Leukoencephalopathies in childhood

Delineation of phenotypes

Tarja Linnankivi

Department of Child Neurology
and
Pediatric Graduate School
Hospital for Children and Adolescents
and
Biomedicum-Helsinki, Neuroscience Program
University of Helsinki
Finland

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Supervisors:

Docent Helena Pihko
Department of Child Neurology
University of Helsinki
Helsinki, Finland

and

Docent Leena Valanne
Helsinki Medical Imaging Center
University of Helsinki
Helsinki, Finland

Reviewers:

Professor Helena Kääriäinen
Department of Medical Genetics
University of Turku
Turku, Finland

and

Docent Oili Salonen
Helsinki Medical Imaging Center
University of Helsinki
Helsinki, Finland

Opponent:

Acting Professor Leena Haataja
Department of Child Neurology
University of Turku
Turku, Finland

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LIST OF ORIGINAL PUBLICATIONS

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I Linnankivi T, Lundbom N, Autti T, Häkkinen A-M, Koillinen H, Kuusi T, Lönnqvist T, Sainio K, Valanne L, Äärimaa T, Pihko H. Five new cases of a recently described leukoencephalopathy with high brain lactate. Neurology 2004; 63:688-692.

II Linnankivi TT, Autti TH, Pihko SH, Somer MS, Tienari PJ, Wirtavuori KO, Valanne LK. 18q-syndrome: brain MRI shows poor differentiation of gray and white matter on T2-weighted images. J Magn Reson Imaging 2003;18:414-419.

III Linnankivi T, Tienari P, Somer M, Kähkönen M, Lönnqvist T, Valanne L, Pihko H. 18q deletions: clinical, molecular and brain MRI findings of 14 individuals. Am J Med Gen 2006;140A:331-339.

IV Linnankivi T, Valanne L, Paetau A, Alafuzoff I, Hakumäki JM, Kivelä T, Lönnqvist T, Mäkitie O, Pääkkönen L, Vainionpää L, Vanninen R, Herva R, Pihko H. Cerebroretinal microangiopathy with calcifications and cysts. Neurology 2006 (in press).

ABBREVIATIONS

AGU	aspartyl glucosaminuria
CACH	childhood ataxia with cerebral hypomyelination
CADASIL	cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy
CDG	congenital disorders of glycosylation
CGH	comparative genomic hybridization
Cho	choline-containing compounds
CMV	cytomegalovirus
CNP	2'3'-cyclic nucleotide 3'-phosphodiesterase
CNS	central nervous system
CP	cerebral palsy
Cr	creatine
CSF	cerebrospinal fluid
CT	computed tomography
DNA	deoxyribonucleic acid
EEG	electroencephalogram
eIF2B	eukaryotic translation initiation factor 2B
FEVR	familial exudative vitreoretinopathy
FISH	fluorescence in situ hybridization
FLAIR	fluid-attenuated inversion recovery
GFAP	glial fibrillary acidic protein
GJA12	gap junction protein α12
H-ABC	hypomyelination with atrophy of the basal ganglia and cerebellum
HERNS	hereditary endotheliopathy with retinopathy, encephalopathy, and deafness
^{1}H-MRS	proton magnetic resonance spectroscopy
HIV	human immunodeficiency virus
IgG	immunoglobulin G
INCL	infantile neuronal ceroid lipofuscinosis
IQ	intelligence quotient
IUGR	intrauterine growth retardation
Lac	lactate
LBSL	leukoencephalopathy with brainstem and spinal cord involvement and elevated white matter lactate
LRP5	low-density lipoprotein receptor-related protein 5
MAG	myelin-associated glycoprotein
Mb	mega base
MBP	myelin basic protein
Mins	myo-inositol
MLC	megalencephalic leukoencephalopathy with subcortical cysts
MLD	metachromatic leukodystrophy
MNGIE	mitochondrial neurogastrointestinal encephalomyopathy

MOG	myelin-oligodendrocyte glycoprotein
MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy
MRSI	magnetic resonance spectroscopic imaging
MS	multiple sclerosis
NAA	N-acetylaspartate
NCV	nerve conduction velocity
NDP	Norrie disease protein
PCR	polymerase chain reaction
PLP	proteolipid protein
PMD	Pelizaeus-Merzbacher disease
PMP22	peripheral myelin protein 22
PNS	peripheral nervous system
P0	myelin protein zero
SCAD	short-chain acyl CoA dehydrogenase
SD	standard deviation
SE	spin echo
SEP	somatosensory evoked potentials
T	tesla
TE	time to echo
TR	time to repeat
UCSC	University of California, Santa Cruz
VOI	volume of interest
VWM	vanishing white matter
X-ALD	X-linked adrenoleukodystrophy

SUMMARY

Awareness of cerebral white matter disorders has markedly increased since the introduction of magnetic resonance imaging (MRI). Although MRI is very sensitive in showing white matter signal abnormalities, the etiology of these changes often remains unknown. During the last decade, several new leukoencephalopathies have been delineated based on characteristic patterns of MRI changes for each of them. Recently, the genetic basis of some of these disorders has been elucidated. However, more than half of children with white matter abnormalities on brain MRI still have no specific diagnosis.

The aim of this study was to classify patients with unknown cerebral white matter abnormalities and to identify new diseases among them. During the course of the study, three subgroups of patients were delineated and examined further.

The study began by survey of 38 patients with undetermined leukoencephalopathy. Neurological, ophthalmological, and neurophysiological examinations were carried out to evaluate the spectrum of clinical manifestations. Biochemical and morphological investigations were performed to exclude known causes of leukoencephalopathies. Brain MRI findings were grouped into seven major categories according to the predominant location of the abnormalities. The largest subcategories were patients with myelination abnormalities (n=20) and patients with predominantly periventricular white matter abnormalities (n=12).

Six patients shared a similar MRI pattern with hemispheric white matter abnormalities combined with changes in selective brain stem and spinal cord tracts. Magnetic resonance spectroscopy (MRS) showed consistently elevated lactate and decreased N-acetylaspartate (NAA) in the abnormal white matter. The patients presented at childhood or adolescence with sensory ataxia, tremor, distal spasticity, and signs of dorsal column dysfunction. The first description of this new phenotype – leukoencephalopathy with brain stem and spinal cord involvement and elevated white matter lactate (LBSL) – was published elsewhere in 2003. A finding not described in the earlier patients was a mild axonal neuropathy, which develops in the later phase of the disease. The etiopathogenesis of this disease is unknown, but elevated white matter lactate in MRS suggests a mitochondrial disorder.

Among the study patients, one child and his two first-degree relatives were found to have a small deletion of chromosome 18q. To better delineate the white matter changes related to 18q deletions, an additional 19 patients (age range 0.7-51 years) with previously diagnosed 18q deletions were investigated clinically and by MRI. The size of the deletion was determined using segregation analysis with microsatellite markers mapping to 18q. All patients with deletions between markers D18S469 (at 18q22.3) and D18S1141 (at 18q23) had abnormal myelination in brain MRI. In infants, the myelination appeared severely delayed, and in older children and adults, the differentiation of the cerebral gray and white matter was poor on T2-weighted images, consistent with dysmyelination.

The last part of the study comprised 13 patients (not included in the overview of unknown leukoencephalopathies) with strikingly similar MRI findings with leukoencephalopathy and progressive calcifications involving the thalami, cerebral and cerebellar white matter, brain stem, basal ganglia, and dentate nuclei. Five of the patients were studied prospectively and medical records of eight patients (of whom seven were deceased) with similar findings were reviewed. The patients showed a spectrum of findings, including progressive cerebral cysts, retinal telangiectasias and angiomas, intrauterine growth retardation, skeletal and hematologic abnormalities, and severe intestinal bleeding. Neurological symptoms were slowly progressive and included spasticity, ataxia, dystonia, seizures, and cognitive decline. The findings of these patients overlapped with features described previously in conjunction with two very rare disorders, namely “Coats plus” syndrome and “leukoencephalopathy with calcifications and cysts”, suggesting that these disorders are related. All six autopsied patients had similar neuropathological findings showing calcifying obliterative microangiopathy. The etiology of these changes is still unknown. The patients included two pairs of siblings, suggesting an autosomal recessive mode of inheritance.

INTRODUCTION

The diagnostics of cerebral white matter disorders was revolutionized by the advent of magnetic resonance imaging (MRI) in the late 1980s. While leukodystrophies were previously defined based on pathological findings, MRI has provided a powerful tool for investigating the cerebral white matter in living individuals.

Within the last 15 years, knowledge of white matter disorders has grown rapidly. The previously known "classical" leukodystrophies have been demonstrated to show characteristic patterns of MRI abnormalities (138). New white matter diseases have been recognized, initially defined by their specific MRI features, and subsequently, gene defects underlying these diseases have been found. Examples of such novel disorders are the vanishing white matter (VWM) disease (52, 67, 129, 135), also called childhood ataxia with cerebral hypomyelination (CACH) (105), and megalencephalic leukoencephalopathy with subcortical cysts (MLC) (68, 137). The pathophysiological mechanisms underlying these disorders are under active investigation.

Cerebral white matter is apparently involved in a large number of inherited metabolic diseases, chromosomal abnormalities, and acquired brain diseases. Despite this growing knowledge, when this study began, more than half of the children with white matter abnormalities on MRI remained without a specific diagnosis (62). This group of unknown leukoencephalopathies consists of rare and heterogeneous disorders, both progressive and nonprogressive (62). Without a diagnosis, predicting future outcome, assigning disease-specific treatment and providing prenatal diagnostics are impossible. Also, in undefined leukoencephalopathy, extensive investigations are often needed, which are exhaustive for the children and family and expensive for the healthcare system. Thus, further studies to classify and characterize white matter disorders are warranted. Categorization of patients into subgroups, based on MRI findings, has been carried out to facilitate determination of new disorders and to enable comparison of the data across centers (62, 133).

Many of the disorders with white matter involvement cannot be placed under the narrow concept of leukodystrophy, which implies inherited demyelinating disorders. The broader terms "white matter disorders" and "leukoencephalopathies" are defined as all conditions in which predominantly or exclusively white matter is affected, irrespective of the underlying histopathologic basis (125). Other important concepts used in this thesis are 1) "demyelination", used when there is loss of myelin, 2) "hypomyelination", which means that too little myelin is formed and this deficiency is permanent, 3) "dysmyelination", used when the process of myelination is disturbed, leading to abnormal, patchy, or irregular myelination and 4) "delayed myelination", used when the process of myelination is retarded but progressing.

REVIEW OF THE LITERATURE

1. White matter

The central nervous system (CNS) comprises gray matter structures and an extensive array of connecting white matter tracts. The white matter is made up of myelinated axons, a large number of glial cells, and the blood vessels that nourish them. The glial cells include oligodendrocytes, which are myelin-forming cells, astrocytes, and microglial cells, which have a phagocytic function. On a dry weight basis, 40-50% of white matter is myelin (8).

1.1 Myelin sheath

Myelin is a multilayered substance that surrounds the axons. It is present both in the central and in the peripheral nervous system (PNS), but the myelin-forming cells and composition of myelin differ between these two systems. In the CNS, myelin is formed by oligodendrocytes. The myelin sheath is an extended, modified oligodendrocyte plasma membrane surrouding a portion of an axon in a spiral fashion (Figure 1). Each oligodendrocyte may wrap up to 40 axons, and on the same axon, adjacent myelin segments belong to different oligodendrocytes. Small unmyelinated regions of axons between two segments of myelin, the nodes of Ranvier, contain a cluster of voltage-gated sodium channels. When the axon membrane is excitated, the electrical impulse does not flow through the high-resistance myelin sheath, but jumps from node to node. The low capacitance of the sheath allows depolarization of the remaining membrane between the nodes. This saltatory conduction of nerve impulses allows the fast nerve conduction that is crucial for functional integration of the CNS [reviewed in (8, 125)].

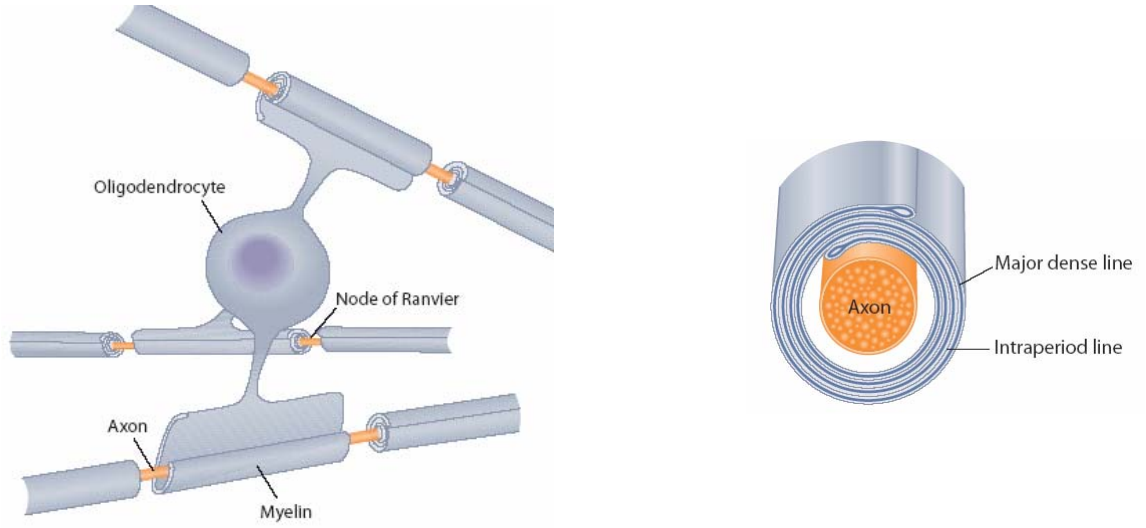

Figure 1. *The relationship between an oligodendrocyte and myelinated nerve fibers in the CNS.*

1.2 Composition of the myelin membrane

The myelin membrane is a lipid bilayer interrupted by proteins (Figure 2). Compared with other membranes, the composition of myelin is unique, with low water content (40%) and a very high lipid content. On a dry weight basis, myelin contains 70% lipids and 30% proteins (8).

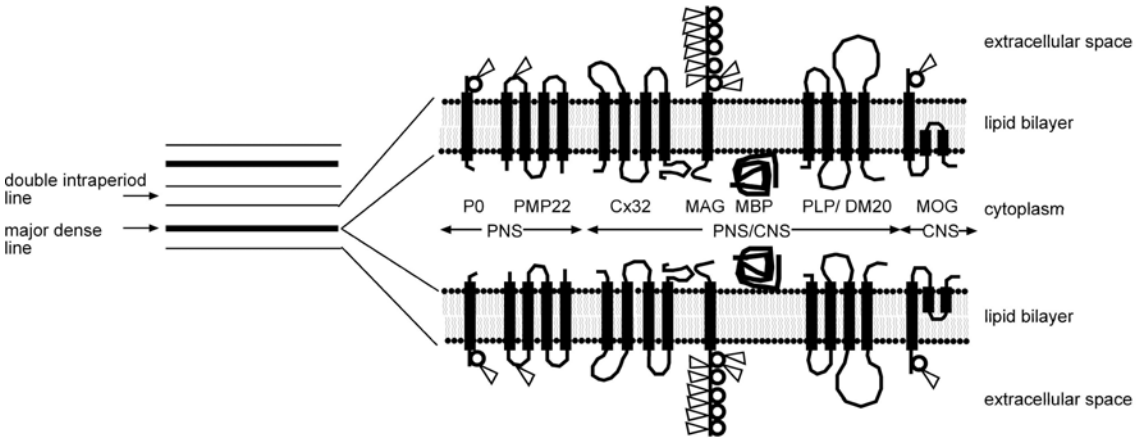

Figure 2. *Composition of myelin. The myelin membrane is composed of repeated layers with protein-lipid-protein-lipid-protein structure. Glycolipids and cholesterol are located at the extracellular faces and phospholipids at the cytoplasmic (intracellular) faces of the lipid bilayer. The presence and relative amounts of myelin proteins differ between the CNS and PNS. In compact CNS myelin, MBP and PLP are the major proteins. While P0 and PMP22 are found only in the PNS, MOG is specific for CNS myelin. The closely apposed external faces of the myelin membrane form the double intraperiod lines.The fused internal faces of the membrane form the major dense lines (see also Fig.1). Circles represent IgG-like domains, and triangles N-linked oligosaccharides of the proteins. P0, myelin protein zero; PMP22, peripheral myelin protein 22; Cx32, connexin 32. Adapted from (8, 98).*

Most myelin proteins are unique to myelin. Each protein is localized at a specific site in the myelin membrane, reflecting its function. The two major proteins of CNS myelin are proteolipid protein (PLP) and myelin basic protein (MBP), accounting for 50% and 30% of the total protein, respectively. Both are found in different isoforms, which are produced by alternative splicing of their genes. MBP is necessary for myelin compaction, adhering the cytoplasmic leaflets of the myelin membrane, while PLP (and its isoform DM20) stabilizes the intraperiod line of the membrane (21). In addition, myelin contains several minor proteins, e.g. glycoproteins MAG (myelin-associated glycoprotein) and MOG (myelin-oligodendrocyte protein). MAG is localized in the periaxonal myelin membrane and participates in signaling between axons and glia. This axon-glia interaction is suggested to be important in, for example, promoting myelin formation (98). MOG is localized on the outside surface of the myelin sheaths and oligodendrocytes. It is proposed to transmit signals from the extracellular environment and to be an important target in autoimmune demyelinating diseases (98). CNP (2'3'-cyclic nucleotide 3'-

phosphodiesterase) is only found in noncompact parts of myelin, e.g. in paranodal loops. CNP is not important for myelin assembly, but is essential for axonal survival (65).

The myelin lipids cholesterol, phospholipids, and glycolipids are not specific to myelin, but are also found in other cellular membranes. However, glycolipids, in particular galactocylceramides and sulfatides, and ethanolamine plasmalogen are highly enriched in myelin. Gangliosides are minor lipids of myelin, more abundantly localized in neuronal membranes. A special feature of myelin lipids is a high amount of monounsaturated long-chain fatty acids, resulting in the membrane being tightly packed and stabile (8).

1.3 Astrocytes

Astrocytes are glial cells found throughout the CNS. They interact with each other and with other glial cells by gap junctions and have multiple functions (63). In the white matter, astrocytes have a role in controlling the onset of myelination by inducing the adhesion of oligodendrocyte processes to axons. They also stimulate myelin formation and remyelination by secreting growth factors (8, 144). Triggered by cell damage, astrocytes proliferate and accumulate glycogen and filaments, a state known as gliosis.

1.4 Process of myelination in the central nervous system

Myelination starts during the fifth month of fetal life at the spinal cord. It proceeds rostrocaudally (from head to tail) in the spinal cord and from the spinomedullary junction to the forebrain in the cerebrum. CNS tracts begin myelinating when they become functional (125). The peak of myelin formation occurs during the first postnatal year, but myelination may progress slowly until 20 years of age in some cortical fibers, especially in associative areas (8). Myelination is a strictly regulated process and requires coordinated expression of genes responsible for synthesis of myelin components (34). An active field of research deals with the complex cellular mechanisms needed to determine how the oligodendrocytes migrate towards the axons and recognize them, how the interaction of glia and axons is initiated, how the spiraling of myelin occurs around an axon, and how the nodes of Ranvier are formed (27, 110). While active myelination is a vulnerable process, mature myelin is quite stable and resistant to alterations.

1.5 Different forms of myelin disturbance

Various types of underlying pathology are found in myelin disorders [reviewed in (131)], and most disorders include a combination of different pathologies.

The complex process of myelin assembly may be disturbed by different genetic and acquired factors. The best-known example of a severe hypomyelinating disorder is

Pelizaeus-Merzbacher disease (PMD), which is caused by defects in the PLP gene (56, 109). A disturbance of myelination may also lead to patchy or irregular myelination. This kind of dysmyelination is seen in, for example, certain disorders of amino acid metabolism such as serine synthesis defects (30). Also, if a predominantly neuronal degenerative disorder has an early infantile onset, the process of active myelination may be disrupted, as in INCL (infantile neuronal ceroid lipofuscinosis) (141) and GM1 and GM2 gangliosidoses (23, 48).

Demyelination of the previously normal myelin sheath is seen in, for example, acquired inflammatory white matter diseases such as multiple sclerosis (MS) (55). Demyelination is also a predominant pathology in "classical" leukodystrophies, including metachromatic leukodystrophy (MLD) and X-linked adrenoleukodystrophy (X-ALD) (43, 82). In these disorders, the composition and degradation of myelin lipids are altered, leading to structurally unstable myelin and eventually to demyelination.

The intramyelinic vacuole formation may result in myelin splitting, with or without concomitant demyelination. This occurs in MLC (136), in organic acidurias, such as Canavan disease (60), and in some mitochondriopathies (88). Moreover, intoxications, e.g. inhalation of heroin vapor (61), may cause myelin splitting.

Damage to oligodendrocytes leads to disturbance of both the formation and maintenance of myelin. This is seen, for instance, in Krabbe disease, where accumulation of the toxic substance psychosine causes rapid and almost complete oligodendrocyte cell death (115).

1.6 Interaction between glial cells and axons after myelination

The myelin sheath and axon are mutually dependent on each other. If the axon is destroyed, the myelin sheath distal to the lesion is lost, a process called Wallerian degeneration. Recently, the long-term maintenance of the normal structure and function of the axon has been shown to be dependent on normal myelin function (46, 110). Evidence for axonal damage has been found in humans and animals with myelin protein deficiencies and in the acquired demyelinating disease MS. This axonal damage also occurs in areas without overt signs of demyelination or damaged oligodendrocytes (15, 32, 39, 47). Moreover, mice lacking a minor myelin protein CNP have no apparent myelin abnormality, but they gradually develop axonal swelling and degeneration (65). Thus, probably the disruption of the axon-glia interaction rather than demyelination is the cause of this axonal degeneration (39).

2. Magnetic resonance in investigating white matter disorders

The most important clinical tools for investigating white matter disorders in living patients are MRI, which shows macroscopic structural changes, and MRS, which reveals biochemical changes in the brain.

2.1 Magnetic resonance imaging (MRI)

MRI is very sensitive in showing signal abnormalities in the white matter. Apart from myelin disturbances (hypomyelination, dysmyelination, and demyelination), damage to other components of the white matter may contribute to the observed signal abnormality. These include edema, seen for instance in conjunction with increased vascular permeability (116), and gliosis, which occurs when astrocytes react to diverse forms of injury (131). Axonal damage may also contribute to white matter signal abnormalities, as is seen in giant axonal neuropathy (19).

T1-weighted images are used to assess the anatomic structures. During the early phases of myelination, small amounts of myelin are better visualized on T1-weighted images. On these images, normally myelinated white matter appears bright and cerebrospinal fluid (CSF) is black. Pathological white matter gives a decreased signal and appears dark on T1-weighted images.

T2-weighted images are superior in showing the pathological processes of white matter. For subjects aged 9-10 months or beyond, T2-weighted images are more useful in evaluating the stage of myelination. Normal myelinated white matter appears dark and CSF is bright on these images. Pathology in the white matter leads to an abnormally high signal on T2-weighted images.

FLAIR (Fluid attenuated inversion recovery) is a T2-weighted sequence, where the signal of the free fluid, e.g. CSF, is nullified. In these images, the CSF is black and myelinated white matter is dark. If white matter is pathologic, it appears hyperintense and especially periventricular lesions are easily distinguished. The cystic nature of the lesions is also revealed by FLAIR images.

As the white matter myelinates, it changes from hypointense (dark) to hyperintense (bright) relative to gray matter on T1-weighted images and from hyperintense to hypointense relative to gray matter on T2-weighted images. These changes in signal intensity are caused by increasing brain lipid concentration and decreasing water content (6). The myelin signal appears earlier on T1-weighted images. In a newborn, myelin is seen in the posterior limbs of the internal capsules and in the dorsal brainstem (Table 1, Figure 3). During the first year of life, progressive myelination is seen, with sensorimotor and visual pathways maturating first and subcortical association areas maturing last (Figure 3)(5, 7). By the age of 24 months, myelination appears essentially mature in MRI (6).

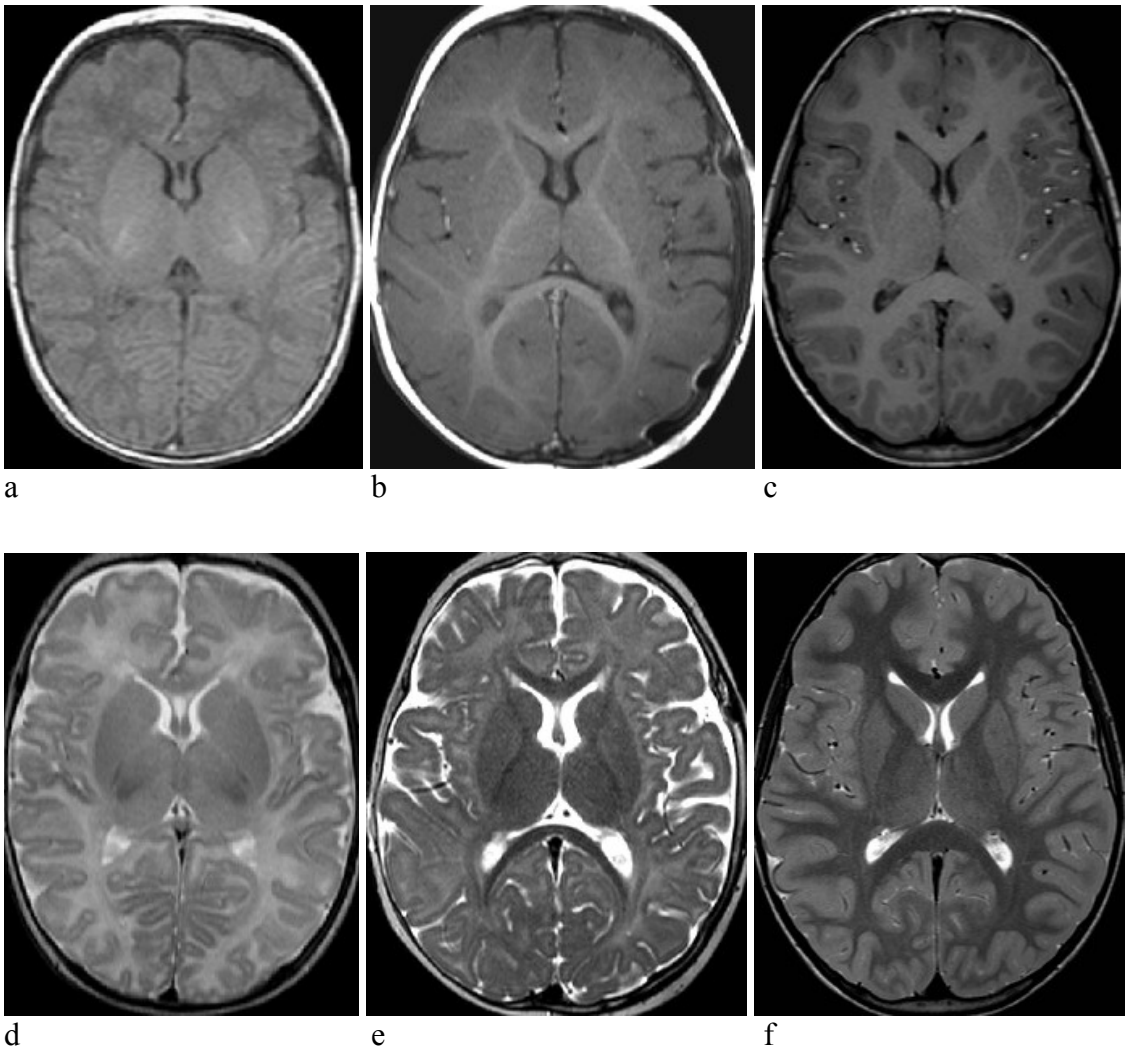

Figure 3. *Progress of myelination on T1-weighted (upper row) and T2-weighted (lower row) MRIs. In a newborn (a) and (d), the myelinated white matter is seen in the posterior limbs of the internal capsule. It appears hyperintense on T1-weighted images and hypointense on T2-weighted images. By the age of 8 months (b) and (e), the deep white matter is hyperintense on T1-weighted images, but the subcortical areas are still mostly unmyelinated. Hypointensity on T2-weighted images lags behind. By 24 months (c) and (f), the brain is fully myelinated on both T1- and T2-weighted images.*

Table 1. *Progress of myelination visible on MRI. Modified from (5, 7).*

Region	T1-weighted images	T2-weighted images
Dorsal brainstem	26-28 gw	27-30 gw
Middle cerebellar peduncle	Birth	Birth-2 mo
Cerebellar white matter	Birth-4 mo	3-5 mo
Posterior limb of the internal capsule		
anterior portion	First month	4-7 mo
posterior portion	Birth	Birth- 2 mo
Anterior limb of the internal capsule	2-3 mo	7-11 mo
Genu corpus callosum	4-6 mo	5-8 mo
Splenium corpus callosum	3-4 mo	4-6 mo
Occipital white matter		
central	3-5 mo	9-14 mo
subcortical	4-7 mo	11-15 mo
Frontal white matter		
central	3-6 mo	11-18 mo
subcortical	7-18 mo	14-30 mo
Centrum semiovale	2-4 mo	7-11 mo

gw, gestational weeks; mo, months

2.2 Magnetic resonance spectroscopy (MRS)

MRS provides a noninvasive method for identifying and quantifying brain metabolites. Several nuclei can be used, but proton MRS (^{1}H-MRS) is the most widely applied method in clinical settings. MRS, like MRI, is based on nuclear magnetic resonance. The nuclei, in ^{1}H-MRS protons, resonate at a slightly different frequency in different chemical environments. From the measured frequency spectrum, one can deduce the chemical composition of the target tissue. Because the concentrations of the investigated metabolites are much lower than the concentration of the water in the tissue, the water signal must be suppressed in ^{1}H-MRS. In single-voxel MRS, only one region of interest is measured. In magnetic resonance spectroscopic imaging (MRSI), multiple spectra covering a larger volume of brain are obtained simultaneously.

The major peaks in the human 1H-MR spectrum are N-acetylaspartate (NAA), choline-containing compounds (Cho), and total creatine (Cr), including creatine and phosphocreatine (Figure 4). Lactate (Lac) is normally not detected, but it is visualized if elevated. NAA is an amino acid found only in the CNS, and it is considered a specific neuroaxonal marker (12). Cho is elevated when membrane turnover is enhanced, both in

anabolic (myelination) (139) and in catabolic (demyelination and tumor growth) (11, 50) conditions. The concentration of Cr is high in glia, and thus, increased Cr is seen in conditions of gliosis (125). Lac increases in anaerobic glycolysis, as in hypoxia or in disorders of mitochondrial energy production. It may also be produced by metabolism of macrophages (73). Thus, elevated Lac is seen in conditions with increased numbers of macrophages, such as in inflammation occurring with active demyelination or in tissue necrosis (11). The major metabolites are visualized in spectra at long echo times (135-288 ms). More peaks are identified using short echo time (20-30 ms), e.g. myo-inositol (MIns). MIns is believed to be an astrocytic marker, and it is increased in conditions of gliosis (102). It is also an osmoregulator (107). However, with short echo times, quantification of peaks is more difficult than with long echo times.

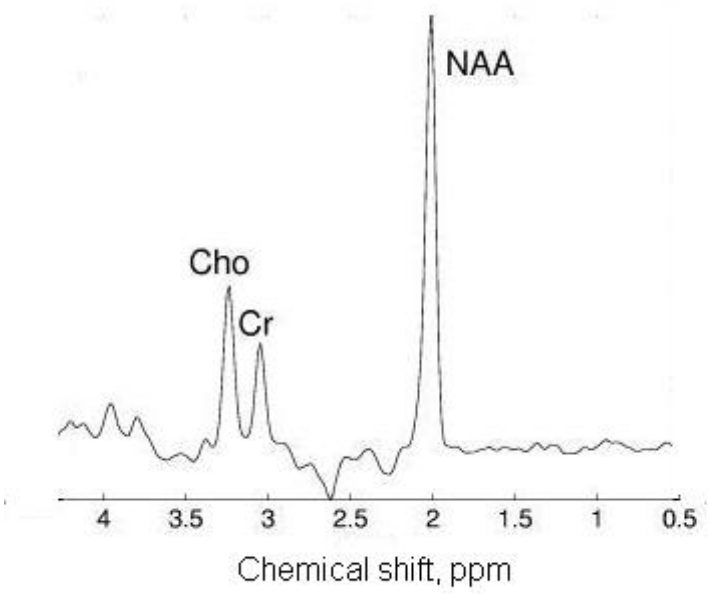

Figure 4. *In an ^{1}H-MR spectrum, each peak represents a proton in a particular compound. The area under each peak is proportional to the number of protons producing that peak. The chemical shifts (differences in resonance frequencies) are expressed in relative units (parts per million, ppm). The major peaks represent N-acetylaspartate (NAA), choline- containing compounds (Cho), and creatine (Cr).*

The MR spectrum changes during brain maturation. The NAA peak increases most rapidly during the first years of life, and this increase is suggested to reflect the formation of dendritic arborization and synaptic connections (96, 139). The prominent Cho peak in young infants probably reflects high membrane synthesis and turnover related to myelin formation. In white matter, Cho concentrations in older children (5-18 years) are about 15% below the levels in infants and young children (0-5 years) but the concentration of Cr remains stable after 1 year of age (96).

A shortcoming of MRS is that it is rather insensitive. The metabolites must have a concentration of at least millimolar range, and they also need to be small and mobile to be

visible in MRS. Macromolecules, such as proteins and membrane components, are "invisible" in MRS . Thus, normal myelin is mostly inaccessible to MRS. However, breakdown products of myelin membranes, like choline, may be detected.

3. Hereditary diseases with cerebral white matter involvement

Cerebral white matter abnormalities are found in many inherited diseases, examples of which are given in Table 2. Among "classical" leukodystrophies, MLD, Krabbe disease, and X-ALD are lysosomal and peroxisomal diseases in which progressive demyelination occurs in both the CNS and the PNS (42, 82, 115). Alexander disease is a primary astrocytic disorder caused by dominant mutations of the glial fibrillary acidic protein (GFAP) gene (16). Canavan disease is an organic acid disorder with spongiform leukoencephalopathy (76), and Pelizaeus-Merzbacher disease is an X-linked hypomyelinating disorder (109), caused by defects in the gene coding for PLP, a major protein of the myelin membrane (56). In recent years, it has become clear that severe leukoencephalopathy may be the predominant MRI finding also in mitochondrial disorders (31, 81, 123).

Some of the new phenotypes of childhood-onset leukoencephalopathies, recently recognized by their specific MRI patterns, are presented briefly here, as well as 18q-syndrome.

3.1 Leukoencephalopathy with vanishing white matter (VWM)

In this peculiar disease, the cerebral white matter slowly disappears. However, the brain does not collapse because the vanished white matter is replaced by fluid. FLAIR images are needed to show the rarefaction and cystic degeneration of the white matter (135).

Clinically, this disorder is characterized by progressive ataxia, followed by spasticity. Mental decline is relatively mild compared with severe motor handicap. Optic atrophy and seizures may occur as well (52, 135). Characteristic are episodes of rapid deterioration which may be provoked by mild head trauma, febrile infections, or stress. These episodes can lead to coma and death, and if the patient survives, the recovery is usually incomplete (135, 143). The most common variant of VWM has its onset in childhood, usually between the ages of 2 and 6 years. In the severe infantile-onset variant, death ensues before 2 years of age. Infantile cases may show multisystem involvement, with growth retardation, cataracts, hepatosplenomegaly, and pancreatic abnormalities (127). The adult-onset variant of VWM may manifest with seizures, cognitive and behavioral abnormalities, or motor deterioration (10, 36). In females, primary or secondary ovarian dysfunction may occur (37, 104).

Table 2. *Examples of hereditary disorders with cerebral white matter abnormalities* (3, 93, 122, 125).

Lysosomal disorders
Metachromatic leukodystrophy
Krabbe disease
GM1 and GM2 gangliosidosis
Multiple sulfatase deficiency
Free sialic acid storage disorders
Aspartylglucosaminuria
Neuronal ceroid lipofuscinoses
Fabry disease
Fucosidosis
Mucopolysaccharidoses
Adult polyglycosan body disease

Peroxisomal disorders
Peroxisome biogenesis defects
X-linked adrenoleukodystrophy and adrenomyeloneuropathy
Bifunctional protein deficiency
Rhizomelic chondrodysplasia punctata
Acyl-CoA oxidase deficiency

Disorders of amino acid and organic acid metabolism
L-2 and D-2 hydroxyglutaric aciduria
Canavan disease
Methylmalonic aciduria
HMG coenzyme A lyase deficiency
Maple syrup urine disease
Nonketotic hyperglycinemia
Hyperhomocysteinemias
Phenylketonuria
Serine synthesis defects
Glutaric aciduria type I
Propionic acidemia
Urea cycle defects

Mitochondrial disorders
Leber's hereditary optic neuropathy
Mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes
Leigh syndrome
Mitochondrial neurogastrointestinal encephalomyopathy
Kearns-Sayre syndrome
Isolated deficiencies of respiratory chain complexes
Pyruvate dehydrogenase complex deficiency
Pyruvate carboxylase deficiency
Multiple carboxylase deficiency

Defects in genes encoding myelin proteins
Pelizaeus-Merzbacher disease

Defects of nuclear DNA repair
Cockayne syndrome
Trichothiodystrophy

Muscular dystrophies
Congenital muscular dystrophies
Myotonic dystrophy

Macro-/microdeletion syndromes
18q- deletions
11q- deletions
6p- deletions
Angelman syndrome

Defects in connexin genes
Oculodentodigital dysplasia
Hypomyelination with mutations in a gene encoding GJA12

Neurocutaneous disorders
Tuberosis sclerosis
Hypomelanosis of Ito
Neurofibromatosis type I

Other
Alexander disease
Vanishing white matter disease
Megalencephalic leukoencephalopathy with subcortical cysts
Hypomyelination with atrophy of the basal ganglia and cerebellum
Leukoencephalopathy with involvement of brain stem and spinal cord and high lactate
Aicardi-Goutières syndrome
Leukoencephalopathy with calcifications and cysts
Giant axonal neuropathy
Cerebrotendinous xanthomatosis
Lowe syndrome
Sjögren-Larsson syndrome
Wilson disease
Sulfite oxidase deficiency and molybdenum cofactor deficiency
Galactosemia
Hereditary diffuse leukoencephalopathy with neuroaxonal spheroids
Dentatorubropallidoluysian atrophy
Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy
Cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy
Nasu-Hakola disease
Pigmentary orthochromatic leukodystrophy
Adult autosomal dominant leukoencephalopathies

VWM is inherited in an autosomal recessive mode and is related to gene defects in eukaryotic translation initiation factor 2B (eIF2B) (67). eIF2B is a protein complex with five subunits encoded by five different genes (EIF2B1-EIF2B5). Missense mutations of any of these genes may cause VWM (129). eIF2B is required for protein synthesis initiation and regulation. Under stress conditions, as in fever, protein synthesis is in healthy individuals downregulated. This regulation is postulated to be deteriorated in VWM, leading to misfolding and denaturation of proteins (127). However, the association between eIF2B mutations and pathophysiological mechanisms of VWM remains poorly understood. Also unknown is why the cerebral white matter is predominantly affected since eIF2B is expressed in all cells of the body (127).

3.2 Megalencephalic leukoencephalopathy with subcortical cysts (MLC)

This autosomal recessive disorder is characterized by infantile onset macrocephaly and extensive cerebral white matter changes, with swelling and subcortical cysts found mainly in temporal regions (137). Considering the severe white matter changes, the initial manifestations are surprisingly mild, with normal or mildly delayed motor development. After an interval of several years, a slowly progressive ataxia and spasticity occur. Most children are wheelchair-dependent at the end of the first decade or in the second decade of life (9, 111, 121, 137). Epilepsy is common and seizure episodes may be triggered by minor head trauma (111). Most patients have normal or low-normal cognitive capacity. In some cases, mild cognitive deterioration occurs (111).

Little is known about the pathogenesis of MLC. Brain biopsy of one patient showed the presence of innumerable vacuoles and intense astrogliosis in the cerebral white matter. Electron microscopy revealed splitting of the outer lamellae of the myelin membrane (136). One gene related to MLC was identified in 2001 and named MLC1 (68). MLC1 encodes a membrane protein of unknown function. In the brain, MLC1 is expressed especially in astrocytic endfeets, but not in oligodendrocytes (117). This expression is concentrated at the blood-brain barrier and at the brain–CSF barrier, suggesting that MLC1 participates in the transport of ions or other substances (14, 117). Thus, astrocytic dysfunction might play a primary role in MLC. Mutations of MLC1 have been found in only 60-70% of patients with typical MRI findings (125) implying that more than one gene is involved. This disorder is more prevalent in an Indian Agarwal community (111) and in Turkey (121) than elsewhere.

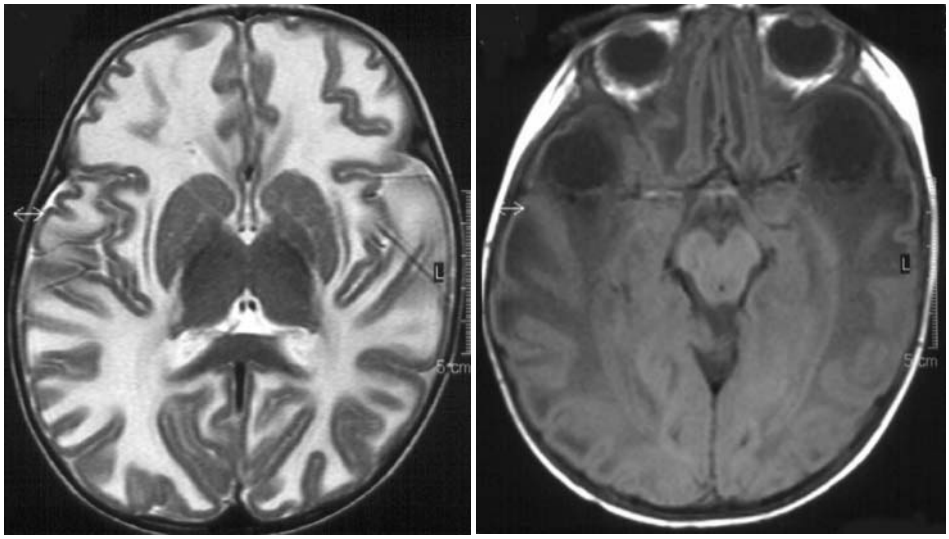

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Figure 5. *Brain MRI of a patient with MLC. The cerebral white matter appears diffusely hyperintense and swollen on a T2-weighted image (a). A T1-weighted image (b) shows the typical subcortical cysts at the temporal lobes.*

3.3 Hypomyelination with atrophy of the basal ganglia and cerebellum (H-ABC)

The diagnostic MRI pattern of this disorder consists of a combination of severe hypomyelination and progressive atrophy of the putamen, caudate nucleus, and cerebellum. Eight patients have been described to date (77, 130).

The majority of these patients had delayed motor development and onset of motor deterioration at the age of 2-6 years. They showed spasticity, ataxia, dysarthria, and prominent extrapyramidal movement abnormalities, including dystonia, choreoathetosis, and rigidity. All patients had learning difficulties or mental retardation, but no marked cognitive decline. In the severe form of the disease, the onset was within the first months of life, manifesting as poor visual contact, hypotonia, and absent motor development (77, 130). No underlying genetic defect has been identified.

3.4. Leukoencephalopathy with brain stem and spinal cord involvement and elevated white matter lactate (LBSL)

The first description of this new leukoencephalopathy published in 2003 (128) included eight patients with slowly progressive pyramidal, cerebellar, and often dorsal column dysfunction. Brain MRI was characterized by inhomogeneous signal abnormalities in periventricular and deep white matter in combination with selective involvement of certain brain stem and spinal cord tracts. The pyramidal tracts were shown to be affected over

their entire length. The sensory tracts, including dorsal columns, medial lemniscus, and corona radiata were also involved over their entire length. In addition, the tracts of the trigeminal nerve and cerebellar connections were selectively involved. MRS showed a significant decrease in NAA, an increase in myoinositol, normal or mildly elevated Cho, and elevated Lac within the white matter of all patients.

The pathophysiology of this disease is unknown, but based on MRS findings and involvement of the entire sensory and pyramidal tracts, the disease process has been proposed to primarily involve white matter axons (128) .

3.5 18q- syndrome

MBP is a major protein of the CNS myelin membrane. Mutations of the MBP gene have not been found in humans. However, the MBP gene region (18q23) is commonly deleted in patients with partial deletions of the long arm of chromosome 18. The phenotype in 18q- syndrome is variable, but neurologic features, including tremor, dyscoordination, oculomotor problems, and mental retardation, are commonly reported, as are short stature, foot deformities, midface hypoplasia, hypotonia, hearing impairment, atretic or stenotic external auditory canals, and genitourinary malformations (41, 72, 78, 113, 147).

Patients with 18q- syndrome are reported to have diverse cerebral white matter abnormalities, including delayed or incomplete myelination, periventricular and focal deep white matter lesions and poor differentiation of gray and white matter (78, 90, 114, 146). A previous study with 16 patients (72) found diffuse or focal white matter abnormalities in 60% of 18q- syndrome patients, but was unable to reveal a constant pattern of findings Another study, by contrast, showed a more consistent pattern, with incomplete myelination as a typical finding (41). In the latter study, a single patient with normal brain MRI had an interstitial deletion of 18q which spared the region for the MBP gene. Thus, haploinsufficiency of the MBP was suggested as the cause of the abnormal myelination (41).

The specificity of these MRI findings has been questioned (38) because white matter abnormalities are found in other chromosomal abnormalities as well, e.g. in partial deletions of chromosomes 11, 22, and 6 and in sex chromosomal disorders (40, 89, 145).

3.6 Leukoencephalopathy with calcifications and cysts

A rare disorder with leukoencephalopathy, extensive brain calcifications, and progressive intracranial cysts has been reported in six patients previously. Findings in brain biopsies have suggested that this is a microangiopathic disorder (64, 83). Similar white matter changes and calcifications, but no cyst formation, have been reported in several patients with bilateral retinal telangiectasias and exudations (“Coats’ disease”) (29, 45, 100, 103, 119). Some of these patients have also had other manifestations, including intrauterine

growth retardation, sparse hair, dysplastic nails, skeletal abnormalities, and pancytopenia, and have often been reported as cases of a "new" syndrome. Based on one patient with overlapping findings (both cysts and Coats' disease and calcifications and leukoencephalopathy), these disorders were proposed to be related (83).

4. Acquired disorders with cerebral white matter involvement

A thorough discussion of acquired white matter disorders (Table 3) is beyond the scope of this thesis. However, congenital cytomegalovirus (CMV) infection may cause white matter abnormalities, which are often confused with hereditary leukoencephalopathies, and this disorder is therefore discussed here.

4.1 Congenital cytomegalovirus infection

Cytomegalovirus is the most common cause of congenital viral infections throughout the world, occurring in about 1% of all live births (1). Approximately 10% of infected infants are symptomatic at birth. Common manifestations are petechiae, hepatosplenomegaly, jaundice, microcephaly, and chorioretinitis. However, 90% of infected infants are asymptomatic at birth, with 5-17% of these infants developing long-term sequelae (99). The long-term problems are nonprogressive and include sensorineural hearing deficit, learning problems, epilepsy, mental retardation, and motor deficits ranging from coordination problems to severe motor handicap.

To diagnose congenital CMV infection, the antibody tests should be performed within the first 3 weeks of life. Thus, in patients who were asymptomatic at birth, the diagnosis usually cannot be confirmed retrospectively. However, in most Western countries, the blood spots of neonates are collected on filter paper (Guthrie cards) and used for screening tests for treatable infantile diseases. Using stored Guthrie cards, CMV DNA can be tested by polymerase chain reaction (PCR). The typical pattern of white matter abnormalities caused by congenital CMV infections was recently elucidated in an elegant study, where congenital CMV infection was retrospectively confirmed by PCR (126). In milder cases, the suggestive MRI pattern was found to consist of multifocal lobar white matter lesions that were most pronounced in the parietal area and relatively spared the immediately periventricular and subcortical white matter (Figure 6). In more severe cases, there may be concomitant gyration abnormalities (polymicrogyria), and the white matter abnormalities may also be diffuse (Figure 7). In particular, abnormalities of the anterior temporal lobe, including swollen white matter and cysts, are suggestive of congenital CMV infection (126).

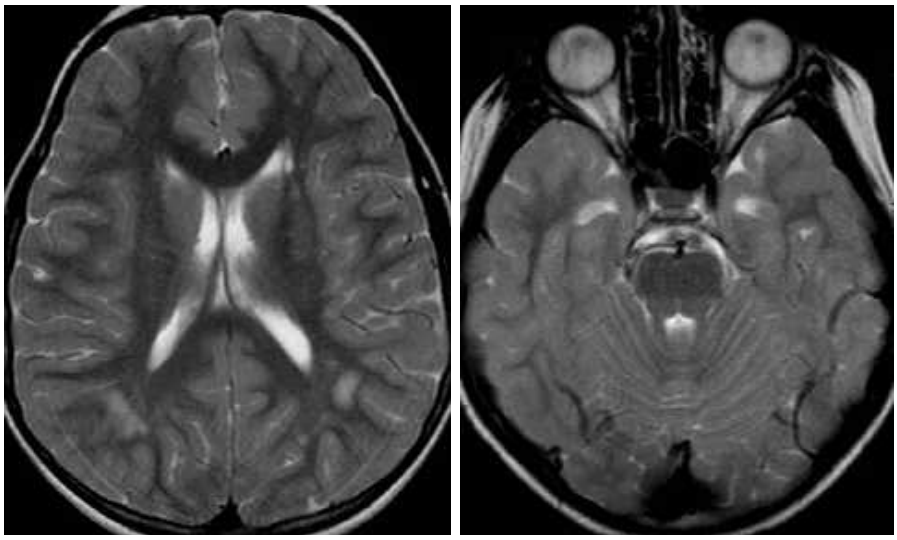

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Figure 6. *T2-weighted images of a 2-year-old girl show mild, patchy, multifocal white matter abnormalities in the lobar white matter (a) and in the anterior temporal lobe (b), a typical pattern for congenital CMV infection.*

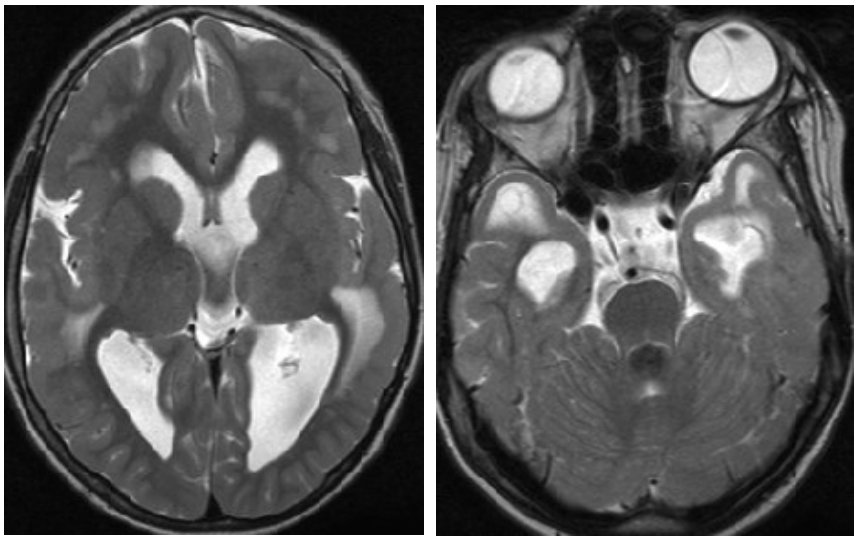

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Figure 7. *T2-weighted images of an 11-year-old girl with static encephalopathy and sensorineural hearing impairment show severe changes typical of congenital CMV infection. The gyration is abnormal (a) and diffuse white matter signal abnormalities are prominent, especially in the temporal lobes (b).*

Table 3. *Acquired disorders with cerebral white matter abnormalities in the pediatric age group.*

Noninfectious inflammatory disorders
Multiple sclerosis and variants
Acute disseminated encephalomyelitis
Systemic lupus erythematosus
Sarcoidosis
Vasculitis

Infectious inflammatory disorders
Subacute HIV encephalitis
Progressive multifocal leukoencephalopathy
Neuroborreliosis
Subacute sclerosing panencephalitis
Congenital cytomegalovirus infection
Brucellosis
Whipple disease
Other infections

Toxic-metabolic disorders
Endogenous and exogenous toxins
Central pontine myelinolysis
Salt intoxication
Vitamin B12 and folate deficiency
Malnutrition
Posterior reversible leukoencephalopathy

Hypoxic-ischemic disorders
Post-hypoxic-ischemic leukoencephalopathy

Traumatic disorders
Diffuse axonal injury

Other
Radiation damage

AIMS OF THE STUDY

The initial aim of this work was to classify patients with unknown cerebral white matter abnormalities and to identify new diseases among them. During the course of the study, three subgroups were delineated and examined further. The following subgoals were set:

a) to characterize clinical and MRI findings in patients with a novel leukoencephalopathy with brain stem and spinal cord involvement and high white matter lactate in MRS (LBSL) (Study I)

b) to determine whether any specific patterns of white matter abnormalities are present in patients with 18q deletions and to correlate these findings with molecularly defined size of the deletion (Studies II and III)

c) to describe clinical and radiological findings in patients with leukoencephalopathy, cerebral calcifications, cysts, and retinal vascular abnormalities (Study IV)

PATIENTS AND METHODS

1. Patients

1.1 Overview of leukoencephalopathies of unknown etiology (unpublished data)

Patients with white matter abnormalities, detected by MRI, were found through the following sources: 1) patients evaluated by MRI at the Department of Child Neurology, Hospital for Children and Adolescents, University of Helsinki, during 2000-2005, 2) patients sent for consultation from other hospitals in Finland or Estonia (n=3) due to white matter abnormalities, and 3) patients traced by reviewing the notes of neuroradiological meetings, held in years 1990-1999 in the above mentioned hospital.

Inclusion criteria were:
1. Sufficient medical records and available MRIs.
2. MRIs showing siqnificant white matter signal intensity abnormalities, including severely delayed myelination (i.e. myelination age of 6-8 months or less at patient's age of over 1 year).
3. In cases with combined pathology of white and gray matter, the white matter abnormality was predominant.

Excluded were cases with
1. A known diagnosis.
2. Only minor white matter involvement (slight delay in myelination or only a few isolated T2 hyperintense spots).
3. Predominantly gray matter involvement. These included cases where marked cerebral atrophy was accompanied by solely white matter volume loss, but not signal abnormality, and cases with severe gyration or migration abnormalities.

Forty-six patients fulfilled the above-mentioned criteria. Of these patients, two were deceased and six were not accessible or were unwilling to participate in further studies. The remaining 38 patients, including five sib-pairs, entered the study.

1.2 Leukoencephalopathy with brainstem and spinal cord involvement and elevated white matter lactate (Study I, unpublished data)

Among patients with undetermined leukoencephalopathy, six patients, including two siblings, with similar clinical and MRI features were identified. Five of the patients are described in Study I, after which one additional patient entered the study.

1.3 18q deletions (Studies II and III)

Among patients with undetermined leukoencephalopathy, one patient and his two first-degree relatives were found to have a partial deletion of the long arm of chromosome 18. An additional 19 patients with 18q deletions, diagnosed previously at our clinic or identified through cytogenetic laboratories, participated. The age range of patients (13 males, 9 females) was 0.7- 51 years, and their 18q deletions were caused by terminal de novo deletions (eight patients), inherited deletions (two families each with an affected parent and two affected children) or unbalanced translocations (eight patients from three families). All patients participated in the MRI study (Studies II and III) but patients with translocations were excluded from phenotype-genotype correlation analyses.

1.4 Leukoencephalopathy with cerebral calcifications (Study IV)

Thirteen patients with leukoencephalopathy combined with extensive cerebral calcifications were not included in the overview of unknown leukoencephalopathies, but were identified through the following sources: 1) they were former or current patients of the Hospital for Children and Adolescents, 2) their MRIs were sent for consultation from other hospitals in Finland, or 3) they were patients with a similar phenotype identified by the Departments of Pediatrics and Pathology, University of Oulu. Five of the patients participated in the prospective study. In addition, medical records and neuropathological specimens of eight patients with a similar phenotype, seven of whom were deceased, were reviewed.

1.5 Ethical aspects

Because most of the subjects were children and/or had developmental problems, they could not provide informed consent. Diseases of this category cannot, however, be investigated in any other patient group and the findings are likely to benefit both these subjects and future patients. Except for the blood samples for DNA studies in patients with 18q deletions, the investigations performed had clinical as well as scientific indications.

The study protocol was approved by the Ethics Committee of the Hospital for Children and Adolescents, University of Helsinki. All parents or patients provided written consent prior to investigations being performed.

2. Methods

2.1 Clinical evaluation

A general physical examination and a neurological examination were performed by the author on all patients. Patients and parents were interviewed and previous clinical data reviewed. All patients of the Overview, Study I, and Study IV underwent an ophthalmologic examination.

2.2 Neurophysiological examinations (Overview and Study I)

Motor and sensory nerve conduction velocity (NCV) studies were available for 36 patients. Studies of 33 patients were performed in our hospital (17 examinations during this study), and three studies recently performed in other hospitals were reviewed. The results were interpreted using age-dependent reference values from the literature (79, 92). The type of neuropathy was judged as predominantly demyelinating in cases with abnormally slow NCVs and predominantly axonal in cases with abnormally low sensory or motor amplitudes, or if NCV was only slightly slowed despite evidence of denervation.

Somatosensory evoked potentials (SEP) of 32 patients were recorded. Thirty studies were performed in our hospital (27 examinations during this study), and two studies performed elsewhere were reviewed. SEPs were recorded with median nerve stimulation in 28 patients and with tibial nerve stimulation in 31 patients (four limb study in 27 patients). Results of EEG studies were available for 37 patients. (25 studies in our hospital, 15 of them during this study, and 12 studies elsewhere).

2.3 Exclusion of known white matter disorders (Overview, Study I)

Previous laboratory work-up of the patients was reviewed and complemented as needed to exclude, as far as possible, hereditary disorders known to be associated with white matter abnormalities (Table 4). If not examined previously, the following investigations were performed on all undiagnosed patients (or one of the siblings): basic chemical and hematological studies (blood count, blood gases, electrolytes, anion gap, glucose, transaminases, creatinine, thyroid function, calcium homeostasis, creatine kinase, ammonia), cholesterol, lactate, pyruvate, total and free carnitines, B12-vitamin, folate, very long chain fatty acids, phytanic acid, vacuolated lymphocytes, desialotransferrin, amino acids in plasma and urine, organic acids, oligosaccharides (including free sialic acid), and glycosaminoglycans in urine. Lysosomal enzyme activities to exclude MLD and Krabbe disease were performed on all patients except for two patients with improving MRI findings. For 17 patients, also lysosomal enzymes related to GM1 and GM2 gangliosidosis were examined. If clinically indicated, further studies were performed,

including blood levels of copper and ceruloplasmine, uric acid, acylcarnitines, biotinidase activity, karyotype, FISH studies (to exclude 18q- syndrome or Angelman syndrome), pyruvate metabolism in cultured fibroblasts, muscle or nerve biopsy, respiratory chain enzyme activites, and DNA studies (to diagnose Salla disease, PMD, VWM, Alexander disease, INCL, or mitochondrial mutations and deletions). In 20 patients, CSF was investigated for cells, glucose, protein, lactate, pyruvate, IgG-index, oligoclonal bands and amino acids. An additional 13 patients had had a previous CSF study, with measurements of at least cells, protein, and lactate.

2.4 Exclusion of other disorders with cerebral calcifications (Study IV)

The extensive cerebral calcifications of patients in Study IV necessitated investigations, including calcium homeostasis, parathyroid function, vitamin D-25, liver function, lactate, pyruvate, B12-vitamin, folate, plasma amino acids, urine organic acids, and antibodies related to coeliac disease.

2.5 MRI studies

Brain MRIs were performed on a 1.5T MRI unit with at least axial T2-weighted, FLAIR, and sagittal T1-weighted images. In the Overview, Study I and Study IV, three patients had only one brain MRI study; all others had two or more MRI studies. Five patients (Study I) also underwent spinal MRI with sagittal T2-weighted fast spin-echo images. Brain MRI was performed only once in 18q- deletion patients: 15 studies were performed prospectively, and in seven cases previous MR images were reviewed.

2.6 MRI classification

MRIs were classifed using a method developed previously (133), but the subgrouping of Category A was slightly modified. The imaging findings were grouped into seven major categories according to the predominant location of the white matter abnormalities, using a scoring list of 68 items (Appendix 1). The categories were:

A. Myelination abnormalities

A1. Severe hypomyelination. No myelin visible on T2- or T1-weighted images

A2. Moderate hypomyelination. No or very little myelin on T2-weighted images, but a substantial amount of myelin signal on T1-weighted images.

A3. Other myelination abnormalities. Severely delayed (myelination age <6-8 months in children >12 months of age), arrested, or irregular myelination (myelination does not progress evenly in all cerebral lobes or zones).

Table 4. *Laboratory work-up in leukoencephalopathies.*

Examination	Relevant in diagnosis of
Blood	
creatine kinase	mitochondrial disorders, metabolic myopathies
ammonia	urea cycle defects, organic acidurias
lactate, pyruvate	mitochondrial disorders, organic acidurias
total and free carnitine	mitochondrial disorders, organic acidurias
B12 vitamine, folate	disorders of B12 and folic acid metabolism
very long chain fatty acids, phytanic acid	peroxisomal disorders (X-ALD, Refsum disease)
cholesterol and cholestanol	cerebrotendinous xanthomatosis
biotinidase activity	biotinidase deficiency
vacuolated lymphocytes	lysosomal storage disorders (AGU, fucosidosis, infantile GM1 gangliosidosis)
amino acids and homocysteine	amino acidopathies, urea cycle defects, hyperhomocysteinemias, mitochondrial disorders
copper, ceruloplasmine	Wilson disease
desialotransferrin	CDG syndromes
urate	molybdenum cofactor deficiency
karyotype and FISH studies	chromosomal abnormalities (18q-, 11q-, 6p- deletions, sex chromosomal disorders, Angelman syndrome)
CSF	
protein	infantile Krabbe disease, MLD, MNGIE
lactate, pyruvate	mitochondrial disorders
IgG index, oligoclonal bands	immune-mediated diseases
amino acids	amino acidopathies (nonketotic hyperglycinemia)
Urine	
oligosaccharides	lysosomal storage disorders (Salla disease, AGU, GM1 and GM2 gangliosidosis, fucosidosis)
glycosaminoglycans	mucopolysaccharidoses (multiple sulfatase deficiency)
organic acids	organic acidurias (Canavan disease, L-2- and D-2-hydroxyglutaric aciduria, glutaric aciduria), hyperhomocysteinemias, mitochondrial disorders, aminoacidopathies
Enzyme activities in fibroblasts or leukocytes	
galactocerebrosidase	Krabbe disease
arylsulfatase A	metachromatic leukodystrophy, multiple sulfatase deficiency
betagalactosidase	GM1 gangliosidosis
hexosaminidase A and B	GM2 gangliosidosis
DNA studies	e.g. Pelizaeus-Merzbacher disease, Alexander disease, Salla disease, VWM, INCL, deletions and mutations of mitochondrial DNA
muscle biopsy	mitochondrial disorders, congenital muscular dystrophies
nerve biopsy	giant axonal neuropathy, other axonal neuropathies
skin biopsy	CADASIL, Salla disease

B. Global cerebral white matter abnormalities

- all cerebral lobes are involved
- all cerebral white matter zones are involved, but minimal sparing of U-fibers is accepted

C. Extensive cerebral white matter abnormalities combined with involvement of the putamen

- frontal predominance or global involvement of cerebral lobes
- not all zones of the cerebral white matter necessarily involved (U-fibers may be spared)

D. Predominantly periventricular white matter abnormalities

E. Predominantly lobar white matter abnormalities

F. Predominantly subcortical white matter abnormalities

G. Predominantly posterior fossa white matter abnormalities

With the majority of patients being children or adolescents, clearly enlarged sulci were considered a sign of atrophy.

MR images were reviewed simultaneously by two radiologists experienced in child neuroradiology (Doctors Valanne and Autti) and one child neurologist (the author), and in case of disagreement, a consensus of interpretation was reached by discussion.

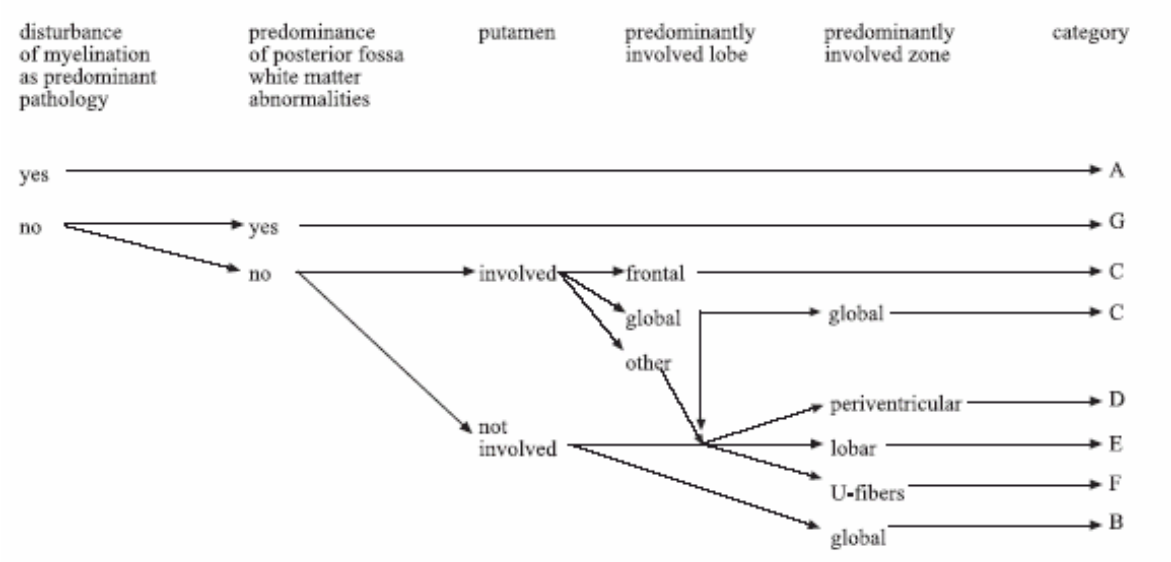


Figure 8. *Decision tree used to categorize patients with unclassified leukoencephalopathies, according to van der Knaap et al.* (133).

2.7 Quantitative MRI signal analysis

To strengthen the visual analysis of MRIs of patients with 18q deletions, the signal intensities of the white matter and of the deep gray matter were measured quantitatively on T2-weighted images in 11 patients and in 11 healthy, age-matched controls, using a previously described method (4). The measured values were related to the signal intensity of the CSF. Three patients under 10 years of age were excluded from the statistical analysis because CSF spaces of the controls were too narrow to be measured. Mann-Whitney U-test was used in statistical analyses.

2.8 MRS

MRS studies were performed with two different 1.5 MR units. 3D proton magnetic resonance spectroscopic imaging (^{1}H-MRSI) was performed on 8 patients (age 3.3-30 years) and a single voxel spectroscopy on 12 patients (age 2-21 years). 3D MRSI consisted of a double spin-echo (SE) spectroscopic sequence with 16 x 16 phase encoding steps (repetition time [TR] 1500 ms; echo time [TE] 288 ms) and eight 10-mm slices with two acquisitions. Volume preselection was 100 x 100 x 80 mm^3. In Study I, the white matter metabolite intensity was sampled as the average of eight voxels. For comparison of signal intensities, the spectra of individuals were corrected for coil loading and volume of interest (VOI) size, and values were given in institutional units. Single voxel spectra were measured from 8 cm^3 volumes in the frontoparietal white matter, with stimulated echo sequences (TR 3000 ms; TE 270 ms).

At the beginning of the study, the length of the MRS examination was 1.5 hours. In agreement with the recommendation of the local ethics committee, MRS was performed only on patients who did not need to be sedated. Towards the end of the study, the length of the MRS had considerably decreased, allowing us to prolong MRI sedation to obtain MR spectra. Six healthy 11- to 23-year-old controls were used for 3D measurements and six healthy 11- to 18-year-old controls for 1D measurements. For practical and ethical reasons, the examination of young, healthy children was not possible. Age-related changes in MRS, from the literature (96, 139), were used to assess the spectra of the younger children.

2.9 Analysis of chromosome 18 deletions (Studies II and III)

Blood samples of patients with 18q deletions and both parents, if available, were obtained and genomic DNA was extracted from the blood leukocytes using a standard method. The size of the deletion was determined using segregation analysis with microsatellite markers mapping to 18q. The order and location of the markers were based on those of the databases of the National Center for Biotechnology information (www.ncbi.nlm.nih.gov) and the UCSC Genome Browser (www.genome.ucsc.edu). A tetranucleotide repeat 5´to the myelin basic protein (MBP) gene was analyzed (MBP-STR-A/B) as previously

described (94, 118). All markers were PCR amplified and for allele determination, the amplified DNA samples were separated on a nondenaturing polyacrylamide gel and run for 8000 Vh. Two patients had large regions of uninformative telomeric markers, and thus, a subtelomeric FISH of 18q was performed to exclude an interstitial deletion. A multicolor FISH was performed on one patient to exclude translocation.

2.10 Neuropathological evaluation (Study IV)

Six patients of Study IV were previously autopsied. Neuropathological samples were re-examined (by Doctors Paetau and Herva), as were brain biopsies of two patients. Immunohistochemical stainings with antibodies against the endothelial marker CD31, the basement membrane marker laminin, and smooth muscle actin were performed in one.

RESULTS

1. Overview of undetermined leukoencephalopathies

1.1. Clinical findings

The onset of symptoms was during the first year of life in 24 patients (63%). The predominant symptoms at presentation (Table 5) differed between infants and older children. Infants presented with hypotonia, nystagmus, "progressive CP" of spastic or dystonic type, seizures, or "encephalopathy" (irritability, hypertonus, poor visual contact). Older children and adolescents, in turn, presented with ataxia, tremor, distal spasticity, developmental delay, or neuropsychiatric symptoms.

Table 5. *Predominant manifestation at presentation.*

Manifestation	**No. of patients (%)**
Spasticity or dystonia	13 (34)
Ataxia	11 (29)
Developmental delay +/- seizures	7 (18)
Severe hypotonia +/- nystagmus	5 (13)
Neuropsychiatric symptoms	2 (5)

The symptoms were progressive in 27 (71%) patients. Six of them had an episode of acute deterioration, often in conjunction with a trivial viral infection, and recovery was only partial. In two patients with severe hypomyelination, the appearance of distal spasticity was taken as a sign of progression, even if ataxia and nystagmus had decreased. Eleven patients (29%) had static neurological signs.

Clinical findings are summarized in Table 6. Over the course of the disease, 58% of patients developed spasticity and 39% ataxia. Seizures occurred initially in five of 38 patients (13%), but epilepsy eventually affected 39% of patients. In 42% of patients, cognitive development was hampered early in the course of the disease and led to mental retardation. Learning disabilities were present in 32% of patients. In 13% of patients, decline in cognitive skills was evident. However, in most cases, motor problems were more pronounced than cognitive deficits.

Ophthalmological investigation revealed squint in 21% and rotatory or pendular nystagmus in 16% of patients. Typically, nystagmus was severe in infancy, but ameliorated during the first years of life. Optic nerve atrophy was observed in three patients (8%), and two patients had severe visual impairment due to central deficits. Subtle

irregularities of retinal pigmentation were present in three patients. One patient had severe myopia.

Audiometry was performed during or prior to the study on 28 patients with adequate co-operation. Three patients had sensorineural hearing impairment. One was diagnosed with 18q- deletion, and another had MRI findings suggestive of intrauterine CMV infection.

NCV studies showed signs of peripheral polyneuropathy in 11 of 36 patients (31%). The type of neuropathy was predominantly demyelinating in four patients and predominantly axonal in seven patients. SEP was abnormal in 20 of 33 patients (61%), showing low or absent cortical responses in 18 patients and delayed CNS conduction in 10 patients. One patient had asymmetric cortical responses and another had abnormally high cortical responses in median nerve stimulation. EEG was abnormal in 27 of 38 patients (71%). A background abnormality was found in 17 patients, accompanied by epileptiform discharges in 11 patients. Eight patients had epileptiform discharges with normal background activity.

Table 6. *Clinical findings of 38 patients with undetermined leukoencephalopathy.*

Clinical findings	**No. of patients (%)**
Truncal hypotonia	22 (58)
Spasticity or dystonia	22 (58)
Cognitive problems:	
Early mental retardation	16 (42)
Learning disorder	12 (32)
Cognitive decline	5 (13)
Ataxia	15 (39)
Seizures	15 (39)
Polyneuropathy	11/36 (29)
Tremor	9 (24)
Dysmorphic features	9 (24)
Squint	8 (21)
Nystagmus	6 (16)
Optic atrophy	3 (8)
Hearing impairment	3 (8)

1.2 MRI classification and comparison with clinical findings

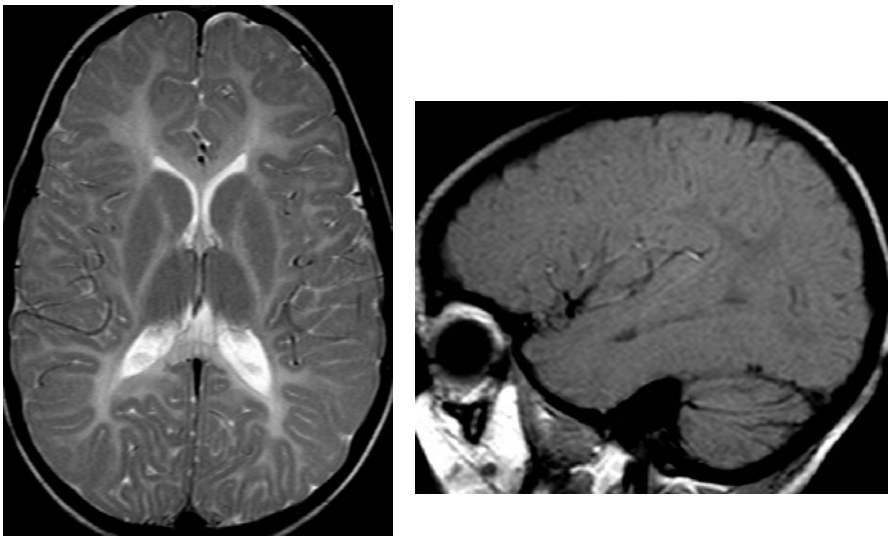
The MRIs were classified into seven categories (Table 7). The largest groups were category A with abnormal myelination (53%) and category D with periventricular abnormalities (32%). No MRIs were grouped into categories F or G (predominantly subcortical or posterior fossa abnormalities).

Table 7. *MRI classification and established diagnoses within different categories.*

Category	No. of patients	Established diagnoses (n)
A1	3	Salla disease (1)
A2	4	H-ABC (1)
A3	13	18q deletion (1)
B	1	
C	1	Alexander disease (1)
D	12	LBSL (6)
E	4	
F	0	
G	0	
Total	38	

H-ABC, hypomyelination with atrophy of the basal ganglia and cerebellum; LBSL, leukoencephalopathy with brainstem and spinal cord involvement and elevated lactate

Category A1. **Severe hypomyelination** (Figure 9). Patients with severe hypomyelination lacked additional lesions in MRI, only minor white matter volume loss was observed in follow-up. The myelin deficiency remained unchanged in repeated examinations. These patients presented in infancy with severe hypotonia and delayed motor development. Two of three patients had severe rotatory or pendular nystagmus, with ataxia manifesting later. Positive psychomotor development was seen in childhood with decreasing nystagmus and ataxia, but in two patients distal spasticity evolved (at ages of 2 and 16 years). All patients were mildly to moderately mentally retarded.



a b

Figure 9. *Category A1. Axial (a) and sagittal (b) image of a 4-year-old boy. White matter signal intensity is high on the T2-weighted image (a) and low on the T1-weighted image (b), consistent with severe hypomyelination.*

Category A2. Moderate hypomyelination (Figure 10). This group had deficient myelination in the cerebrum. The cerebellum showed a better state of myelination in three of four patients. Additional findings were cerebellar atrophy in two patients, one of whom also had cortical atrophy. Two patients had basal ganglia lesions (calcifications in one and atrophy in one patient). Clinically, all four patients in this category had slowly progressive neurological signs. Two of four patients presented in infancy with hypotonia, nystagmus, and psychomotor retardation, with a later onset of spasticity. Two patients (siblings) had always been clumsy. They presented at mid-childhood with ataxia and tremor, and mild cognitive decline became evident during follow-up.

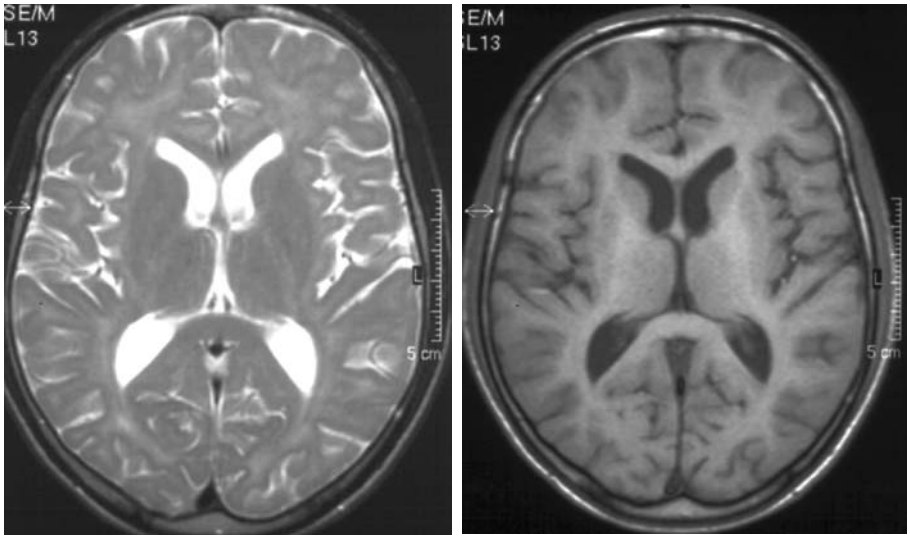


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Figure 10. *Category A2. Moderate hypomyelination. Axial T2-weighted image (a) of a 17-year-old boy shows deficient myelination. On T1-weighted images (b), the white matter signal is high, indicating that small amounts of myelin are present.*

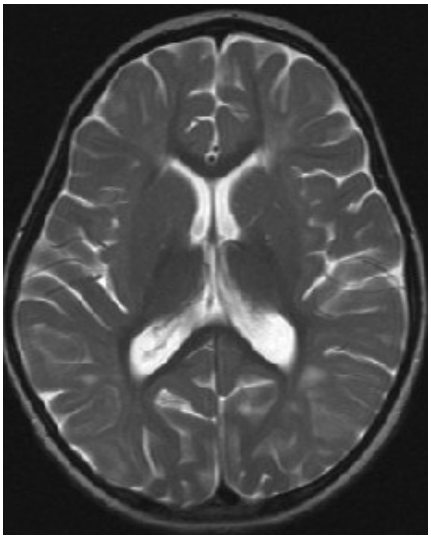
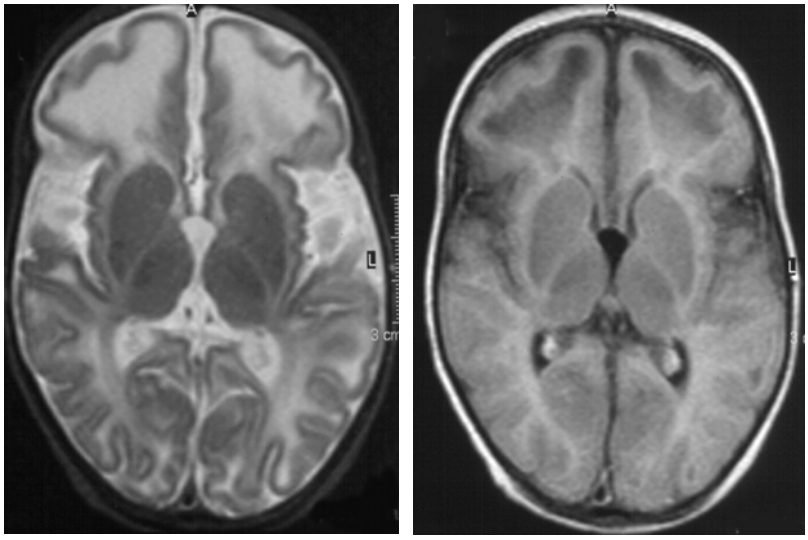


Figure 11. *Category A3. An axial T2-weighted image of a 4-year-old girl shows patchy and irregular myelination.*

Category A3. Delayed, arrested, or irregular myelination (Figure 11). Both imaging and clinical findings were very heterogeneous in this group. In addition to myelination abnormality, other types of pathology were common. Nine of 13 patients (69%) had atrophic findings; cerebellar atrophy in seven and cortical atrophy in five patients, with white matter volume loss in three of them. One patient had basal ganglia calcifications. The most common clinical

manifestations within this group were mental retardation, truncal hypotonia, spasticity, epilepsy, and ataxia. Two patients showed substantial progress in myelination during follow-up, thus representing delayed myelination. These patients had static neurologic signs (infantile-onset epilepsy and learning disability with dysmorphism).

Category B. Global involvement of cerebral white matter (Figure 12). The MRIs of only one girl belonged to this group. She presented at the age of 1 month with irritability, developmental regression, and dystonia, subsequently developing epileptic spasms, optic atrophy, and demyelinating polyneuropathy.



a b

Figure 12. *Category B. Axial images of a 3-month-old girl. The cerebral white matter is globally hyperintense on the T2-weighted image (a) and partly hypointense on the FLAIR image (b), consistent with rarefaction.*

Category C. Frontal white matter involvement with basal ganglia abnormalities (Figure 13). This pattern is consistent with Alexander disease. The one child in this group presented with prolonged seizure, psychomotor regression, and spasticity at the age of 9 months. She was normocephalic.

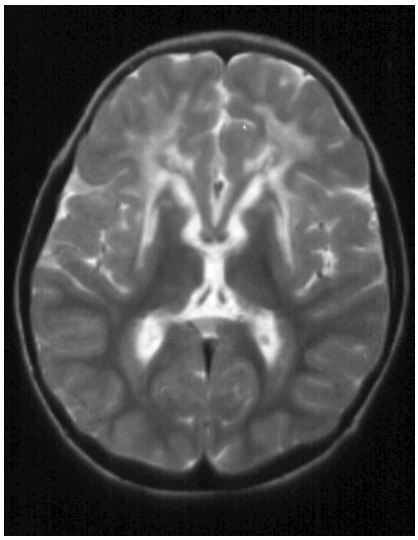


Figure 13. *Category C. An axial T2-weighted image of a 4-year-old girl. The white matter abnormality is predominantly frontal. The basal ganglia are atrophic and show signal abnormalities.*

Category D. Predominantly periventricular white matter abnormalities (Figure 14). Six patients in this category showed a unique imaging pattern, described in detail in Study I. The remaining six patients had heterogeneous MRI- and clinical findings, with additional basal ganglia abnormalities (calcifications or atrophy) in two, cortical atrophy in two, and cerebellar atrophy in one patient. Three patients had reduced white matter volume. Three of six patients, including two siblings, presented with acute neurologic deterioration during infectious disease.

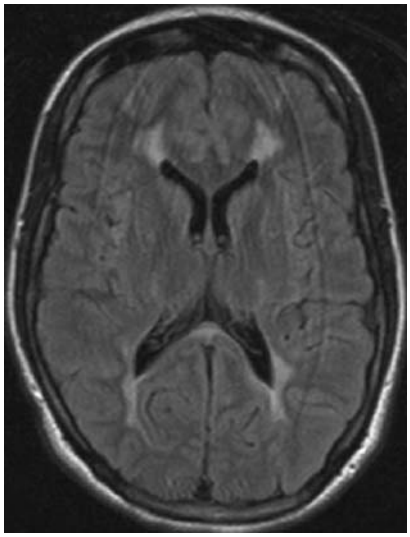


Figure 14. *Category D. An axial FLAIR image shows hyperintensity predominantly in the periventricular areas.*

Category E. Predominantly lobar white matter abnormalities (Figure 15) without additional lesions elsewhere. All patients in this category had static neurological signs, including delayed psychomotor development, neuropsychiatric symptoms, dysmorphic features, and/or sensorineural hearing impairment.

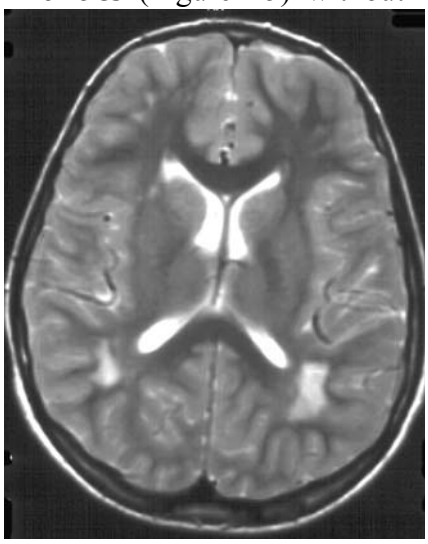


Figure 15. *Category E. An axial T2-weighted image of an 8-year-old girl shows hyperintense, patchy lesions in the lobar white matter. The subcortical fibers and periventricular areas are spared.*

1.3 MRS findings

MRS was performed only once in all 20 patients studied, and thus, changes related to the stage of the disease could not be assessed. ^{1}H-MR white matter spectra were normal in only two patients with hypomyelination (Table 8). Patients with LBSL (# 14-18 in Table 8) showed a consistent pattern of findings, with decreased NAA and presence of lactate, discussed in detail in Study I. The findings of the other patients were more variable. Cho was increased in 10 patients, with a concomitant increase in Cr in eight patients. NAA was decreased in 13 patients. One patient with dysmyelination had distinct findings, with severely decreased Cho and mildly decreased NAA and Cr. A Lac peak was seen in eight patients.

Table 8. *Findings of 1H-MR spectra from the affected white matter (long echo time).*

Patient	MRI category	Age at MRS	NAA	Cr	Cho	Lac
1	A1	4y10m	=	=	=	-
2	A2	3y4m	↓↓	↑	↑↑	+
3	A2	12y	(↑)	↑	=	-
4	A2	20y	=	=	=	-
5	A3	4y8m	(↓)	(↓)	↓↓	-
6	A3	5y10m	↓	=	=	-
7	A3	8y7m	=	↑↑	↑	+
8	A3	16y	↓↓	(↓)	(↑)	-
9	C	4y	↓↓	=	↑↑	++
10	D	1y9m	=	↑↑	=	-
11	D	2y1m	=	↑	↑↑	-
12	D	3y8m	=	↑↑	↑↑	-
13	D	7y4m	↓↓	=	↑↑	-
14	D	9y	↓	=	=	+
15	D	12y	↓↓	=	↑	+
16	D	13y	↓	=	=	+
17	D	16y	↓	=	=	+
18	D	30y	↓↓	=	=	+
19	E	13y	↓	↑	↑	-
20	E	16y	↓	↑	↑	-

(↑)/(↓) possible increase/decrease; ↑/↓ mild increase/decrease; ↑↑/↓↓ severe increase/decrease

1.4 Diagnosed cases and results of laboratory examinations

During the study, a specific diagnosis was established in 10 patients (Table 7).
1. Salla disease was diagnosed in one patient (Category A1), previously missed due to erroneously normal findings of urinary oligosaccharides (70).
2. One patient with hypomyelination (Category A2) developed atrophy of the putamen, caudate nucleus, and cerebellum, a MRI pattern consistent with H-ABC (130).
3. One patient (Category A3) with a partial deletion of chromosome 18q was diagnosed using a prometaphase study and 18q subtelomere FISH.
4. A mutation of the glial fibrillary acidic protein (GFAP) gene was found in the only patient in Category C, confirming the clinical diagnosis of Alexander disease.
5. Six patients with uniform clinical and MRI findings (LBSL) are described in Study I.

DNA studies for Pelizaeus-Merzbacher disease in males with severe hypomyelination were normal. Despite a MRI pattern consistent with VWM disease for the only patient in category B, no mutations of the five subunits of eIF2B genes were found. In category E,

one patient had an imaging pattern highly suggestive of congenital CMV infection. She had also suffered neonatal thrombocytopenia and had sensorineural hearing impairment consistent with congenital CMV infection, but retrospectively, the diagnosis could not be confirmed.

One patient in Category D had a homozygous SCAD (short-chain acyl CoA dehydrogenase) gene variation which explained her elevated urinary ethylmalonic acid, but whether this caused all her neurologic findings is uncertain since asymptomatic individuals also may carry this variation (28).

Five patients in categories C and D had a slightly increased IgG index in the CSF. In patients with elevated lactate or pyruvate levels, further studies for mitochondrial disorders were inconclusive. A muscle biopsy was performed on 8 patients during the study and had been performed previously on 9 patients. It was normal in 10 patients, showed signs of neurogenic damage in three patients, and revealed mild steathosis in two patients. Two patients had a few clusters of compact mitochondria, warranting further studies of mitochondrial disorders. Sural nerve biopsies (3 patients) showed normal findings for one patient and axonal neuropathy for two patients.

2. Leukoencephalopathy with brain stem and spinal cord involvement and elevated white matter lactate (LBSL) (Study I)

2.1 Clinical findings

Six patients with uniform MRI and clinical findings were identified among patients with undetermined leukoencephalopathies. The children learned to walk unsupported at the age of 10-18 months, but three of them had always had a slightly unsteady gait (Table 9). Neurologic symptoms manifested at the age of 3-10 years, with tremor or ataxic gait. The symptoms were slowly progressive. By adolescence, distal spasticity and/or deficits in proprioception and vibration sense were evident. In four patients, the main disability was due to sensory ataxia, while distal spasticity predominated in one patient. The adult patient had a 20-year history of symptoms. She needed support in walking since the age of 20. At 31 years of age, she had severe sensory ataxia, but was still ambulant. In one patient (#3), MRI was performed at the age of 6 years because of nocturnal seizures. He was still asymptomatic and had a normal neurologic examination at the age of 13 years. The general cognitive level of the patients was normal, but three patients had learning problems.

Table 9. *Clinical findings of six patients with LBSL.*

Characteristic	**Patient**					
	1	2	3	4	5	6
Affected/ unaffected siblings	0/1	1/0 (Pat. 3)	1/0 (Pat. 2)	0/1	0/2	0/2
Age/gender	30/f	16/m	13/m	12/f	11/f	15/m
Initial symptoms /age at onset	ataxia, tremor/10y	always unsteady, tremor/ 2.5y	seizures /6y	always unsteady, ataxia, tremor/3y	always unsteady, ataxia, tremor/9y	tremor, headache /10y
Progression of symptoms	dysarthria/ 11y distal spasticity/ 15y	ataxia, distal spasticity /16y	-	dysarthria/ 10y distal spasticity/ 12y	-	ataxia, distal spasticity /14y
Romberg sign	++	+	-	+	+	(+)
Sensory function	vibration sense↓/16y	vibration sense↓/ 16y	normal	vibration sense ↓↓ position sense ↓	vibration sense↓ position sense↓	normal
Deep tendon reflexes	↑	↑	normal	↓	↑	↑
Babinski sign	+/13y	+/16y	-	+/12y	-	+/14y
Cognitive function	visuo-spatial deficits	normal	normal	learning problems	normal	visuo-spatial deficits
Axonal neuropathy	+	-	-	+	+	+
Medianus SEP	low cortical responses	normal	normal	normal	normal	normal
Tibialis SEP	delayed central conduction	low cortical responses	normal	delayed central conduction	delayed central conduction	normal

2.2 Neurophysiological findings

Nerve conduction examination was mildly abnormal in four of six patients (Table 9), consistent with predominantly sensory axonal neuropathy in three of them and predominantly motor axonal neuropathy in one patient. SEPs with tibial nerve stimulation showed delayed central conduction in three patients and low cortical responses in one. SEPs with median nerve stimulation were normal, except in one patient who had low cortical amplitudes. Two patients had midtemporal spikes in EEG; in the others, EEG was normal.

2.3 MRI and MRS findings

All patients had lesions in periventricular and deep white matter. They were hyperintense in both T2 and FLAIR images and varied from patchy and inhomogeneous to confluent. The subcortical U-fibers were spared. The posterior limbs of the internal capsules and corona radiata were affected in all patients and the posterior part of the corpus callosum in five patients, while the anterior part was totally or mostly spared. The subcortical cerebellar white matter was affected in four patients.

In the brainstem, the pyramidal tracts, mesencephalic trigeminal tracts, and superior and inferior cerebellar peduncles were involved, and in some cases also the anterior spinocerebellar tracts and transverse pontine fibers. In follow-up, the brainstem abnormalities progressed slightly in three patients, and minor white matter volume loss occurred in two patients.

The spinal cord was hyperintense in T2-weighted images. In two patients with axial images, this hyperintensity could be localized to the dorsal columns and to the lateral corticospinal tracts.

In all five patients studied by ^{1}H-MRS, the frontoparietal white matter spectra showed elevated Lac, reduced NAA and normal Cr. Cho was elevated in only one patient. Cortical and thalamic gray matter spectra were normal.

3. Deletions of chromosome 18q (Studies II and III)

3.1 Clinical findings in patients with terminal and interstitial deletions of 18q (Study III)

The phenotype of patients with 18q deletions was highly variable. The clinical findings are summarized in Table 10 and presented in detail in Study III. Among common problems in infancy were hypotonia, delayed psychomotor development, nystagmus, recurrent

respiratory infections, and atopic disorders. Most patients had slightly dysmorphic facial features and deformities or anomalies of the hands or feet. Half of the patients had atresia or stenosis of the external auditory canals, and 43 % had hearing impairment. During childhood, common problems were motor clumsiness, wide-based gait, poor coordination, and difficulties in balance and visuomotorics. Only a few patients had epilepsy with complex partial and generalized seizures. Cognitive function ranged from normal IQ to severe mental retardation. The two affected parents had visuo- and fine motor deficiencies, but IQ within the normal range. The neurologic symptoms were nonprogressive.

EEG had been performed on 11 individuals. Three had epileptiform activity and five had minor background abnormality. Motor and sensory conduction velocities of the peripheral nerves, investigated in two patients, were normal despite very weak deep tendon reflexes.

Table 10. *Most common clinical features in patients with terminal and interstitial 18q deletions.*

Feature	**No. of patients**
Small for gestational age	7/14
Height below –2 SD	7/14
Facial dysmorphism (flat midface, down-turned corners of mouth, malformed earlobes, hypotelorism, short neck)	12/14
Aural atresia/stenosis	7/14
Palatal abnormalities (cleft lip/palate, bifid uvula)	4/14
Cryptorchidism or micropenis	5/8
Hand anomalies (proximal thumbs, tapering fingers)	9/14
Foot deformities (overriding toes, club foot, pes cavus, vertical talus)	8/14
Hyperextensible joints	6/14
Hernias (umbilical or inguinal)	4/14
Frequent respiratory tract infections	8/14
Atopic disorders	8/14
IgA deficiency	2/7
Motor clumsiness/dyscoordination	12/14
Hypotonia	10/14
Dysmyelination	10/14
Mental retardation	8/14
Weak/absent deep tendon reflexes	7/14
Nystagmus	6/14
Hearing impairment	6/14
Oral dyspraxia	5/14
Squint	4/14
Autistic features	4/14

3.2 Phenotypic variation within families

Interestingly, manifestations of 18q- deletions varied between members of the same family, as demonstrated by Family I (Table 11). Despite similar-sized deletions, the presence of e.g. palatal and aural abnormalities, foot deformities, and mental retardation was variable. However, the dysmyelination phenotype did not vary within families. Evidence for mosaicism, which could explain this variation, was not detected in 50 analyzed metaphases of the mother.

Table 11. *Phenotypic variation within a family (Family I, Study III)*

Feature	**Son**	**Son**	**Mother**
Age, years	2.8	12	36
Height, SD	-2.5	-1.8	-2.8
Facial dysmorphism	+	+	—
Aural atresia/stenosis	+	+	—
Cleft lip/palate or bifid uvula	+	+	—
Hand anomaly	+	+	—
Foot deformity	—	+	+
Umbilical or inguinal hernia	—	+	+
Atopic disorders	+	+	+
Hypothyreosis	—	—	+
Dyscoordination	+	+	+
Hypotonia	+	+	—
Dysmyelination	+	+	+
Mental retardation	++	++	—
Nystagmus	+	+	+
Hearing impairment	+	+	—
Strabismus	+	+	+
Autistic features	—	+	—
Tremor	—	—	+

3.3 MRI findings (Studies II and III)

Myelination was abnormal in 18 of 22 patients: in all patients with terminal 18q-deletions and in one patient with an interstitial 18q deletion. In the youngest patient (aged 8 months), this appeared as severely delayed myelination. In older children and adults, the cerebral white matter was diffusely hyperintense on T2-weighted images, which led to poor differentiation of gray and white matter. A topographical variation in the extent of myelination was observed; lower frontal and temporal areas were better myelinated than upper frontal, parietal, and occipital areas. Moreover, 72% of the patients (13/18) had T2 hyperintensity in the posterior limbs of the internal capsules, and 67% (12/18) had poor differentiation of the cerebellar gray and white matter. Mild atrophic findings were seen

supratentorially in three and infratentorially in three patients. Additional findings present in single patients were a porencephalic cyst and a focal mass lesion.

The abnormality of the signal intensity, observed in visual analysis, was confirmed by quantitative measurement of signal intensities. The measured signal intensities of the occipital and frontal white matter were significantly higher ($p=0.0008$) in the patients than in the controls. Signal intensities of the caudate nuclei, putamina, and thalami were also significantly increased in patients ($p=0.0157$, $p=0.0063$, $p=0.0016$, respectively).

Four patients with interstitial deletions (Family II and Patient 14) had a normal myelination pattern in MRI. In one of them, a few nodular periventricular heterotopias were observed.

3.4 Molecular study – size of deletions and comparison with phenotype (Study III)

In two patients, who cytogenetically appeared to have terminal deletions, molecular study showed heterozygosity for the distal markers of 18q. This implies that they had interstitial deletions. The size of the deletions varied from 7.7 Mb to 29.4 Mb, and only a small common region (approximately 1.1 Mb) was deleted in every patient.

In phenotype-genotype comparison, the patients with unbalanced translocations were excluded. All patients with an interstitial deletion and normal myelination spared a region between markers D18S469 (69.5 Mb) and D18S1141 (75.0 Mb). This area was deleted in every patient with abnormal myelination. This critical region for dysmyelination includes the gene for MBP, which was specifically tested.

Individuals with atretic or stenotic auditory canals, palatal abnormalities, nystagmus, and IgA deficiency shared deletions between markers D18S812 (68.4 Mb) at q22.3 and D18S1141 (75.0 Mb) at q23. Individuals with cryptorchidism shared a deletion between markers D18S64 (55.6 Mb) at q21.32 and D18S469 (69.5 Mb) at q22.3. Results of the molecular analysis of the key patients in determining the critical area for dysmyelination are illustrated in Figure 16. More detailed information about segregation analysis is given in Study III.

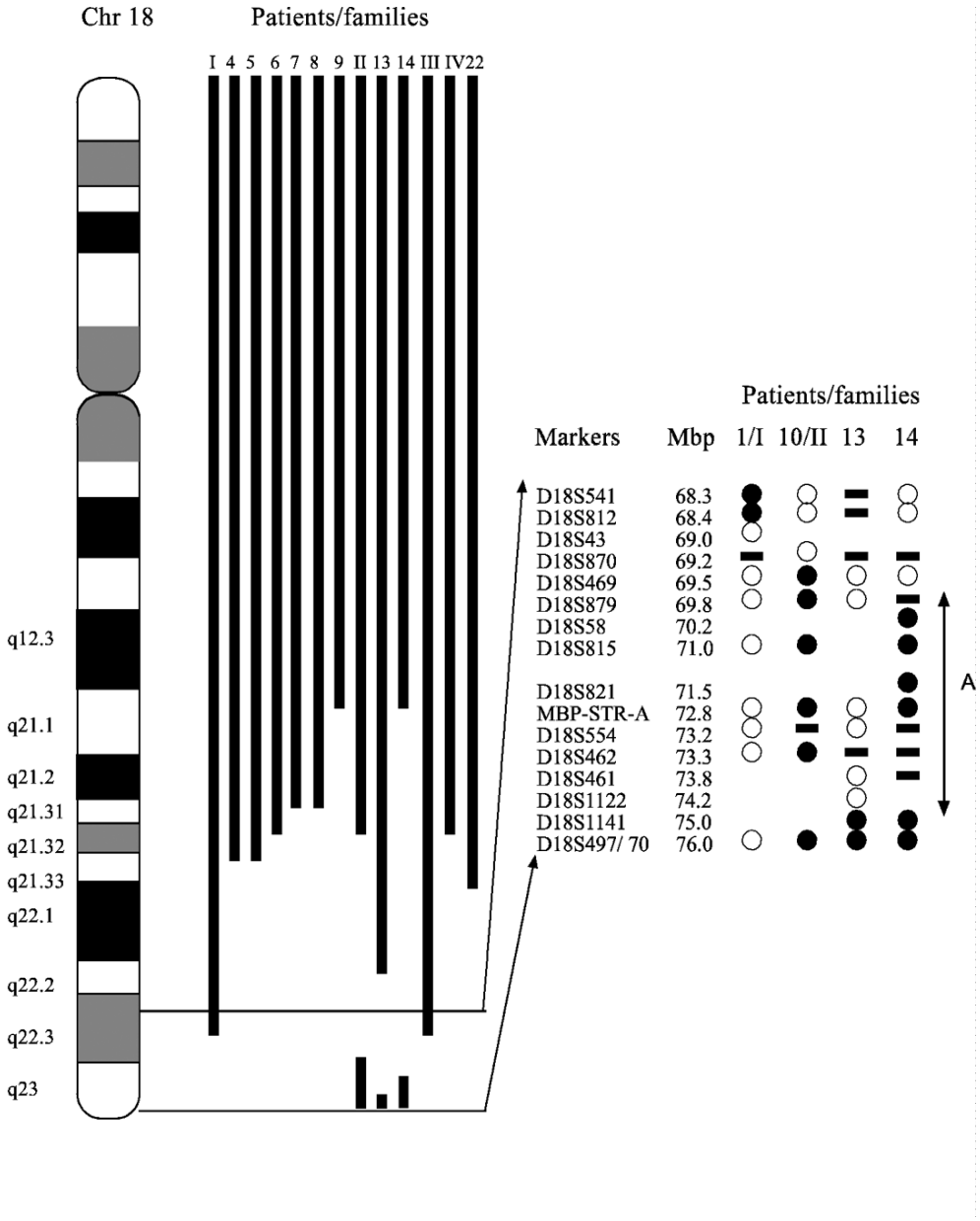


Figure 16. *A schematic presentation of 18q deletions of 22 patients, assessed by molecular analysis (left side of the figure). The Roman numerals refer to families (Family I: Patients 1-3; Family II: Patients 10-12; Family III, Patients 15-18 and Family IV, Patients 19-21) and the Arabic numerals to individual patients. The results of segregation analysis of the key patients in determining the critical region for dysmyelination (region A, between markers D18S469 and D18S1141) are presented on the right side of the figure. Myelination was abnormal in all patients, except in Patients 10-12 (Family II) and Patient 14. A closed circle indicates that marker alleles from both parents segregated to the patient (i.e. no deletion). An open circle indicates that only one allele segregated to the patient (deletion), and a dash indicates that segregation of the marker was uninformative in the family.*

4. Leukoencephalopathy with calcifications, cysts, and retinal vascular abnormalities (Study IV)

4.1 Family history

The study included 13 patients from 11 families. The patients had altogether 18 unaffected siblings. Patients 3 and 4 (siblings) had a stillborn brother with severe intrauterine growth retardation (IUGR). In addition, the brother of Patient 7 was born on the 31st week of gestation with severe IUGR and had several anomalies (radius aplasia, missing thumbs, syndaktylias, meatus atresia, cleft palate, and cataracts). He died on the first day of life due to respiratory distress syndrome and intracranial hemorrhage. The parents were healthy and nonconsanguineous. A segregation analysis has not yet been carried out.

4.2 Clinical findings

The patients showed a spectrum of neurological, ophthalmological, skeletal, gastrointestinal, and hematological findings, which are summarized in Table 12.

Eleven of 13 patients suffered from IUGR, and five were born by Cesarean section due to poor growth, placental ablation, Rhesus immunization, or abnormal cardiotocography. One patient (#7) had malformations (patent ductus arteriosus, meatal atresia, cleft lip, rudimentary thumbs, and syndactyly).

The patients presented at the age of 6 months to 14 years with ophthalmological or neurological symptoms. The most common ocular complaints, squint, decreased vision, leukocoria, and painful eye were related to vitreoretinal bleeding, retinal detachment, and glaucoma. Neurologic presentation was with either seizures or spasticity.

The most common neurological symptoms were partial epilepsy, hemiparesis, ataxia, and cognitive decline. In some cases, enlarging cysts caused increased intracranial pressure and needed shunting. Two patients had migraine-like headaches. The symptoms were slowly progressive. In eight patients who had been followed-up for more than 10 years, the disease had progressed to severe spasticity, bulbar symptoms and anarthria within 11-20 years.

Eleven patients had anomalies of retinal vasculature, bilaterally in nine cases. In eight patients, these were angioma-like excrescences of small vessels which caused preretinal and vitreous hemorrhages. Seven patients had retinal telangiectasias, often associated with subretinal lipid exudation reminiscent of Coats' disease. In all five prospectively studied patients, the retina peripheral to the angiomatous nodules and telangiectatic vessels was avascular. Eight patients developed retinal detachment, six patients neovascular glaucoma, and three patients complicated cataracts. These changes led to uni- or bilateral blindness in

nine patients. The retinal vasculature appeared normal in two patients with adolescence-onset symptoms.

Table 12. *Characteristics of 13 patients with leukoencephalopathy and cerebral calcifications.*

	Patients												
	Early onset												**Late onset**
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Gender	f	f	m	f	f	m	f	f	f	m	f	m	m
Age at onset, y	0.9	0.5	0	0.5	0.5	5.0	0.6	1.0	1.0	2.0	4.0	14	14
Present age, y (age at death)	6	4	(5)	10	(13)	(16)	(20)	(22)	20	(27)	(19)	16	(43)
IUGR	+	+	+	+	+	+	+	+	+	+	-	+	na
Cerebral calcifications	+	+	+	+	+	+	+	+	+	+	+	+	+
Leuko-encephalo-pathy	+	+	+	+	+	+	+	+	na	+	+	+	+
Intracranial cysts	-	+	+	+	-	-	+	-	-	-	-	-	+
Retinal angiomas or telangiectasias	+	+	+	+	+	+	+	+	+	+	+	-	-
Sparse hair	+	+	-	-	-	+	+	+	+	-	na	-	-
Skeletal changes	-	+	na	-	+	+	+	+	+	+	na	+	na
Intestinal bleeding	-	-	-	-	+	+	-	+	+	+	+	-	-
Hematologic abnormality	-	-	-	-	+	+	-	+	+	+	+	-	-
Neuro-pathological examination	-	-	+	-/ bb	+	+	-	+	-	+	+	-	-/ bb

[a]only computed tomography (CT) available; na, data not available; bb, brain biopsy only

Two of 11 patients with IUGR reached normal growth postnatally. Three patients showed some catch-up growth, after which the height and proportional weight increased linearly 2-3 SD and 15-30% below the mean, respectively. In six patients, growth velocity gradually slowed from the age of 2-14 years, and the last growth measured had declined to 3-5.5 SD below the mean.

Six patients developed severe anemia and needed blood transfusions since the age of 11-20 years. The underlying cause of the anemia remained obscure. Anemia was hypochromic and macrocytic. Bone marrow examinations, performed on four patients, showed increased erythropoiesis, and, in three patients, megaloblastic changes were seen. One patient had fragmented erythrocytes, suggestive of mechanical hemolysis due to microangiopathy. Three patients also had thrombocytopenia, which resolved after splenectomy. Occult intestinal bleeding probably partly caused iron deficiency because recurrent intestinal bleeding began in these six patients within a few years. Endoscopies revealed no obvious reason for the bleeding, but showed mild inflammatory changes and, in one patient, partial villous atrophy. However, in another patient gut biopsy revealed telangiectatic vessels. Two patients developed hepatic insufficiency and esophageal varices.

Patient 3 died unexpectedly during sleep at the age of 5 years. He had suffered prolonged apneic epileptic seizures, which possibly was the cause of death. Seven patients died at the age of 13-43 years. The immediate cause of death of these severely handicapped patients was pneumonia, fulminant intestinal bleeding, or multiorgan failure.

4.3 Radiological findings

The most striking radiological findings were extensive brain calcifications. They involved the thalami, the cerebral white matter, and the brain stem of all patients, and the basal ganglia of all but one patient. Calcifications of white matter were predominantly situated subcortically. The cerebellar white matter was affected in eight patients and the dentate nuclei in the five oldest patients. Calcifications were evident both in CT and in MRI, especially in T2*-weighted images, but also in T2- and T1-weighted spin-echo images. The brain CT performed on two patients during the neonatal period was normal.

Diffuse or patchy white matter changes, hyperintense on T2-weighted MRI images, occurred most commonly in the periventricular region, but sometimes extended subcortically. The internal capsules were always involved. Brain stem lesions were seen in eight patients, and the cerebellar white matter and the corpus callosum were involved in six and four patients, respectively. All patients had signal changes in the thalami and four patients in the basal ganglia. In some cases, the lesions spread along the white matter tracts, resembling vasogenic edema, and these changes partly resolved during follow-up.

Contrast enhancement appeared at the outer margin of the abnormal white matter and around the calcified lesions in the thalami and the basal ganglia. Five patients had

parenchymal cysts, either singly or multilobularly. They were situated mainly in the thalamic region, but occurred also in the brain stem and in the parietal and frontal lobes. In some cases, the cysts appeared several years after presentation, and new cysts formed during follow-up.

Various skeletal changes in radiographs were found in six of seven patients. Five patients had metaphyseal changes, including sclerosis and mild flaring, which were most pronounced in the distal femur and proximal tibia. The long bones appeared osteopenic in six patients. Additional findings in some patients were short femoral neck, abnormal molding of the ulna and radius, thin fibula and humerus, mild vertebral end plate deformity, and subnormal widening of the interpeduncular distance in the lumbar spine. One patient had hypo- or aplasia of the first metacarpal bones and the thumbs, hypoplasia of the 4th right metatarsal bone, and fusion of 4th and 5th left metatarsals. Two patients had pathological fractures. The areal bone mass density was reduced in all three patients studied. The Z-scores for the lumbar spine were -2.0, -1.8, and -1.2, and those for the left femoral neck were -0.5, -1.1, and -1.7.

4.4 Laboratory findings

The hematological abnormalities were described above. With respect to the etiology of the calcifications, the laboratory findings were uninformative (details in Study IV). Investigations of calcium homeostasis and parathyroid function, serologic studies for Toxoplasma and Cytomegalovirus, and karyotype (five patients) as well as mitochondrial work-up: blood and CSF lactate and pyruvate, urinary organic acids, muscle biopsy (three patients), activities of respiratory chain enzymes, and mutation analysis of the mitochondrial DNA (two patients) were particularly unrevealing.

4.5 Neuropathology

All autopsied patients had similar findings, with only variation in the extent of changes. Calcifications were seen along the walls of small vessels both in the deeper cortical laminae and in the white matter and deep nuclei. In subcortical and gyral white matter, the extensive calcifications often followed the shape of the U-fibers. Massive calcifications were seen in the thalami, basal ganglia, pontine basis, and in dentate nuclei. A very typical finding was a cystically degenerated focus with calcified obliterated vessels at the border between the pontine tegmentum and basis. Within the calcified areas, numerous small vessels showed thickened, hyalinized walls. Their lumina were substantially reduced or obliterated, and the internal elastic membrane could not be seen in the abnormal vessels. Angiomatous proliferation of these vessels was present in severely affected areas. Immunohistochemical stainings with antibodies against the endothelial marker CD31, the basement membrane marker laminin, and smooth muscle actin showed that the hyalinized thickening of the vessel walls was mainly outside the subendothelial basement membrane. The partly degenerated and fragmented vascular smooth muscle fibers were displaced

peripherally. The white matter surrounding these lesions showed rarefaction, gliosis, and myelin loss. In areas devoid of vascular changes, the white matter appeared intact. No changes consistent with inflammation or infection were found. Gliosis and similar thick-walled vessels were seen in brain biopsies from tissue around the cyst during neurosurgical operations (Patients 4 and 13).

4.6 Other autopsy findings

Two of the six autopsied patients had abnormalities of intestinal vasculature, including distinct nodules with thick-walled vessels or dilated vessels. In two patients, arteries of the portal area were thick-walled, one patient had hepatic cirrhosis, and one had a small liver. Two patients had mild glomerular sclerosis.

DISCUSSION

1. Overview

1.1 Types of leukoencephalopathies found

Childhood-onset cerebral white matter disorders are a heterogeneous group of rare diseases. Even though this study cannot be considered epidemiologic, the data allow some conclusions to be drawn on occurrence of "new" recognizable phenotypes among undefined leukoencephalopathies in Finland. LBSL was the most common group in patients entering the study due to undetermined leukoencephalopathy, thus appearing to be among the more common childhood-onset leukoencephalopathies in our country. More cases will likely be identified as awareness of this disorder increases.

Vanishing white matter disease has been estimated to be among the more prevalent leukoencephalopathies elsewhere, with an incidence similar to MLD (1:40 000) (125). However, no patients with VWM were found in this study. Only one child had an MRI pattern suggestive of VWM disease but she did not have mutations of genes related to VWM. This phenotype thus seems to be rare in Finland.

Leukoencephalopathy with calcifications, cysts, and retinal vascular abnormalities is considered a very rare disease. Thirteen Finnish patients with this phenotype, born within the last five decades, were identified in Study IV. Compared with the approximately 15 similar patients previously reported in the literature, this disorder appears to be more prevalent in Finland than elsewhere.

Other leukoencephalopathies, diagnosed in single patients were Alexander disease, 18q-deletion, and H-ABC. We also found one case of Salla disease, missed in previous studies.

1.2 Usefulness of MRI categorization

Categorization of MRI findings in patients with undetermined leukoencephalopathies helps in planning the most appropriate etiologic investigations. Examples of known disorders within each category are given in Table 13 [reviewed in (133)].

Few diseases are known to produce a pattern of severe hypomyelination. The classical X-linked disease causing hypomyelination in males is Pelizaeus-Merzbacher disease. In Finland, however, exclusion of Salla disease belongs to the first line of investigations. In Salla disease, most patients have only supratentorial hypomyelination, but some patients, like the one diagnosed in this study, may have severe hypomyelination involving also the cerebellar white matter (59, 70, 112). Recently, mutations in a gene encoding GJA12, a

gap junction protein, were found in patients with severe hypomyelination (122). In addition to symptoms typical of all hypomyelinating disorders, hypotonia and nystagmus, these patients had facial weakness. GJA12 mutations were not excluded in our patients. Severe hypomyelination may also be seen in trichothiodystrophy, which clinically differs from the above-mentioned disorders, with characteristic congenital ichtyosis and brittle hair (91, 95). In H-ABC disease, severe or moderate hypomyelination is accompanied by atrophy of the cerebellum and basal ganglia, especially the putamen (130). The atrophic findings develop gradually, emphasizing the importance of follow-up imaging. At present, diagnosis is based on MRI.

In patients with mild to moderate hypomyelination, severely delayed myelination or irregular myelination, a much wider diagnostic approach must be considered. A large variety of metabolic disorders, such as amino acidopathies and organic acidurias (30, 93), fucosidosis (125), AGU (3), Cockayne syndrome (66), and biotinidase deficiency (49), may interfere with myelination. In addition to primarily white matter disorders, early-onset neuronal degenerative disorders may disturb myelination (23, 48, 141) .In patients in category A3 who developed severe cerebral atrophy in follow-up, an underlying neuronal disorder is likely. Diagnosing patients with chromosome 18q deletions in this category is important to avoid unnecessary extensive metabolic investigations. In particular, 18q-syndrome should be sought in patients with dysmorphic features, meatal atresia, or poor growth (Studies II and III). It is noteworthy that small terminal deletions and deletions caused by unbalanced translocations may be missed even with high-resolution G-banding. In these cases, subtelomeric FISH of 18q, array CGH (comparative genomic hybridization), or molecular analysis may be used to establish the diagnosis. Acquired disorders, e.g. B12 deficiency and hypothyroidism, may also interfere with the process of myelination (74, 106).

Global involvement of cerebral white matter is seen in a few diseases. MLC is a disorder characterized by macrocephaly and subcortical cysts (137). VWM disease shows typical findings, with cystic rarefaction of the white matter (135). Globally affected, swollen white matter may also be seen in merosin-deficient congenital muscular dystrophy (22).

Predominantly frontal white matter abnormalities in conjunction with basal ganglia signal changes are typical of Alexander disease, which today can be confirmed using genetic methods (16, 17, 132).

Many leukoencephalopathies initially involve predominantly the periventricular white matter. This pattern is seen, for instance, in LBSL [Study I, (128)], MLD (93), X-ALD (71), and some hyperhomocysteinemias (97). Increased periventricular signal is also seen in some patients with Angelman syndrome (personal observation). Because patients with Angelman syndrome present with ataxia, a symptom typical of leukoencephalopathies, this MRI finding may misdirect the investigations to metabolic diseases.

Table 13. *Examples of known diseases within MRI categories A-G (for definition of categories, see p.32)*

MRI category	Examples of disorders within the category
A1 (A2)	Pelizaeus-Merzbacher disease, Salla disease, trichothiodystrophy, mutations in a gene encoding GJA12, H-ABC, Cockayne syndrome
(A2) A3	18q- deletions, amino acidopathies, organic acidurias, galactosemia, fucosidosis, AGU, infantile-onset neuronal degenerative disorders
B	MLC, VWM, merosin-deficient congenital muscular dystrophy
C	Alexander disease
D	LBSL, MLD, X-ALD
E	Chromosomal disorders, congenital CMV infections
F	Kearns-Sayre syndrome, Canavan disease, L2- and D2-hydroxyglutaric aciduria
G	Adrenomyeloneuropathy, cerebrotendinous xanthomatosis

Isolated, multifocal, or confluent lobar white matter lesions are seen, for instance, in conjunction with several chromosomal abnormalities (40, 89, 145) and in congenital CMV infections.

Predominant involvement of the subcortical white matter is rare. It occurs in cerebral organic acidurias L2-and D2-hydroxyglutaric aciduria and Canavan disease (60, 108), and in Kearns-Sayre syndrome (69). This pattern should not be confused with delayed myelination presenting with unmyelinated subcortical white matter.

Finally, when the lesions are located predominantly in the cerebellar white matter, adrenomyeloneuropathy (71, 93) and cerebrotendinous xanthomatosis (33) should primarily be considered.

Often MRIs taken early in the course of the disease are most useful in showing the specific patterns of involvement. In some cases, only follow-up images reveal the characteristic features, as in H-ABC, discussed above. Early- and late-onset forms of diseases may have different MRI manifestations, e.g. the childhood cerebral form of X-linked adrenoleukodystrophy shows characteristic parieto-occipital periventricular changes,

while later onset adrenomyeloneuropathy may present with abnormalities of the cerebellar white matter and corticospinal tracts (125).

The MRI categories differed with respect to clinical manifestations. While patients with severe hypomyelination presented in infancy, the patients with periventricular lesions usually presented in childhood or adolescence An "encephalitis-like" presentation was most commonly seen in patients with periventricular lesions. One-third of all patients had static neurologic signs, and this was most common in Category E, with isolated lobar changes. Many chromosomal disorders and congenital CMV infection are known to produce lobar white matter changes, and our patients in category E may have had some of these disorders, although the diagnosis could not be verified. Milder disorders with static signs might be underrepresented in this study because patients sent for consultation from other hospitals most probably represented the more severe and progressive cases in the spectrum of undetermined leukoencephalopathies.

1.3 Usefulness of MRS investigations

Not surprisingly, the MRS findings were heterogeneous in patients with unknown leukoencephalopathy and revealed mostly process-related abnormalities reflecting active myelin turnover and axonal loss within the affected white matter. We found no disease-specific metabolic patterns, like increased NAA, consistent with Canavan disease (2) or peaks, which are not normally present, such as those seen in maple syrup urine disease (57). Half of the patients (Overview) had findings suggestive of active ongoing demyelination, often with concomitant gliosis. Also two patients (siblings) in category E, who had clinically stable disease, had slightly abnormal MRS findings, suggesting low-grade demyelination.

In hypomyelinating disorders, MRS findings of normal or decreased Cho have been reported previously (39, 53, 85, 95). In this study, variable findings were observed. Three of four patients with severe or moderate hypomyelination (categories A1 and A2) had normal Cho concentration and normal or near-normal NAA concentrations, arguing against active demyelination. In one patient with hypomyelination plus severe atrophic findings, MRS was suggestive of active demyelination and axonal loss. Interestingly, only one girl with irregular myelination (Figure 11) had severely decreased Cho, which could be due to a profound disturbance of myelin build-up, similar to that seen in PMD (53).

Evidence for axonal injury within the white matter was found in 65% of patients. While in demyelinating disorders reduced NAA occurs late in the course of the disease, in LBSL (Study I) evidence for axonal injury was the initial finding and only one patient showed signs of demyelination (increased Cho). This suggests a different pathophysiology, with primary axonal involvement. All LBSL patients had elevated Lac, discussed further below. Highly elevated Lac was also seen in a patient with Alexander disease, consistent with previous findings (17). The finding of Lac in MRS warrants work-up for mitochondrial disorders, including muscle biopsy. However, elevated Lac is not specific

to mitochondrial disorders, also being related to active demyelination, inflammation, and tissue necrosis (11, 73).

We used mainly long echo time spectra, which are more reliable when quantitation of peaks is important, as in assessment of brain maturity or brain injury. However, more peaks are identified in short echo time spectra, which has proved to be more informative in neurometabolic disorders (5, 20, 80, 134), and this might have yielded more useful data in our study patients. MRS findings may also depend on the stage of the disease, and longitudinal studies were not performed. Furthermore, the lack of young control patients hampers the interpretation of our MRS data and may make the results less valid. Obviously, more thorough MRS studies are needed in the future for this patient group.

1.4 Usefulness of other clinical and neurophysiological investigations

In addition to careful neurologic investigation, ophthalmologic examination proved to be important. Nystagmus, optic atrophy, and squint occurred relatively often in patients with undetermined leukoencephalopathy (Overview). Fundus examination of patients with cerebral calcifications is especially important because it may reveal retinal angiomas and telangiectasias, as was seen in patients in Study IV, or cataracts and pigmentary retinopathy typical of Cockayne syndrome (84).

Measurement of nerve conduction velocities is necessary because clinical signs of peripheral neuropathy may be overshadowed by spasticity and neuropathy may exist despite brisk deep tendon reflexes. Even though in classical leukodystrophies the neuropathy is typically demyelinating, patients in the present study (Overview, Study I) more often had findings suggestive of predominantly axonal polyneuropathy. Somatosensory evoked potentials were abnormal in the majority of patients with undetermined leukoencephalopathies, showing delayed CNS conduction or absent cortical reponses. Abnormal SEP correlated well with clinically evident sensory disturbance. SEP with tibial nerve stimulation showed abnormalities more frequently and earlier than SEP with median nerve stimulation, probably because the measured sensory tract is longer in tibial SEP. While in Pelizaeus-Merzbacher disease markedly prolonged cortical latencies or absent cortical responses are a consistent finding (86), the two males with PMD-like severe hypomyelination (but without PLP mutations) in this study had normal SEP. Thus, in hypomyelinating disorders, a normal tibial SEP is suggestive of a diagnosis other than PMD.

2. Leukoencephalopathy with brain stem and spinal cord involvement and elevated white matter lactate (LBSL)

A specific MRI pattern and consistent clinical features defined this disorder. This disease was first reported by van der Knaap et al. in 2003 (128), but the pattern was found independently in this study, confirming that this is a separate entity with constant findings.

In this disorder, designated as LBSL, the hemispheric patchy or confluent white matter abnormalities are combined with involvement of selective brain stem and spinal cord tracts. On a systemic level, the long sensory tracts (dorsal columns, medial lemniscus, and corona radiata) and the pyramidal tracts (internal capsules, pyramidal tracts in the brain stem, and lateral corticospinal tracts within the spinal cord) are involved over their entire length.

Clinical correlates of this involvement are sensory ataxia, tremor, and impaired vibration sense and proprioception. Distal spasticity is usually less prominent, but in some patients it may be a predominant problem. The symptoms manifest from early infancy to adolescence and are slowly progressive. The clinical impression of dorsal column dysfunction was supported by evoked potential studies, which showed impaired central conduction of the sensory stimuli. A new finding was development of a mild axonal neuropathy, shown by NCV studies.

The etiopathogenesis of this disease is unknown. The MRS findings might give some clues. MRS showed consistently elevated Lac, normal or elevated Cho, and decreased NAA in the abnormal white matter, consistent with a previous report. Van der Knaap et al. (128) proposed that axonal degeneration could be a primary pathology because the entire long motor and sensory tracts are involved and there is a consistent loss of NAA. Increased Cho, suggesting demyelination, was only occasionally found and might be secondary to axonal disease. The constant elevation of Lac is also interesting and could point towards a mitochondrial disorder. This is supported by mild exercise intolerance and worsening of symptoms during infections, features reported by several patients. Also NCV studies suggested development of axonal neuropathy, a feature seen in several mitochondrial disorders (87, 140). Even though the muscle histology and respiratory chain enzyme complex activities were normal, a mitochondrial etiology remains a possibility. However, apart from mitochondrial disorders, elevated Lac is seen in MRS with several white matter disorders, e.g. in Alexander and Krabbe disease (17, 18) and in MS, where it is related to active demyelination and inflammation (11).

Our patients possibly represent an autosomally recessively inherited disease entity because there were affected siblings and patients were of both sexes, born to healthy parents.

3. Deletions of chromosome 18q

The 18q- syndrome is an example of a static disorder among leukoencephalopathies. The phenotype in patients with 18q deletions was variable, consistent with previous reports (26, 113). This clinical heterogeneity is partly due to variation in the size of the deletions. However, with respect to most clinical manifestations, the phenotype varied between patients with deletions of apparently similar size and even between members of the same family. Thus, other factors, such as effects of other genes, allelic variation of the haploid portion of 18q, or environmental variables, also modulate the phenotype. Moreover, imprinted genes in the region might influence the phenotype, but a previous study found no evidence supporting this in 18q- deletions (75).

With regard to dysmyelination phenotype, the situation was more straightforward. A uniform MRI pattern – a poor differentiation of gray and white matter, consistent with dysmyelination – was found in all patients with deletions of the critical region in 18q22.3-18q23. In infants, this appeared as severely delayed myelination. This phenotype was absent in all patients retaining that region. The region associated with dysmyelination was approximately 5.5 Mb. One previous study, where a single patient with an interstitial deletion had a normal MRI, delineated this critical region to an even smaller area of 2 Mb (41). The four patients in our study, who had normal brain myelination and interstitial deletions, spared the same 2 Mb region, confirming that result. This critical region includes several genes, of which the MBP gene is the logical candidate for the dysmyelinating phenotype because it is a major protein of CNS myelin, important for myelin formation and compaction (8). However, this remains to be proved, as no patient with a deletion of exclusively the MBP gene is known. Growth hormone deficiency, found to some degree in 72% of the 18q- children (51), has also been proposed to contribute to dysmyelination in combination with hemizygosity of MBP (25). This dysmyelination pattern differed from white matter abnormalities reported in other chromosomal abnormalities, where focal isolated or confluent hyperintense white matter lesions on T2-weighted images are typical findings (40, 89, 145).

We also searched for any correlation of other phenotypic features with the size and localization of the deletion, but due to the small number of patients, no firm conclusions can be drawn. However, congenital atresia or stenosis of the external ear canals was found in 50% of the cases and was associated with deletions between markers D18S812 (at 18q22.3) and D18S1141 (at q23). This finding is consistent with two recent reports (35, 142), which, using array CGH, defined the critical region for congenital aural atresia to be located on 18q22.3-23. The gene responsible for aural atresia is unknown.

4. Leukoencephalopathy with calcifications, cysts, and retinal vascular abnormalities

The patients of Study IV had a spectrum of manifestations – progressive extensive brain calcifications, cerebral cysts, leukoencephalopathy, retinal vascular abnormalities, and skeletal abnormalities – which overlapped with previous reports on ”Coats' plus” syndrome or ”leukoencephalopathy, calcifications, and cysts” (Table 14), suggesting that all of these conditions are related. Recurrent episodes of intestinal bleeding, a feature not reported earlier, occurred in nearly half of the patients.

Table 14. *Previously reported patients with cerebral calcifications and leukoencephalopathy who are reminiscent of our patients.*

Feature	Revesz et al. 1992	Labrune et al. 1996			Goutieres et al. 1999		Sazgar et al. 2002		Tolmie 1988 + Crow et al. 2004				Nagae-Poetscher et al. 2004		
Gender	m	f	m	f	f	m	m	m	f	f	f	m	m	f	f
Age at onset	6m	11y	4y	3m	11m	3y	9m	1m	3y	1y	2.y	3y	2y	2y	nm
IUGR	+	nm	nm	nm	+	+	+	+	+	+	+	+	nm	-	+
Retinal angiomas or telangiectasias	+	-	-	-	+	+	+	+	+	+	+	+	+	-	-
Cerebral calcifications	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cerebral cysts	-	+	+	+	-	-	-	-	-	-	-	-	+	+	+
Leukoencephalopathy	+	+	+	+	+	+	nm	nm	+	+	+	+	+	+	+
Hair or nail abnormality	+	-	nm	nm	-	-	+	+	+	+	+	+	+	nm	nm
Osteopenia/ skeletal changes	nm	-	nm	nm	-	-	+	+	+	+	+	-	nm	nm	nm
Pancytopenia	+	-	-	-	-	-	-	+.	-	-	-	-	-	-	-
Hepatopathy	-	-	nm	nm	-	-	-	+	nm	nm	nm	-	-	-	-
Cognitive decline	+	+	dd	+	+	-	-	-	-	-	-	-	+	+	+

nm, not mentioned; dd, developmental delay

The findings in neuropathologic examinations suggest that the primary abnormality in this disorder is microangiopathy. Gradual obliteration of the cerebral small vessels could lead to dystrophic calcification via slow necrosis and then to formation of cysts. Strokes, common to other inherited microangiopathies, e.g. CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy) (54, 124) and HERNS (hereditary endotheliopathy with retinopathy, encephalopathy and deafness) (58) did not occur. The extensive white matter abnormalities seem to represent secondary changes. The distribution of calcifications is reminiscent to that seen in mitochondrial disorders, but mitochondrial work-up of some of our patients and others (64, 83) was unremarkable.

Retinal small vessels were abnormal in patients with early-onset disease. Both telangiectasias and angiomas were found. The ophthalmological abnormalities were not limited to changes seen in Coats' disease, and thus the previous designation “Coats' plus” might be misleading. A unifying feature in all five prospectively studied patients was

avascular retina peripheral to angiomas and telangiactasias. A potential explanation for this is obliteration of retinal microvessels or, alternatively, incomplete vascularization of the retina during development.

Components of the Wnt signaling pathway (LRP5, Frizzled-4, and NDP gene), involved in retinal vascular development (24) would be interesting candidates for a mutation search. Mutations in these genes are associated with familial exudative vitreoretinopathy (FEVR) and Norrie's disease, vitreoretinal disorders in which abnormal vascularization of the peripheral retina is a key pathological feature (101, 120). FEVR, in particular, is characterized by retinal telangiectasias, exudation, and vitreoretinal folds resembling the findings seen in our patients. Mutations in the X-chromosomal NDP gene are responsible for Norrie's disease, some cases of FEVR, and, apparently, some cases of sporadic Coats' disease (13). LRP5 is also an important modifier of bone density (44), an interesting point regarding osteopenia in our patients. However, intracranial calcifications or cysts, so prominent in this disorders, have not been associated with mutations in these genes.

These patients may represent an autosomally recessively inherited disorder. However, since the causative gene has not been identified, it is also possible that this phenotype turns out to be genetically heterogeneous. We have started molecular genetic studies of this disorder.

5. Conclusions

Leukoencephalopathies in childhood represent a heterogeneous group of disorders. When categorized based on MRI findings, the largest subgroups were patients with myelination abnormalities and patients with predominantly periventricular white matter abnormalities.

Two recently described disorders were further delineated in this study:

Leukoencephalopathy with brain stem and spinal cord involvement and elevated white matter lactate (LBSL). This disorder was clinically characterized by slowly progressive sensory ataxia, tremor, distal spasticity, and dorsal column dysfunction. Development of an axonal neuropathy was a new finding.

Leukoencephalopathy with calcifications, cysts, and retinal vascular abnormalities. This study suggests that the previously reported rare cases of "leukoencephalopathy with calcifications and cysts" and "Coat's plus" are manifestations of the same disorder. All autopsied patients had similar neuropathological findings showing calcifying obliterative microangiopathy.

We have started genealogic and molecular genetic studies of these two disorders with the aim of identifying the genes underlying these clinically defined diseases.

MRI findings in 18q- deletions were as follows:

A typical MRI finding is a poor differentation of the cerebral gray and white matter on T2-weighted images. This pattern is associated with deletions between markers D18S469 (at 18q22.3) and D18S1141 (at 18q23). Haploinsufficiency of myelin basic protein is suggested to be responsible for the observed dysmyelination phenotype.

The majority of patients with leukoencephalopathies remain without a diagnosis. Because these disorders are rare, delineation of new diseases requires long-term studies and multi-institutional collaboration.

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REFERENCES

1. **Alford CA, Stagno S, Pass RF, and Britt WJ.** Congenital and perinatal cytomegalovirus infections. *Rev.Infect.Dis.* 12 Suppl 7: S745-53, 1990.

2. **Austin SJ, Connelly A, Gadian DG, Benton JS, and Brett EM.** Localized 1H NMR spectroscopy in Canavan's disease: a report of two cases. *Magn.Reson.Med.* 19: 439-445, 1991.

3. **Autti T, Raininko R, Haltia M, Lauronen L, Vanhanen SL, Salonen O, Aronen HJ, Wirtavuori K, and Santavuori P.** Aspartylglucosaminuria: radiologic course of the disease with histopathologic correlation. *J.Child Neurol.* 12: 369-375, 1997.

4. **Autti T, Raininko R, Vanhanen SL, Kallio M, and Santavuori P.** MRI of the normal brain from early childhood to middle age. II. Age dependence of signal intensity changes on T2-weighted images. *Neuroradiology* 36: 649-651, 1994.

5. **Barkovich AJ.** Magnetic resonance techniques in the assessment of myelin and myelination. *J.Inherit.Metab.Dis.* 28: 311-343, 2005.

6. **Barkovich AJ.** *Pediatric neuroimaging.* Philadelphia: Lippincott Williams & Wilkins, 2000, p. 850.

7. **Barkovich AJ, Kjos BO, Jackson DE, Jr, and Norman D.** Normal maturation of the neonatal and infant brain: MR imaging at 1.5 T. *Radiology* 166: 173-180, 1988.

8. **Baumann N and Pham-Dinh D.** Biology of oligodendrocyte and myelin in the mammalian central nervous system. *Physiol Rev* 81: 871-927, 2001.

9. **Ben-Zeev B, Gross V, Kushnir T, Shalev R, Hoffman C, Shinar Y, Pras E, and Brand N.** Vacuolating megalencephalic leukoencephalopathy in 12 Israeli patients. *J.Child Neurol.* 16: 93-99, 2001.

10. **Biancheri R, Rossi A, Di Rocco M, Filocamo M, Pronk JC, van der Knaap MS, and Tortori-Donati P.** Leukoencephalopathy with vanishing white matter: an adult onset case. *Neurology* 61: 1818-1819, 2003.

11. **Bitsch A, Bruhn H, Vougioukas V, Stringaris A, Lassmann H, Frahm J, and Bruck W.** Inflammatory CNS demyelination: histopathologic correlation with in vivo quantitative proton MR spectroscopy. *AJNR Am.J.Neuroradiol.* 20: 1619-1627, 1999.

12. **Bjartmar C, Battistuta J, Terada N, Dupree E, and Trapp BD.** N-acetylaspartate is an axon-specific marker of mature white matter in vivo: a biochemical and immunohistochemical study on the rat optic nerve. *Ann.Neurol.* 51: 51-58, 2002.

13. **Black GC, Perveen R, Bonshek R, Cahill M, Clayton-Smith J, Lloyd IC, and McLeod D.** Coats' disease of the retina (unilateral retinal telangiectasis) caused by somatic mutation in the NDP gene: a role for norrin in retinal angiogenesis. *Hum.Mol.Genet.* 8: 2031-2035, 1999.

14. **Boor PK, de Groot K, Waisfisz Q, Kamphorst W, Oudejans CB, Powers JM, Pronk JC, Scheper GC, and van der Knaap MS.** MLC1: a novel protein in distal astroglial processes. *J.Neuropathol.Exp.Neurol.* 64: 412-419, 2005.

15. **Brady ST, Witt AS, Kirkpatrick LL, de Waegh SM, Readhead C, Tu PH, and Lee VM.** Formation of compact myelin is required for maturation of the axonal cytoskeleton. *J.Neurosci.* 19: 7278-7288, 1999.

16. **Brenner M, Johnson AB, Boespflug-Tanguy O, Rodriguez D, Goldman JE, and Messing A.** Mutations in GFAP, encoding glial fibrillary acidic protein, are associated with Alexander disease. *Nat.Genet.* 27: 117-120, 2001.

17. **Brockmann K, Dechent P, Meins M, Haupt M, Sperner J, Stephani U, Frahm J, and Hanefeld F.** Cerebral proton magnetic resonance spectroscopy in infantile Alexander disease. *J.Neurol.* 250: 300-306, 2003.

18. **Brockmann K, Dechent P, Wilken B, Rusch O, Frahm J, and Hanefeld F.** Proton MRS profile of cerebral metabolic abnormalities in Krabbe disease. *Neurology* 60: 819-825, 2003.

19. **Brockmann K, Pouwels PJ, Dechent P, Flanigan KM, Frahm J, and Hanefeld F.** Cerebral proton magnetic resonance spectroscopy of a patient with giant axonal neuropathy. *Brain Dev.* 25: 45-50, 2003.

20. **Brockmann K, Bjornstad A, Dechent P, Korenke CG, Smeitink J, Trijbels JM, Athanassopoulos S, Villagran R, Skjeldal OH, Wilichowski E, Frahm J, and Hanefeld F.** Succinate in dystrophic white matter: a proton magnetic resonance spectroscopy finding characteristic for complex II deficiency. *Ann.Neurol.* 52: 38-46, 2002.

21. **Campagnoni AT and Skoff RP.** The pathobiology of myelin mutants reveal novel biological functions of the MBP and PLP genes. *Brain Pathol.* 11: 74-91, 2001.

22. **Caro PA, Scavina M, Hoffman E, Pegoraro E, and Marks HG.** MR imaging findings in children with merosin-deficient congenital muscular dystrophy. *AJNR Am.J.Neuroradiol.* 20: 324-326, 1999.

23. **Chen CY, Zimmerman RA, Lee CC, Chen FH, Yuh YS, and Hsiao HS.** Neuroimaging findings in late infantile GM1 gangliosidosis. *AJNR Am.J.Neuroradiol.* 19: 1628-1630, 1998.

24. **Clevers H.** Wnt signaling: Ig-norrin the dogma. *Curr.Biol.* 14: R436-7, 2004.

25. **Cody JD, Semrud-Clikeman M, Hardies LJ, Lancaster J, Ghidoni PD, Schaub RL, Thompson NM, Wells L, Cornell JE, Love TM, Fox PT, Leach RJ, Kaye CI, and Hale DE.** Growth hormone benefits children with 18q deletions. *Am.J.Med.Genet.A.* 137: 9-15, 2005.

26. **Cody JD, Ghidoni PD, DuPont BR, Hale DE, Hilsenbeck SG, Stratton RF, Hoffman DS, Muller S, Schaub RL, Leach RJ, and Kaye CI.** Congenital anomalies and anthropometry of 42 individuals with deletions of chromosome 18q. *Am.J.Med.Genet.* 85: 455-462, 1999.

27. **Coman I, Barbin G, Charles P, Zalc B, and Lubetzki C.** Axonal signals in central nervous system myelination, demyelination and remyelination. *J.Neurol.Sci.* 233: 67-71, 2005.

28. **Corydon MJ, Vockley J, Rinaldo P, Rhead WJ, Kjeldsen M, Winter V, Riggs C, Babovic-Vuksanovic D, Smeitink J, De Jong J, Levy H, Sewell AC, Roe C, Matern D, Dasouki M, and Gregersen N.** Role of common gene variations in the molecular pathogenesis of short-chain acyl-CoA dehydrogenase deficiency. *Pediatr.Res.* 49: 18-23, 2001.

29. **Crow YJ, McMenamin J, Haenggeli CA, Hadley DM, Tirupathi S, Treacy EP, Zuberi SM, Browne BH, Tolmie JL, and Stephenson JB.** Coats' plus: a progressive familial syndrome of bilateral Coats' disease, characteristic cerebral calcification, leukoencephalopathy, slow pre- and post-natal linear growth and defects of bone marrow and integument. *Neuropediatrics* 35: 10-19, 2004.

30. **de Koning TJ, Jaeken J, Pineda M, Van Maldergem L, Poll-The BT, and van der Knaap MS.** Hypomyelination and reversible white matter attenuation in 3-phosphoglycerate dehydrogenase deficiency. *Neuropediatrics* 31: 287-292, 2000.

31. **de Lonlay-Debeney P, von Kleist-Retzow JC, Hertz-Pannier L, Peudenier S, Cormier-Daire V, Berquin P, Chretien D, Rotig A, Saudubray JM, Baraton J, Brunelle F, Rustin P, Van Der Knaap M, and Munnich A.** Cerebral white matter disease in children may be caused by mitochondrial respiratory chain deficiency. *J.Pediatr.* 136: 209-214, 2000.

32. **De Stefano N, Guidi L, Stromillo ML, Bartolozzi ML, and Federico A.** Imaging neuronal and axonal degeneration in multiple sclerosis. *Neurol.Sci.* 24 Suppl 5: S283-6, 2003.

33. **De Stefano N, Dotti MT, Mortilla M, and Federico A.** Magnetic resonance imaging and spectroscopic changes in brains of patients with cerebrotendinous xanthomatosis. *Brain* 124: 121-131, 2001.

34. **de Vellis J.** Developmental and hormonal regulation of gene expression in oligodendrocytes. *Ann.N.Y.Acad.Sci.* 605: 81-89, 1990.

35. **Dostal A, Nemeckova J, Gaillyova R, Vranova V, Zezulkova D, Lejska M, Slapak I, Dostalova Z, and Kuglik P.** Identification of 2.3-Mb Gene Locus for Congenital Aural Atresia in 18q22.3 Deletion: A Case Report Analyzed by Comparative Genomic Hybridization. 27: 427-432, 2006.

36. **Fogli A, Schiffmann R, Bertini E, Ughetto S, Combes P, Eymard-Pierre E, Kaneski CR, Pineda M, Troncoso M, Uziel G, Surtees R, Pugin D, Chaunu MP, Rodriguez D, and Boespflug-Tanguy O.** The effect of genotype on the natural history of eIF2B-related leukodystrophies. *Neurology* 62: 1509-1517, 2004.

37. **Fogli A, Rodriguez D, Eymard-Pierre E, Bouhour F, Labauge P, Meaney BF, Zeesman S, Kaneski CR, Schiffmann R, and Boespflug-Tanguy O.** Ovarian failure related to eukaryotic initiation factor 2B mutations. *Am.J.Hum.Genet.* 72: 1544-1550, 2003.

38. **Gabrielli O, Coppa GV, Carloni I, and Salvolini U.** 18q- syndrome and white matter alterations. *AJNR Am.J.Neuroradiol.* 19: 398-399, 1998.

39. **Garbern JY, Yool DA, Moore GJ, Wilds IB, Faulk MW, Klugmann M, Nave KA, Sistermans EA, van der Knaap MS, Bird TD, Shy ME, Kamholz JA, and Griffiths IR.** Patients lacking the major CNS myelin protein, proteolipid protein 1, develop length-dependent axonal degeneration in the absence of demyelination and inflammation. *Brain* 125: 551-561, 2002.

40. **Garcia-Cazorla A, Sans A, Baquero M, Garcia-Bargo MD, Arellano M, Poo P, Gean E, and Campistol J.** White matter alterations associated with chromosomal disorders. *Dev.Med.Child Neurol.* 46: 148-153, 2004.

41. **Gay CT, Hardies LJ, Rauch RA, Lancaster JL, Plaetke R, DuPont BR, Cody JD, Cornell JE, Herndon RC, Ghidoni PD, Schiff JM, Kaye CI, Leach RJ, and Fox PT.** Magnetic resonance imaging demonstrates incomplete myelination in 18q- syndrome: evidence for myelin basic protein haploinsufficiency. *Am.J.Med.Genet.* 74: 422-431, 1997.

42. **Gieselmann V.** Metachromatic leukodystrophy: recent research developments. *J.Child Neurol.* 18: 591-594, 2003.

43. **Gieselmann V, Franken S, Klein D, Mansson JE, Sandhoff R, Lullmann Rauch R, Hartmann D, Saravanan VP, De Deyn PP, D'Hooge R, Van Der Linden AM, and Schaeren-Wiemers N.** Metachromatic leukodystrophy: consequences of sulphatide accumulation. *Acta Paediatr.Suppl.* 92: 74-9, 2003.

44. **Gong Y, Slee RB, Fukai N, Rawadi G, Roman-Roman S, Reginato AM, Wang H, Cundy T, Glorieux FH, Lev D, Zacharin M, Oexle K, Marcelino J, Suwairi W, Heeger S, Sabatakos G, Apte S, Adkins WN, Allgrove J, Arslan-Kirchner M, Batch JA, Beighton P, Black GC, Boles RG, Boon LM, Borrone C, Brunner HG, Carle GF, Dallapiccola B, De Paepe A, Floege B, Halfhide ML, Hall B, Hennekam RC, Hirose T, Jans A, Juppner H, Kim CA, Keppler-Noreuil K, Kohlschuetter A, LaCombe D,**

Lambert M, Lemyre E, Letteboer T, Peltonen L, Ramesar RS, Romanengo M, Somer H, Steichen-Gersdorf E, Steinmann B, Sullivan B, Superti-Furga A, Swoboda W, van den Boogaard MJ, Van Hul W, Vikkula M, Votruba M, Zabel B, Garcia T, Baron R, Olsen BR, Warman ML, and Osteoporosis-Pseudoglioma Syndrome Collaborative Group. LDL receptor-related protein 5 (LRP5) affects bone accrual and eye development. *Cell* 107: 513-523, 2001.

45. **Goutieres F, Dollfus H, Becquet F, and Dufier JL.** Extensive brain calcification in two children with bilateral Coats' disease. *Neuropediatrics* 30: 19-21, 1999.

46. **Greer JM and Lees MB.** Myelin proteolipid protein--the first 50 years. *Int.J.Biochem.Cell Biol.* 34: 211-215, 2002.

47. **Griffiths I, Klugmann M, Anderson T, Yool D, Thomson C, Schwab MH, Schneider A, Zimmermann F, McCulloch M, Nadon N, and Nave KA.** Axonal swellings and degeneration in mice lacking the major proteolipid of myelin. *Science* 280: 1610-1613, 1998.

48. **Grosso S, Farnetani MA, Berardi R, Margollicci M, Galluzzi P, Vivarelli R, Morgese G, and Ballestri P.** GM2 gangliosidosis variant B1 neuroradiological findings. *J.Neurol.* 250: 17-21, 2003.

49. **Grunewald S, Champion MP, Leonard JV, Schaper J, and Morris AA.** Biotinidase deficiency: a treatable leukoencephalopathy. *Neuropediatrics* 35: 211-216, 2004.

50. **Gupta RK, Cloughesy TF, Sinha U, Garakian J, Lazareff J, Rubino G, Rubino L, Becker DP, Vinters HV, and Alger JR.** Relationships between choline magnetic resonance spectroscopy, apparent diffusion coefficient and quantitative histopathology in human glioma. *J.Neurooncol.* 50: 215-226, 2000.

51. **Hale DE, Cody JD, Baillargeon J, Schaub R, Danney MM, and Leach RJ.** The spectrum of growth abnormalities in children with 18q deletions. *J.Clin.Endocrinol.Metab.* 85: 4450-4454, 2000.

52. **Hanefeld F, Holzbach U, Kruse B, Wilichowski E, Christen HJ, and Frahm J.** Diffuse white matter disease in three children: an encephalopathy with unique features on magnetic resonance imaging and proton magnetic resonance spectroscopy. *Neuropediatrics* 24: 244-248, 1993.

53. **Hanefeld FA, Brockmann K, Pouwels PJ, Wilken B, Frahm J, and Dechent P.** Quantitative proton MRS of Pelizaeus-Merzbacher disease: evidence of dys- and hypomyelination. *Neurology* 65: 701-706, 2005.

54. **Haritoglou C, Hoops JP, Stefani FH, Mehraein P, Kampik A, and Dichgans M.** Histopathological abnormalities in ocular blood vessels of CADASIL patients. *Am.J.Ophthalmol.* 138: 302-305, 2004.

55. **Hickey WF.** The pathology of multiple sclerosis: a historical perspective. *J.Neuroimmunol.* 98: 37-44, 1999.

56. **Inoue K.** PLP1-related inherited dysmyelinating disorders: Pelizaeus-Merzbacher disease and spastic paraplegia type 2. *Neurogenetics* 6: 1-16, 2005.

57. **Jan W, Zimmerman RA, Wang ZJ, Berry GT, Kaplan PB, and Kaye EM.** MR diffusion imaging and MR spectroscopy of maple syrup urine disease during acute metabolic decompensation. *Neuroradiology* 45: 393-399, 2003.

58. **Jen J, Cohen AH, Yue Q, Stout JT, Vinters HV, Nelson S, and Baloh RW.** Hereditary endotheliopathy with retinopathy, nephropathy, and stroke (HERNS). *Neurology* 49: 1322-1330, 1997.

59. **Kleta R, Morse RP, Orvisky E, Krasnewich D, Alroy J, Ucci AA, Bernardini I, Wenger DA, and Gahl WA.** Clinical, biochemical, and molecular diagnosis of a free sialic acid storage disease patient of moderate severity. *Mol.Genet.Metab.* 82: 137-143, 2004.

60. **Kolker S, Mayatepek E, and Hoffmann GF.** White matter disease in cerebral organic acid disorders: clinical implications and suggested pathomechanisms. *Neuropediatrics* 33: 225-231, 2002.

61. **Kriegstein AR, Shungu DC, Millar WS, Armitage BA, Brust JC, Chillrud S, Goldman J, and Lynch T.** Leukoencephalopathy and raised brain lactate from heroin vapor inhalation ("chasing the dragon"). *Neurology* 53: 1765-1773, 1999.

62. **Kristjansdottir R, Uvebrant P, Hagberg B, Kyllerman M, Wiklund LM, Blennow G, Flodmark O, Gustavsson L, Ekholm S, and Mansson JE.** Disorders of the cerebral white matter in children. The spectrum of lesions. *Neuropediatrics* 27: 295-298, 1996.

63. **Kurosinski P and Gotz J.** Glial cells under physiologic and pathologic conditions. *Arch.Neurol.* 59: 1524-1528, 2002.

64. **Labrune P, Lacroix C, Goutieres F, de Laveaucoupet J, Chevalier P, Zerah M, Husson B, and Landrieu P.** Extensive brain calcifications, leukodystrophy, and formation of parenchymal cysts: a new progressive disorder due to diffuse cerebral microangiopathy. *Neurology* 46: 1297-1301, 1996.

65. **Lappe-Siefke C, Goebbels S, Gravel M, Nicksch E, Lee J, Braun PE, Griffiths IR, and Nave KA.** Disruption of Cnp1 uncouples oligodendroglial functions in axonal support and myelination. *Nat.Genet.* 33: 366-374, 2003.

66. **Leech RW, Brumback RA, Miller RH, Otsuka F, Tarone RE, and Robbins JH.** Cockayne syndrome: clinicopathologic and tissue culture studies of affected siblings. *J.Neuropathol.Exp.Neurol.* 44: 507-519, 1985.

67. **Leegwater PA, Vermeulen G, Konst AA, Naidu S, Mulders J, Visser A, Kersbergen P, Mobach D, Fonds D, van Berkel CG, Lemmers RJ, Frants RR, Oudejans CB, Schutgens RB, Pronk JC, and van der Knaap MS.** Subunits of the translation initiation factor eIF2B are mutant in leukoencephalopathy with vanishing white matter. *Nat.Genet.* 29: 383-388, 2001.

68. **Leegwater PA, Yuan BQ, van der Steen J, Mulders J, Konst AA, Boor PK, Mejaski-Bosnjak V, van der Maarel SM, Frants RR, Oudejans CB, Schutgens RB, Pronk JC, and van der Knaap MS.** Mutations of MLC1 (KIAA0027), encoding a putative membrane protein, cause megalencephalic leukoencephalopathy with subcortical cysts. *Am.J.Hum.Genet.* 68: 831-838, 2001.

69. **Lerman-Sagie T, Leshinsky-Silver E, Watemberg N, Luckman Y, and Lev D.** White matter involvement in mitochondrial diseases. *Mol.Genet.Metab.* 84: 127-136, 2005.

70. **Linnankivi T, Lönnqvist T, and Autti T.** A case of Salla disease with involvement of the cerebellar white matter. *Neuroradiology* 45: 107-109, 2003.

71. **Loes DJ, Fatemi A, Melhem ER, Gupte N, Bezman L, Moser HW, and Raymond GV.** Analysis of MRI patterns aids prediction of progression in X-linked adrenoleukodystrophy. *Neurology* 61: 369-374, 2003.

72. **Loevner LA, Shapiro RM, Grossman RI, Overhauser J, and Kamholz J.** White matter changes associated with deletions of the long arm of chromosome 18 (18q-syndrome): a dysmyelinating disorder?. *AJNR Am.J.Neuroradiol.* 17: 1843-1848, 1996.

73. **Lopez-Villegas D, Lenkinski RE, Wehrli SL, Ho WZ, and Douglas SD.** Lactate production by human monocytes/macrophages determined by proton MR spectroscopy. *Magn.Reson.Med.* 34: 32-38, 1995.

74. **Lovblad K, Ramelli G, Remonda L, Nirkko AC, Ozdoba C, and Schroth G.** Retardation of myelination due to dietary vitamin B12 deficiency: cranial MRI findings. *Pediatr.Radiol.* 27: 155-158, 1997.

75. **Maiwald R, Overhauser J, and Laccone F.** No evidence for imprinting in distal 18q. *J.Med.Genet.* 37: 152-156, 2000.

76. **Matalon R and Michals-Matalon K.** Recent advances in Canavan disease. *Adv.Pediatr.* 46: 493-506, 1999.

77. **Mercimek-Mahmutoglu S, van der Knaap MS, Baric I, Prayer D, and Stoeckler-Ipsiroglu S.** Hypomyelination with Atrophy of the Basal Ganglia and Cerebellum (H-ABC). Report of a New Case. *Neuropediatrics* 36: 223-226, 2005.

78. **Miller G, Mowrey PN, Hopper KD, Frankel CA, and Ladda RL.** Neurologic manifestations in 18q- syndrome. *Am.J.Med.Genet.* 37: 128-132, 1990.

79. **Miller RG and Kuntz NL.** Nerve conduction studies in infants and children. *J.Child Neurol.* 1: 19-26, 1986.

80. **Moolenaar SH, van der Knaap MS, Engelke UF, Pouwels PJ, Janssen-Zijlstra FS, Verhoeven NM, Jakobs C, and Wevers RA.** In vivo and in vitro NMR spectroscopy reveal a putative novel inborn error involving polyol metabolism. *NMR Biomed.* 14: 167-176, 2001.

81. **Moroni I, Bugiani M, Bizzi A, Castelli G, Lamantea E, and Uziel G.** Cerebral white matter involvement in children with mitochondrial encephalopathies. *Neuropediatrics* 33: 79-85, 2002.

82. **Moser H, Dubey P, and Fatemi A.** Progress in X-linked adrenoleukodystrophy. *Curr.Opin.Neurol.* 17: 263-269, 2004.

83. **Nagae-Poetscher LM, Bibat G, Philippart M, Rosemberg S, Fatemi A, Lacerda MT, Costa MO, Kok F, Costa Leite C, Horska A, Barker PB, and Naidu S.** Leukoencephalopathy, cerebral calcifications, and cysts: new observations. *Neurology* 62: 1206-1209, 2004.

84. **Nance MA and Berry SA.** Cockayne syndrome: review of 140 cases. *Am.J.Med.Genet.* 42: 68-84, 1992.

85. **Nezu A, Kimura S, Takeshita S, Osaka H, Kimura K, and Inoue K.** An MRI and MRS study of Pelizaeus-Merzbacher disease. *Pediatr.Neurol.* 18: 334-337, 1998.

86. **Nezu A.** Neurophysiological study in Pelizaeus-Merzbacher disease. *Brain Dev.* 17: 175-181, 1995.

87. **Nikali K, Suomalainen A, Saharinen J, Kuokkanen M, Spelbrink JN, Lönnqvist T, and Peltonen L.** Infantile onset spinocerebellar ataxia is caused by recessive mutations in mitochondrial proteins Twinkle and Twinky. *Hum.Mol.Genet.* 14: 2981-2990, 2005.

88. **Oldfors A, Fyhr IM, Holme E, Larsson NG, and Tulinius M.** Neuropathology in Kearns-Sayre syndrome. *Acta Neuropathol.(Berl)* 80: 541-546, 1990.

89. **Ono J, Harada K, Hasegawa T, Sakurai K, Kodaka R, Tanabe Y, Tanaka J, Igarashi T, Nagai T, and Okada S.** Central nervous system abnormalities in chromosome deletion at 11q23. *Clin.Genet.* 45: 325-329, 1994.

90. **Ono J, Harada K, Yamamoto T, Onoe S, and Okada S.** Delayed myelination in a patient with 18q- syndrome. *Pediatr.Neurol.* 11: 64-67, 1994.

91. **Ostergaard JR and Christensen T.** The central nervous system in Tay syndrome. *Neuropediatrics* 27: 326-330, 1996.

92. **Parano E, Uncini A, De Vivo DC, and Lovelace RE.** Electrophysiologic correlates of peripheral nervous system maturation in infancy and childhood. *J.Child Neurol.* 8: 336-338, 1993.

93. **Patay Z.** Diffusion-weighted MR imaging in leukodystrophies. *Eur.Radiol.* 15: 2284-2303, 2005.

94. **Pihlaja H, Rantamäki T, Wikström J, Sumelahti ML, Laaksonen M, Ilonen J, Ruutiainen J, Pirttilä T, Elovaara I, Reunanen M, Kuokkanen S, Peltonen L, Koivisto K, and Tienari PJ.** Linkage disequilibrium between the MBP tetranucleotide repeat and multiple sclerosis is restricted to a geographically defined subpopulation in Finland. *Genes Immun.* 4: 138-146, 2003.

95. **Porto L, Weis R, Schulz C, Reichel P, Lanfermann H, and Zanella FE.** Tay's syndrome: MRI. *Neuroradiology* 42: 849-851, 2000.

96. **Pouwels PJ, Brockmann K, Kruse B, Wilken B, Wick M, Hanefeld F, and Frahm J.** Regional age dependence of human brain metabolites from infancy to adulthood as detected by quantitative localized proton MRS. *Pediatr.Res.* 46: 474-485, 1999.

97. **Powers JM, Rosenblatt DS, Schmidt RE, Cross AH, Black JT, Moser AB, Moser HW, and Morgan DJ.** Neurological and neuropathologic heterogeneity in two brothers with cobalamin C deficiency. *Ann.Neurol.* 49: 396-400, 2001.

98. **Quarles RH.** Myelin sheaths: glycoproteins involved in their formation, maintenance and degeneration. *Cell Mol.Life Sci.* 59: 1851-1871, 2002.

99. **Raynor BD.** Cytomegalovirus infection in pregnancy. *Semin.Perinatol.* 17: 394-402, 1993.

100. **Revesz T, Fletcher S, al-Gazali LI, and DeBuse P.** Bilateral retinopathy, aplastic anaemia, and central nervous system abnormalities: a new syndrome? *J.Med.Genet.* 29: 673-675, 1992.

101. **Robitaille J, MacDonald ML, Kaykas A, Sheldahl LC, Zeisler J, Dube MP, Zhang LH, Singaraja RR, Guernsey DL, Zheng B, Siebert LF, Hoskin-Mott A, Trese MT, Pimstone SN, Shastry BS, Moon RT, Hayden MR, Goldberg YP, and Samuels ME.** Mutant frizzled-4 disrupts retinal angiogenesis in familial exudative vitreoretinopathy. *Nat.Genet.* 32: 326-330, 2002.

102. **Ross B and Bluml S.** Magnetic resonance spectroscopy of the human brain. *Anat.Rec.* 265: 54-84, 2001.

103. **Sazgar M, Leonard NJ, Renaud DL, Bhargava R, and Sinclair DB.** Intracranial calcification, retinopathy, and osteopenia: a new syndrome? *Pediatr.Neurol.* 26: 324-328, 2002.

104. **Schiffmann R, Tedeschi G, Kinkel RP, Trapp BD, Frank JA, Kaneski CR, Brady RO, Barton NW, Nelson L, and Yanovski JA.** Leukodystrophy in patients with ovarian dysgenesis. *Ann.Neurol.* 41: 654-661, 1997.

105. **Schiffmann R, Moller JR, Trapp BD, Shih HH, Farrer RG, Katz DA, Alger JR, Parker CC, Hauer PE, and Kaneski CR.** Childhood ataxia with diffuse central nervous system hypomyelination. *Ann.Neurol.* 35: 331-340, 1994.

106. **Schoonover CM, Seibel MM, Jolson DM, Stack MJ, Rahman RJ, Jones SA, Mariash CN, and Anderson GW.** Thyroid hormone regulates oligodendrocyte accumulation in developing rat brain white matter tracts. *Endocrinology* 145: 5013-5020, 2004.

107. **Schweinsburg BC, Taylor MJ, Videen JS, Alhassoon OM, Patterson TL, and Grant I.** Elevated myo-inositol in gray matter of recently detoxified but not long-term abstinent alcoholics: a preliminary MR spectroscopy study. *Alcohol.Clin.Exp.Res.* 24: 699-705, 2000.

108. **Seijo-Martinez M, Navarro C, del Rio MC, Vila O, Puig M, Ribes A, and Butron M.** L-2-hydroxyglutaric aciduria - Clinical, neuroimaging, and neuropathological findings. *Arch.Neurol.* 62: 666-670, 2005.

109. **Seitelberger F.** Neuropathology and genetics of Pelizaeus-Merzbacher disease. *Brain Pathol.* 5: 267-273, 1995.

110. **Sherman DL and Brophy PJ.** Mechanisms of axon ensheathment and myelin growth. *Nat.Rev.Neurosci.* 6: 683-690, 2005.

111. **Singhal BS, Gorospe JR, and Naidu S.** Megalencephalic leukoencephalopathy with subcortical cysts. *J.Child Neurol.* 18: 646-652, 2003.

112. **Sonninen P, Autti T, Varho T, Hämäläinen M, and Raininko R.** Brain involvement in Salla disease. *AJNR Am.J.Neuroradiol.* 20: 433-443, 1999.

113. **Strathdee G, Zackai EH, Shapiro R, Kamholz J, and Overhauser J.** Analysis of clinical variation seen in patients with 18q terminal deletions. *Am.J.Med.Genet.* 59: 476-483, 1995.

114. **Sturm K, Knake S, Schomburg U, Wakat JP, Hamer HM, Fritz B, Oertel WH, and Rosenow F.** Autonomic seizures versus syncope in 18q- deletion syndrome: a case report. *Epilepsia* 41: 1039-1043, 2000.

115. **Suzuki K.** Globoid cell leukodystrophy (Krabbe's disease): update. *J.Child Neurol.* 18: 595-603, 2003.

116. **Szigeti K, Sule N, Adesina AM, Armstrong DL, Saifi GM, Bonilla E, Hirano M, and Lupski JR.** Increased blood-brain barrier permeability with thymidine phosphorylase deficiency. *Ann.Neurol.* 56: 881-886, 2004.

117. **Teijido O, Martinez A, Pusch M, Zorzano A, Soriano E, Del Rio JA, Palacin M, and Estevez R.** Localization and functional analyses of the MLC1 protein involved in megalencephalic leukoencephalopathy with subcortical cysts. *Hum.Mol.Genet.* 13: 2581-2594, 2004.

118. **Tienari PJ, Wikström J, Sajantila A, Palo J, and Peltonen L.** Genetic susceptibility to multiple sclerosis linked to myelin basic protein gene. *Lancet* 340: 987-991, 1992.

119. **Tolmie JL, Browne BH, McGettrick PM, and Stephenson JB.** A familial syndrome with coats' reaction retinal angiomas, hair and nail defects and intracranial calcification. *Eye* 2: 297-303, 1988.

120. **Toomes C, Bottomley HM, Jackson RM, Towns KV, Scott S, Mackey DA, Craig JE, Jiang L, Yang Z, Trembath R, Woodruff G, Gregory-Evans CY, Gregory-Evans K, Parker MJ, Black GC, Downey LM, Zhang K, and Inglehearn CF.** Mutations in LRP5 or FZD4 underlie the common familial exudative vitreoretinopathy locus on chromosome 11q. *Am.J.Hum.Genet.* 74: 721-730, 2004.

121. **Topcu M, Saatci I, Topcuoglu MA, Kose G, and Kunak B.** Megalencephaly and leukodystrophy with mild clinical course: a report on 12 new cases. *Brain Dev.* 20: 142-153, 1998.

122. **Uhlenberg B, Schuelke M, Ruschendorf F, Ruf N, Kaindl AM, Henneke M, Thiele H, Stoltenburg-Didinger G, Aksu F, Topaloglu H, Nurnberg P, Hubner C, Weschke B, and Gartner J.** Mutations in the gene encoding gap junction protein alpha 12 (connexin 46.6) cause Pelizaeus-Merzbacher-like disease. *Am.J.Hum.Genet.* 75: 251-260, 2004.

123. **Valanne L, Ketonen L, Majander A, Suomalainen A, and Pihko H.** Neuroradiologic findings in children with mitochondrial disorders. *AJNR Am.J.Neuroradiol.* 19: 369-377, 1998.

124. **van den Boom R, Lesnik Oberstein SA, Ferrari MD, Haan J, and van Buchem MA.** Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy: MR imaging findings at different ages--3rd-6th decades. *Radiology* 229: 683-690, 2003.

125. **van der Knaap MS and Valk J.** *Magnetic Resonance of Myelination and Myelin Disorders.* Berlin, Heidelberg: Springer-Verlag, 2005.

126. **van der Knaap MS, Vermeulen G, Barkhof F, Hart AA, Loeber JG, and Weel JF.** Pattern of white matter abnormalities at MR imaging: use of polymerase chain

reaction testing of Guthrie cards to link pattern with congenital cytomegalovirus infection. *Radiology* 230: 529-536, 2004.

127. **van der Knaap MS, van Berkel CG, Herms J, van Coster R, Baethmann M, Naidu S, Boltshauser E, Willemsen MA, Plecko B, Hoffmann GF, Proud CG, Scheper GC, and Pronk JC.** eIF2B-related disorders: antenatal onset and involvement of multiple organs. *Am.J.Hum.Genet.* 73: 1199-1207, 2003.

128. **van der Knaap MS, van der Voorn P, Barkhof F, Van Coster R, Krageloh-Mann I, Feigenbaum A, Blaser S, Vles JS, Rieckmann P, and Pouwels PJ.** A new leukoencephalopathy with brainstem and spinal cord involvement and high lactate. *Ann.Neurol.* 53: 252-258, 2003.

129. **van der Knaap MS, Leegwater PA, Konst AA, Visser A, Naidu S, Oudejans CB, Schutgens RB, and Pronk JC.** Mutations in each of the five subunits of translation initiation factor eIF2B can cause leukoencephalopathy with vanishing white matter. *Ann.Neurol.* 51: 264-270, 2002.

130. **van der Knaap MS, Naidu S, Pouwels PJ, Bonavita S, van Coster R, Lagae L, Sperner J, Surtees R, Schiffmann R, and Valk J.** New syndrome characterized by hypomyelination with atrophy of the basal ganglia and cerebellum. *AJNR Am.J.Neuroradiol.* 23: 1466-1474, 2002.

131. **van der Knaap MS.** Magnetic resonance in childhood white-matter disorders. *Dev.Med.Child Neurol.* 43: 705-712, 2001.

132. **van der Knaap MS, Naidu S, Breiter SN, Blaser S, Stroink H, Springer S, Begeer JC, van Coster R, Barth PG, Thomas NH, Valk J, and Powers JM.** Alexander disease: diagnosis with MR imaging. *AJNR Am.J.Neuroradiol.* 22: 541-552, 2001.

133. **van der Knaap MS, Breiter SN, Naidu S, Hart AA, and Valk J.** Defining and categorizing leukoencephalopathies of unknown origin: MR imaging approach. *Radiology* 213: 121-133, 1999.

134. **van der Knaap MS, Wevers RA, Struys EA, Verhoeven NM, Pouwels PJ, Engelke UF, Feikema W, Valk J, and Jakobs C.** Leukoencephalopathy associated with a disturbance in the metabolism of polyols. *Ann.Neurol.* 46: 925-928, 1999.

135. **van der Knaap MS, Barth PG, Gabreels FJ, Franzoni E, Begeer JH, Stroink H, Rotteveel JJ, and Valk J.** A new leukoencephalopathy with vanishing white matter. *Neurology* 48: 845-855, 1997.

136. **van der Knaap MS, Barth PG, Vrensen GF, and Valk J.** Histopathology of an infantile-onset spongiform leukoencephalopathy with a discrepantly mild clinical course. *Acta Neuropathol.(Berl)* 92: 206-212, 1996.

137. **van der Knaap MS, Barth PG, Stroink H, van Nieuwenhuizen O, Arts WF, Hoogenraad F, and Valk J.** Leukoencephalopathy with swelling and a discrepantly mild clinical course in eight children. *Ann.Neurol.* 37: 324-334, 1995.

138. **van der Knaap MS, Valk J, de Neeling N, and Nauta JJ.** Pattern recognition in magnetic resonance imaging of white matter disorders in children and young adults. *Neuroradiology* 33: 478-493, 1991.

139. **van der Knaap MS, van der Grond J, van Rijen PC, Faber JA, Valk J, and Willemse K.** Age-dependent changes in localized proton and phosphorus MR spectroscopy of the brain. *Radiology* 176: 509-515, 1990.

140. **Van Goethem G, Luoma P, Rantamäki M, Al Memar A, Kaakkola S, Hackman P, Krahe R, Lofgren A, Martin JJ, De Jonghe P, Suomalainen A, Udd B, and Van Broeckhoven C.** POLG mutations in neurodegenerative disorders with ataxia but no muscle involvement. *Neurology* 63: 1251-1257, 2004.

141. **Vanhanen SL, Raininko R, and Santavuori P.** Early differential diagnosis of infantile neuronal ceroid lipofuscinosis, Rett syndrome, and Krabbe disease by CT and MR. *AJNR Am.J.Neuroradiol.* 15: 1443-1453, 1994.

142. **Veltman JA, Jonkers Y, Nuijten I, Janssen I, van der Vliet W, Huys E, Vermeesch J, Van Buggenhout G, Fryns JP, Admiraal R, Terhal P, Lacombe D, van Kessel AG, Smeets D, Schoenmakers EF, and van Ravenswaaij-Arts CM.** Definition of a critical region on chromosome 18 for congenital aural atresia by arrayCGH. *Am.J.Hum.Genet.* 72: 1578-1584, 2003.

143. **Vermeulen G, Seidl R, Mercimek-Mahmutoglu S, Rotteveel JJ, Scheper GC, and van der Knaap MS.** Fright is a provoking factor in vanishing white matter disease. *Ann.Neurol.* 57: 560-563, 2005.

144. **Volterra A and Meldolesi J.** Astrocytes, from brain glue to communication elements: the revolution continues. *Nat.Rev.Neurosci.* 6: 626-640, 2005.

145. **Wardinsky TD, Weinberger E, Pagon RA, Clarren SK, and Thuline HC.** Partial deletion of the long arm of chromosome 11 [del(11)(q23.3----qter)] with abnormal white matter. *Am.J.Med.Genet.* 35: 60-63, 1990.

146. **Weiss BJ, Kamholz J, Ritter A, Zackai EH, McDonald-McGinn DM, Emanuel B, and Fischbeck KH.** Segmental spinal muscular atrophy and dermatological findings in a patient with chromosome 18q deletion. *Ann.Neurol.* 30: 419-423, 1991.

147. **Wertelecki W and Gerald PS.** Clinical and chromosomal studies of the 18q-syndrome. *J.Pediatr.* 78: 44-52, 1971.

APPENDIX

Structure or finding no.	Anatomic structure or finding
1	Periventricular white matter involved?
2	Lobar white matter involved?
3	U-fibers involved?
4	Predominantly involved white matter zone: periventricular?
5	Predominantly involved white matter zone: lobar?
6	Predominantly involved white matter zone: U-fibers?
7	Global involvement of all white matter zones?
8	White matter of the frontal lobe involved?
9	White matter of the parietal lobe involved?
10	White matter of the occipital lobe involved?
11	White matter of the temporal lobe involved?
12	Predominantly involved lobe: frontal?
13	Predominantly involved lobe: parietal?
14	Predominantly involved lobe: occipital?
15	Predominantly involved lobe: temporal?
16	Global involvement of all lobes?
17	Cerebellar white matter involved?
18	Posterior fossa white matter predominantly involved?
19	Middle cerebellar peduncles involved?
20	Pyramidal tracts involved?
21	Central tegmental tracts involved?
22	Medulla oblongata involved?
23	Other brainstem tracts involved?
24	Hilus of the dentate nucleus involved?
25	Inner rim of the corpus callosum involved?
26	Outer rim of the corpus callosum involved?
27	Anterior part of the corpus callosum involved?
28	Middle part of the corpus callosum involved?
29	Posterior part of the corpus callosum involved?
30	Anterior limb of the internal capsule involved?
31	Posterior limb of the internal capsule involved?
32	External and extreme capsules involved?
33	Confluent abnormalities?
34	Isolated multifocal abnormalities?
35	Homogeneous abnormalities?
36	Symmetrical abnormalities?
37	Low signal intensity on intermediate-weighted images?
38	Myelination delayed?
39	Myelination arrested in an early stage?
40	Myelination abnormal and irregular?
41	Abnormality in myelination predominant pathologic finding?
42	Cerebral cortex involved?
43	Caudate nucleus involved?

44	Putamen involved?
45	Globus pallidus involved?
46	Thalamus involved?
47	Dentate nucleus involved?
48	Cerebellar cortex involved?
49	White matter volume loss?
50	Ventricular enlargement?
51	Enlarged subarachnoid spaces?
52	Atrophy of cerebellar vermis?
53	Atrophy of cerebellar hemispheres?
54	Subcortical cysts?
55	Intraparenchymal cysts?
56	Frontal location cysts?
57	Parietal location cysts?
58	Occipital location cysts?
59	Temporal location cysts?
60	White matter swelling?
61	Calcium depositions?
62	Stripe-like pattern on sagittal images?
63	Iron depositions?
64	Other extra characteristics?
65	Change over time: improvement?
66	Change over time: stationary?
67	Change over time: worsening?
68	No second MRI obtained?

Source (133).