Megalencephalic leukoencephalopathy with subcortical cysts: chronic white matter oedema due to a defect in brain ion and water homoeostasis

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Megalencephalic leukoencephalopathy with subcortical cysts (MLC) is characterised by chronic white matter oedema. The disease has an infantile onset and leads to slow neurological deterioration in most cases, but, surprisingly, some patients recover. The first disease gene, MLC1, identified in 2001, is mutated in 75% of patients. At that time, nothing was known about MLC1 protein function and the pathophysiology of MLC. More recently, HEPACAM (also called GLIALCAM) has been identified as a second disease gene. GlialCAM serves as an escort for MLC1 and the chloride channel CLC2. The defect in MLC1 has been shown to hamper the cell volume regulation of astrocytes. One of the most important consequences involves the potassium siphoning process, which is essential in brain ion and water homoeostasis. An understanding of the mechanisms of white matter oedema in MLC is emerging. Further insight into the specific function of MLC1 is necessary to find treatment targets.

Introduction

Megalencephalic leukoencephalopathy with subcortical cysts (MLC) is an infantile-onset inherited disease of the brain white matter. The disease was recognised independently in India and the Netherlands.1,2 MLC has also been called van der Knaap disease,3,4 but if one prefers eponyms, van der Knaap–Singhal disease would be more appropriate. Two affected siblings with a severe variant had been described before,5 in whom we later confirmed the diagnosis by DNA analysis. The core phenotype was described in the first two reports of MLC.1,2 Patients develop macrocephaly in infancy. Brain MRI shows diffuse cerebral white matter signal abnormalities and swelling of the abnormal white matter. By contrast, most patients initially have no or only mild neurological signs. MLC is very rare and there have been no formal studies of its incidence, which seems to be highest in the Turkish population1,5 and Indian Agrawal community.6,7 As is the case for diseases with a low carrier frequency, consanguinity and inbreeding contribute to its occurrence.

During the past few years, insight into the molecular basis and disease mechanisms of MLC has increased rapidly. MLC is now known to be a disease with a defect in brain ion and water homoeostasis and, with that, a defect in brain volume regulation. The result is chronic white matter oedema and slowly progressive neurological deterioration, but some patients have a surprising recovery. In this Review we discuss new research findings and provide background information that is necessary to understand which processes are affected by the MLC-related defect. Understanding of disease mechanisms of MLC is pertinent for insight into brain oedema.

Clinical phenotypes: classic and remitting phenotypes and variation

The classic phenotype is most common and has an autosomal recessive mode of inheritance. There are two associated genes: MLC1 and HEPACAM (also called GLIALCAM). Classic MLC related to recessive MLC1 mutations has been called MLC1 (MIM number 604004).12 Classic MLC related to recessive HEPACAM mutations has been called MLC2A (MIM number 613925).12

At birth, infants with classic MLC are healthy. In the first year of life, patients develop macrocephaly in the absence of other neurological signs.1,2,13,14 After the first year, head growth rate becomes normal, with a plot of head circumference that runs parallel to and above the 98th percentile for healthy children.1 Some patients have a borderline macrocephaly,2,11 but more often the macrocephaly is striking.1 Most children are mildly delayed in achieving unsupported walking and their gait is unstable.1,2,11,13 After several years, patients with MLC develop slowly progressive cerebellar ataxia and, to a lesser degree, spasticity.1,12,13,14 They most often become wheelchair-dependent as teenagers. Cognitive capacities are initially either normal or mildly decreased.1,11,13,14 Autism is observed in some patients.2 Mild cognitive deterioration becomes apparent over the years, but communication and social skills remain relatively unaffected.12 Some patients develop extrapyramidal movement abnormalities with dystonia and athetosis.12 Almost all children have occasional epileptic seizures, easily controlled with drugs,1 but status epilepticus can occur.2,16 Minor head trauma can induce temporary deterioration, most often with seizures or status epilepticus, prolonged unconsciousness lasting days to months, or acute motor deterioration with gradual improvement.12,14 No systematic study of the lifespan of patients with MLC has been done. The earliest death known to us is at age 15 years due to status epilepticus (personal observation), but many living patients in their 40s and 50s are known.1,2,20

Some patients have a more severe clinical course and maintain the ability to walk without support for only a
short time or never achieve it. Other patients have a more benign clinical course. As a young adult they can have normal cognitive and motor function and only borderline macrocephaly. Some patients pursue a career and still walk without support in their 40s. Striking differences in disease severity can occur among patients with the same mutation and sibling patients. Unaffected siblings and parents are healthy and do not have macrocephaly.

Patients have been recognised who initially have a typical clinical picture, but subsequently improve and lack signs of deterioration. This phenotype is related to autosomal dominant mutations in HEPACAM, and has been assigned MIM number 613926. Patients with MLC2B develop macrocephaly within the first year of life. Their initial development is normal or mildly delayed. Subsequently, motor capabilities can become normal or patients can remain clumsy, but not spastic or ataxic. IQ can be normal or mildly decreased. About half of the patients with a cognitive deficit have autism. Most patients retain macrocephaly, but in some head circumference becomes normal. Often one of the parents has macrocephaly, sometimes a history of transient macrocephaly as a child. Similar patient was described in the 1980s, before MLC was recognised as a disease.

Investigations
Laboratory findings
Laboratory investigations are unrevealing. No biochemical marker of the disease has been found in urine, blood, or CSF.

Neurophysiology
Peripheral motor and sensory nerve conduction velocities and electromyograms are normal. Evoked potentials are initially normal. Brainstem auditory evoked potentials usually remain normal. In classic MLC, somatosensory and visual evoked potentials deteriorate over the years, with prolonged latencies and abnormal cortical responses. Electroencephalograms are initially normal. Subsequently, in the classic phenotype, background slowing occurs and sharp waves, spikes, and spike-wave complexes with variable location are seen. Abnormal photoparoxysmal responses are present in some patients, but not in all. No systematic neurophysiological data are available for patients with the remitting phenotype.

MRI and spectroscopy
Most patients undergo MRI of the brain in the first year because of rapidly increasing macrocephaly. MRI shows diffuse cerebral white matter abnormality with swelling of the abnormal white matter, compressing the ventricles and subarachnoid spaces (figure 1A). On FLAIR images, part of the cerebral white matter might have slightly lower signal intensity than the cortex, related to the very high water content of the white matter. Diffusion-weighted imaging shows increased diffusivity with high apparent diffusion coefficient values in the cerebral white matter and reduced anisotropy, indicative of increased size of the water spaces. Subcortical cysts are invariably present in the anterior temporal region, frequently also in frontal and parietal regions (figure 1A). In infants, the anterior temporal white matter can be rarefied, but not yet cystic.

Central structures including the corpus callosum and anterior limb of the internal capsule are usually preserved. The occipital periventricular and subcortical white matter is better preserved than the rest of the cerebral hemispheric white matter. The posterior limb of the internal capsule is usually partly affected, either in the lateral part or as two lines with a spared line in the middle. The brainstem and cerebellar white matter are typically mildly abnormal in signal and never swollen. Cortical and central grey matter structures are not affected.

Over time, the abnormal cerebral white matter becomes less severely swollen and atrophy ensues, leading to widened lateral ventricles and subarachnoid spaces (figure 1B, D). Prominent atrophy is usually seen only in adults. The subcortical cysts can become larger and more numerous over the years. Contrast enhancement is not a feature of the disease at any stage, indicating that the blood–brain barrier is gresso modo intact.

We have observed less prominent white matter abnormalities in exceptional patients with DNA-confirmed classic MLC and clinically mild disease (personal observations). In such children, early MRI shows the typical diffuse cerebral white matter abnormalities with swelling, whereas follow-up MRI shows improvement or even normalisation of part of the cerebral white matter and decreased swelling. The subcortical white matter remains abnormal and the characteristic subcortical cysts remain. No systematic study has been done on the subject, but we and others have the impression that more severe changes on MRI, especially in patients older than a few years, are associated with more severe disease.

In patients with the remitting clinical phenotype, striking improvement and normalisation of the initial MRI abnormalities occur (figure 2). In retrospect, early MRIs also show less severe abnormalities than do those of patients with the classic phenotype. Cysts in the anterior temporal region are typically but not invariably present, but there are no subcortical cysts elsewhere. The cerebellar white matter is typically normal from the outset. Subsequently, the signal abnormality and swelling decrease, first in the pericentral and occipital regions, spreading to the remainder of the cerebral white matter. The last white matter abnormalities are seen in subcortical regions (figure 2F). Subcortical cysts decrease in size and...
disappear (figure 2A–C). Most improvement occurs between 1 year and 4 years. We have documented completely normal MRIs on long-term follow-up, although follow-up of some patients has not been long enough to document normalisation. We suspect that, in the end, normalisation occurs in all patients with the remitting phenotype.

Proton magnetic resonance spectroscopy (MRS) of the cerebral white matter has been reported only in patients with the classic phenotype. It reveals a reduction of all signals per volume, indicative of high water content. In severely affected white matter, metabolites might be undetectable. Relative to creatine, N-acetylaspartate is decreased and myoinositol is increased, indicating axonal damage and gliosis. With increasing age, the abnormalities become more pronounced.

Pathology

Few pathological data are available for MLC. Most information comes from four brain biopsies. In all biopsies, the cortex was normal, apart from astrocytosis of the molecular layer, a common finding in patients with chronic epilepsy. A brain biopsy from the frontal lobe in a 13-year-old patient revealed countless small or larger vacuoles in the white matter, accompanied by fibrillary astrogliosis, without signs of inflammation. This brain biopsy was carefully prepared to avoid artifacts of swelling or shrinking. The lining of the vacuoles was positive for myelin basic protein. Electron microscopy revealed that the vacuoles were covered by membranes with a layered structure with major dense lines and intraperiod lines (figure 3A, B), confirming their myelinic nature. The separation of the myelin lamellae was invariably at the intraperiod line that is continuous with the extracellular space. Most vacuoles were covered by five layers and some had a multilamellar border over part of their extent with the typical periodicity of myelin, suggesting that the splitting occurred at the intraperiod line in the outer part of myelin sheaths. Vacuoles covered by multiple myelin lamellae over their entire extent, located in the middle of myelin sheaths, and vacuoles bordering axons, located in the inner part of myelin sheaths, were not observed. No appreciable decrease in myelin content was observed. In the same brain biopsy, vacuoles were also found in the endfeet of astrocytic processes ending on capillaries (figure 3C). Intramyelinic vacuoles within the white matter have also been reported by others. Two studies mention enlarged extracellular spaces. Myelin sheaths were abnormally thin in three biopsies.
disease gene was found, called KIAA0027 or WKL1, which was renamed MLC1. Many different mutations have been published (appendix), which are distributed over the entire gene. There are several founder effects for the MLC1 gene. The most common founder mutation is in the Indian Agrawal community, in which all patients share an insertion of one base-pair (c.135dupC), causing a frame shift and premature stop (p.Cys46LeufsX34). Founder effects have also been described in Jewish, Lebanese, and Japanese patients (appendix). There is no evidence of a genotype–phenotype correlation. The full phenotypic range is seen among patients from the Agrawal community, who are homozygous for the same founder mutation.

From the beginning, it was clear that MLC1 mutations were found in only 70–80% of patients with MLC. Several informative MLC families in whom no MLC1 mutations were found excluded linkage of the disease with the locus on chromosome 22qtel, indicating that there had to be at least one other gene for MLC. Studies in several laboratories failed to identify a second disease gene, suggesting further genetic heterogeneity. In a candidate gene approach, CLCN2 encoding the chloride channel CLC2 was analysed. No pathogenic mutations were found.

Recently, two different phenotypes were shown among non-MLC1-mutated patients with MLC, substantiating the idea of genetic heterogeneity. Some of these patients had the classic phenotype, but more than half had the remitting phenotype. A proteomic approach showed a consistent association between MLC1 and a protein called GlialCAM. Analysis of the gene HEPACAM revealed two mutations with recessive inheritance in patients with the classic phenotype, and one mutation with dominant inheritance in patients with the remitting phenotype (appendix). Recessive HEPACAM mutations were spread over the entire extracellular region of GlialCAM, whereas dominant mutations were clustered in the first immunoglobulin domain. There was no overlap between dominant and recessive mutations; they were consistently different, but could affect the same residue.

There is no evidence that there is another gene for MLC. No informative MLC families have been reported that exclude linkage of the disease with both the MLC1 and HEPACAM loci.

### Association of MLC1 and HEPACAM with psychiatric disorders

In 2001, an association between a p.Leu309Met mutation in MLC1 and autosomal dominant catatonic schizophrenia (MIM 605419) was reported in one large pedigree. Several subsequent studies did not confirm an association between heterozygous MLC1 variants and schizophrenia. In 2005, however, an association of the MLC1 gene with schizophrenia and bipolar disorder was shown, again suggesting an involvement of MLC1 as a susceptibility gene for schizophrenia and bipolar disorder. A further study suggested an association between MLC1 variants and periodic catatonia, a non-remitting subtype of schizophrenia with bipolar course, but not with schizophrenia or bipolar disorder in general. Interestingly, blood gene expression profiles of unmedicated patients with a major depressive disorder led to identification of a set of seven genes that serves as a molecular signature of major depressive disorder, and MLC1 was one of these. Also interesting is that patients with the remitting phenotype of MLC1 had the reconstituted HEPACAM mutations frequently have autism.

### Proteins, their functions, and effect of mutations

MLC1 protein

When MLC1 was identified as a gene associated with MLC, nothing was known about the function of the MLC1 protein. All species that produce myelin have the MLC1 protein, whereas species that do not produce myelin do not have the gene. MLC1 is a plasma membrane protein. Northern blot shows that it is expressed...
mainly in the brain and white blood cells. The expression level is much higher in the brain. Immunohistochemical staining of brain tissue shows that MLC1 is located at the blood–brain and CSF–brain barriers: in distal astroglial processes around blood vessels in the cortex and white matter (figure 4A), in distal astroglial processes at the glial limiting membrane of the pia, and in ependymal cells. Oligodendrocytes and microglia do not express MLC1.

The striking similarity in brain MRI abnormalities between MLC and congenital muscular dystrophy with merosin deficiency and the histological evidence of myelin vacuolation in both disorders drew our attention. Merosin is part of the dystrophin-glycoprotein complex that links cytoskeletal proteins to the extracellular matrix. In the brain, one of its locations is the interface between astrocytic endfeet and the perivascular basal lamina, where it anchors endfeet to the basal lamina. Evidence of colocalisation of MLC1 and members of the dystrophin-glycoprotein complex was found in immunohistochemistry at light microscopic level and in coimmunoprecipitation using cross-linking reagents, but coimmunoprecipitation studies without cross-linking failed to confirm the interaction. Electron microscopy with immunogold staining for MLC1 showed that the protein is present in astrocytic junctions, but not at the vascular basal lamina (figure 3D). In these astrocyte junctions, MLC1 has been shown to colocalise with components of tight (ZO-1) and adherent (β-catenin) junctions. Double immunogold electron microscopy for MLC1 and β dystroglycan, a member of the dystrophin-glycoprotein complex, provided final proof that MLC1 does not colocalise with the dystrophin-glycoprotein complex at astrocytic endfeet (figure 3D).

Aminoacid sequence analysis reveals a weak similarity with several channels and transporters. MLC1 contains an internal aminoacid sequence repeat, as found in several ion channel proteins. These data, in combination with the localisation of MLC1 at astrocytic endfeet at blood–brain and CSF–brain barriers and the highly increased water content of the cerebral white matter in MLC, suggested that MLC1 could have a channel or transporter function, involved in brain water and ion homeostasis.

Water homeostasis and osmotic balance are vital in the brain. Within the bony casket of the skull, any significant brain swelling leads to herniation of the brain and death of the patient. Astrocytes are central in the process of brain volume regulation. They are highly sensitive to changes in extracellular osmolality and can display prominent cell volume changes as part of the osmoregulatory process. Even small osmotic perturbations induce a transmembrane flow of ions and water in astrocytes that rapidly restores the osmotic equilibrium and induces temporary swelling or shrinkage of astrocytes, followed by regulatory volume decrease or increase, respectively, to normalise cell volume. Regulatory volume decrease involves activation of ion channels and transporters that allow effluxes of potassium, chloride, organic osmolites, and water. Volume-regulated anion channels (VRACs) play an important part in the regulatory volume decrease. VRACs are activated by water fluxes and changes in cell shape and volume. VRAC function is probably dependent on multiple channels, which could be different for different cell types. The molecular identity of most of these channels is unknown.

An association has been found between MLC1 expression and chloride currents in different cell types, including lymphoblasts and astrocytes, the main cell

Figure 4: Immunohistochemistry and immunocytochemistry
MLC1 immunoreactivity (A, B) is found in astrocytic processes surrounding blood vessels in the normal human brain (A), but no MLC1 immunoreactivity is found in the brain from a patient with MLC (B). Immunocytochemistry in astrocytes shows that normal localisation of GlialCAM (red, C), MLC1 (green, coexpressed with GlialCAM, D), and CLC2 (green, coexpressed with GlialCAM, E) is seen in junctions of astrocytic processes (overlap in yellow, D, E). By contrast, the presence of an MLC-causing HEPACAM mutation (G89D, p.Gly89Asp) leads to mislocalisation of GlialCAM (red, F), MLC1 (green, G), and CLC2 (green, H).
types that express MLC1 (figure 5A–D). These currents are greatly increased by cell swelling induced by hypo-osmotic pretreatment (figure 5C, D). Ion substitution experiments, current–voltage profile, the activation of the currents by hypo-osmotic pretreatment, and sensitivity to inhibition by specific ion channel blockers indicate that the MLC1-related chloride current is a VRAC activity. Further studies of the regulatory volume decrease after cell swelling in lymphoblasts and astrocytes confirm that a defect in MLC1 reduces the rate of the regulatory volume decrease (figure 5E, F). Notably, the regulatory volume decrease is slower and not abolished in the absence of normal MLC1, indicating that it is not exclusively dependent on MLC1 function. MLC is not an immediately life-threatening disease. Complete abolition of the regulatory volume decrease would not be compatible with life. These studies left open the question of whether MLC1 is a VRAC, a component of a VRAC, or a protein that activates a VRAC, either directly or indirectly.

Recent studies suggest that MLC1 could be part of a multiprotein complex that interacts with TRPV4, a channel that mediates hypo-osmosis-induced cytosolic calcium increase, which is essential for activation of the regulatory volume decrease after cell swelling. MLC1 mutations have been suggested to reduce MLC1 interactions with the complex and abolish the TRPV4-mediated intracellular calcium influx in astrocytes induced by hypo-osmosis. The decreased influx of calcium in response to cell swelling could account for the decreased VRAC activation and decreased regulatory volume decrease in MLC. Importantly, these studies were not based on specific coimmunoprecipitation using MLC1 antibodies, but on less specific ouabain-chromatography. Additionally, all experiments were done with overexpression of normal and mutant MLC1 in astrocytoma tumour cells, and overexpression can cause artifactual results. Further studies are necessary to clarify the possible role of TRPV4 in MLC, including experiments in cells with reduced MLC1 expression and different cell types, in particular cultured primary astrocytes.

**GliaCAM protein**

In 2005, a novel gene was identified, designated *HEPACAM*, encoding a hepatic cell adhesion molecule (*HEPACAM*). In 2008, the protein was renamed glial cell adhesion molecule (GliaCAM), because liver expression is very low and it is predominantly expressed in glia of the nervous system. Cell adhesion molecules (CAMs) are transmembrane proteins at the cell surface. They interact with other cell adhesion molecules on the adjacent cell surface and with proteins in the extracellular matrix. Inside the cell, they interact with the actin cytoskeleton, which is the key structure in the maintenance of cell integrity and shape and in the movement of cells. Cell adhesion is crucial for the formation and maintenance of cellular architecture and for processes such as migration, proliferation, differentiation, apoptosis, and survival. CAMs also play a crucial part in modulating cytoplasmic signalling cascades by capturing and integrating signals from the extracellular environment.
GlialCAM has the typical structure of immunoglobulin-like cell adhesion molecules (IgCAMs), with two extracellular immunoglobulin-like domains, a transmembrane segment, and a cytoplasmic tail. IgCAMs are particularly abundant in the nervous system. During development, IgCAMs are involved in neuronal and glial migration, process guidance, target recognition, and synapse formation. In the mature nervous system, they participate in the maintenance and function of neuronal networks, the integrity of the ependymal lining, and in the formation and stability of myelin sheaths around axons.

Within the human brain, GlialCAM expression is seen mainly within white matter tracts and ependymal cells. Within the white matter, it is present mainly in astrocytic endfeet around blood vessels. Additionally, GlialCAM is localised inside axons, in contact regions between myelin and axons, on the outside of myelin sheaths, and in oligodendrocytes. Immunogold electron microscopy with double staining for GlialCAM and MLC1 show that they colocalise in astrocyte–astrocyte junctions at astrocytic endfeet (figure 3E). Both MLC1 and GlialCAM are associated with caveolae, which are small, flask-like invaginations of the plasma membrane, present in most adherent cells. Caveolae are important in the compartmentalisation of lipid and protein components that function in signal transduction, biosynthetic transport functions, endocytosis, and transcytosis.

The function of GlialCAM is unknown. It has been studied mainly in the context of different types of cancer. Studies of MLC indicate that one of the GlialCAM functions is to act as an MLC1 β subunit necessary for its trafficking to cell–cell junctions. GlialCAM is necessary for the correct localisation of MLC1, but the expression and localisation of GlialCAM are independent of MLC1. In the presence of mutant MLC1, GlialCAM is mislocalised (figure 4), but mutant MLC1 does not change GlialCAM localisation. The facts that GlialCAM is not obligatorily associated with MLC1 and that GlialCAM is expressed in cell types that do not express MLC1, such as oligodendrocytes, suggest that it has other functions.

Chloride channel CLC2

The chloride channel encoded by CLCN2 belongs to the CLC family of chloride channels or transporters. It is almost ubiquitously expressed and is also abundant in the brain. Within the brain, CLC2 is, like MLC1 and GlialCAM, localised in astrocyte–astrocyte junctions at astrocytic endfeet around blood vessels and in Bergmann glia. Additionally, CLC2 is, like GlialCAM, located in myelinated fibre tracts. Hypotonic cell swelling can activate CLC2. Glial CLC2 is important in maintaining extracellular ion homoeostasis in the brain.

Clen2 knockout mice display severe degeneration of the retina and testes, leading to blindness and male infertility. Additionally, they have highly increased brain white matter water content with vacuole formation in the outer myelin lamellae, similar to what is seen in the brains of patients with MLC. Homozygous Clcn2 mutant mice also display myelin vacuolation. Strikingly and similar to the earlier stages of MLC in patients, the mice lack overt neurological deficits despite extensive myelin vacuolation of the brain white matter.

No CLCN2 mutations were found in patients with MLC who did not have MLC1 mutations. No direct protein interaction was found between MLC1 and CLC2. The Clcn2 knockout mouse showed no changes in GlialCAM or MLC1 expression and localisation. GlialCAM has been shown to be a CLC2 binding partner. GlialCAM targets CLC2 to astrocyte–astrocyte junctions, whereas HEPACAM mutations, as identified in patients with MLC, abolish this targeting (figure 4). GlialCAM greatly increases CLC2-mediated chloride currents, changing properties of activation and rectification (appendix). GlialCAM has a much more restricted expression pattern than does CLC2 and GlialCAM is clearly not an obligate binding partner of CLC2, but an auxiliary subunit that associates with CLC2 only in glial cells.

Mutations and their effects

MLC1 mutations, both missense and null, lead to the same result: major reduction or absence of the plasma membrane expression of MLC1 (figure 4B). This similarity probably accounts for the absence of genotype–phenotype correlation.

All known MLC-causing HEPACAM mutations, both dominant and recessive, affect the extracellular part of GlialCAM and not its transmembrane and intracellular part. MLC-causing HEPACAM mutations lead to a GlialCAM-trafficking defect. Partial or complete deletion of the intracellular GlialCAM domain does not change the plasma membrane localisation. Apparently, the intracellular domain is needed for cell–matrix modulation, but is dispensable for the membrane localisation; only disruption of membrane localisation leads to MLC.

The effect of dominant versus recessive GlialCAM mutations has been studied at the cellular level. The defects in trafficking of MLC1 and GlialCAM caused by recessive mutations in GlialCAM can be rescued by coexpression of the normal protein, but the trafficking defects caused by dominant mutations in GlialCAM cannot be rescued by coexpression of the normal protein. These results suggest that the difference between the recessive progressive MLC phenotype and dominant remitting phenotype is not a protein dosage effect. At present, why some GlialCAM mutations behave as recessive and others as dominant, and why dominant mutations are associated with a much more benign disease course than recessive mutations, is unclear.

All dominant mutations identified thus far are located in a
putative pocket of the immunoglobulin variable domain and have been suggested to disrupt interactions with GlialCAM itself and other molecules, but recessive mutations might affect the same domain.22 The clinical and MRI phenotypes related to recessive MLC1 and HEPACAM mutations are indistinguishable,21,22 suggesting that the molecular basis of MLC is either in all cases dependent on deficiency of cell-surface expression and function of MLC1 or on a defect in the interaction of MLC1 and GlialCAM or the interaction of these proteins with a third partner, such as CLC2. In view of the presumed functional roles of GlialCAM as a cell adhesion molecule in cell growth, differentiation, motility, and adhesion and its widespread expression compared with that of MLC1, there is no evidence that these functions are affected in patients with MLC and HEPACAM mutations is surprising. A possible explanation is that apart from bringing MLC1 to astrocyte–astrocyte junctions, GlialCAM functions are redundant and can be executed by other proteins. Another possibility is that other HEPACAM mutations or mutations in other domains are associated with a different, as yet unidentified, disease.

The role of CLC2 in this context is unclear. There is no evidence that CLCN2 mutations underlie MLC or any other human disease.41,52 In view of the widespread expression of CLC2 in the human body, CLCN2 mutations could lead to a disease dominated by non-neurological manifestations and a brain white matter disease could be present, but overlooked because of lack of overt neurological signs, as happened in the Clcn2 knockout mouse. HEPACAM mutations, as identified in patients with MLC, disrupt the localisation of both MLC1 and CLC2. The data available at present suggest that MLC1–GlialCAM and CLC2–GlialCAM are different complexes, although they could be functionally related. In view of the functional association of GlialCAM with both MLC1 and CLC2, why the disease associated with HEPACAM mutations is not more severe than the disease associated with MLC1 mutations is unclear.

Pathophysiology

MLC is a disease of chronic brain white matter oedema with accumulation of water in vacuoles between myelin lamellae and astrocytic endfeet. MLC1 has recently been
shown to have a role in the regulatory volume decrease after cell swelling. 113

Action potential firing causes continuous shifts of ions and associated osmotic water, necessitating tight control of water and ion homeostasis in the brain. Astrocytes are central to this process. They are the most abundant cell type of the so-called pangial syncytium. This is a vast network of interconnected glia, consisting of astrocytes, oligodendrocytes, and ependymal cells, which are extensively interlinked by gap junctions. Gap junctions are composed of connexin proteins and form channels across the intercellular space. The pangial syncytium is essential for long-distance disposal of excess axonally derived potassium and water in a process called potassium siphoning. 97–98

In myelinated axons, depolarisation-related sodium influx occurs at nodes of Ranvier; potassium efflux occurs in paranodal and internodal axonal regions and water transport between myelin layers at successive intercellular spaces (figure 6). 99 Electrical and osmotic gradients are the driving forces for this process, which prevents potassium-induced osmotic swelling of myelin. 99

If any molecular component of the pangial syncytium is compromised, hampered siphoning of excess potassium related to neuronal activity leads to osmotic myelin swelling. 99 Kir4.1 knockout mice 99 and double-knockout mice for gap junction proteins connexin32 and connexin47 99 display myelin vacuolation. Disruption of CLC2 similarly leads to brain myelin vacuolation in mice. 114,115 Interestingly, no myelin vacuolation in the optic nerve is observed in Kir4.1 knockout mice, if action potential generation is blocked with tetrodotoxin, 99 and in Clcn2 knockout mice, which are blind because of retinal degeneration, 116 emphasising the importance of the siphoning process during neuronal activity. In human beings, mutations in connexin32 are associated with stress-provoked episodes of brain dysfunction with MRI evidence of acute, reversible myelin vacuolation. 100,101 We hypothesise that in MLC one of the most important consequences of the defect in the astrocytic regulatory volume decrease, caused by disrupted volume-regulated chloride channel activity, is that the disposal of excess potassium and water associated with action potential firing through the astrocytic syncytium is hampered, necessitating slightly steeper electrical and osmotic gradients, explaining the presence of extra water in the form of vacuoles in myelin and astrocytic endfeet.

**Diagnosis and differential diagnosis**

In MLC, rapidly increasing macrocephaly is the most common reason for presentation in the first or second

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<td>Diffuse cerebral leukoencephalopathy, no grey matter lesions, anterior temporal cysts</td>
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<tr>
<td>Merosin-deficient congenital muscular dystrophy</td>
<td>Infancy</td>
<td>Severe muscle weakness</td>
<td>Diffuse cerebral leukoencephalopathy, no grey matter lesions, occasionally anterior temporal cysts</td>
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<td>Alexander disease</td>
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<td>Early onset associated with severe course; later onset associated with slower course</td>
<td>Leukoencephalopathy with frontal preponderance, abnormalities of basal nuclei, contrast enhancement, sometimes cystic white matter degeneration</td>
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<td>Canavan disease</td>
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<td>GM, and GM, gangliosidoses, infantile form</td>
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<td>Diffuse cerebral leukoencephalopathy, abnormalities of basal nuclei and thalamus</td>
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Table: Differential diagnosis of megalencephalic leukodystrophies
year of life. Such patients undergo CT or MRI, which rules out hydrocephalus and shows profound cerebral white matter abnormalities with swelling. The severity of the white matter disease contrasts with the presence of no or only mild clinical signs at this age. The diagnosis of MLC is not difficult in typical cases and can be made on the basis of clinical and MRI criteria (panel). If no early MRI is available in patients with remitting MLC, the MRI might show only limited or no white matter abnormalities and the diagnosis can be easily missed.

The combination of macrocephaly and diffuse cerebral white matter abnormalities can be seen in Alexander disease, Canavan disease, L-2-hydroxyglutaric aciduria, congenital muscular dystrophy with merosin deficiency, and sometimes in infantile-onset GM1 and GM2 gangliosidosis.12,101,103 These disorders can be differentiated by clinical signs, MRI findings, and laboratory tests (table).12,13,101,103

Treatment
Understanding of MLC disease mechanisms is increasing, but is still incomplete. The most important missing link is that, although it has become clear that MLC1 is involved in brain volume regulation through astrocytes, its exact function in this process has not been identified. There is no easy treatment option. Trials with diuretic drugs have failed (unpublished observations). The transient MLC phenotype in patients with a dominant HEPACAM mutation suggests that there is a window of time in which rescue of MLC1 function can prevent the disease or modify its course.

With our present knowledge, two therapeutic strategies could be envisioned. Pharmacological strategies aimed at increasing cell membrane expression of MLC1 and GlialCAM could be beneficial, although whether expression of mutant proteins would compensate for the functional deficit is not known. Alternatively, activation of ion channels that have a reduced function in MLC, such as VRACs, or perhaps TRPV4 or CLC2, could be useful. Additional experiments are needed to investigate these hypotheses.

Conclusions
MLC is the first known human disease with a genetic defect in brain ion and water homeostasis and volume regulation by astrocytes. The defect results in chronic brain white matter oedema. The excess water is located in vacuoles within myelin sheaths and, to a lesser extent, astrocytic endfeet. Most patients have slow neurological deterioration, but some patients show surprising improvement or recovery, clinically and on MRI, suggesting that the disease might be accessible for treatment.

Brain oedema is a major problem in neurology. Whereas other organs have space to swell without causing a life-threatening situation, brain oedema rapidly leads to coma and death. Combating of brain oedema is of major importance in many acute and subacute situations, including cerebral infarction, trauma, and tumours. MLC can be regarded as a natural disease model of brain oedema, and the discovery of successful strategies for the treatment of MLC will probably have implications for the treatment of certain types of brain oedema. Further studies should focus on the precise role of MLC1 dysfunction in the development of oedema. Such studies could reveal therapeutic options for patients with MLC and yield information that is relevant for other conditions.

References for this Review were identified by searches of PubMed between 1969 and May, 2012, and references from relevant articles. The search terms “leukoencephalopathy”, “MLC”, “MLC1”, “HEPACAM”, “GlialCAM”, “ClC-2”, and “CLCN2” were used. There were no language restrictions. The final reference list was generated on the basis of relevance to the topics covered in this Review.


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