decreases OPC proliferation (Barres & Raff, 1993) and pathological or non-physiological electrical activity enhances OPC proliferation and differentiation or the rate of myelin development (Li et al., 2010; Goldsberry et al., 2011). Neuronal activity may also promote adaptive myelination after development. Optogenetic stimulation of mouse cortical neurons results in OPC proliferation, differentiation and myelin remodelling with accompanying changes in behavioural function (Gibson et al., 2014). The same may occur in the adult human brain, as suggested by studies on the effects of training and learning (possibly including learning a foreign language and a new job as adults, author’s note) showing structural white matter changes within the relevant neural circuit (Roberts et al., 2013; Scholz et al., 2009; Sampaio-Baptista et al., 2013; Schlegel et al., 2012; Wang & Yang, 2014).

Intriguingly, recent in vitro studies revealed two distinct modes of myelination by oligodendrocytes, one independent of neuronal activity and the other dependent on neuronal action potentials. NRG1 switches oligodendrocytes between these two myelination programmes by making oligodendrocyte lineage cells more sensitive to glutamate released from active axons and consequently increasing myelination. Such interaction of NRG1 and glutamatergic signalling may also provide a
mechanism by which myelination is focussed on the axons of active neurons (Lundgaard et al., 2013).

Given the above, an intriguing possibility is that abnormal neuronal activity in genetic diseases affecting the human cortex may as well impact on myelination. Indeed, myelination is perturbed in infantile-onset lysosomal neuronal storage disorders as gangliosidoses and neuronal ceroid lipofuscinosis (NCL), and in the white matter underlying the dysplastic cortex in Zellweger syndrome. Cln8-deficient mice modelling NCL show delayed myelination and increased OPC numbers, suggesting a defect in OPC maturation (Kuronen et al., 2012). Analysis of brain tissue from patients with GM1 and GM2 gangliosidosis reveals failed myelin development and paucity of oligodendrocyte lineage cells, which may be compatible with a defect in OPC proliferation or survival (Haberland et al., 1973; van der Voorn et al., 2004).

Pathology and pathologic classification of genetic white matter disorders

The first pathologic classification of white matter disorders dates back to 1956, when Charles Poser and Ludo van Bogaert proposed a histologic systematization of the heterogeneous group of ‘diffuse cerebral scleroses’, chronic progressive diseases mainly affecting children characterized by diffuse myelin loss that until then had been grouped under the clinical umbrella of ‘Schilder’s disease’ (Poser & van Bogaert, 1956; Zeman & Whieldon, 1961). Poser introduced the concept of dysmyelination to differentiate those conditions in which normally constituted myelin is destroyed (myelinoclastic diseases) from those in which myelin does not form properly (Poser, 1957). Incorporating etiologic and prognostic implications with histologic features, Poser and van Bogaert proposed that diffuse cerebral scleroses fall into one of three categories: the myelinoclastic (demyelinating) group, closely related to multiple sclerosis; the dysmyelinating group of the leukodystrophies, probably related to some inherited enzymatic defect; and the leukoencephalitides, possibly of viral origin. Subsequently, the concept of dysmyelination was extended to include also those diseases in which myelin formation is delayed or arrested, as well as those in which the maintenance of already formed myelin appears to be disturbed (Poser 1961, 1968 & 1978).

The pathologic classification currently in use has added one category and separates primary diseases of myelin into three major types: demyelinative, dysmyelinative, and myelinolytic. Quoting James M. Powers (contribution in Naidu, 1999): “The prototypic demyelinative disease is multiple sclerosis (MS), included in
This category because of its inflammatory/immune-mediated destruction of biochemically normal myelin. These are dynamic lesions in which the acute or early lesions are characterized by glial (astrocytic and microglial) activation and perivascular astrocytic cuffs, followed by myelin-oligodendrocyte loss with macrophage infiltration and phagocytosis of myelin, followed by perivascular cuffs of macrophages filled with myelin debris and few lymphocytes, leading finally to a chronic fibrillary astrocytic scar with variable axonal loss. Leukodystrophies, primarily X-ALD, MLD and GLD are included in the dysmyelinative category and share an inherited (genetic) defect in myelin or myelin-forming cells. They generally do not manifest a true inflammatory response, and the myelin destruction appears to be owing to a biochemical abnormality in myelin (X-ALD, in part, MLD) or a molecular defect in the myelin-forming cells (GLD), particularly the oligodendrocyte. Hypomyelinative types, typified by PMD, are also included in this category. Myelinolytic diseases are distinct from the others because their lesions display a spongy or vacuolar myelinopathy owing to intralamellar edema. They may be genetic, such as Canavan’s disease (and MLC, author’s note), or acquired. Their separation has practical implications in that these lesions appear to be less detrimental to myelin, at least in the early stages, and more correctable or reversible. ALD and Canavan’s disease bridge the demyelinative-dysmyelinative and dysmyelinative-myelinolytic category, respectively."

This classification has the major value of categorizing white matter disorders according to the main mechanism of white matter injury and recognizing the possibility that different pathomechanisms may contribute to determine a single disease. However, the choice of terms could be questioned, also at the light of the new insights into the pathogenesis of some diseases. The word ‘dysmyelination’ by itself implies some kind of aberration in the myelination process, which is considered intrinsic to oligodendrocytes and/or myelin. While the term still applies to very early onset diseases interfering de facto with developmental myelin deposition, it is more difficult to employ in case of adult onset variant of the same condition. Krabbe disease is one such example. Infantile Krabbe disease typically displays marked paucity of myelin throughout the entire brain. In adult-onset Krabbe disease, by contrast, white matter degeneration appears limited to the corticospinal tracts. The term ‘myelinolytic’ is also questionable. Etymologically (Gr. λυσις, meaning breakdown or decomposition, as in cytolysis or hydrolysis), it implies dissolution or destruction of the white matter. However, no white matter destruction takes place in some of the disorders included in this category, which are rather characterized by intramyelinic edema and vacuolization. Again, a
classification should reflect the knowledge of its time. A new classification of genetic white matter disorders based on different criteria than the ones exposed above is perhaps also needed. The contribution of cell types other than oligodendrocytes driving myelin dysfunction or degeneration, including astrocytes and neurons, could also be considered.

Mechanisms of white matter injury

As stated above, the current pathologic classification reflects the main pathogenetic mechanisms that target the brain white matter. Different pathomechanisms may occur in the same condition either simultaneously or in sequence. The best characterized pathomechanism is probably demyelination, which results from direct insults targeting oligodendrocytes and myelin. This kind of injury is often referred to as primary demyelination to distinguish it from secondary demyelination (or Wallerian degeneration), in which myelin degenerates as a consequence of primary axonal loss (Ellison et al., 2004). It should be noted, however, that this distinction may be very difficult to achieve in demyelinating as well as dysmyelinating conditions. In both instances, loss or lack of myelin may coincide temporally with early axonal degeneration, raising the question of which cell type is truly primarily affected. This is the case in multiple sclerosis, but also VWM and some infantile onset primary neuronal disorders as gangliosidoses and NCL.

Depending on the developmental stage and pathomechanism involved, three main early patterns of tissue injury can be differentiated that eventually result in myelin loss (Bradl & Lassmann, 2010; Simons et al., 2014; Fern et al., 2014): (1) When oligodendrocytes and myelin are simultaneously targeted by the pathogenic insult, complete loss of myelin sheaths occurs while oligodendrocytes may either rapidly succumb by necrosis or transiently survive to then be cleared by apoptosis. This process characterizes dysmyelinating storage disorders. (2) Primary injuries involving the oligodendrocytes and not directly affecting the myelin sheaths, including some metabolic disturbances, result in incomplete loss of myelin. This pattern of tissue damage results from loss of individual oligodendrocytes with their respective myelin sheath. (3) Energy deficiency to the white matter leads to ‘dying back oligodendrogliopathy’, in which the damage progresses centripetally along the oligodendrocytic processes to last reach the cell body inducing caspase-independent apoptotic like death. This injury pattern implies an initial selective loss of distally enriched proteins (CNP and myelin-associated glycoprotein [MAG]) that
can be monitored with immunohistochemistry. Of note, the myelin (and axons) surrounding larger blood vessels may be remarkably spared.

Hypomyelination may result from deficiencies in or misfolding of key myelin proteins. The general pathomechanisms underlying this pattern of white matter injury are still poorly understood. The prototypic hypomyelinating leukoencephalopathy is PMD due to mutations in the \textit{PLP1} gene. PLP1 misfolding mutations result in oligodendrocytic loss due to ER stress-induced apoptosis, while duplications lead to oligodendrocyte arrested maturation, dysfunction and loss due to protein accumulation into the late endosomal/lysosomal compartment (Torii et al., 2014). Notably, PLP losses (null mutations) do not significantly affect oligodendrocytic numbers or myelination, but rather cause late-onset axonal degeneration (see above) (Griffiths et al., 1998; Rosenbluth et al., 2006). Arrested oligodendrocyte maturation also underlies hypomyelination due to hypothyroidism (see above) (Ibarrola & Rodriguez-Pena, 1997; Gupta et al., 1995; Jagannathan et al., 1998). The pathogenetic mechanisms leading to hypomyelination in other genetic disorders are largely unknown, also due to the rarity of the single conditions and scarcity of human tissue. However, it is conceivable that a reduction in the numbers of myelinating oligodendrocytes, either by cell loss, arrested maturation or both, may be a final common pathogenetic pathway leading to hypomyelination.

The so-called myelinolytic disorders are characterized by intramyelinic fluid accumulation with lamellar splitting along the intraperiod line (extracellular compartment) of the myelin sheath. It is conceivable that a general pathomechanism underlies myelinolysis, but lack of human tissue for many conditions prevents confirmation of this hypothesis. In Canavan disease, it has been suggested that intramyelinic edema results from abnormal water accumulation due to an osmotic-hydrostatic defect. Such defect has been hypothetically ascribed to astrocytes (Baslow & Guilfoyle, 2009 & 2013). Indeed, other genetically defined myelinolytic diseases are due to mutations in either astrocyte-specific gene products (MLC) or in gap junctions acting as water channels across the myelin sheaths. In these conditions, intramyelinic edema could therefore be a general manifestation of failed potassium siphoning and lost ion-water homeostasis. Whether this also applies to other genetic disease with ‘toxic’ intramyelinic edema such as disorders of organic acids, amino acids and urea cycle metabolism is not known.
Pathology of genetic white matter disorders

The pathologic characterization of genetic white matter disorders is largely limited to post-mortem material (Powers & De Vivo, 2002). On macroscopic examination (fig. 5), many conditions display similar macroscopic features that reflect the longstanding, diffuse involvement of the white matter. Typically, the brain is small and the white matter is atrophic with thinning of the corpus callosum and optic nerves and secondary dilation of the lateral ventricles. The white matter is stiff and discoloured (hence the first designation of diffuse cerebral sclerosis) and the U-fibers are often spared. The cortex and deep gray structures are generally better preserved or even apparently normal. A predominant involvement of frontal or posterior brain areas is typical of AxD and X-ALD, respectively.

Notable exceptions to the general gross appearance of white matter disorders are the cavitating leukoencephalopathies. In VWM, the cerebral white matter varies from gelatinous to cystic to frankly cavitary to totally disappeared, but the corpus callosum, internal capsule and anterior commissure are generally relatively preserved. In infantile AxD, the affected frontal white matter often shows early cystic degeneration. Cysts can also be expected in the subcortical white matter especially of the temporal lobe in MLC and RNASET2-deficient leukodystrophy, and deeper in the hemispheric white matter in some mitochondrial leukoencephalopathies (NDUFS1, SDHAF1 and APOPT1 mutations). The white matter is grayish to indistinguishable from the gray matter in hypomyelinating disorders. Lacunae or larger infarcts can be appreciated in angiopathic white matter disorders, including cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), AGS variants and some mitochondrial diseases as mitochondrial encephalopathy with lactic acidosis and stroke-like episodes (MELAS).

At light microscopy examination, the typifying features of genetic white matter disorders are myelin pallor with reduced myelin staining and eventually oligodendrocytic loss, relative sparing of axons and reactive to fibrillary astrocytosis. Spongy or vacuolated myelin due to intramyelinic edema (myelin splitting occurring at the intraperiod line) especially affecting the subcortical white matter is characteristic of myelinolytic leukoencephalopathies like Canavan disease and MLC. Macrophages, either scattered throughout the white matter or clustered around venules, are seen in many demyelinating and dysmyelinating conditions (see below), but are typically rare to absent in hypomyelinating and myelinolytic diseases. Reactive gliosis (isomorphic or anisomorphic) is prominent in
hypomyelinating disorders and mitochondrial diseases, but meagre in VWM and myelinolytic conditions. Astrocytes may display typical morphological changes, including the numerous Rosenthal fibres of AxD (and Labrune disease), the blunt coarse cells of VWM and the abundant Alzheimer type II astrocytes of Canavan disease. With the exception of X-ALD, inflammatory cells other than macrophages are generally insignificant in dysmyelinative and hypomyelinating conditions compared to demyelinating diseases as MS.

Careful survey of macrophages and of the myelin debris they contain is especially useful in dysmyelinating disorders (fig. 6). In this category of diseases, myelin is initially formed and then degenerates because it is biochemically abnormal (X-ALD, MLD), following the storage of toxic metabolites in oligodendrocytes (Krabbe disease), or for still unknown reasons (sudanophilic leukodystrophies in general). Breakdown of normal myelin yields galactolipids (sulfatide and cerebroside) and cholesterol; the former are rapidly degraded and are virtually not appreciable, while cholesterol is esterified by phagocytic cells where it persists for longer times and can be visualized with lipid stains as the oil red O. In lysosomal leukodystrophies galactolipids accumulate because of an enzymatic defect and become visible on
carbohydrate stains. Cerebroside, accumulating in Krabbe disease, is positive with the periodic acid Shiff (PAS) stain; sulfatide, stored in MLD, is PAS-positive and also metachromatic due to its anionic sulphate groups. In this, recognition of lipid components of the myelin debris helps both in addressing towards an underlying biochemical defect and in dating the duration of the lesions. The protein composition of the myelin debris also aids in determining the duration and degree of activity of the lesions, and is especially useful in demyelinating conditions.

Proteins that are contained in small amounts in the myelin sheath such as MOG or MAG are cleared much faster than proteins that are more abundant like PLP or MBP. Additionally, macrophages and their content may display characteristic morphologic features. Macrophages are typically striated and vacuolated in X-ALD, granular in MLD and pigmented in adult-onset leukoencephalopathy with neuroaxonal spheroids and pigmented glia. Multinucleated giant macrophages (globoid cells) are pathognomonic of Krabbe disease.

White matter lesions in microangiopathic leukoencephalopathies have no specific features and reflect the acute or chronic ischemia of the tissue. However, they may progress to white matter macrocystic degeneration, as in Labrune’s disease, AGS and MELAS. Perivascular mineralization to large calcifications are seen in AGS, leukoencephalopathy with calcifications and cysts, Cockayne syndrome and cerebrotendinous xanthomatosis. Involvement of the cortex especially the depth of the gyri and of selected neuronal populations may be seen in leukoencephalopathies due to energy defect, including microangiopathic conditions and mitochondrial diseases. Malformations of cortical development to frank cortical dysplasia may be encountered in some mitochondrial leukoencephalopathies (pyruvate dehydrogenase deficiency) and in peroxisomal biogenesis defects (Zellweger syndrome); the exact relation between cortical changes and white matter pathology in these cases is still undefined. Associated atrophy of the cerebellum and striatum suggests hypomyelination with atrophy of the basal ganglia and cerebellum (HABC).

Pathologic examination of biopsy specimens is usually not performed in white matter disorders, apart from selected cases of rapidly progressive demyelination that cannot be diagnostically resolved on MRI grounds.
Figure 6. Common and different microscopic features of genetic white matter disorders. Reduced myelin stain with relative sparing of the U-fibers (A, MBP, Krabbe disease), relative preservation of axons (B, Bodian, AxD), reactive astrogliosis (C, GFAP, MLD) and macrophages containing myelin debris (arrow in D, Kluver-PAS, X-ALD) are common to many conditions. Other features are distinctive, including metachromasia of storage material (E, Toluidine blue, MLD), multinucleated (globoid) cells (F, H&E, Krabbe disease), infiltration of inflammatory cells (G, H&E, X-ALD), Rosenthal fibers (H, H&E, AxD) and spongy myelin degeneration (I, Kluver, Canavan disease). Abbreviations are listed on page 7.

White matter potential for repair: remyelination and tissue remodelling

Remyelination is the process by which myelin sheaths are restored to demyelinated axons, re-enabling saltatory conduction and trophic support to axons.
and resolving functional deficits (Franklin & ffrench-Constant, 2008). CNS remyelination is thus a truly regenerative process that, under permissive conditions, may be extremely effective. Efficient remyelination occurs after acute focal myelin loss, especially in younger individuals. In absence of remyelination, axonal degeneration ensues.

The remyelination process largely recapitulates developmental myelination (recapitulation hypothesis of remyelination) (Franklin & Hinks, 1999; Fancy et al., 2011). As developmental myelination, remyelination involves the same key steps of OPC activation, proliferation, migration and terminal differentiation, interaction with unmyelinated axons, expression of myelin genes and synthesis, wrapping and compaction of myelin. However, notable differences between remyelination and developmental myelination exist. The strict correlation between myelin sheath thickness and length and axon diameter typical of developmental myelination is lost during remyelination, which typically results in thinner and shorter sheath segments that are roughly constant independent of the axon diameter (Blakemore, 1974; Ludwin & Maitland, 1984). This is reflected by an increase of the G-ratio, i.e. the ratio between axonal diameter and myelinated fiber diameter. The mechanisms underlying this discrepancy remain unclear. Experimental work suggests that many axonal signals and signalling pathways controlling myelin parameters in developmental myelination (including NRG1, Notch and PI3K/Akt/mTOR) may be dispensable during remyelination (Brinkmann et al., 2008; Stidworthy et al., 2004; Harrington et al., 2010).

Remyelination involves the generation of new myelinating oligodendrocytes that are mostly derived from local OPCs (Prayoonwiwat & Rodriguez, 1993; Sim et al., 2002; ffrench-Constant & Raff, 1986; Dawson et al., 2003; Sim et al., 2006; Zawadzka et al, 2010). Adult OPCs differ from their perinatal counterparts in many aspects, including cell cycle time, motility rate, growth factors responsiveness and antigenic markers (Wolswijk & Noble, 1989; Fancy et al., 2011). Noteworthy, CNS remyelination may also be mediated by Schwann cells, the myelin forming cells of the peripheral nervous system (Itoyama et al., 1983; Dusart et al., 1992; Duncan & Hoffmann, 1997). Schwann cells involvement in CNS remyelination has been observed in animal models and in human demyelinating diseases characterized by a lack of reactive astrogliosis (Blakemore, 1975; Zawadzka et al, 2010). Transgenic fate mapping studies showed that the majority of these cells are actually derived from OPCs (Crang et al., 2004; Zawadzka et al., 2010), revealing an unanticipated differentiation potential of these CNS precursors in the adult brain.
The extrinsic and intrinsic signals regulating remyelination largely overlap with those involved in developmental myelination. OPC activation following injury involves the upregulation of many genes encoding transcription factors associated with oligodendrocyte generation during development (Olig2, Nkx2.2) (Fancy et al., 2004; Sim et al., 2002; Watanabe et al., 2004; Talbott et al., 2005; Shen et al., 2008). Both during development and regeneration PDGF and IGF-1 act as major OPC mitogens, whereas FGF-2 regulates the balance between OPC recruitment and differentiation (Woodruff et al., 2004; Fruttiger et al., 1999; Carson et al., 1993; Mason et al., 2003; Murtie et al., 2005; Zhou et al., 2006; Armstrong et al., 2002). Lingo-1, Notch and the canonical Wnt pathway play a negative regulatory role in remyelination similar to that in myelination (Mi et al., 2005 & 2009; Seifert et al., 2007; Stidworthy et al., 2004; Fancy et al., 2009). The transcription factor Olig1 plays key roles in early OPC development and in OPC differentiation during remyelination (Xin et al., 2005; Arnett et al., 2004).

The efficiency of remyelination declines with age due to age-associated changes in both the extrinsic environmental signals to which OPCs are exposed in remyelinating lesions and intrinsic determinants of OPC behaviour (Franklin & ffrench-Constant, 2008; Crawford et al., 2013). For example, overexpression of mitogens as PDGF in aged animals increases OPC recruitment to demyelinated areas, but does not improve remyelination (Woodruff et al., 2004). This suggests that OPC differentiation into myelin-forming cells is a major limiting step associated with ageing. Remyelination may also fail when myelin loss occurs in the context of a maladaptive immune response, as in chronic lesions of multiple sclerosis. In these conditions, the environment may contain elements that are inhibiting or lack elements that would promote remyelination (Franklin & ffrench-Constant, 2008; Crawford et al., 2013). Indeed, as developmental myelination, remyelination relies on the tight interaction of a multitude of extrinsic and intrinsic signals that regulate the behaviour of the oligodendrocyte lineage through the different stages of the process. The efficiency of remyelination may therefore depend on the presence or absence of regulatory factors, but also on the coordinated timing at which these factors come into play. If any of the stages of remyelination is delayed or dysregulated, oligodendrocytes may miss the critical time window in which the signalling environment is promotive to successful remyelination (Franklin, 2002). Arresting the remyelination process leaves the axons chronically deprived of the trophic support of myelin and prone to degeneration. In case of a disease also featuring early axonal damage, failure to remyelinate naked axons may further exacerbate the extent of axon degeneration.
Several studies have investigated the role of astrocytes in remyelination (Lundgaard et al., 2014). Again, these have been mostly focussed on multiple sclerosis and its models. Transplantation of astrocytes into demyelinated lesions may enhance remyelination by endogenous OPCs (Franklin et al., 1991). Similar to myelination in development, astrocytic signals also regulate remyelination (Barnett & Linington, 2012). However, astrocytic secreted factors have both promoting and inhibiting roles in relation to remyelination (Nair et al., 2008). Astrocytes have recently been implicated in the regulation of oligodendrocytic iron metabolism, which may be vital for oligodendrocytes also in the context of remyelination (Schulz et al., 2012). Gap junction coupling between astrocytes and oligodendrocytes is necessary for myelination and myelin maintenance (Odermatt et al., 2003; Orthmann-Murphy et al., 2007; Tress et al., 2012). The observation that expression of the oligodendrocyte connexin (Cx)47 and astrocyte Cx43 is downregulated in chronic demyelinated multiple sclerosis lesions suggests that oligodendrocyte-astrocyte gap junctions are also needed for effective remyelination (Markoullis et al., 2012). Upon injury, astrocytes become reactive and participate in tissue remodelling by increasing ECM protein and chondroitin sulphate proteoglycan production and deposition. In the case of severe and/or chronic injury, this contributes to the formation of a ‘glial scar’. Following chronic inflammation-induced demyelination, ECM remodelling also coincides with inhibition of OPC differentiation, oligodendrocyte process outgrowth and remyelination (Back et al., 2005; Harlow & Macklin, 2014; Pendleton et al., 2013). The balance between opposing ‘pathologic’ effects of astrogliosis and normal ‘physiologic’ functions of astrocytes is one major factor determining if astrocytes hamper or sustain remyelination in demyelinating diseases. In this respect, inhibition of astrogliosis has been suggested as a potential strategy to allow remyelination to occur.

In agreement with their role in developmental myelination and myelin remodelling in the adult brain, axons and neuronal activity can also influence remyelination. As illustrated above, oligodendrocytes possess two distinct myelination programmes, one independent of neuronal activity and the other dependent on neuronal action potentials via NRG and glutamatergic signalling on oligodendrocytic NMDA receptors (Lundgaard et al., 2013). In vivo mouse experiments show that remyelination of axons in demyelinated lesions is NMDA receptor dependent (Lundgaard et al., 2013). This suggests enhancement of NRG and NMDAR dependent remyelination as a therapeutic approach for promoting recovery after demyelination.
Remyelination in genetic leukoencephalopathies is much less studied. The endogenous potential for repair is likely to be limited in those diseases that result from intrinsic abnormalities of the oligodendrocyte leading to dysmyelination or hypomyelination. Nonetheless, a regenerative attempt could take place, and fail. Translating knowledge on remyelination (and remyelination failure) from multiple sclerosis and its models to genetic leukoencephalopathies is challenging and probably not entirely valid. Disease-specific mechanisms underlying myelin loss and possibly influencing repair potential must be taken into account. In multiple sclerosis, for example, the immune response not only is responsible for the inflammatory attack on myelin, but also has a beneficial permissive role in creating the conditions for remyelination and repair to take place (Franklin & Kotter, 2008). A similar scenario could theoretically be envisaged for inflammatory demyelinating leukodystrophies like X-ALD, but not for VWM where inflammation is typically lacking. Availability only of human tissue obtained at end-stage disease together with absence of relevant animal models (see below) are also major limitations to the recognition of the regenerative potential of genetic white matter disorders.

**In vitro and in vivo models of myelination and genetic white matter disorders**

The development of a method to grow OPCs from mixed glial cultures (McCarthy & de Vellis, 1980) has allowed the possibility to study OPC differentiation and maturation into myelin-forming cells, in ‘spontaneous’ conditions as well as after genetic manipulation or upon administration of biomolecules. Human and rodent OPCs can be isolated with shake-off techniques or by magnetic-activated cell sorting, and used for studies on cell viability and functionality, cell migration and cell differentiation (Barateiro & Fernandes, 2014; Dincman et al., 2012). However, this *in vitro* model does not allow communication between OPCs and neurons, which is essential for proper myelination. Oligodendrocyte-neuron co-cultures permit studying the molecular basis of myelination and axoglial signalling without interference of other glial cells (Laursen et al., 2009). Myelinating culture systems employing OPCs, neurons and astrocytes allow investigation of the astrocytic contribution to myelination and oligodendrocyte-neuron communication (Sorensen et al., 2008). In these culture systems, participating cell types can be selectively genetically manipulated. A major disadvantage of all *in vitro* models is the loss of the tissue architecture and cell-cell and cell-matrix interactions present in the brain. *Ex vivo* organotypic slice culture models overcome these issues. Organotypic slices from the cerebellum are usually employed to investigate changes in
myelination (Jaeger et al., 1988; Notterpeck et al., 1993). The choice of this brain structure is based on the fact that the cerebellar white matter is easily recognizable and contains high numbers of oligodendrocyte lineage cells (Zhang et al., 2011). Moreover, as the cerebellum myelines postnatally, slice cultures of neonatal animals reproduce reliably the in vivo myelination process (Suzuki et al., 2012). Organotypic slices from non-myelinating animals as the shiverer mouse can be efficiently myelinated by 'transplanted' wild-type OPCs (Bin et al., 2012). Ex vivo slices from mice models of genetic white matter disorders have also been employed to address the issue of specific pathogenetic mechanisms, including oxidative damage in X-ALD (Fourcade et al., 2008).

In vitro and ex vivo culture models however all lack the temporal and spatial signalling existing in vivo, as well as any interaction with the immune system and blood circulation. Only in vivo models allow investigation of brain development and pathology within a complete organism. Among these, zebrafish has oligodendrocyte lineage cells and myelin homologous to mammals and presents the advantages of developing very rapidly and being transparent as embryos, simplifying in vivo imaging of myelin and myelination (Buckley et al., 2008). However, several aspects of zebrafish nervous system organization, including a different myelin composition compared to mammals, dictate circumspection when translating data inter-species. Said this, zebrafish has emerged as a promising genetic model system for the study of myelination and node of Ranvier formation (Pogoda et al., 2006) and as attractive option for addressing selected pathomechanisms of genetic white matter disorders, including Krabbe disease, MLC and RNASET2-related leukencephalopathy (Zizioli et al., 2014; Sirisi et al., 2014; Haud et al., 2011).

Of all the other in vivo animal models, the most used is the mouse. The fundamental mechanisms underlying oligodendrocyte development and myelination are closely conserved between mouse and human, making this a suitable model system for studies on myelination and myelin pathology.

Mouse models with perturbed growth factor pathways and/or targeted ablation of receptors provided detailed insight into the molecular regulation of induction, proliferation and differentiation of OPCs (Rowitch, 2004). These studies revealed the crucial role of PDGF and FGF in OPC development (Richardson et al., 1988; Bansal et al., 1996; Fortin et al., 2005). Manipulation of transcription factors as OLIG2, NKX2-2 and SOX10 has elucidated the molecular machinery underlying cell specification and maturation (Bansal et al., 1996; Li & Richardson, 2008;
Rowitch, 2004). Spontaneously occurring and genetically engineered mouse models abnormally expressing major myelin proteins or lipids shed light on myelin composition and the mechanisms of myelin sheath formation. The shiverer and jimpy mutants, for example, allowed the identification of MBP and PLP as crucial components of the myelin sheath and enlightened their roles in myelin wrapping and compaction (Lunn et al., 1995). Targeted knockout of enzymes responsible for myelin lipid synthesis revealed a role for lipids in the stabilization of newly formed myelin (Coetzee et al., 1996; Kassmann & Nave, 2008). Immune-mediated, toxic, viral and genetic models of demyelination are used to understand the manifold aspects of MS and investigate the mechanisms of remyelination (Franklin & ffrench-Constant, 2008; Ransohoff, 2012). More recently, myelin mutants have also been employed as hosts to assess the myelination potential of isolated neural cells. Transplantation of rodent and human glial progenitors into non-myelinating animals as shiverer demonstrated these cells’ ability to migrate throughout the host brain and form myelin, in some instances in amounts sufficient to reverse the host phenotype (Ben-Hur & Goldman, 2008; Low et al., 2009; Windrem et al., 2008).

By contrast, generation of genetically manipulated mouse models of hereditary degenerative white matter disorders has often been disappointing. Mouse models of X-ALD, MLD, AGS, VWM and AxD, for instance, show biochemical or otherwise pathologic signs of disease, but have intact myelin and also lack the expected clinical phenotype (Lu et al., 1997; Hess et al., 1996; Behrendt & Roers, 2014; Geva et al., 2010; Hagemann et al., 2006). The absence of myelin pathology in these models is a major limit to their use in studies on pathophysiology and treatment. Greater quantity and complexity of the human brain with its unique regions and functions probably contributes to their limited success. Expansion of the cerebral hemispheric white matter is an evolutionary achievement of primates and humans. Mice have barely any white matter in the telencephalon outside the corpus callosum. Processes as oligodendrocyte development and myelination proceed at different pace in mice and humans and comparisons are neither easy, nor linear (Jakovcevski et al., 2009). Mice have a life span of approximately 2 years, only a fraction of the normal human life span. Also, maturation and ageing progress in the two species at totally different rates (Demetrius, 2005; Flurkey et al., 2007). Overall, mice appear to model primate oligodendrocyte and myelin developmental mechanisms much better than more complex degenerative myelin diseases. Generation of valid mouse models recapitulating the human disease is difficult. When achieved, study of these models in parallel with human brain tissue
should provide the better chance to obtain data that are both pathophysiologically powerful and trustfully translatable across species.

**Aims**

Oligodendrocytes, astrocytes, microglia, and neurons interact intimately. Consequently, the cellular pathologies of genetic white matter disorders are very complex. Indeed, the identification of novel disease genes, which has accelerated rapidly in recent years, has facilitated -often unexpected- insights into the biology and the interactions within the brain white matter. Genetic white matter disorders vary considerably regarding the affected genes and the pathophysiology. As illustrated in Table 1, only a subset of the mutated genes encodes myelin proteins. Many genetic leukoencephalopathies are caused by mutations that do not affect ‘classical’ myelin gene products, or even affect proteins considered to be expressed specifically in cells other than oligodendrocytes. AxD and MLC are due to mutations in the astrocytes-specific gene products GFAP, and MLC1 and GlialCAM (Brenner et al., 2001; Leegwater et al., 2001; Lopez-Hernandez et al., 2011). Hypomyelination and congenital cataract is caused by mutations in FAM126A encoding the primarily neuronal protein hyccin (Zara et al., 2006; Gazzero et al., 2012). Hereditary diffuse leukoencephalopathy with spheroids is caused by mutations affecting the gene encoding the colony stimulating factor 1 receptor (CSF1R), considered exclusively expressed in microglia (Rademakers et al., 2011; Erblich et al., 2011). Importantly, such prior knowledge may infer an unjustified bias in the search for the pathomechanism. For example, astrocytes are considered the primary site of pathology in AxD. Here, white matter degeneration coincides with the appearance of Rosenthal fibers, astrocytic aggregates of the mutant intermediate filament protein GFAP (Mignot et al., 2004; Sawaish et al., 2009). However, whether astrocytic Rosenthal fibers indeed contribute to the white matter degeneration has not been satisfactorily shown. Overviewing the evolution of understanding of white matter physiology and pathology, it is clear that it is important to revise old concepts and definitions and that an unbiased approach is necessary to achieve further, truly new insights.

The aims of this thesis are to ascertain 1) if a cellular pathology perspective can be applied to the neuropathological evaluation of genetic white matter disorders; 2) if this approach can help categorize the major neuropathological findings in individual diseases; 3) if this approach can identify common pathomechanisms driving white matter dysfunction and degeneration; 4) if this information may be used to assess
the white matter repair potential; and 5) if this approach may contribute to a novel classification of genetic white matter disorders, which recapitulates all the above information.

To do this, we will compare and contrast the neuropathology of selected genetic white matter disorders of different aetiology, including VWM (chapter 2), MLC and CIC-2 related disease (chapter 3), a hypomyelination syndrome (chapter 4), and leukoencephalopathies due to vascular changes (chapter 5). Some of the diseases described are novel conditions. When available, the hypotheses formulated for human diseases will be verified and further investigated on their animal models using a combined evolutionary-developmental-pathological approach. A general discussion follows (chapter 6).
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