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MINI-SYMPOSIUM: LEUKODYSTROPHIES DUE TO ASTROYCTIC DYSFUNCTION

Genetic defects disrupting glial ion and water homeostasis in the brain

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Abstract

Electrical activity of neurons in the brain, caused by the movement of ions between intracellular and extracellular compartments, is the basis of all our thoughts and actions. Maintaining the correct ionic concentration gradients is therefore crucial for brain functioning. Ion fluxes are accompanied by the displacement of osmotically obliged water. Since even minor brain swelling leads to severe brain damage and even death, brain ion and water movement has to be tightly regulated. Glial cells, in particular astrocytes, play a key role in ion and water homeostasis. They are endowed with specific channels, pumps and carriers to regulate ion and water flow. Glial cells form a large panglial syncytium to aid the uptake and dispersal of ions and water, and make extensive contacts with brain fluid barriers for disposal of excess ions and water. Genetic defects in glial proteins involved in ion and water homeostasis disrupt brain functioning, thereby leading to neurological diseases. Since white matter edema is often a hallmark disease feature, many of these diseases are characterized as leukodystrophies. In this review we summarize our current understanding of inherited glial diseases characterized by disturbed brain ion and water homeostasis by integrating findings from MRI, genetics, neuropathology and animal models for disease. We discuss how mutations in different glial proteins lead to disease, and highlight the similarities and differences between these diseases. To come to effective therapies for this group of diseases, a better mechanistic understanding of how glial cells shape ion and water movement in the brain is crucial.

INTRODUCTION

Electrical activity in the brain relies on the existence of ionic concentration gradients over the neuronal membrane. This, together with the presence of specific voltage- and ligand-gated ion channels in the neuronal membrane, allows neurons to rapidly and specifically change their membrane potential in a propagative manner, a process that stands at the basis of all our thoughts and actions. The maintenance of ionic gradients at rest by means of ion pumps, together with the energy required for restoring ion fluxes associated with action potentials and synaptic transmission, comprises a large part of total brain energy consumption (61, 67). Predictably, the collapse of these ionic concentration gradients has deleterious consequences for brain function.

Activity dependent ion fluxes are associated with the movement of osmotically obliged water. The encasement of the brain by a hard skull, although crucially protecting the brain from damage by impact, greatly limits its tolerance for volume changes: acute brain swelling quickly leads to severe damage or death. Together these observations underline the fundamental importance of ion and water homeostasis for brain functioning.

Glial cells are crucial for the homeostatic regulation of ion and water flow in the brain. They form an extensive network, the so-called panglial syncytium, which consists of myelinating oligodendrocytes and astrocytes coupled to each other through gap junctions (114) (Figure 1). Additionally, astrocytes make extensive specialized connections in the form of perivascular, subependymal or subpial endfeet, which are part of the blood–brain and brain–cerebrospinal fluid barriers. Activity-dependent uptake of ions and water into the panglial syncytium is thought to aid the dispersal and homeostasis of ions and water over large areas of the brain, thereby dampening the impact of local increases in neuronal activity. The extensive coupling through endfeet to fluid reservoirs that are devoid of neuronal elements allows for safe "dumping" of excess ions and water.

In this review, we start by highlighting pathways involved in glial ion and water homeostasis during neuronal activity. We focus mainly on regulation of the extracellular K^+ concentration, since disrupted K^+ regulation is a clear cause of neuronal network dysfunction. Next we summarize genetic diseases characterized by defects in glial proteins crucial for ion and water homeostasis. Finally, we will compare these different diseases and outline important clinical and mechanistic similarities and differences.

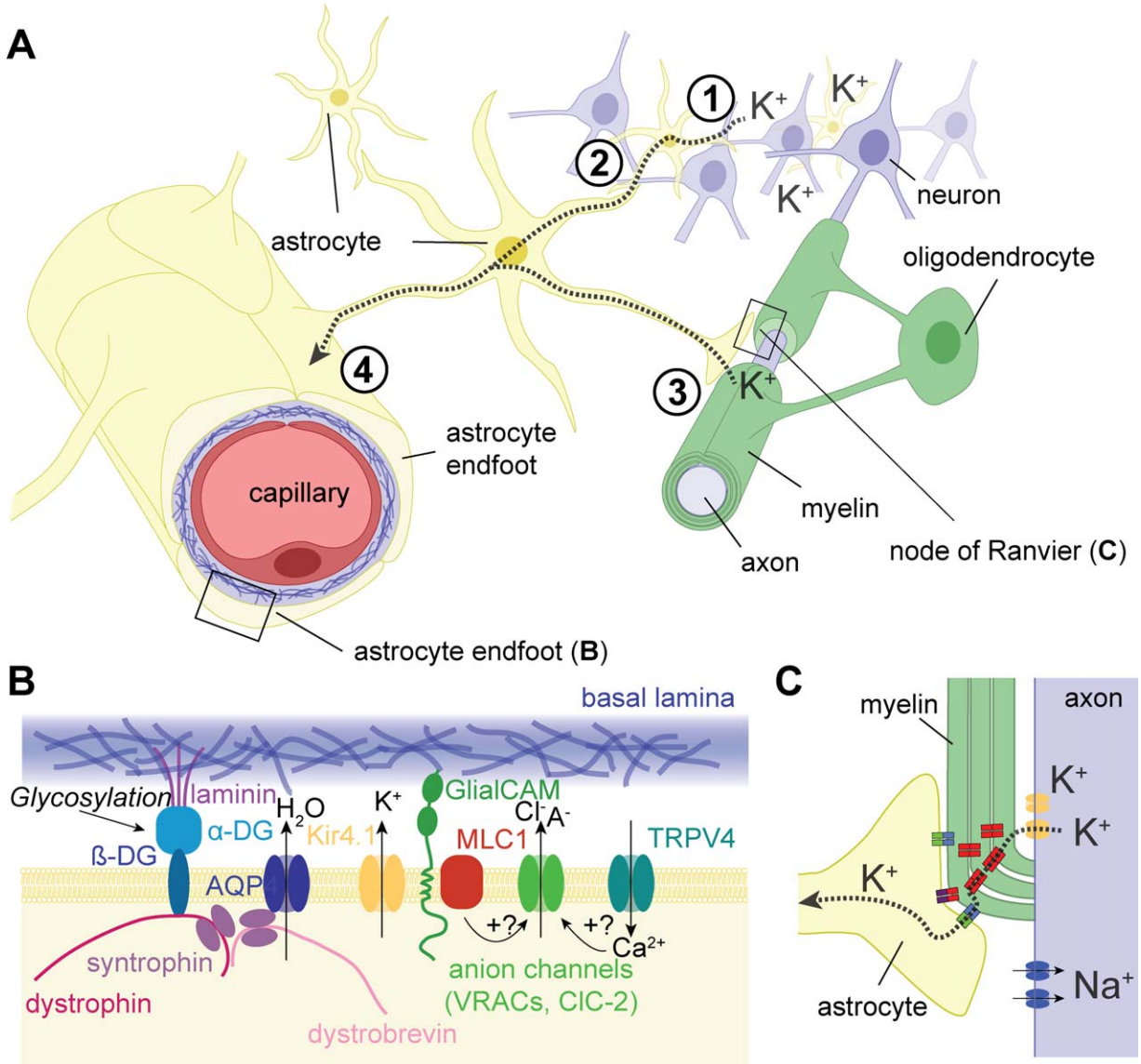

Figure 1. *Ion and water homeostasis in the panglial syncytium.* **A**. The panglial syncytium, consisting of astrocytes (yellow) and oligodendrocytes (green), coupled to neurons (blue). Increases in extracellular K^+ during neuronal activity are buffered by uptake into astrocytes (1), dispersal to neighboring glia through gap junction coupling (2) and subsequent release of K^+ and accompanying H_2O into the perivascular space from astrocyte endfeet (4; also see B). Action potential derived K^+ released by axons underneath the myelin sheath is transported through subsequent myelin layers into perinodal astrocytes (3; also see C), from where it can again move toward perivascular astrocyte endfeet (4). **B**. Proteins involved in ion and water homeostasis in astrocyte endfeet. These include members of the dystrophin associated glycoprotein complex (left), coupled to AQP4 water channels and Kir4.1 K^+ channels. GlialCAM, MLC1, VRACs and ClC-2 Cl^- channels are important for volume regulation. Mechanisms underlying activation of volume regulation are enigmatic, but Ca^{2+} influx through TRPV4 channels might play an important role. **C**. Upon action potential propagation, Na^+ flows in at the node of Ranvier while K^+ is released into the periaxonal space. To prevent buildup of K^+ and accompanying water into the periaxonal space K^+ is presumably taken up into myelin through as yet unidentified mechanisms. Subsequent myelin wraps are coupled by homotypic gap junctions containing Cx32 subunits (red). Coupling of the outermost myelin layer to perinodal astrocyte endfeet is achieved through heterotypic gap junctions [Astrocyte: Cx43 (green) and Cx30 (purple); Oligodendrocyte: Cx32 (red) and Cx47 (blue)]. This allows the flow of K^+ into the panglial syncytium (dotted line).

ION AND WATER HOMEOSTASIS DURING NEURONAL ACTIVITY

Neuronal action potential firing comprises the depolarizing influx of Na^+ into the axon and the somatodendritic region of the neuron, followed by a compensatory repolarizing efflux of K^+ into the extracellular space. Action potentials trigger the release of neurotransmitters such as glutamate and GABA into the synaptic cleft, followed by Na^+ and K^+ fluxes through postsynaptic glutamate receptors in excitatory synapses or by Cl^- fluxes through $GABA_A$

receptors in inhibitory synapses. The accumulation of both K^+ and neurotransmitters in the extracellular space poses a threat to neuronal network functioning, as it can quickly lead to neuronal depolarization, thereby enhancing neuronal excitability and increasing the risk for epileptic seizures or spreading depression waves (132). Ideally, neurons would restore equilibrium following neuronal activity by pumping back displaced Na^+ and K^+ ions and by reuptake and recycling of neurotransmitters. However, especially during high frequency neuronal activity, additional uptake and dispersal mechanisms are necessary for temporary buffering.

Astrocytes play an essential role in the clearance of extracellular K^+ (Figure 1A). There are two separate principles for how astrocytes do this (77, 155): astrocytes clear extracellular K^+ through uptake and accumulation into neighboring astrocytes, keep it in transient storage, and subsequently release it back into the extracellular space. Alternatively, astrocytes spatially buffer K^+ by uptake at one location coupled to release of K^+ at a different location. Importantly, the first principle leads to (temporary) accumulation of K^+ in astrocytes. The second principle does so at most locally, but not overall, since for each entering K^+ ion another one leaves the astrocyte syncytium at a location away from the site of neuronal activity.

The concept of K^+ spatial buffering by glia was introduced by Orkand *et al* (104). Spatial buffering is a passive process, which does not require active ion pumps. It strongly depends on electrical coupling of glial cells in a syncytium, which ensures that these cells are largely isopotential. Under these conditions, a local rise in extracellular K^+ will locally raise the K^+ equilibrium potential to above the resting membrane potential, thereby leading to an inward driving force for K^+ and, given the high permeability of the glial membrane to K^+, to passive K^+ influx. To close this current loop, K^+ is redistributed through the panglial syncytium. This will in turn lead to an increased intracellular K^+ concentration in regions not exposed to high neuronal activity, where the equilibrium potential in turn is lowered to below the resting membrane potential by the increased intracellular K^+, and where K^+ efflux occurs.

Spatial buffering strongly depends on the existence of a large glial syncytium. First, this maintains the membrane potential below the K^+ reversal potential during local rises in K^+. Second, it directly couples active sites where K^+ flows into the syncytium to sites of normal K^+ concentration, where K^+ flows out. Although swelling of astrocytes should not occur during spatial buffering, the process can induce a redistribution of water with local swelling of glia at the site of neuronal activity coupled to shrinkage at far away sites (66, 96).

In contrast to spatial buffering, active uptake and accumulation of K^+ requires activity of glial ion carriers, mainly the Na^+/K^+-ATPase and $Na^+/K^+/Cl^-$ cotransporter. Like spatial buffering, it is greatly aided by gap junction coupling of glia, which increases the accumulation capacity during local extracellular K^+ rises. The uptake of K^+ is accompanied by intracellular Cl^- accumulation, to guarantee electric neutrality. It leads to a net increase in intracellular osmolytes, and is expected to cause a significant swelling of astrocytes (89, 154).

Although the occurrence and relative contribution of K^+ spatial buffering and K^+ uptake have been disputed, the current consensus is that both processes operate in the brain. Their exact contribution probably differs between different brain regions and during different activity conditions (77, 155).

The clearance mechanisms for extracellular K^+ described above apply to grey matter, where neurons release K^+ directly into the extracellular space. The situation is different when considering ion and water homeostasis around myelinated axons in grey and especially white matter. For myelinated axons, the main location of Na^+ influx is the node of Ranvier, while K^+ efflux is mainly localized to the periaxonal space, underneath the myelin sheath (114). Tight junctions at the paranodal region prevent action potential-derived K^+ to reach the node of Ranvier, keeping it trapped under the myelin sheath. To prevent accumulation of K^+ in the internode upon repetitive action potential firing, which would cause depolarization of the axon and possible osmotic myelin vacuolization, an exit pathway for K^+ is necessary. This exit pathway comprises gap junctions connecting the successive myelin loops, mainly at the paranodal region (114). The outer surface of the myelin sheath in turn is gap junctionally coupled to periaxonal astrocyte processes (73). From here, K^+ can be transported through the panglial syncytium and released following similar routes as described above (Figure 1A,C).

ASTROCYTE VOLUME REGULATION

As described above, accumulation of K^+ into astrocytes during neuronal activity causes astrocyte swelling, although the underlying mechanism is still disputed (79, 80, 89). In addition, synaptic glutamate release activates uptake by glutamate transporters on perisynaptic astrocytes (14), which is coupled to Na^+ and water influx and adds to activity-dependent astrocyte swelling (78, 88, 123). Swelling of astrocytes can increase neuronal excitability through reduction of the extracellular space, which elevates extracellular neurotransmitter concentration and increases ephaptic interactions between neurons (94). Therefore, it is important that astrocyte swelling is counteracted.

Like most cells in the body, swelling leads to activation of a homeostatic process in astrocytes called regulatory volume decrease (RVD), by which astrocytes attempt to restore their original volume to prevent cellular damage. RVD in astrocytes has mainly been studied in isolated cultured cells. Exposure of these cells to either hypo-osmolar medium or to a high extracellular K^+ concentration causes swelling and subsequent RVD. This RVD must involve either active transport or the opening of volume-sensitive channels to mediate efflux of ions and organic osmolytes accompanied by water. A key player in RVD is the volume-regulated anion channel (VRAC).

Although the molecular identity of VRACs has long been elusive, the likely pore-forming subunits were recently identified as members of the leucine-rich repeat containing protein 8 family (LRRC8A-E) (112, 153). VRACs are ubiquitously expressed in all cells of the body, open upon cell swelling, and lead to an efflux of anions such as Cl^- and organic osmolytes such as taurine. To maintain electroneutrality, the efflux of negatively charged molecules must be accompanied by efflux of cations such as K^+ through nearby K^+ channels. The efflux of ions pulls osmotically obliged water out of the cell, thereby enforcing volume decrease. Water efflux can be facilitated by aquaporin water channels, such as Aquaporin-4 (AQP4) in the brain (Figure 1B).

VRAC activity in astrocytes can be regulated in various ways. The channel formed by LRRC8 subunits is directly sensitive to

ionic strength, and a lowering of intracellular ionic strength, as would occur during cell swelling, leads to channel opening (138). Additionally, evidence indicates that VRAC activity is modulated by a number of signaling cascades which are activated by cell swelling (72).

VRACs are likely not the only anion channels involved in astrocyte volume regulation. Another possible player is the Cl^- channel ClC-2. This channel is also sensitive to volume changes, and opens upon cell swelling (48, 56). Astrocytes express functional ClC-2 channels (90, 106).

Finally, volume-sensitive transient receptor potential vanilloid 4 (TRPV4) channels are likely involved in volume regulation in astrocytes (12). Opening of these channels during cell swelling leads to an intracellular Ca^{2+} transient which might be necessary for activating cellular signaling cascades activating the RVD process (13, 72). An interaction between TRPV4 and AQP4 seems necessary for volume regulation (13), although the nature of this interaction is of yet unresolved.

Membrane proteins involved in regulation of astrocyte volume are localized to astrocyte endfeet. These endfeet contain a large number of AQP4 water channels in the form of so-called square or orthogonal arrays (95, 99). AQP4 channels are closely associated with Kir4.1 K^+ channels (95) and TRPV4 channels (13). Additionally, the essential VRAC subunit LRRC8A (29) and the ClC-2 Cl^- channel (20, 41) are all highly enriched in the endfeet membrane (Figure 1B).

THE CONSEQUENCES OF GENETIC DEFECTS ON BRAIN ION AND WATER HOMEOSTASIS

As described above, ion and water homeostasis in the brain is crucial for neuronal functioning as well as for brain volume regulation. We have outlined several important components for this process, the location of which is highlighted in Figure 1: (i) The presence of ion pumps and channels for the fast buffering of activity-dependent extracellular K^+ increases; (ii) extensive gap junction coupling of the panglial syncytium (astrocytes to astrocytes and oligodendrocytes to astrocytes) to maintain isopotentiality and for the dispersal of ions and water over large areas; (iii) an exit route to facilitate removal of axonally released K^+ and water from underneath the myelin sheath and (iv) the presence of exit routes for ions, osmolytes and water from perivascular, subependymal and subpial astrocyte endfeet. As expected, genetic disruption of any of these components hampers brain ion and water homeostasis and leads to neurological disease.

Initial diagnosis of genetic diseases with defective ion and water homeostasis is often based on the clinical picture and brain MRI, followed by confirmation using DNA testing. Patterns of MRI abnormalities are different for each disorder. Water diffusion, which can be assessed by diffusion tensor imaging, can be increased or decreased depending on the size of water spaces. At the cellular level, disturbances in ion and water homeostasis can lead to chronic or transient cell swelling and myelin vacuolization, which, if severe and longstanding, could result in myelin loss. In the following section we will describe several genetic diseases in which brain ion and water homeostasis by glia is disturbed, grouped by the type of protein that is dysfunctional in these diseases.

GAP JUNCTION PROTEINS

The integrity of the panglial syncytium, and thereby its ability to homeostatically regulate ion and water balance over large areas, critically depends on coupling of glial cells by means of gap junctions. Coupling of astrocytes to their neighbors allows for dispersal of ions and water over long distances. Coupling of oligodendrocytes to astrocytes enables the flow of ions and water away from myelinated axons. Intramyelinic gap junctions are necessary to allow for removal of ions and water underneath the myelin sheath.

Gap junctions are tight intercellular channels formed by two opposing hemichannels, each being a hexamer of connexin proteins (52). When the opposing hemichannels are of similar connexin composition, they are called homotypic gap junctions; if the hemichannels differ they are called heterotypic. To date 21 connexin proteins have been identified, of which eleven are expressed in the brain. Connexin proteins are often named based on the molecular weight of the protein (eg, Cx32, Cx26). The distribution of different connexins differs between cell-type and subcellular location (52).

The oligodendrocyte gap junctions that link subsequent myelin layers are homotypic gap junctions composed of Cx32 subunits (73) (Figure 1C). In a similar way, homotypic Cx32 channels couple subsequent myelin layers formed by Schwann cells in the peripheral nervous system (10). Coupling of the outermost myelin layer to neighboring astrocytes is achieved through heterotypic gap junctions. These can consist of Cx43, Cx30 or Cx26 on the astrocytic side, with Cx47 or Cx32 on the oligodendrocytic side (116, 156) (Figure 1C). Astrocytes form large connected networks with neighboring astrocytes through gap junctions mainly consisting of homotypic Cx43 or Cx30 gap junctions (52).

In addition to their importance for ion and water fluxes through the panglial syncytium, glial gap junctions facilitate essential metabolic support to glial cells and neurons by allowing the efficient movement of glucose and its metabolites derived from the blood stream throughout the panglial syncytium (119).

X-linked Charcot–Marie–Tooth disease

Deleterious mutations in genes encoding connexins are associated with different leukodystrophies (1). Mutations in *GJB1*, the gene encoding Cx32, lead to X-linked Charcot–Marie–Tooth disease (CMTX) (15). As mentioned above, Cx32 is expressed in Schwann cells and oligodendrocytes. CMTX mainly manifests itself as a peripheral neuropathy characterized by myelin vacuolization and demyelination, underlining the importance of Cx32-mediated coupling of subsequent myelin layers in myelinating Schwann cells. Additionally, patients may suffer from acute episodes of CNS dysfunction, which are often triggered by exertion, return from high altitude, or minor infections. Clinically, these episodes are characterized by transient ataxia, dysarthria and weakness (2). Exceptional cases, in which *GJB1* mutations lead to persistent CNS dysfunction, mainly ataxia, dysarthria and spasticity, have also been described (41, 131). Persistent signs described in one patient are present in all affected male family members (41). This suggests

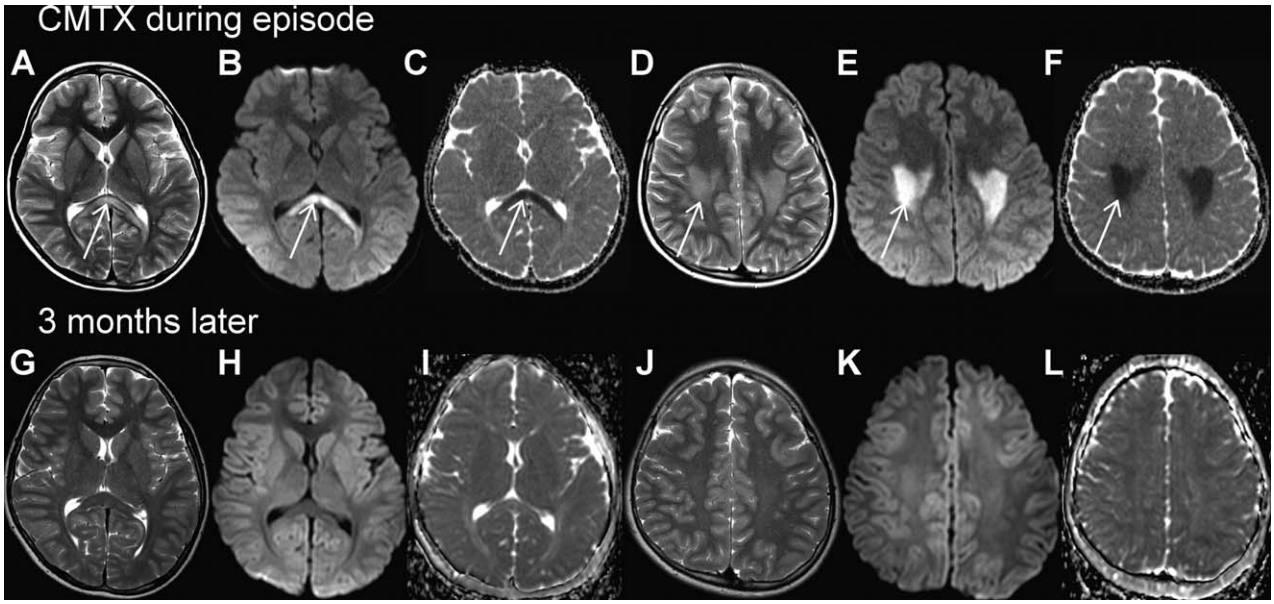

Figure 2. *MRI during and after an episode in a teenage boy with CMTX.* Axial T2-weighted images (**A, D, G, J**), diffusion-weighted images (**B, E, H, K**) and ADC maps (**C, F, I, L**). During the episode (A–F), signal abnormalities are seen in the splenium of the corpus callosum (arrow in A) and centrum semiovale (arrow in D) with evidence of restricted diffusion on the diffusion-weighted images (high signal indicated by the arrow in B and E) and confirmed by low values on the ADC maps (low signal indicated by the arrow in C and F). Three months later (G–L), the abnormalities have disappeared. The latter images also serve as normal for the subsequent figures.

that the transient vs. persistent nature of CNS dysfunction in CMTX depends on the specific mutation.

During an episode of CNS dysfunction, MRI shows mild signal abnormalities with profound diffusion restriction, preferentially in the central or posterior part of the centrum semiovale, the splenium of the corpus callosum, posterior limb of the internal capsule and middle cerebellar peduncles (Figure 2A–F). These MRI abnormalities disappear after the episode (120) (Figure 2G–L). MRI from a patient with persistent CNS dysfunction shows diffuse mild signal abnormality and mildly restricted diffusion of all brain white matter structures, with more pronounced changes in the posterior limb of the internal capsule, splenium of the corpus callosum, cerebral peduncles and middle cerebellar peduncles, very similar to what is seen in the case of mutations involving the Cl^- channel ClC-2 (see below). No pathology is available documenting the transient or permanent brain white matter abnormalities in CMTX, but the profound diffusion restriction of white matter in MRI suggests myelin microvacuolization (41).

Similar to patients, mice lacking Cx32 show a clear peripheral neuropathy (93, 122). The CNS phenotype of these mice is more subtle, but it includes thinner myelin sheaths, altered neuronal membrane properties and dysfunctional synaptic inhibition (137). Myelin vacuolization in the CNS has not been reported in these mice.

Pelizaeus–Merzbacher-like disease

Recessive mutations in *GJC2*, encoding Cx47, are associated with a leukodystrophy called Pelizaeus–Merzbacher-like disease (PMLD) (28, 143, 158). Cx47 is expressed by oligodendrocytes. In contrast to Cx32, it does not form intramyelinic gap junctions, but rather is involved in oligodendrocyte-astrocyte coupling. PMLD is similar in clinical presentation to classic Pelizaeus–Merzbacher disease, which is caused by mutations in the gene encoding myelin protein proteolipid protein 1. PMLD patients have severely impaired motor development, spasticity and cognitive impairment. More severe forms have been described (16), as well as milder forms presenting as hereditary spastic paraplegia (SPG44) (3, 105).

In classic PMLD as well as in SPG44, MRI shows signal abnormalities compatible with diffuse hypomyelination and striking pons involvement (Figure 3). In some patients the periventricular and deep cerebral white matter is much better myelinated than the directly subcortical white matter (134). No pathology is available for either disease.

Mice lacking Cx47 or homozygous for a PMLD causing mutation show sparse central myelin vacuolization, cystic spaces in white matter structures and sparse astrogliosis. Clinically they display a transient motor phenotype (103, 142). These problems resolve with age, suggesting effective compensation by other connexins. In line with this, mice lacking both Cx47 and Cx32, hampering possible compensation, suffer from profound central myelin abnormalities. They show thin, vacuolated or absent myelin sheaths, develop severe action tremor and epilepsy, and die by 5–10 weeks of age (93, 103, 142).

Oculodentodigital dysplasia

Mutations in *GJA1*, the gene encoding Cx43, which in the brain is highly expressed in astrocytes, leads to oculodentodigital dysplasia (ODDD) (106). ODDD is characterized by abnormalities of the eyes, teeth and fingers, underlining the importance of gap junctions in other tissues. Neurological signs are typically relatively mild and include ataxia, epilepsy and loss of vision and hearing (38, 84).

On MRI, ODDD patients show mild signal changes in the cerebral white matter (84, 126). In our experience, the MRI findings are

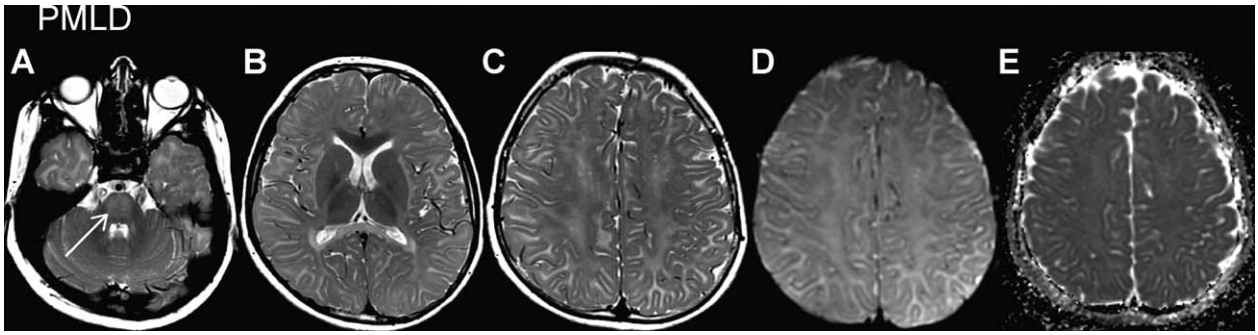

Figure 3. *MRI in a 10-year-old boy with PMLD.* Axial T2-weighted images (**A–C**), diffusion-weighted image (**D**) and ADC map (**E**). The mildly increased T2-signal of the pons (arrow in A) and cerebral white matter (B, C) are indicative of hypomyelination. There are no clear diffusion abnormalities (D, E).

compatible with mild hypomyelination (Figure 4). Neuropathology from ODDD patients is not available.

When the astrocyte gap junction protein Cx43 is knocked out specifically from astrocytes in the brain, mice show increased propagation of spreading depressions (140), compatible with disturbed ion and water homeostasis in these mice. No white matter abnormality has been described. In contrast, when both astrocyte gap junctions Cx43 and Cx30 are removed from astrocytes, pronounced edema and vacuolization of white matter are observed (87).

GLIAL K^+ CHANNELS

For the passive flow of K^+ through the panglial syncytium, correct expression and localization of K^+ channels is critical. Glial cells express large numbers of K^+ channels (125). By far the largest contributors to K^+ conductance in astrocytes are Kir4.1 K^+ channels. These channels are highly enriched in astrocyte endfeet (63, 96). They play a key role in the rapid uptake and redistribution of K^+ in the panglial syncytium (77).

SeSAME/EAST syndrome and an autism-epilepsy phenotype

In humans, loss of function mutations in the *KCNJ10* gene, encoding Kir4.1, give rise to SeSAME/EAST syndrome. This is an autosomal recessive disorder, characterized by early onset seizures, sensorineural deafness, ataxia, mental retardation and electrolyte imbalance (21, 124). The electrolyte imbalance indicates renal dysfunction in the disease (117). Heterozygous gain of function mutations in *KCNJ10* have been associated with seizures, intellectual disability and autism spectrum disorder (127, 128).

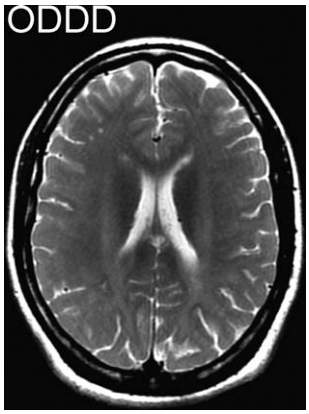

Figure 4. *MRI in a 10-year-old girl with ODDD.* The axial T-2weighted image reveals a mildly increased T2-signal of the cerebral white matter, indicative of hypomyelination.

On MRI, patients with SeSAME/EAST syndrome show cerebellar hypoplasia, sometimes with a thin corpus callosum or spinal cord (37). No MRI abnormalities are seen in patients with a gain of function *KCNJ10* mutation (128). Therefore, in contrast to what would be expected, no white matter abnormalities on MRI have been reported in these patients. Neuropathology is not available.

In striking contrast with the human disease and in line with the importance of Kir4.1 K^+ channels for extracellular K^+ homeostasis in the brain white matter, full Kir4.1 knockout mice display severe dysmyelination, myelin vacuolization, motor dysfunction and death around 3 weeks after birth (98). Astrocyte specific Kir4.1 knockout mice show a similar phenotype of myelin vacuolization, with ataxia, seizures and early death (43). Furthermore, both Kir4.1 lacking oligodendrocytes (98) and astrocytes (43) show a depolarized membrane potential and a strongly reduced membrane conductance. As a consequence, K^+ buffering is compromised in these mice (33, 43, 58). The reason for the phenotypic difference between mutant Kir4.1 mice and patients is unclear (but see discussion of species differences below).

NA^+/K^+-ATPASE

The Na^+/K^+-ATPase is present at the membrane of all cells in the body to maintain transmembrane ionic gradients. It hydrolyzes one molecule of ATP to exchange 3 Na^+ ions for 2 K^+ ions. For a functional pump, an α and a β subunit are required. Several isoforms of each subunit have been identified, which can assemble in a variety of different configurations (19). In the brain, the astrocytic Na^+/K^+-ATPase is crucial for active uptake of K^+ (81). Both $\alpha2$ and $\beta1$ and $\alpha2$ and $\beta2$ subunit pairs seem to coexist in astrocytes (135). Expression of the $\alpha3$ subunit is restricted to neurons (26), where it is important for preventing elevation of intracellular Na^+ and for powering Na^+ coupled secondary transport (65).

Familial hemiplegic migraine type 2 and other paroxysmal disorders

Dominant mutations in *ATP1A2*, encoding the $\alpha2$ subunit of the human Na^+/K^+-ATPase, are the cause of familial hemiplegic

migraine type 2 (FHM2) (39, 148). Clinically, FHM2 is characterized by episodes of migraine with aura, associated with hemiparesis. Episodes can be triggered by mild head trauma, and incomplete recovery can lead to permanent mental retardation (151). Patients with FHM2 often suffer from additional manifestations, such as seizures, anxiety and developmental disability (25, 151). Some patients manifest with alternating hemiplegia of childhood (5, 11, 157) or episodic ataxia (34).

MRI for the diseases described above is typically normal. However, during an episode of migraine in FHM2 MRI reveals transient grey matter edema restricted to one hemisphere (9, 151). White matter abnormalities have not been described. Neuropathology is not available.

Multiple mouse models carrying mutations in the α2 subunit of the Na^+/K^+-ATPase are available. These recapitulate features of the diseases described above (69). Mouse models for FHM2, which display heightened anxiety and seizures (68, 75), reveal that the threshold for induction of cortical spreading depression is reduced in these mice. Cortical spreading depression is linked to disrupted K^+ homeostasis and thought to underlie migraine episodes (83). Furthermore, studies in FHM2 mouse models show that the clearance of K^+ and glutamate following neuronal activity is defective in these mice (31).

WATER CHANNELS

AQP4 water channels are highly expressed in astrocyte endfeet, and play a crucial role in brain ion and water homeostasis (97). Several studies indicate hampered K^+ homeostasis in loss-of-function AQP4 mutants (*Aqp4*-null or *Syntrophin*-null mice) (8, 18, 57, 136), underlining the interconnection of water and ion fluxes in astrocytes. Additionally, these mutants have chronic brain edema (59), and show a seizure phenotype. Paradoxically the threshold for induced seizures is increased in mutants (17), but once initiated, seizures are more severe than in wildtype mice (18). Therefore, the presence of AQP4 at astrocyte-fluid barriers appears to serve an important physiological role. The channel also poses a potential risk, as it facilitates the formation of edema under conditions such as stroke by enabling the rapid influx of water from the blood stream into astrocytes (91).

No human disease has been associated with mutations in the *AQP4* gene, although attempts have been made to identify such mutations (133, 152).

THE BASEMENT MEMBRANE AND THE DYSTROPHIN ASSOCIATED GLYCOPROTEIN COMPLEX

The basement membrane is a layer of extracellular matrix proteins providing support for neighboring cells. In the brain, the basement membrane surrounding blood vessels and directly underneath the pia enables anchoring of astrocyte endfeet. The strong polarization of astrocytes crucially depends on the presence of the correct basement membrane components. Additionally, it requires endfeet expression of the dystrophin associated glycoprotein complex (DAGC), a multiprotein complex that connects the cell cytoskeleton to the basement membrane (Figure 1B). Composition of the DAGC differs between tissues. A key component is α-dystroglycan, a highly glycosylated extracellular protein that binds the extracellular matrix protein laminin-alpha2 (merosin), which is part of the basal lamina. β-Dystroglycan is a transmembrane protein linking extracellular α-dystroglycan to intracellular dystrophin. Dystrophin in turn links to the actin cytoskeleton in most cell types and to dystrobrevin. In astrocytes, loss of key DAGC components leads to mislocalization of important endfoot proteins involved in ion and water homeostasis. For example, knockout mice for dystrophin (51, 144) or α-syntrophin (7) show reduced AQP4 localization to endfeet, and the same likely holds for other proteins in the endfoot complex.

Congenital muscular dystrophies with brain involvement

Genetic disruption of components of the basement membrane or the DAGC in astrocytes can lead to neurological disease. Many of these diseases are primarily known as congenital muscular dystrophies (CMDs), because of overlap in basement membrane and DAGC components between muscle and brain. Diseases can be subdivided into those due to extracellular matrix protein dysfunction and those due to dysfunction of membrane receptors for the extracellular matrix. Examples falling into the first category are CMD with merosin deficiency (CMD type 1A, MDC1A), caused by mutations of the gene encoding the laminin subunit alpha2 (*LAMA2*) (62), and an encephalopathy caused by mutations in the laminin subunit beta1 (*LAMB1*) (113). The second group includes Fukuyama type CMD (FCMD), Walker–Warburg syndrome (WWS), and muscle–eye–brain disease (MEBD). WWS, MEBD and FCMD are so-called "dystroglycanopathies," and underlying mutations mainly affect genes encoding enzymes involved in the glycosylation of α-dystroglycan (55). Improper glycosylation hampers binding of α-dystroglycan to extracellular matrix components, thereby disrupting the DAGC.

Most CMDs have an early disease onset and a static or slowly progressive course. Patients show generalized hypotonia and muscular weakness at birth, and most patients do not achieve independent ambulation (92). Surprisingly, patients with *LAMB1* mutations do not show obvious muscle dysfunction, although *LAMB1* is expressed in skeletal muscle (113). Eye abnormalities are invariably present in WWS and MEBD, less frequently in FCMD, sometimes observed in *LAMB1*-mutated patients, and not observed in MDC1A. Whereas MDC1A is associated with normal or mildly impaired cognitive ability (109), dystroglycanopathies are characterized by severe mental deficiency (92). Seizures are common in dystroglycanopathies (91). Seizures also occur in *LAMB1* patients (113, 141), while MDC1A is associated with epilepsy in an estimated 6%–8% of patients (109, 112).

MRI from MDC1A patients (Figure 5) is characterized by extensive or diffuse cerebral white matter abnormalities, with a swollen appearance of the abnormal white matter (109, 149). Structural brain abnormalities, mainly cerebellar hypoplasia and occipital agyria, have occasionally been reported (108). Anterior temporal subcortical cysts are sometimes present (149). *LAMB1*-mutated patients (Figure 6) also show diverse brain malformations including agyria and cerebellar hypoplasia, together with diffusely swollen white matter suggesting myelin vacuolization and in some cases with subcortical cysts (113, 141). Neuropathology from a 4-month-old MDC1A patient confirmed abnormal cortical gyration; as

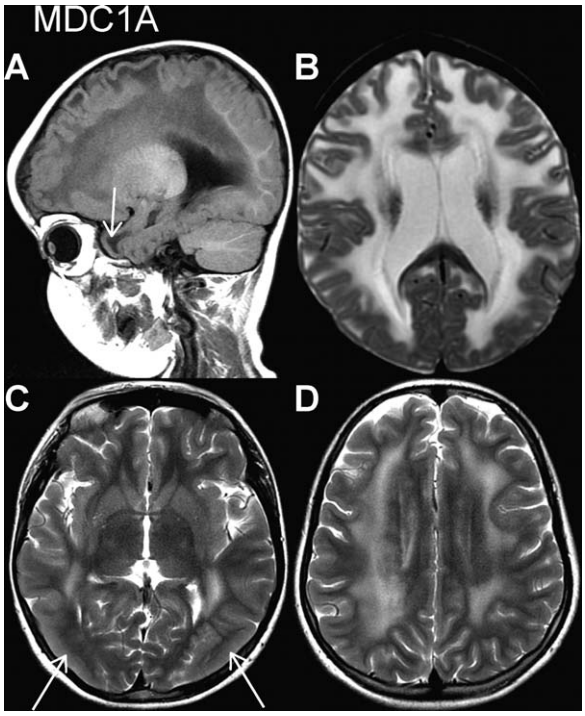

Figure 5. *MRI in MDC1A*. The sagittal T1-weighted image in a 2-year-old girl shows a small anterior temporal cyst (arrow in **A**). The axial T2-weighted image reveals diffuse and prominent T2-hyperintensity of the cerebral white matter, with also swelling of the abnormal white matter (**B**). Axial T2-weighted images in a 15-year-old boy shows milder and more limited white matter abnormalities (**C, D**). Note the occipital agyria (arrows in C).

expected at this young age, white matter defects were not observed (139). Neuropathological examination of a patient that was later genetically confirmed to have MDC1A showed diffuse myelin vacuolization (22, 47). Neuropathology from *LAMB1*-mutated patients is not available.

MRI from dystroglycanopathy patients is characterized by striking structural abnormalities. Pachygyric polymicrogyria or lissencephaly type II of the cerebral cortex, cerebellar cortical dysplasia, pons hypoplasia, cerebellar vermis hypoplasia and small subcortical cerebellar cysts are typical of WWS, MEBD and FCMD (Figure 7). An important difference between dystroglycanopathies is that the cerebral cortical dysplasia is less severe and more variable in MEBD as compared with WWS. Furthermore, cerebral white matter abnormalities in MEBD are absent or focal, more extensive in FCMD, whereas they are diffuse in WWS. The abnormal white matter often has a swollen aspect, in WWS sometimes strikingly so. The cerebral white matter abnormalities tend to improve over time in FCMD (Figure 8) and MEBD, but remain severe in WWS (Figure 7). In WWS cysts may occur in the cerebral subcortical white matter. Hydrocephalus is often observed in WWS, but rare in MEBD and FCMD (4, 35, 44, 145, 149). Neuropathology from dystroglycanopathy patients is consistent with disrupted neuronal migration in these diseases. An early neuropathological study identified important differences between WWS and FCMD (76). In WWS, the brain is severely malformed, shows lissencephaly type II, and severely hypoplastic cerebellum. In FCMD the general CNS configuration is better preserved, with diffuse or focal polymicrogyria and sometimes few pachygyric lesions. The cerebellum is not hypoplastic but focally polymicrogyric (76). Neuropathology from MEBD patients shows coarse gyri with an abnormally nodular cortical surface, and a total disorganization of cerebral and cerebellar cortices (60). Regarding the cerebral white matter, it is strikingly abnormal, poorly myelinated, gliotic, spongy and often strikingly edematous with occasionally cavitations in WWS (50). In FCMD and MEBD, the cerebral white matter changes vary in severity. Myelin paucity and gliosis are especially seen in younger children, whereas in older patients the white matter may be more normal.

These findings show that the basement membrane and DAGC is not only critical for muscle function, but that it also is crucial for organization of astrocyte endfeet in the brain. Disruption appears to result in two distinct defects: (i) structural defects, most prominently cortical dysplasia, which is likely related to breaching of the glia limitans, and (ii) white matter edema, presumably due to disturbed ion and water homeostasis because of endfeet dysfunction. The prominence of these two phenotypes differs for different diseases. Brain abnormalities in MDC1A are dominated by swollen cerebral white matter and myelin vacuolization. Cortical dysplasia may occur, but if so, it is typically limited to the occipital region. Patients with *LAMB1* mutations show both cortical dysplasia and

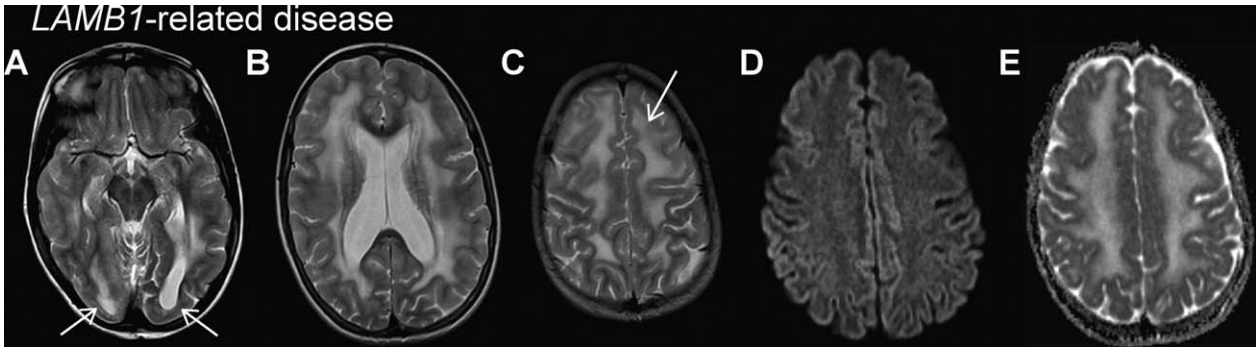

Figure 6. *MRI in LAMB1-related disease in a teenage girl*. Axial T2-weighted images (**A–C**), diffusion-weighted image (**D**) and ADC map (**E**). The axial T2-weighted images show extensive and prominent T2-hyperintensity of the cerebral white matter, with also some swelling of the abnormal white matter (arrow in C). Note the occipital agyria (arrows in A). The diffusion-weighted image shows a low signal of the cerebral white matter (D), while the ADC map shows high values (E), indicating increased diffusion.

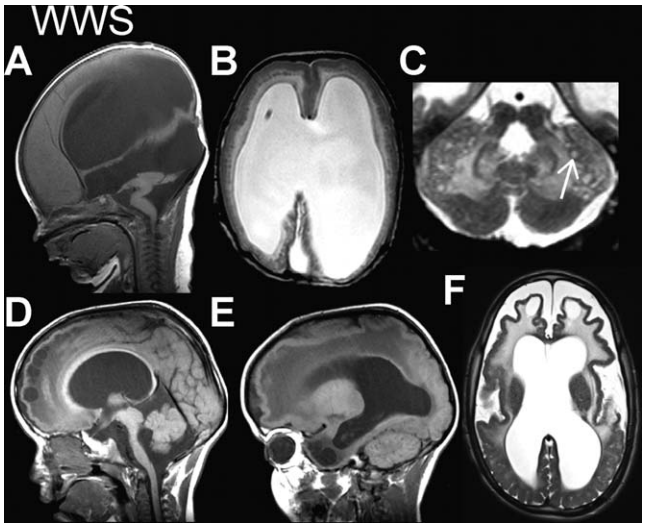

Figure 7. *MRI in WWS*. The mid-sagittal T1-weighted image (A) in a 3-month-old boy with *ISPD* mutations (**A, B**) shows a dysplastic brain stem and very small cerebellum. The axial T2-weighted image of this patient reveals lissencephaly type II cortical dysplasia (B). In a 10-year-old girl with *LARGE* mutations (**C–F**), the mid-sagittal T1-weighted image (D) shows a dysplastic brain stem and small cerebellum. The axial T2-weighted image through the cerebellum (C) reveals also cerebellar cortical dysplasia and numerous subcortical cysts (arrow). There is a diffuse polymicrogyric pachygyria of the cerebral cortex, best seen in E. Cerebral subcortical cysts are present in the frontal (D, F) and anterior temporal (E) areas. The cerebral white matter is diffusely abnormal in signal (F).

white matter abnormalities. In dystroglycanopathies the cortical dysplasia is severe. White matter abnormalities are present, but more variable, depending on the specific CMD. These differences underline the fact that involvement of different components of the basement membrane and DAGC in brain functioning is complex and not yet fully understood.

MLC1 AND GLIALCAM

MLC1 is a membrane protein of unknown function (23). Intriguingly, the *MLC1* gene is present in all species that produce myelin, but absent in those that do not (23). MLC1 is expressed in the brain as well as in all types of leukocytes. Within the brain, it is exclusively expressed in astrocytes, where it localizes to astrocyte endfeet at brain–fluid barriers. MLC1 associates with the DAGC, and was shown to undergo a direct interaction with Kir4.1 channels (6, 22). GlialCAM is an immunoglobulin-like cell adhesion molecule, which acts as a chaperone for MLC1 (30). It also mainly localizes to astrocyte endfeet. However, GlialCAM expression is not restricted to astrocytes; it is also present in axons, on the outside of myelin sheaths and in oligodendrocytes (49, 85). The role of these two proteins in astrocyte endfeet is not yet fully understood.

MLC1 has been shown to interact with a variety of other proteins potentially involved in brain ion and water homeostasis. These include, among others, the Na^+/K^+-ATPase, TRPV4, caveolin-1 and Kir4.1 (27). Furthermore, recently an interaction of GlialCAM with connexin-43 was described (159). These findings underline the potential importance of MLC1 and GlialCAM for brain ion and water homeostasis.

Megalencephalic leukoencephalopathy with subcortical cysts

Megalencephalic leukoencephalopathy with subcortical cysts (MLC) (127, 144) is an infantile-onset leukodystrophy. The disease is caused by recessive mutations in *MLC1* (82) or by recessive or dominant mutations in *GLIALCAM* (also called *HEPACAM*) (85).

Clinically, MLC patients typically present with macrocephaly in their first year of live. After an interval of a variable number of years slow motor deterioration follows with ataxia and spasticity. Patients often become wheelchair dependent as teenagers (147). Cognitive capacities are normal or mildly decreased. Autism is often observed in patients with dominant *GLIALCAM* mutations (148). An early onset of epileptic seizures is common (160). Seizures are typically easily controlled by medication. Mild head trauma is an important provoking factor for seizures, and status epilepticus occurs relatively often in MLC patients (46). The clinical picture of patients with recessive *MLC1* mutations (classic MLC or MLC1) or recessive *GLIALCAM* mutations (MLC2A) is indistinguishable. However, patients with dominant *GLIALCAM* mutations have a remitting disease course (MLC2B) where macrocephaly is present in the first year of live but where no deterioration occurs and patients improve (85, 148).

On MRI (Figure 9), MLC is characterized by chronic diffuse cerebral white matter edema and the presence of subcortical cysts in anterior temporal, frontal and parietal regions (147). The MRI pattern is almost indistinguishable from that seen in MDC1A patients (146) (Figure 5). On follow-up most patients show decrease of the cerebral white matter swelling and slowly progressive atrophy. By contrast, patients with MLC2B show major improvement or normalization upon MRI follow-up (148). Pathology in brains from MLC patients reveals extensive myelin vacuolization as well as vacuolization of astrocyte endfeet (147).

Our knowledge of MLC has been greatly enhanced by the use of animal models for the disease. Both *Mlc1*-null mice and *Glialcam*-null mice have been studied (29, 45, 64). Pathological examination confirms a high brain water content and progressive myelin

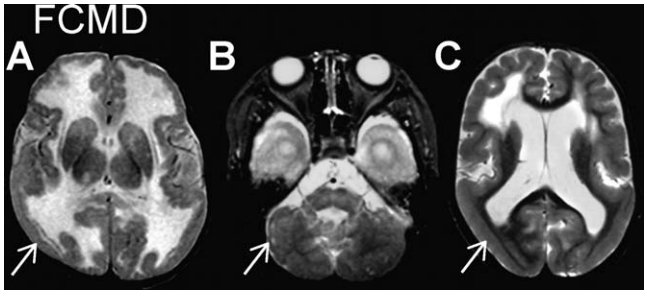

Figure 8. *MRI in FCMD*. The axial T2-weighted images at 5 months (**A, B**) show diffuse cerebral white matter signal abnormalities, while at 7 years (**C**) the white matter abnormalities are more limited in extent. There is an extensive cortical dysplasia, which in the occipital region looks like agyria/lissencephaly type II (arrows in A and C). Within the cerebellum small subcortical cysts are present (arrow in B). The pons is dysplastic and small (B).

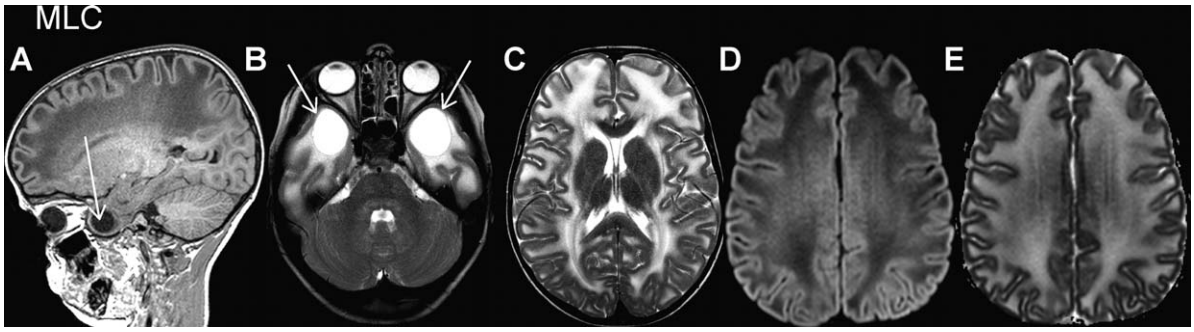

Figure 9. *MRI in a 6-year-old girl with MLC.* The sagittal T1-weighted image shows an anterior temporal cyst (arrow in **A**). The axial T2-weighted images (**B, C**) show diffuse, prominent T2-hyperintensity of the cerebral white matter, with some swelling of the abnormal white matter. The anterior temporal cysts are large on both sides (arrows in B). The diffusion-weighted image shows a low signal of the cerebral white matter (**D**), while the ADC map shows high values (**E**), indicating increased diffusion.

vacuolization, and shows that that swelling of astrocyte endfeet is a primary occurrence in the disease (29, 45). Immunohistochemistry in MLC animal models shows that if either *Mlc1* or *Glialcam* is defective, endfeet localization for both is disturbed (29, 45, 64, 130). Although intriguing, this finding is not fully supported by studies on human tissue: one study showed no change in either the localization or the expression of GlialCAM in brain tissue from a patient with recessive *MLC1* mutations (86), while the same research group more recently suggested a disturbed localization of GlialCAM in cerebellar Bergmann glia from another patient with recessive *MLC1* mutations (130). The reason for this discrepancy is unclear, but it highlights that caution is necessary when translating results from animal models to humans.

Important insights into the pathophysiology of MLC have come from the discovery that the disease is accompanied by defective VRAC currents. This was first recognized in lymphoblasts from patients with *MLC1* mutations (118). Both RVD upon exposure to hypotonic solution and swelling activated VRAC activity were disturbed in patient lymphoblasts. HEK293, HeLa or Sf9 cells transfected with wildtype MLC1 showed enhanced VRAC activity, which was absent upon transfection with patient mutated MLC1 (118). Disrupted VRAC activity and disturbed RVD have also been observed in astrocytes prepared from *Mlc1*-null mice (45) and in astrocytes treated with MLC1 or GlialCAM siRNA (30, 118). This suggests that defective astrocyte volume regulation, leading to disturbed ion and water homeostasis, is central in MLC.

Cl^- CHANNELS

As mentioned earlier, anion channels like VRACs and the ClC-2 Cl^- channel play an important role in volume regulation. ClC-2 Cl^- channels share their localization in astrocyte endfeet with MLC1 and GlialCAM (41). Intriguingly, GlialCAM was identified as an auxiliary subunit for ClC-2 Cl^- channels (71). It greatly enhances ClC-2 mediated currents and changes their functional properties. MLC patient mutations in *GLIALCAM* disrupt this effect on ClC-2 mediated currents (71). Additionally the localization of ClC-2 in astrocyte endfeet and along Bergman glial processes is disrupted in both *Mlc1*-null and *Glialcam*-null mice (29, 45, 67).

CLCN2-related leukoencephalopathy

Initial screens for *CLCN2* as a candidate leukodystrophy gene in humans were negative (121), and a proposed link between *CLCN2* mutations and epilepsy has been disproven (100). However, a recent study identified six leukodystrophy patients with recessive loss-of-function *CLCN2* mutations (41). This leukodystrophy has been called *CLCN2*-related leukoencephalopathy (41). Clinically, these patients presented with variable mild neurological features including cerebellar ataxia, spasticity, chorioretinopathy with visual field defects, optic neuropathy, cognitive defects and headaches (41). Later, two patients with a subclinical leukodystrophy and recessive *CLCN2* mutations were identified (42, 54). Male infertility is another feature of the disease. The patients described above suggest that *CLCN2*-related leukoencephalopathy is a rare disease with variable age of onset and with a wide range of clinical presentations, but until now invariably mild.

MRI (Figure 10) from patients with *CLCN2*-related leukoencephalopathy shows evidence of myelin microvacuolization and signal abnormalities mainly in the posterior limbs of the internal capsules, cerebral peduncles in the midbrain, central tegmental tracts and pyramidal tracts in the pons, and middle cerebellar peduncles (41). Neuropathology is not available from patients.

Studies in *Clcn2* knockout mice, which preceded the discovery of the leukodystrophy, reveal progressive and wide-spread myelin vacuolization in the CNS (20). The mice do not display obvious neurological deficits, but have severe retinal degeneration (24) and a decreased conduction velocity in neurons of the central auditory pathway (20). Strikingly, the myelin vacuolization was not observed in the electrically silent optic nerve.

The similar localization of ClC-2, MLC1 and GlialCAM, as well as the tight interaction between the three proteins, would suggest a similarity between *CLCN2*-related leukoencephalopathy and MLC. However, the clinical and MRI picture for the two diseases differs tremendously (compare Figures 9 and 10). The main affected regions on MRI are almost complementary: while in MLC the cerebral white matter is mainly affected and corpus callosum, internal capsule and brainstem structures are relatively preserved, the latter are mainly affected in *CLCN2*-related leukoencephalopathy. Also, while large fluid-filled intramyelinic vacuoles are evident in the MLC brain, *CLCN2*-related leukoencephalopathy is characterized by white matter microvacuolization (41). In contrast to MLC,

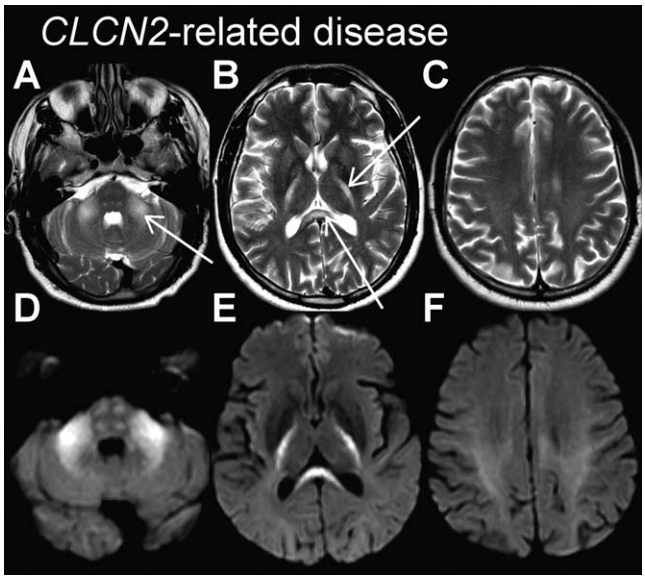

Figure 10. *MRI in CLCN2-related disease in a 40-year-old patient.* Axial T2-weighted images (**A–C**) and matching diffusion-weighted images (**D–F**) show signal abnormalities and diffusion restriction in the middle cerebellar peduncles (arrow in A), posterior limb of the internal capsule and splenium of the corpus callosum (arrows in B). The cerebral white matter shows minimal signal abnormalities and hardly any diffusion restriction (C, F).

CLCN2-related leukoencephalopathy is typically later in its clinical onset, and does not involve macrocephaly in the first year of live. Expression of ClC-2 in other cells then astrocytes, such as oligodendrocytes (64), might explain such differences, but future studies are necessary to address this hypothesis. Therefore, the interplay of MLC1, GlialCAM and ClC-2 is an intriguing puzzle.

COMPARING DISEASES WITH DEFECTIVE ION AND WATER HOMEOSTASIS

As described above, genetic disruption of proteins involved in brain ion and water homeostasis is the cause of several human diseases. Most of these proteins are primarily expressed in glial cells in the brain, highlighting the importance of glia in ion and water homeostasis. When comparing these different diseases it is important to identify commonalities and differences between them.

Edematous white matter due to myelin vacuolization, as observed in MRI and neuropathology, occurs in most diseases described above. These prominently include MLC, MDC1A, *LAMB1*-related encephalopathy, the dystroglycanopathies, *CLCN2*-related leukoencephalopathy and CMTX (with episodes of white matter edema being transient in most patients). The diversity of responsible proteins (an astrocyte protein of unknown function, two basal lamina components, the DAGC, a Cl^- channel and an oligodendrocyte gap junction subunit respectively) highlights the fact that both astrocytes and oligodendrocytes are crucial for activity dependent myelin integrity.

The size of water spaces in the white matter, determined by MRI diffusion tensor imaging or neuropathology, allows a distinction between myelin macrovacuolization and microvacuolization. Macrovacuolization, as seen in MLC, MDC1A, *LAMB1*-related disease and the dystroglycanopathies, is always chronic. Over time, the swelling often diminishes. In MLC caused by dominant *GLIALCAM* mutations it can even completely resolve over a period of years. In MLC, MDC1A, *LAMB1*-related disease and WWS macrovacuolization can be accompanied by appearance of subcortical cysts. White matter microvacuolization can also be chronic, such as in *CLCN2*-reated leukoencephalopathy. However, transient episodes of myelin microvacuolization in CMTX resolve within months, showing that microvacuolization can be rapidly reversed. The reason for heterogeneity in the nature and dynamics of myelin vacuolization is unclear and requires further investigation.

The MRI phenotype of the leukodystrophies PMLD and ODDD indicate white matter hypomyelination. The absence of clear diffusion abnormalities in the white matter on MRI in any stage of the disease is not in line with disturbed ion and water homeostasis. This makes it unlikely that the hypomyelination in these diseases is a secondary consequence of disturbed ion and water homeostasis. Therefore, it shows that gap junctions have multiple roles apart from their involvement in ion and water homeostasis (40, 53, 101).

In addition to white matter swelling, all CMDs can show some degree of abnormality in neuronal migration, due to disruption of the glia limitans. This ranges from MDC1A, where agyria is absent or mild and limited to the occipital region, to dystroglycanopathies characterized by extensive cortical dysplasia. The reason for this range of severities is unclear but likely relates to the developmental and regional importance of different basement membrane and DAGC components. If cortical dysplasia or other structural brain abnormalities are present, the related neurological dysfunction typically dominates the clinical picture.

In contrast to the leukodystrophies mentioned above, disturbances in glial ion and water homeostasis in SeSAME/EAST syndrome and FHM2 do not lead to white matter abnormalities. Instead, in FHM2 episodes of migraine are associated with transient gray matter edema.

It is not well understood why the regional pattern of brain abnormalities differs so much over different diseases. This is inconsistent with a simplified view in which some brain white matter tracts are more vulnerable to edema than others. Instead it suggests regional heterogeneity in the molecular pathways involved in ion and water homeostasis. In line with this, recent studies highlight regional heterogeneity in astrocyte populations in the brain (32, 102).

Clinically, unifying observations for the group of diseases described here are a high occurrence of seizures or epilepsy, motor dysfunction, presence of headaches or migraine, and no or mild cognitive disabilities. Paroxysmal symptoms can be related to triggers. Episodes of CNS dysfunction in CMTX are triggered by exertion, return from high altitude or minor infections. Seizures in MLC or migraine episodes in FHM2 can be triggered by mild head trauma. Interestingly, animal studies show that closed head injury leads to a rise in extracellular K^+ (74), and both epilepsy and migraine have been linked to impaired clearance of K^+ from the extracellular space (36, 110). This highlights the fact that disrupted K^+ clearance plays a key role in pathogenic effects of disturbed ion and water homeostasis.

Another common feature unifying the highlighted diseases is that the clinical manifestations related to the white matter disease are generally relatively mild, although exceptions occur. A likely

explanation for this is redundancy in proteins involved in brain ion and water homeostasis, to ensure functioning of a homeostatic process that is conditional for life. This is in line with animal studies: As described above mutant mice lacking a single gap junction subunit often show no or only a minor phenotype, while double mutations lead to a more severe and often lethal phenotype.

USING ANIMAL MODELS TO STUDY ION AND WATER HOMEOSTASIS

Our understanding of ion and water homeostasis in the brain, as well as its disruption in disease, has been greatly aided by the use of animal experiments. Especially for rare diseases, where patient material is not available or scarce, animal models can be of great help. Additionally, using animal models allows the study of intact brain tissue *in vivo* or *in vitro*, with subcellular resolution. Such studies are invaluable for our mechanistic understanding of brain diseases.

When performing comparative studies, species differences should always be taken into account. Transgenic mouse models for a multitude of diseases have been studied. However, compared with humans mice seem more resilient to disturbances in ion and water homeostasis. Potential explanations for species differences are the much shorter life span of most model animals compared with humans. This gives reason for caution especially when studying slowly progressive diseases evolving over years to decades, as most diseases associated with disrupted ion and water homeostasis are. Additionally, differences in brain anatomy might underlie species differences. For example, the cerebral hemispheric white matter is most severely affected in MLC patients, but mice have only very limited amounts of cerebral white matter. Furthermore, differences at molecular level may cause species-specific findings. While GlialCAM is closely associated with ClC-2 in mice, the association is most likely different in humans. Finally, different compensatory mechanisms might be present in different species, which could explain why the phenotype of specific mouse mutants sometimes contrasts with the human situation. For example, *GJA1* mutations lead to a leukodystrophy in humans but not in knockout mice. The opposite is true for Kir4.1 knockout mice, which have a severe leukodystrophy and die in the first weeks of life (43), while patients with SeSAME/EAST syndrome show no MRI signs of leukodystrophy and a milder clinical phenotype (37). Therefore, although animal models allow for studying disease mechanisms with unprecedented resolution, caution is necessary when designing and interpreting animal experiments.

CONCLUSION

In this review we have highlighted mechanisms underlying brain ion and water homeostasis by glia. Integrating results from MRI patterns, genetic studies, neuropathology and animal models for disease has greatly aided our understanding of ion and water homeostasis in health and disease. We know that coupling of astrocytes and oligodendrocytes into an extensive panglial syncytium is crucial for this process. Additionally, the integrity of astrocyte endfeet and the presence of proteins regulating astrocyte volume at this location are indispensable for ion and water homeostasis. Disruption of any of the components described here leads to neurological disease. We discussed several such diseases, including multiple leukodystrophies. Strikingly, all of these diseases are characterized by defects in proteins that are mainly expressed in glial cells in the brain. A better understanding of the interactions between glia and neurons in the healthy brain, and how these interactions are disrupted in disease, will undoubtedly help with the development of novel approaches to treat these diseases.

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