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Brain Connexins in Demyelinating Diseases: Therapeutic Potential of Glial Targets

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Abstract

Several demyelinating syndromes have been linked to mutations in glial gap junction proteins, the connexins. Although mutations in connexins of the myelinating cells, Schwann cells and oligodendrocytes, were initially described, recent data have shown that astrocytes also play a major role in the demyelination process. Alterations in astrocytic proteins directly affect the oligodendrocytes' ability to maintain myelin structure, and associated astrocytic proteins that regulate water and ionic fluxes, including aquaporins, can also regulate myelin integrity.

Here we will review the main evidence from human disorders and transgenic mouse models that implicate glial gap junction proteins in demyelinating diseases and the therapeutic potential of some of these targets.

INTRODUCTION

1. Composition and Function of myelin in the Nervous System

Myelination is essential for brain function in mammals, as it speeds up transmission of neural information. Several sheaths of myelin surround every single axon. This creates an insulating layer of fat with regular discontinuities called nodes of Ranvier. These nodes concentrate the necessary machinery to propagate action potentials and allow the electrical signals to travel in a saltatory manner to reach other cells located hundred of mms away within milliseconds (Sherman and Brophy, 2005).

Although, in principle, the concept of layers of lipid membranes for insulation sounds simple, myelin formation and organization is a rather complex process. Apart from the unique lipid composition of its plasma membrane, several proteins exclusive to myelin serve as structural support within the myelin membranes. Proteolipid protein (PLP) and myelin-associated glycoprotein (MAG) are some of the main integral proteins in myelin (Nave, 2010) although their exact role is still elusive. In the intracellular space, myelin basic protein (MBP), one of the most critical myelin proteins, creates a framework for attachment, not only of lipids, but also of diverse membrane proteins including ionic channels, transporters, gap junctions, as well as cytoskeletal proteins, and signaling molecules.

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The complexity of white matter organization suggests that myelin contributes not only to insulation, but also to signaling within the myelinating cell and axon. For example, the intimate neuro-glial interaction obtained through myelination has proven crucial for axonal integrity and survival. In addition, myelination also allows energy savings by concentrating critical ionic channels in a very restricted area of the axons, thereby reducing the amount of ATP consumed in restoring ionic gradients after every action potential (Nave, 2010).

Many different human disorders have been described to date that affect either the production or the maintenance of myelin. Some of these demyelination pathologies have been linked to a particular group of proteins - the connexins (Cxs) - that form intercellular gap junction channels with adjacent cells, connecting their cytoplasm. These channels allow the exchange of ions and small metabolites up to 1kDa in size and contribute to cooperative metabolism among cells, electrical coupling and spatial buffering (Bruzzone et al., 1996). Alterations in connexins present in the myelinating glial cells (forming intercellular junctions in oligodendrocytes and autaptic -within themselves- in Schwann cells) all promote demyelination diseases. Interestingly, connexins present in the astrocytes, the major macroglial cell type in the nervous system and not traditionally associated with the myelination process, also contribute to some myelin pathologies.

Here, we will discuss the evidence that supports a role for connexins and related proteins present in both oligodendrocytes and astrocytes in myelin disorders. We will also discuss putative signaling mechanisms that could be involved and the potential for therapeutic intervention based on these targets.

2. Oligodendrocyte-mediated demyelination: connexins

Oligodendrocytes in the central nervous system (CNS) and Schwann cells in the peripheral nervous system (PNS) are the cells involved in synthesizing, organizing and wrapping myelin around the nerves. One single oligodendrocyte can wrap many axons, giving a web-like appearance to these cells in the white matter (Nave, 2010). Oligodendrocytes and Schwann cells express 3 different connexins: Cx47, Cx32 and Cx29, although only the first two are believed to form gap junction channels (Ahn et al., 2008). Whereas Cx47 forms extensive gap junctions with astrocytes in soma and outer myelinated fibers, Cx32 is most abundant within the layers of myelin itself (“reflexive” or “autologous” gap junctions), between loops of the myelin sheath in individual oligodendrocytes and Schwann cells (Kamasawa et al., 2005), although it can also form gap junctions with other astrocytic connexins (Figure 1). These more direct pathways between the myelin layers allow a much shorter route for metabolite exchange.

Several human disorders are caused by defects in oligodendrocyte connexins. Below we discuss some of the information we have learnt from the study of human diseases as well as transgenic mice models.

Charcot Marie Tooth disease (CMT1X) / X-linked progressive peripheral neuropathy - Cx32 in Schwann cells (PNS)—Mutations in the gene that encodes Cx32 cause X-linked Charcot-Marie-Tooth disease, a peripheral neuropathy characterized by loss of myelinated fibers accompanied by axonal alterations, axon regeneration and altered myelin thickness and loosening (reviewed in Kleopa, 2011). Although CMT1X is primarily a peripheral neuropathy that starts in distal limbs, there are also some CNS manifestations that may include cerebellar ataxia or encephalopathy under stress conditions (Kleopa, 2011).

Demyelination in leukodystrophies - Pelizaeus-Merzbacher-like disease or milder spastic paraplegia – Cx47 and Sox10—The term leukodystrophy refers to a

group of disorders that result from abnormalities in myelin, with failure to form at all as in Pelizaeus Merzbacher disease (PMD) or to maintain it as in Krabbe disease (Duncan et al., 2011).

In most cases, mutations in oligodendrocyte proteins are causative of the disease (proteolipid protein – PLP - in PMD; the lysosomal enzyme galactocerebrosidase – GALC gene- in Krabbe disease, to cite a few). In 20–50% of the cases of PMD, however, no recognized mutation in the PLP gene is found.

In Pelizaeus-Merzbacher-like disease (PMLD), mutations in the gap junction protein Cx47 are the main abnormality identified to cause severe CNS myelin deficiency (Uhlenberg et al., 2004). Patients with PMLD often exhibit nystagmus, impaired motor development, ataxia and progressive spasticity and sometimes, mild peripheral neuropathy.

Mutations in the Cx gene produce much more severe phenotypes than eliminating the entire Cx47 gene altogether in mice (see below). An explanation for this could be the redundant nature of the connexin family members, as oligodendrocytes also express two other connexins that can compensate for the absence of Cx47. In the case that the connexin is present but not fully functional, this compensatory mechanism is halted. Deficiencies may then arise due to improper trafficking of the mutant connexin proteins to the membrane and/or faster degradation”.

Recently, a new mutation affecting the Cx47 promoter has been described in a patient with mild PMLD. This mutation affects the binding site of the high mobility group transcription factor SOX10 (Osaka et al., 2010), and SOX10 mutations also cause hypomyelination (Pingault et al., 1998). These facts strengthen the notion that proper transcription of the Cx47 gene is critical for CNS myelination.

Demyelination in mouse models with mutant oligodendrocyte connexins – Cx32 and Cx47—To help decipher the underlying mechanism by which connexins influence myelination and further explore the involvement of connexins in demyelination, several mouse models have been created that can replicate the human pathologies.

Mice lacking Cx32 or Cx47 are viable, and no obvious demyelination is observed (Nelles et al., 1996; Odermatt et al., 2003; Menichella et al., 2003) except for some myelin vacuolation. However, Cx32/Cx47 double deficient mice exhibit much more severe nerve fiber vacuolation, loss of myelin sheaths, oligodendrocyte cell death and ultimately death before two months of age (Odermatt et al., 2003; Menichella et al., 2003).

Similarly, mice transgenic for some disease-linked human Cx32 mutations show demyelinated peripheral neuropathy as the one observed in CMT1X (Sargiannidou et al., 2009). Interestingly, the localization of the two other connexins expressed in oligodendrocytes or Schwann cells, Cx47 or Cx29, is not affected in any of these mice, suggesting that the loss of one single connexin in myelin may be partly compensated by an increase in another one (Li et al., 2008).

3. Astrocyte-mediated demyelination: connexins, AQP4 and potassium

Although demyelination appears more related to defects in the myelinating cells and the myelin structure itself, several recent reports suggest a critical role for astrocytes in certain demyelinating diseases, highlighting the importance of these cells in all cellular brain interactions. Astrocytes are the most abundant cell type in the nervous system, and provide metabolic and structural support to neurons, regulate potassium and water homeostasis, glucose uptake and are also implicated in modulating certain aspects of neuronal function

(Allaman et al., 2011; Nedergaard and Verkhratsky, 2012). Fibrous astrocytes, the astrocytes present in white matter, are unique because they contain higher GFAP expression, have fewer and thinner processes and do not organize in domains like the protoplasmic astrocytes in gray matter (Oberheim et al., 2009), suggesting a more structural rather than functional role. We will next review the main evidence that implicate astrocytic gap junction proteins in demyelinating diseases (Table 1).

Demyelination in Leukodystrophies –Alexander’s disease and Vanishing White Matter – GFAP and other unknown proteins—

A very particular picture emerges from another leukodystrophy: Alexander’s disease, because it is the only known human disorder caused by mutations in an astrocytic protein, glial fibrillary acidic protein (GFAP), the main intermediate filament protein in mature astrocytes. In early onset type I Alexander’s disease, extensive cerebral white matter abnormalities occur, with frontal predominance of periventricular and perivascular lesions. Type 2 Alexander’s disease has, primarily, an adult onset and affects mostly the bulbospinal system (Sawaichi et al., 2009). At the molecular and cellular level, Alexander’s disease is characterized by massive protein accumulations called Rosenthal fibers in astrocytes, large aggregates of GFAP mutant protein accompanied by two heat shock proteins HSP27 and α B-crystallin. These Rosenthal fibers are often abundant in fibrous astrocytes of subcortical white matter. Other effects on astrocytes include reduced levels of glutamate transporter GLT1 and increased lipid peroxidation and iron accumulation, indicative of oxidative stress. Although mutant astrocytes are the main cause of the disease in 90% of the cases and become very reactive, there is loss of axons and myelin in variable regions. The very highly reactive astrocytes are, paradoxically, spared of cell death (Brenner et al., 2008).

Similar to Alexander’s disease, hypomyelination/vanishing white matter syndrome (VWM) and Canavan disease are also linked to defective astrocytes but much more indirectly. In VWM, one of the leukodystrophies most prevalent in childhood, mutations in the eukaryotic translation initiation factor 2B (eIF2B) have been identified as the cause of the disease. Despite the ubiquitous function of this housekeeping protein on controlling protein synthesis, it is surprising that VWM patients exhibit mostly a neuropathy characterized by the presence of diffuse, spongiform myelin, which is dramatically diminished (Bugiani et al., 2010). Most intriguingly, oligodendrocytes are increased in number (van Haren et al., 2004), whereas astrocytes are decreased and the few left present an atypical appearance (Dietrich et al., 2005). Thus, this gliopathy has been proposed to combine severe myelin deficiency with an inability of astrocytic gliosis to contain the extended damage (Bugiani et al., 2010).

The mechanisms by which altered astrocytes in these conditions promote white matter changes and affect oligodendrocytes and axons are presently unknown, although it is tempting to speculate that connexin proteins might be important mediators of these effects.

In the next sections we will review some of the evidence that implicate these proteins in astrocyte-mediated demyelination.

Demyelination mediated by astrocytic gap junction proteins in mouse models

– **Cx43 and Cx30**—Astrocytes establish abundant gap junctions among themselves via two main connexins, Cx43 and Cx30 (Nagy et al., 2001). Given that oligodendrocyte connexins are not expressed in astrocytes and that astrocytes are barely coupled among themselves in white matter of young mice (Maglione et al., 2010), it is somewhat surprising that astrocytic connexins are involved in demyelinating diseases. Although as many as 77% of the cells displaying gap junctional coupling in white matter form among oligodendrocytes (via Cx47) or within themselves (via Cx32), heterotypic coupling has also been detected

between oligodendrocytes and astrocytes, albeit in lesser amounts (Maglione et al., 2010). Some evidence indicates that these heterotypic gap junctions form via Cx43/Cx47 on one hand and Cx30/Cx32 on the other (Li et al., 2008; Maglione et al., 2010).

Still, these astro-oligodendrocyte gap junctions have enough significance to strongly affect myelination. Mutations in Cx43 or Cx30 alone do not show signs of abnormal myelination (Nakase et al., 2004; Dere et al., 2003). However, double mutants for Cx43/Cx30 show glial “edema” and myelin vacuolation in white matter, with the hippocampus CA1 region the only area where obvious pathology is found in grey matter (Lutz et al., 2009). Some sensorimotor and spatial memory deficits are also observed in these double knockout mice with no effect on life span. To address the true impact of the astrocytic connexins, Magnotti et al. (2011) developed mice mutant for one oligodendrocyte connexin and one astrocytic connexin: dkoCx43/Cx47 or dkoCx43/Cx32 (Figure 2). Surprisingly, only the Cx43/Cx32 dKO exhibited myelin vacuolation accompanied by little effect on oligodendrocytes but marked increased in astrocytic cell death. This translated into seizure activity and early mortality. Thus, astrocytic connexins may indirectly impact myelin integrity by compromising astrocytic survival.

Osmotic demyelination syndrome – Cx43 and Cx47—Osmotic demyelination syndrome (ODS) occurs during the process of trying to correct chronic hyponatremia. During hyponatremia (low sodium levels outside the cells), the fall in serum osmolarity driven by the fall in sodium levels causes water to move into the cells, posing the risk of cerebral edema. In an attempt to correct this imbalance, there may be too rapid osmotic changes that translate into neurological symptoms known as ODS. This syndrome is characterized by demyelinating lesions in the CNS, especially in the pons, and is preceded by massive death of astrocytes (Gankam et al., 2011). In the early events of the process (12 hours after correction of hyponatremia has been initiated), there is substantial downregulation of astrocytic Cx43 on the one hand, and oligodendrocytic Cx47 on the other. None of these changes are observed in the noncorrected hyponatremic brain (Gankam et al., 2011). Changes in the blood brain barrier have been also reported.

Demyelination in neuromyelitis optica - alterations in astrocytic aquaporin-4 (AQP4)—Neuromyelitis optica (NMO) is an inflammatory autoimmune disease usually restricted to the optic nerve and spinal cord and is characterized by extensive myelin loss in both grey and white matter areas (Hinson et al., 2010). A specific autoantibody, neuromyelitis optica-immunoglobulin G (NMO-IgG) that binds the water channel protein AQP4 has been identified as the primary cause of this pathology (Lennon et al., 2004). AQP4 is the main water regulator of astrocytes and is not expressed in oligodendrocytes. Thus, alterations in this astrocytic protein directly affect the capability of oligodendrocytes to maintain myelin integrity. The exact mechanism for this effect is not clear although complement activation, down-regulation of AQP4 and of the glutamate transporter EAAT2 and death of oligodendrocytes all occur in this disorder (Hinson et al., 2008; Marignier et al., 2010).

Balo’s disease and multiple sclerosis (MS) – AQP4 alterations—An antibody-independent selective loss of AQP4 is also observed in multiple sclerosis (MS) and Balo’s disease. MS is an autoimmune disease where the body’s immune system attacks the myelin sheath causing nerve damage. Balo’s disease is a variant of MS in that the demyelinating tissue forms concentric layers (Kira, 2011). Similar to NMO, these two diseases also feature extensive loss of AQP4 and highly hypertrophic astrocytes. Most importantly, unpublished observations suggest that these three disorders also share disappearance of connexins (Kira, 2011). This evidence points again to a role for astrocytic proteins in the maintenance of myelination.

Interaction of AQP and connexins was first reported in the lens (Yu and Jiang, 2004) where mutations in members of these two families of proteins (Cx50 and AQP0) independently cause human congenital cataracts (reviewed in Huang and He, 2010). Liu et al. (2011) have recently demonstrated that AQP0 has a direct role in the regulation of functional gap junction channels by influencing cell-cell adhesion. In brain astrocytes, AQP4 knockdown produced a strong downregulation of Cx43 with concomitant reduction in cell coupling (Nicchia et al., 2005). However, these effects were exclusive of mouse astrocytes with no similar alterations in human or rat cells. Thus, although it is still not clear how consistent the interaction between connexins and aquaporins is among different connexin types and different species, and the relevance of these interactions to human disorders remains uncertain, it is possible to suggest that a functional relationship between the AQP water channels and the connexins might explain the effects of AQP4 defects in demyelination.

Megalencephalic leukoencephalopathy with subcortical cysts (MLC) – MLC and GlialCAM proteins—Further evidence that supports a critical role for the astrocytic regulation of water and ion fluxes in the maintenance of myelin integrity comes from this leukodystrophy. In MLC, myelin vacuolation is accompanied by increased water content of the brain (van der Knaap et al., 1995, 1996). Mutations have been found in the MLC1 protein, a transmembrane astrocytic protein with low degree of homology to ion channels, and in the GlialCAM adhesion protein (Leegwater et al., 2001; López-Hernández et al., 2011). Recently, GlialCAM has been identified as a binding partner of the Cl⁻ channel CIC-2 (Jeworutzki et al., 2012) and is responsible to target this channel to astrocytic cell junctions, endfeet around vessels and myelin. Interestingly, MLC1 and the actin-binding protein zonula occludens 1 (ZO-1) colocalize in humans (Duarri et al., 2011) and ZO-1 also interacts physically with some connexins (Li et al., 2004).

Further studies on the inter-dependence between connexins, aquaporins, ion channels and cell-cell junction proteins will help determine the impact of these proteins in demyelination.

4. Could astrocytic connexins and interacting proteins be a new family of targets for therapeutic intervention in myelinating diseases?

Connexins and inflammation—An interesting aspect of NMO and related disorders is the involvement of a robust T-cell response against major myelin proteins. This, in turn, initiates a cascade of CNS inflammation, with increased levels of T-helper-1 (Th1), interferon γ (IFN γ) and of the cytokines IL-17 and IL-8 (Kira, 2011) that correlate positively with lesion size. Sharma et al. (2010) have also shown involvement of an inflammation step in a model of lipopolysaccharide (LPS)-induced demyelination. Injection of LPS into white matter triggers initial microglial activation, followed by a strong astrocytic reaction. This, in turn, is accompanied by loss of AQP-4 and connexins and consequent myelin degeneration.

Inflammation can converge negatively on gap junctions in several ways. For example, reactive glia release cytokines, like tumor necrosis factor- α (TNF- α) and interleukin-1 β (IL-1 β), that affect connexin expression and gap junction permeability in astrocytes (Meme et al., 2006). Interestingly, these same cytokines regulate oppositely hemichannel activity increasing membrane permeability (Retamal et al., 2007). In a model of spinal cord injury, O'Carroll et al., (2008) used mimetic peptides to block hemichannel activity and found that these peptides were able to reduce swelling, the number of reactive GFAP⁺ astrocytes, and the loss of NeuN⁺ neurons. Huang et al. (2012) have, in addition, shown that Cx43/Cx30 mediated ATP release is implicated in the post-traumatic inflammation after spinal cord damage. The involvement of Cx43/Cx30 in reduction of inflammation during injury does not seem to apply to all inflammatory processes as deletion of these two connexins did not

impact the extent of inflammation in a mice model of experimental autoimmune encephalomyelitis (Lutz et al., 2012).

It is possible that an initial inflammatory step that alters gap junction communication precedes connexin downregulation, alterations in connexin-mediated astro-oligodendrocyte interactions and, ultimately, loss of myelin with subsequent reduction of inflammatory damage. This cascade of events may be secondary to the initial damage in an attempt to contain further inflammation and would place inflammation as an important causative aspect to keep in mind as a trigger for CNS demyelination when astrocyte dysfunction is involved. It also highlights the importance of hemichannel blocking agents as putative therapeutic agents against myelination alterations.

Connexins and potassium channels—The functional role of astrocytic connexins in the brain has been always elusive given the lack of reagents that exclusively block gap junctional channels. New studies in mutant mice deficient for the two astrocytic connexins have allowed a better insight on how the absence of Cx30 and Cx43 affect brain structure and function. Walraff et al. (2006) has found that astrocytic coupling allowed for a rapid removal of extracellular potassium after neuronal activity and aided its redistribution to areas of lower concentration (potassium “siphoning”). Similarly,

Pannash et al. (2011) have found that the network of Cx30/Cx43-coupled astrocytes modulate synaptic transmission in the hippocampus by removing extracellular glutamate and potassium after synaptic activity. The size of the astrocytic network, a direct correlation of gap junctional coupling, seems crucial to control synaptic strength.

Recently, the concept of “translational” channelopathies is taking shape as more and more disorders present deficits based on secondary changes in ionic channels, more specifically, sodium and potassium ionic channels. The upregulation and downregulation of ionic channels as a consequence of disease is post-translational in nature but can determine the progression of the disease. For example, levels of the sodium channel Nav1.8 are altered in MS and both Kv1.1 and Kv1.2 potassium channels have also abnormal levels in the demyelinated axons of the *shiverer* mouse, another model of demyelination (reviewed in Waxman, 2001).

Given that potassium dysregulation plays a critical role in several of the connexin-mediated models of demyelination, it is reasonable to speculate that these channels are changed in parallel with connexin alterations.

Similarly, we must not forget that this astrocytic network of gap junctions is crucial for glucose uptake from the perivascular endfeet for its subsequent diffusion towards neurons to sustain their synaptic activity and even their epileptic activity (Rouach et al., 2008). Disruptions to connexins will certainly impact the efficiency of the astroglial network to provide energy metabolites to myelinated axons.

Demyelination and adhesion—Vacuolation in animals with deficient gap junctions have been attributed to the inability of the cells to maintain proper fluid exchange, abnormal signal transduction across myelin or to a lack of adhesion to other proteins (Odermatt et al., 2003). In fact, several reports have suggested that gap junctions can behave as adhesion molecules independently of channel formation (Lin et al, 2002; Cotrina et al., 2008). The role of connexins as adhesion molecules is further supported by the elucidation of their interacting partners, like ZO-1 and the scaffolding protein MUPP1, both abundant proteins in numerous mammalian tight junctions and affected after loss of Cx47 (Li et al., 2008).

Coincidentally, ZO-1 also interacts with the protein MLC1, mutations of which are responsible for demyelination in MLC disease (see above).

CONCLUSIONS

Demyelinating disorders linked to mutations in connexins and related astrocytic proteins have in recent years uncovered a wealth of new data suggesting that gap junction proteins play much more important roles in human disease than previously acknowledged. The precise mechanism by which deficient gap junction communication alters myelin formation and maintenance, and why some axonal fibers are more affected than others are questions that remain to be answered. However, the discovery of new mutations in proteins that are indirectly related to the connexin family is quickly reshaping our view on how glial proteins affect myelin integrity and function. Myelin gap junctions seem to be in a unique position not only to regulate metabolite trafficking to and from the myelin sheath, but also to guarantee myelin structure and proper compaction by regulating ionic and water fluxes.

In the context of the disease, regulatory reciprocal loops of inflammation/gap junctions exist in some models of injury that open the possibility of therapeutic intervention by targeting connexin proteins and/or downstream signaling cascades, such as ATP release.

Further studies on the interdependence between connexins, aquaporins, ion channels, the glio-vascular interface, and cell-cell junctions and on how astrocytic proteins interact, in turn, with the myelinating cells, oligodendrocytes and Schwann cells, will lead us into a new exciting time to understand the role of glial cells in myelination.

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References

- Ahn M, Lee J, Gustafsson A, Enriquez A, Lancaster E, Sul JY, Haydon PG, Paul DL, Huang Y, Abrams CK, Scherer SS. Cx29 and Cx32, two connexins expressed in myelinating glia, do not interact and are functionally distinct. *J Neurosci Res.* 2008; 86:992–1006. [PubMed: 17972320]
- Allaman I, Bélanger M, Magistretti PJ. Astrocyte-neuron metabolic relationships: for better and for worse. *Trends Neurosci.* 2011; 34:76–87. [PubMed: 21236501]
- Brenner, M.; Goldman, JE.; Quinlan, RA.; Messing, A. Alexander disease: a genetic disorder of astrocytes. In: Parpura, V.; Haydon, P., editors. *Astrocytes in (patho)physiology of the nervous system.* Springer; 2009. p. 591-648.
- Bruzzone R, White TW, Paul DL. Connections with connexins: the molecular basis of direct intercellular signaling. *Eur J Biochem.* 1996; 238:1–27. [PubMed: 8665925]
- Bugiani M, Boor I, Powers JM, Scheper GC, van der Knaap MS. Leukoencephalopathy with Vanishing White Matter: a review. *J Neuropathol Exp Neurol.* 2010; 69:987–996. [PubMed: 20838246]
- Cotrina ML, Lin JHC, Nedergaard M. Adhesive properties of connexin hemichannels. *Glia.* 2008; 56:1791–1798. [PubMed: 18649405]
- Dere E, De Souza-Silva MA, Frisch C, Teubner B, Söhl G, Willecke K, Huston JP. Connexin30-deficient mice show increased emotionality and decreased rearing activity in the open-field along with neurochemical changes. *Eur J Neurosci.* 2003; 18:629–638. [PubMed: 12911759]
- Dietrich J, Lacagnina M, Gass D, Richfield E, Mayer-Pröschel M, Noble M, Torres C, Pröschel C. EIF2B5 mutations compromise GFAP+ astrocyte generation in vanishing white matter leukodystrophy. *Nat Med.* 2005; 11:277–283. [PubMed: 15723074]
- Duarri A, López de Heredia M, Capdevila-Nortes X, Ridder MC, Montolio M, López-Hernández T, Boor I, Lien C, Hagermann T, Messing A, Gorecki DC, Scheper GC, Martínez A, Nunes V, Van

- der Knaap MS, Estévez R. Knockdown of MLC1 in primary astrocytes causes cell vacuolation: a MLC disease cell model. *Neurobiol Dis.* 2011; 43:228–238. [PubMed: 21440627]
- Duncan ID, Kondo Y, Zhang S-C. The myelin mutants as models to study myelin repair in the leukodystrophies. *Neurotherapeutics.* 2011; 8:607–624. [PubMed: 21979830]
- Gankam F, Nicaise C, Soupart A, Boom A, Schiettecatte J, Pochet R, Brion JP, Decaux G. Astrocytes are an early target in osmotic demyelination syndrome. *J Am Soc Nephrol.* 2011; 22:1834–1845. [PubMed: 21885671]
- Hinson SR, Roemer SF, Lucchinetti CF, Fryer JP, Kryzer TJ, Chamberlain JL, Howe CL, Pittock SJ, Lennon VA. Aquaporin-4-binding autoantibodies in patients with neuromyelitis optica impair glutamate transport by down-regulating EAAT2. *J Exp Med.* 2008; 205:2473–2481. [PubMed: 18838545]
- Hinson SR, Mckeon A, Lennon VA. Neurological autoimmunity targeting aquaporin-4. *Neuroscience.* 2010; 168:1009–1018. [PubMed: 19699271]
- Huang B, He W. Molecular characteristics of inherited congenital cataracts. *Eur J Med Gen.* 2010; 53:347–357.
- Huang C, Han X, Li X, Lam E, Peng W, Lou N, Torres A, Yang M, Garre JM, Tian G-F, Bennett MVL, Nedergaard M, Takano T. Critical role of Connexin 43 in secondary expansion of traumatic spinal cord injury. *J Neurosci.* 2012; 32:3333–3338. [PubMed: 22399755]
- Jeworutzki E, López-Hernández T, Capdevila-Nortes X, Sirisi S, Bengtsson L, Montolio M, Zifarelli G, Amedo T, Müller C, Schulte U, Nunes V, Martínez A, Jentsch TJ, Gasull X, Pusch M, Estévez R. GlialCAM, a protein defective in a leukodystrophy, serves as a CIC-2 Cl⁻ channel auxiliary subunit. *Neuron.* 2012; 73:951–961. [PubMed: 22405205]
- Kamasawa N, Sik A, Morita M, Yasumura T, Davidson KGV, Nagy JI, Rash JE. Connexin-47 and Connexin-32 in gap junctions of oligodendrocyte somata, myelin sheaths, paranodal loops and Schmidt-Lanterman incisures: implications for ionic homeostasis and potassium siphoning. *Neuroscience.* 2005; 136:65–86. [PubMed: 16203097]
- Kira J. Autoimmunity in neuromyelitis optica and opticospinal multiple sclerosis: Astrocytopathy as a common denominator in demyelinating disorders. *J Neurol Sci.* 311:69–77. [PubMed: 21962794]
- Kleopa KA. The role of gap junctions in Charcot-Marie-Tooth Disease. *J Neurosci.* 2011; 31:17753–17760. [PubMed: 22159091]
- Leegwater PA, Yuan BQ, van der Steen J, Mulders J, Könst AA, Boor PK, Mejaski-Bosnjak V, van der Maarel SM, Frants RR, Oudejans CB, Schutgens RB, Pronk JC, van der Knaap MS. Mutations of MLC1 (KIAA0027), encoding a putative membrane protein, cause megalencephalic leukoencephalopathy with subcortical cysts. *Am J Hum Genet.* 2001; 68:831–838. [PubMed: 11254442]
- Lennon VA, Wingerchuk DM, Kryzer TJ, Pittock SJ, Lucchinetti CF, Fujihara K, Nakashima I, Weinshenker BG. A serum auto-antibody marker of neuromyelitis optica: distinction from multiple sclerosis. *Lancet.* 2004; 364:2106–2112. [PubMed: 15589308]
- Li X, Ionescu AV, Lynn BD, Lu S, Kamasawa N, Morita M, Davidson KG, Yasumura T, Rash JE, Nagy JI. Connexin47, connexin29 and connexin32 coexpression in oligodendrocytes and Cx47 association with zonula occludens-1 (ZO-1) in mouse brain. *Neuroscience.* 2004; 7:523–538.
- Li X, Penes M, Odermatt B, Willecke K, Nagy JI. Ablation of Cx47 in transgenic mice leads to the loss of MUPP1, ZONAB and multiple connexins at oligodendrocyte-astrocyte gap junctions. *Eur J Neurosci.* 2008; 28:1503–1517. [PubMed: 18973575]
- Lin JHC, Takano T, Cotrina ML, Arcuino G, Kang J, Liu S, Gao Q, Jiang L, Lichtenberg-Frate H, Hsubrich S, Willecke K, Goldman SA, Nedergaard M. Connexin 43 enhances de adhesivity and mediates the invasion of malignant glioma cells. *J Neurosci.* 2002; 22:4302–4311. [PubMed: 12040035]
- Liu J, Xu J, Gu S, Nicholson BJ, Jiang JX. Aquaporin 0 enhances gap junction coupling via its cell adhesion function and interaction with connexin 50. *J Cell Sci.* 2011; 124:198–206. [PubMed: 21172802]
- López-Hernández T, Ridder MC, Montolio M, Capdevila-Nortes X, Polder E, Sirisi S, Duarri A, Schulte U, Fakler B, Nunes V, Scheper GC, Martínez A, Estévez R, van der Knaap MS. Mutant GlialCAM causes megalencephalic leukoencephalopathy with subcortical cysts, benign familial

- macrocephaly, and macrocephaly with retardation and autism. *Am J Hum Genet.* 88:422–432. [PubMed: 21419380]
- Lutz SE, Zhao Y, Gulinello M, Lee CS, Raine CS, Brosnan CF. Deletion of astrocyte connexins 43 and 30 leads to a dysmyelinating phenotype and hippocampal CA1 vacuolation. *J Neurosci.* 2009; 29:7743–7752. [PubMed: 19535586]
- Lutz SE, Raine CS, Brosnan CF. Loss of astrocyte connexins 43 and 30 does not significantly alter susceptibility or severity of acute experimental autoimmune encephalomyelitis in mice. *J Neuroimmunol.* 2012; 245:8–14. [PubMed: 22342190]
- Maglione M, Tress O, Haas B, Karraam K, Trotter J, Willecke K, Kettenmann H. Oligodendrocytes in mouse corpus callosum are coupled via gap junction channels formed by connexin 47 and connexin 32. *Glia.* 2010; 58:1104–1117. [PubMed: 20468052]
- Magnotti LM, Goodenough DA, Paul DL. Deletion of oligodendrocyte Cx32 and astrocyte Cx43 causes white matter vacuolation, astrocyte loss and early mortality. *Glia.* 2011; 59:1064–1074. [PubMed: 21538560]
- Marignier R, Nicolle A, Watrin C, Touret M, Cavagna S, Varrin-Doyer M, Cavillon G, Rogemond V, Confavreux C, Honnorat J, Giraudon P. Oligodendrocytes are damaged by neuromyelitis optica immunoglobulin G via astrocyte injury. *Brain.* 2010; 133:2578–2591. [PubMed: 20688809]
- Meme W, Calvo C, Froger N, Ezan P, Amigou E, Koulakoff A, Giaume C. Proinflammatory cytokines released from microglia inhibit gap junctions in astrocytes: potentiation by β -amyloid. *FASEB J.* 2006; 20:494–496. [PubMed: 16423877]
- Menichela DM, Goodenough DA, Sirkowski E, Scherer SS, Paul DL. Connexins are critical for normal myelination in the CNS. *J Neurosci.* 2003; 23:5963–5973. [PubMed: 12843301]
- Nagy JI, Li X, Rempel J, Stelmack G, Patel D, Staines WA, Yasumura T, Rash JE. Connexin 26 in adult rodent central nervous system: demonstration of astrocytic gap junctions and colocalization with connexin30 and connexin 43. *J Comp Neurol.* 2001; 441:302–323. [PubMed: 11745652]
- Nakase T, Söhl G, Theis M, Willecke K, Naus CC. Increased apoptosis and inflammation after focal brain ischemia in mice lacking connexin43 in astrocytes. *Am J Pathol.* 2004; 164:2067–2075. [PubMed: 15161641]
- Nave K. Myelination and support of axonal integrity by glia. *Nature.* 2010; 468:244–252. [PubMed: 21068833]
- Nedergaard M, Verkhratsky A. Artifact versus reality – how astrocytes contribute to synaptic events. *Glia.* 2012; 1002:2228
- Nelles E, Butzler C, Jung D, Temme A, Gabriel HD, Dahl U, Traub O, Stumpel F, Jungermann K, Zielasek J, Toyka KV, Dermietzel R, Willecke K. Defective propagation of signals generated by sympathetic nerve stimulation in the liver of connexin32-deficient mice. *Proc Natl Acad Sci USA.* 1996; 93:9565–9670. [PubMed: 8790370]
- Nicchia GP, Srinivas M, Li W, Brosnan CF, Frigeri A, Spray DC. New possible roles for aquaporin-4 in astrocytes: cell cytoskeleton and functional relationship with connexin43. *FASEB J.* 2005; 19:1674–1686. [PubMed: 16103109]
- Oberheim NA, Takano T, Han X, He W, Lin JHC, Wang F, Xu Q, Wyatt JD, Pilcher W, Ojemann JG, Ransom BR, Goldman SA, Nedergaard M. Uniquely hominid features of adult human astrocytes. *J Neurosci.* 2009; 29:3276–3287. [PubMed: 19279265]
- O'Carroll SJ, Alkadhi M, Nicholson LFB, Green CR. A Cx43 mimetic peptide reduces swelling, astrogliosis and neuronal cell death after spinal cord injury. *Cell Comm Adhes.* 2008; 15:27–42.
- Odermatt B, Wellershaus K, Wallraff A, Seifert G, Degen J, Euwens C, Fuss B, Büssow H, Steinhäuser C, Willecke K. Connexin 47 (Cx47)-deficient mice with enhanced green fluorescent protein reporter gene reveal predominant oligodendrocytic expression of Cx47 and display vacuolized myelin in the CNS. *J Neurosci.* 2003; 23:4549–4559. [PubMed: 12805295]
- Osaka H, Hamanoue H, Yamamoto R, Nezu A, Sasaki M, Saitsu H, Kurosawa K, Shimbo H, Matsumoto N, Inoue K. Disrupted SOX10 regulation on GJC2 transcription causes Pelizaeus-Merzbacher-Like disease. *Ann Neurol.* 2010; 68:250–254. [PubMed: 20695017]
- Pannasch U, Vargová L, Reingruber J, Ezan P, Holcman D, Giaume C, Syková E, Rouach N. Astroglial networks scale synaptic activity and plasticity. *PNAS.* 2011; 108:8467–8472. [PubMed: 21536893]

- Pingault V, Bondurand N, Kuhlbrodt K, et al. SOX10 mutations in patients with Waardenburg-Hirschsprung disease. *Nat Genet.* 1998; 18:171–173. [PubMed: 9462749]
- Retamal MA, Froger N, Palacios-Prado N, Ezan P, Sáez PJ, Sáez JC, Giaume C. Cx43 Hemichannels and gap junction channels in astrocytes are regulated oppositely by proinflammatory cytokines released from activated microglia. *J Neurosci.* 2007; 27:13781–13792. [PubMed: 18077690]
- Rouach N, Koulakoff A, Abudara V, Willecke K, Giaume C. Astroglial metabolic networks sustain hippocampal synaptic transmission. *Science.* 2008; 322:1551–1555. [PubMed: 19056987]
- Sargiannidou I, Vavlitou N, Aristodemou S, Hadjisavvas A, Kyriacou K, Scherer SS, Kleopa KA. Connexin 32 mutations cause loss of function in Schwann cells and oligodendrocytes leading to PNS and CNS myelination defects. *J Neurosci.* 2009; 29:4736–4749. [PubMed: 19369543]
- Sawaishi Y. Review of Alexander disease: beyond the classical concept of leukodystrophy. *Brain Dev.* 2009; 31:493–498. [PubMed: 19386454]
- Sharma R, Fisher M, Bauer J, Felts PA, Smith KJ, Misu T, Fujihara K, Bradl M, Lassmann H. Inflammation induced by innate immunity in the central nervous system leads to primary astrocyte dysfunction followed by demyelination. *Acta Neuropat.* 2010; 120:223–236.
- Sherman DL, Brophy PJ. Mechanisms of axon ensheathment and myelin growth. *Nat Rev Neurosci.* 2005; 6:683–690. [PubMed: 16136172]
- Uhlenberg B, Schuelke M, Rüschendorf F, Ruf N, Kaindl AM, Henneke M, Thiele H, Stoltenbug-Didinger G, Aksu F, Topaloglu H, Nürnberg P, Hübner C, Weschke B, Gärtner J. Mutations in the gene encoding gap junction protein $\alpha 12$ (connexin 46.6) cause Pelizaeus-Merzbacher-Like disease. *Am J Hum Genet.* 2004; 75:251–260. [PubMed: 15192806]
- Van der Knaap MS, Valk J, Barth PG, Smit LM, van Engelen BG, Tortori Donati P. Leukoencephalopathy with swelling in children and adolescents: MRI patterns and differential diagnosis. *Neuroradiology.* 1995; 37:679–686. [PubMed: 8748906]
- Van der Knaap MS, Barth PG, Vrensen GF, Valk J. Histopathology of an infantile-onset spongiform leukoencephalopathy with a discrepantly mild clinical course. *Acta Neuropathol.* 1996; 92:206–212. [PubMed: 8841668]
- Van Haren K, van der Voorn P, Peterson DR, van der Knaap MS, Powers JM. The life and death of oligodendrocytes in Vanishing White Matter disease. *J Neuropathol Exp Neurol.* 2004; 63:618–630. [PubMed: 15217090]
- Walraff A, Köhling R, Heinemann U, Theis M, Willecke K, Steinhäuser C. The impact of astrocytic gap junctional coupling on potassium buffering in the hippocampus. *J Neurosci.* 2006; 26:5438–5447. [PubMed: 16707796]
- Waxman SG. Transcriptional channelopathies: an emerging class of disorders. *Nature Rev Neurosci.* 2001; 2:652–659. [PubMed: 11533733]
- Yu XS, Jiang JX. Interaction of major intrinsic protein (aquaporin-0) with fiber connexin. 2004

Figure 1. Schematic of the connexins involved in formation of astrocytic, oligodendrocytic and astro-oligodendrocytic gap junctions

Astrocyte-astrocyte gap junctions are composed primarily of Cx43 (red), with a minor contribution of Cx30. Oligodendrocyte-oligodendrocyte gap junctions are primarily assembled by Cx32 (green), whereas gap junctions connecting astrocytes and oligodendrocytes are composed of a mixture of Cx43/Cx47 or Cx30/Cx32.

Figure 2. Histopathology (H&E staining) of gray and white matter of mice brain lacking the astrocyte and oligodendrocyte connexins

Low power views of sagittal sections of control mice (A,D), dKO of Cx32-Cx43 (B,E) or dKO of Cx32-Cx47 (C,F). Double mutants for astrocyte and oligodendrocyte connexins show myelin vacuolation in the corpus callosum (B,C) and cerebellar white matter (E,F) compared to controls (A,D).

Table 1

Description of the main demyelinated disorders related to connexin/astrocytic proteins

Disease	Protein involved	Reference in text
<i>Oligodendrocyte/Schwann cells proteins</i>		
Charcot-Marie-Tooth Disease (CMT1X)	Cx32	Kleopa 2011
Pelizaeus-Merbacher-like disease (PMLD)	Cx47, SOX10	Uhlenberg et al., 2004; Osaka et al., 2010
<i>Astrocytic proteins or proteins that affect astrocytes</i>		
Alexander's disease	GFAP	Brenner et al., 2008; Sawaichi et al., 2009
Vanishing White matter Syndrome (VWM)	elf2B	Bugiani et al., 2010
Osmotic demyelination syndrome	Cx43 and Cx47	Gankam et al., 2011
Neuromyelitis optica	AQP4	Lennon et al., 2004
Balo's disease and Multiple Sclerosis	AQP4	Kira, 2011
Megalencephalic leukoencephalopathy with subcortical cysts (MLC)	MLC GlialCAM	Leegwater et al., 2001 López-Hernández et al., 2011