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REVIEW ARTICLE

The potential roles of aquaporin 4 in amyotrophic lateral sclerosis

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Abstract

Aquaporin 4 (AQP4) is a primary water channel found on astrocytes in the central nervous system (CNS). Besides its function in water and ion homeostasis, AQP4 has also been documented to be involved in a myriad of acute and chronic cerebral pathologies, including autoimmune neurodegenerative diseases. AQP4 has been postulated to be associated with the incidence of a progressive neurodegenerative disorder known as amyotrophic lateral sclerosis (ALS), a disease that targets the motor neurons, causing muscle weakness and eventually paralysis. Raised AQP4 levels were noted in association with vessels surrounded with swollen astrocytic processes as well as in the brainstem, cortex, and gray matter in patients with terminal ALS. AQP4 depolarization may lead to motor neuron degeneration in ALS via GLT-1. Besides, alterations in AQP4 expression in ALS may result in the loss of blood–brain barrier (BBB) integrity. Changes in AQP4 function may also disrupt K^+ homeostasis and cause connexin dysregulation, the latter of which is associated to ALS disease progression. Furthermore, AQP4 suppression augments recovery in motor function in ALS, a phenomenon thought to be associated to NGF. No therapeutic drug targeting AQP4 has been developed to date. Nevertheless, the plethora of suggestive experimental results underscores the significance of further exploration into this area.

Keywords AQP4 · Amyotrophic lateral sclerosis · Target · Therapy

Introduction

Aquaporins (AQPs) are plasma membrane water-channel proteins that are critical in maintaining water homeostasis in cells of the central nervous system (CNS). There have been at least 13 types of water-channel proteins (AQP1–13) that have been characterized across various mammalian species. The roles of AQP in physiological and pathological conditions have been hotly debated and researched. In addition to regulating water balance, it has been suggested that AQPs are entrenched in the pathophysiology of cerebral edema [1–3], seizures [4, 5], hepatoencephalopathy [6], and brain tumors [7]. Astrocyte is heavily involved in maintaining cerebral homeostasis and is the most abundant non-neuronal glial cells in the brain. Of late, they have frequently been found to feature in several different neurological diseases [8]. Growing evidence indicates that cerebral aquaporin 4 (AQP4), a major AQP isoform in the brains of human adults that are specifically localized in astrocytes, is responsible for mediating astrocytic function in various neuropathologies such as fluid and ion regulation [9–11], release and uptake of potassium by astrocytes [12], glial scarring and astrocytic migration [13, 14], neural signal transduction [15], proinflammatory factor secretion [16], and astrocyte-to-astrocyte cell communication [17].

Amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig's disease, is a progressive neurodegenerative disease involving the motor neurons that culminate in muscle weakness and paralysis. Motor neuron functionality and

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integrity are highly dependent on the astrocytic environment. Astrocytic podocytes line the blood–brain barrier (BBB) and are rich in AQP4, further highlighting its potential role in the pathogenesis and progression of ALS. Initial literature first suggesting the relationship between AQP4 and ALS dates back several years and has since expanded tremendously, with multiple current studies strongly supporting the role of AQP4 in ALS [18]. This paper focuses the discussion on the potential function of AQP4 in ALS as well as the underlying neurobiology (Fig. 1).

Alterations of AQP4 expression in ALS

Motor neuronal death in ALS is a product of astrocytic dysfunction causing disrupted glial homeostasis [19, 20]. AQP4 contributes towards cerebral homeostasis by regulating the microenvironment astrocytic end-feet processes [21]. A previous study investigating the role of the Kir4.1 channels in the spinal cord of superoxide dismutase 1 (SOD1) mutated transgenic mice noted raised astrocytic expressions of the AQP4 protein [22]. Nicaise et al. [23] were successful in confirming and localizing areas of raised AQP4 expressions in rat ALS models. The authors demonstrated that AQP4 mRNA and proteins were abundant in the spinal cord gray matter of rats genetically modified to overexpress mutated human SOD1. There were no changes in AQP4 expression in the white matter of these rats. Intriguingly, it has also been reported that AQP4 expression is increased in the brainstem [24] and cortex [24] in the terminal stages of ALS in SOD1 transgenic mice models.

Studies on atrophied human muscles afflicted with ALS demonstrated suppressed AQP4 mRNA and protein levels, suggesting the AQP4 expression is influenced by neuronal stimulation [25]. The authors demonstrated that while all neurogenic muscular atrophy displayed decreases in AQP4 mRNA, this decrease was much greater in cases of ALS. Based on this finding, it can be suggested that muscular denervation itself impacts AQP4 expression regardless of the site of neuronal injury, i.e., anterior horn cell or peripheral nerve. Both the transcriptional regulation by AQP4 or other aquaporin family proteins as well as the effects of innervation on AQP4 regulation deserves further clarification. Figure 2 summarizes the main changes in the pathophysiology of ALS those regulated by AQP4, as described in the following text.

AQP4 depolarization potentially facilitates motor neuron degeneration in ALS via GLT-1

Previous studies on α-syntrophin knockout mice models have suggested that AQP4's ability to maintain water homeostasis is primarily an effect of AQP4 polarization [26]. Anchoring of AQP4 onto the perivascular end-feet membranes is dependent on the protein α-syntrophin. The absence of this crucial protein in α-syntrophin knockout mice shifted AQP4 localization away from end-feet regions, leading to impaired ion and water balance [27]. Haj-Yasein et al. [28] demonstrated using AQP4 knockout mice that the extracellular cerebral volume is tightly controlled by perivascular AQP4. Losing perivascular AQP4 may therefore result in several cerebral disorders. Dai et al. [26] observed that AQP4 was localized perivascularly in astrocytic end-feet prior to the onset of spinal cord disease [29–31]. The development of ALS is also known to be associated with increased expression of depolarized AQP4 [26], suggesting that this form of AQP4 may be a key pathological characteristic in ALS onset and progression. It is possible that continuous AQP4 depolarization contributes to dysfunctional water homeostasis in the spinal cord, leading to neuronal swelling and impairment of function in the spinal cord in ALS [26].

Dysfunctional glutamate homeostasis leading to excitotoxicity has also been pinpointed as a potential contributor of motor neuron degeneration [32]. Glutamate transporter 1 (GLT-1) is a significant glutamate transporter expressed on astrocytes that is responsible for a large proportion of functional glutamate uptake in the CNS [33]. Absent, decreased, and impaired GLT-1 function leading to dysregulated uptake of glutamate have been noted in astrocytes of the spinal cord and motor cortex in patients with ALS [34, 35] and $SOD1^{G93A}$ rodents [36, 37]. AQP4 has been documented to play a part in the regulation of GLT-1 function and expression [38]. AQP4 deficiency has been shown to downregulate GLT-1 expression and subsequently reduce glutamate uptake in cortical astrocytes [39]. AQP4 and GLT-1 may function as a complex in astrocyte cell membranes that maintains neuronal integrity [39–41]. However, further research is necessary in exploring the mechanisms of AQP4 suppression and its impact on GLT-1 expression as well as if the co-localization of these molecules is important in ALS. Further studies are required to elucidate the precise mechanism between GLT-1 and AQP4 interactions in ALS.

Changes in AQP4 function result in dysfunctional K^+ homeostasis

Changes in routine laboratory tests were currently found to be directly associated with the odds of death or tracheostomy in ALS patients [42]. Motor axonal hyperexcitability is an important component in ALS pathophysiology and is mediated by widespread axonal ion channel dysfunction that encompasses slow and fast potassium (K^+) channel conductance suppression as well as stimulation of sodium (Na^+) conductance [43, 44]. These dysfunctions lead to motor axonal excitability that is thought to contribute towards motor neuron

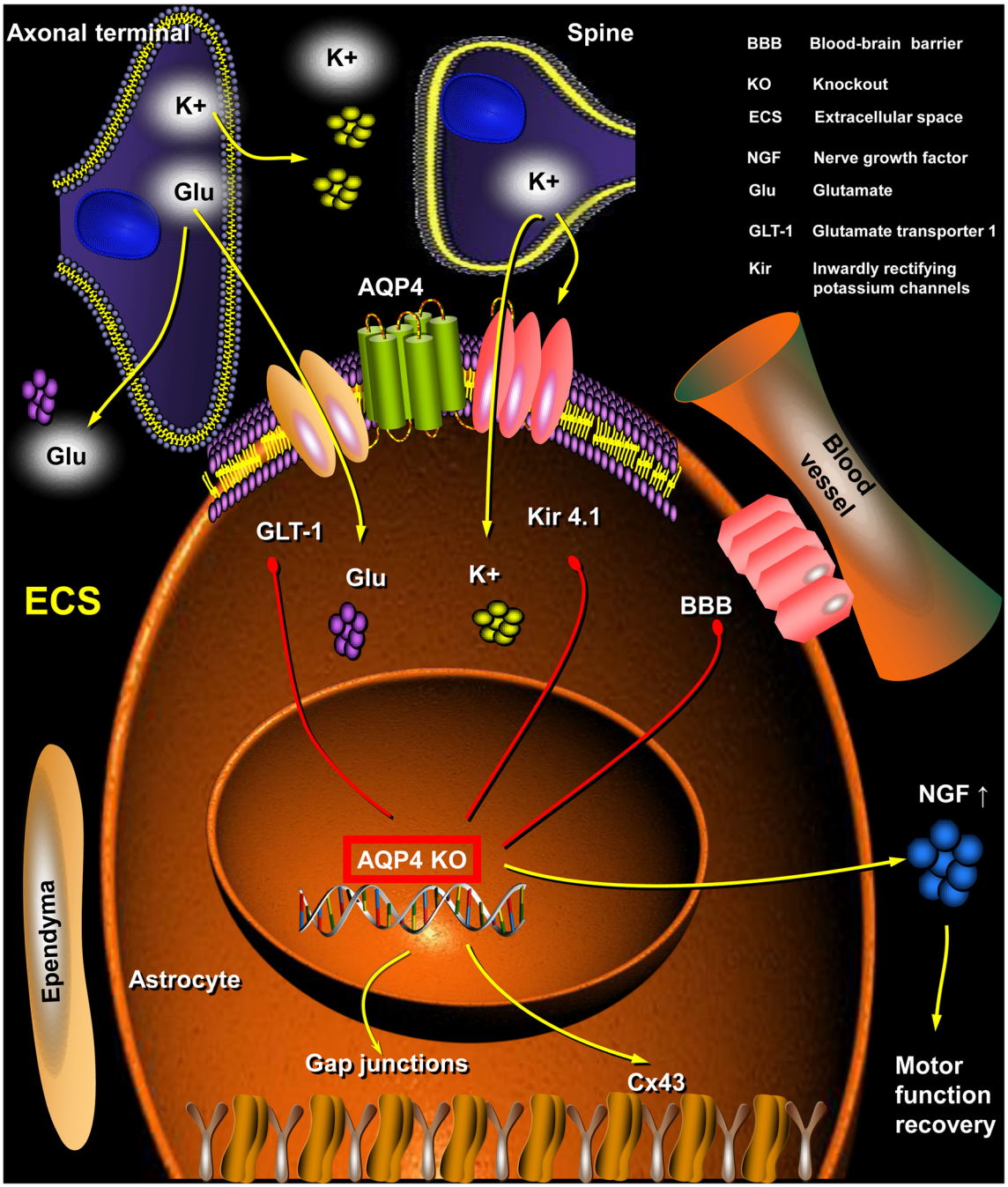

Fig. 1 The potential roles of AQP4 in amyotrophic lateral sclerosis (ALS). AQP4 depolarization may contribute to motor neuron degeneration in ALS via glutamate transporter 1 (GLT-1). Changes in AQP4 function may also disrupt K^+ homeostasis through inwardly rectifying potassium channels (Kir) channels. AQP4 has been found to colocalize with Kir4.1 and GLT-1. Besides, alterations in AQP4 expression in ALS may result in loss of blood–brain barrier (BBB) integrity, which is an early hallmark of ALS. AQP4 dysfunction may cause dysregulations of Gap junctions which are associated with disease progression of ALS. AQP4 suppression enhances motor function recovery in ALS in an NGF-dependent manner

degeneration and clinical characteristics of ALS such as fasciculations [42, 45].

Astrocytes monitor cerebral ion balance by clearing surplus K^+ present in the aftermath of neuronal activity, a process that is carried out by inwardly rectifying potassium channels (Kir) located specifically in astrocyte end-feet membranes [46]. The study of Bataveljić et al. [47] was the first to examine Kir4.1 and AQP4 in cultured cortical astrocytes harvested from the cortices and brainstems of $SOD1^{G93A}$ ALS mice models, demonstrating decreased Kir4.1 and increased AQP4 expressions in these regions. AQP4 and Kir4.1 have been colocalized [48], hinting that these channels may work in

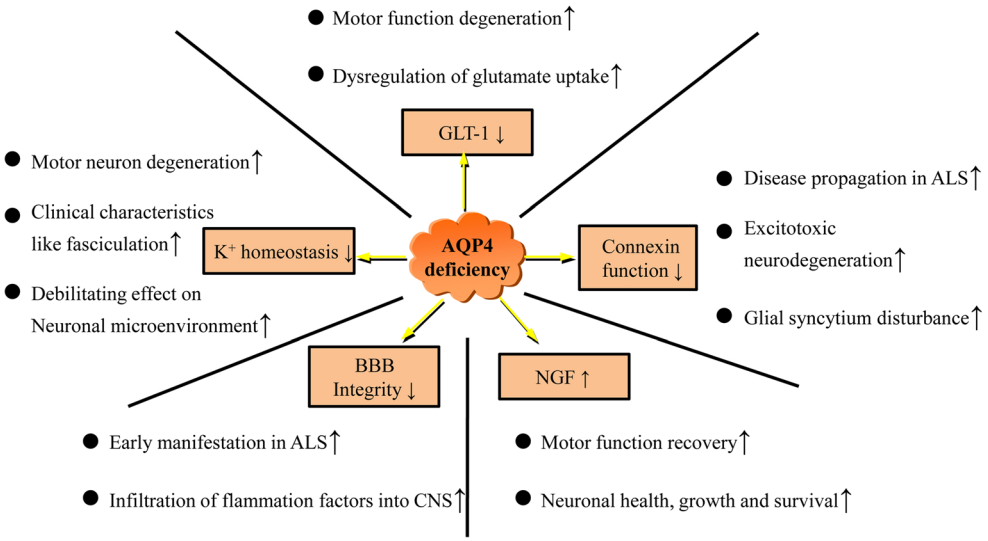

Fig. 2 Summarization of AQP4-mediated pathophysiological changes in ALS

synergy to preserve cerebral osmoregulation [49]. Bataveljić et al. [47] also documented a reduction in Kir4.1 currents and protein levels in cultured cortical astrocytes. Similar findings on spinal cord specimens [22, 23] suggest that both AQP4 overexpression and Kir4.1 downregulation are present throughout the CNS. Altered expressions of these proteins were also demonstrated in in vitro experiments done on cultured ALS astrocytes, further confirming the nature of Kir electrophysiology. In conclusion, alterations in ion channel expression seen in ALS exert a debilitating effect on neuronal microenvironment, imparting deleterious effects on neuronal function and survival.

Alterations in AQP4 expression in ALS may result in loss of BBB integrity

Studies on ALS pathophysiology strongly suggest the presence of BBB disruption. Zlokovic et al. [50] hypothesize that BBB damage is a pivotal event that causes peripheral inflammatory cells and cytokine infiltration into the CNS, triggering and propagating motor neuron damage in ALS. BBB damage is noted to be present early in the disease in ALS animal models [51, 52]. Zhong et al. [52] suggest that BBB disruption could stem from altered tight junction protein expression and a more permeable endothelial layer. Similar conclusions were drawn by Garbuzova-Davis et al., [53] who demonstrated swollen and degenerated endothelial cells, basement membranes, and astrocyte podocytes using blood–spinal cord barrier ultrastructure analysis carried out on SOD1 mice models.

AQP4 seems to influence brain neuroinflammation because of its important role in maintaining BBB integrity and permeability. It is important that the expression of the AQP4 gene regulated by genetic polymorphisms may influence blood flow and fluid balance, and consequently the extent of neuroinflammation. Besides, it is known that S100B is a primary product of astrocytes and exhibits cytokine-like activities. Increased production of S100B and its release from activated glial cells may act as a cytokine and interfere with neurodegeneration [54]. Interestingly, the risk variants in the AQP4 gene, including the T allele of rs1058424, A allele of rs335929, A allele of rs3763043, and TAA haplotype, have been found to be associated with elevated S100B level [55]. The increased interest in AQP4 was derived from its potential use as a therapeutic target in patients with ALS, because inhibitors of the TAA haplotype of AQP4 are expected to protect the brain from persistent neuroinflammation and BBB damage. The use of specific haplotypes as potential biomarkers might be a benefit to clarify different subgroups of patients and identify the potential aquaporin modulators in the management of ALS.

The CNS is shielded from the circulation by the BBB, which restricts the entry of serum proteins, including autoantibodies, into CSF or brain tissue. However, the BBB is not an absolute barrier, but allows the establishment of equilibrium of immunoglobulins between serum and CSF in the range of 1:500–1:1000 [56], and surprisingly, the AQP4-specific antibodies (AQP4-abs) from the circulation were found to be able to enter the CNS via meningeal or parenchymal vessels [57]. The antibodies against AQP4, even in very small amounts, can disturb the astrocyte/endothelial cell interaction and induce further BBB damage [58, 59]. High-affinity AQP4-abs can enter the CNS on their own, bind to astrocytes, and cause loss of AQP4 reactivity, and also employ different mechanisms for lesion formation, which might be related to the progression of ALS [57].

However, there are several controversies that have arisen surrounding BBB dysfunction and AQP4. Experiments on AQP4 knockout mice by Zhou et al. [60] demonstrated damaged cerebral microvascular structures which included swollen perivascular astrocytic podocytes and open tight junctions.

The authors suggested that AQP4 is a crucial component in preserving integrity of the BBB and that any reductions or absence of AQP4 may severely impact astrocytic and endothelial function in the BBB. However, it should be noted that these AQP4 knockout mice failed to demonstrate gross anatomic abnormalities. Other studies using the Nanjing AQP4 knockout mice showed severe BBB dysfunction [60], showing that the damaged BBB became freely permeable to large molecules such as horseradish peroxidase (HRP). Conversely, a study by Saadoun et al. [61] using these same Nanjing AQP4 knockout mice uncovered no differences in BBB permeability between wild-type (WT) and AQP4 knockout mice, indicating that perhaps technical artifacts may have influenced the data of Zhou et al. Saadoun et al. also suggest that that the only established difference in cerebral characteristics in AQP4 knockout mice is an expanded extracellular space compared with WT mice. One caveat that comes with this is that studies by Zhou et al. utilized Nanjing AQP4 knockout mice while Saadoun et al. utilized San Francisco AQP4 knockout mice. Nevertheless, Eilert-Olsen et al. [62] also found that AQP4 deletion caused no alterations and did not alter the ultrastructure of capillary endothelial cell ultrastructure, vascular permeability to HRP and Evans blue albumin dyes, or expressions of tight junction proteins. The authors concluded that the expressions of proteins involved in maintaining perivascular glial scaffolding were reduced in the presence of AQP4 deletion without affecting the endothelial barrier. These results were obtained using C57BL/6J mice, which concur with findings in experiments utilizing CD1 mice by Saadoun et al. The findings of Zhou et al., who also used CD1 mice, were not replicated. Feng et al. [63] also produced results contradictory to those of Zhou et al. Thus, taken together, evidence suggesting that AQP4 is crucial in preserving the BBB remains scarce, indicating the need for further research. Table 1 summarizes comparisons of BBB-related characteristics of mice models from two different origins.

AQP4 dysfunction causing connexin dysregulation in the disease progression of ALS

Cells communicate with one another via gap junctions (GJs), which are direct channels between two cells that grant passage to secondary messengers such as calcium ions and various small molecules. Connexins form heterotypic or homotypic GJs between adjacent astrocytes or between astrocytes and oligodendrocytes [64]. Oligodendrocytes express Cx47 and Cx32, while astrocytes mainly express connexin (Cx30) and Cx48 [65]. There has been a paucity of literature surrounding the role of connexins in motor neuron disease. Díaz-Amarilla et al. [66] characterized enhanced Cx43 immunoreactivity in aberrant astrocyte phenotypes extracted from SOD1 transgenic mice. These same aberrant astrocytes were found to induce motor neuron death when co-cultured with healthy neurons, leading to the hypothesis that glial activation and subsequent excitotoxic neurodegeneration was triggered by increased Cx43 expression present on these astrocytes [66]. Observations of the anterior horns of the spinal cords of mSOD1-Tg mice by Cui et al. [67] found suppressed levels of Cx32 and Cx47, indicating that an altered Cx expression may lead glial syncytium disturbances and therefore enhancing disease propagation in ALS. GJ proteins on astrocytes and oligodendrocytes of the anterior horns of the spinal cord in mSOD1-Tg mice were found to be the most markedly disrupted in the terminal stages of ALS.

Table 1 Published BBB-related characteristics in AQP4-/- mice generated by two different origins

Characteristic	Assessment	Tissue	Effect of AQP4 deletion	Mice origins	References
Cerebral vessels	Gross anatomy	Brain	No effect	San Francisco AQP4-/- mice	Mainly et al. 2000
Cerebral vessels	Electron microscopy	Cerebral cortex	No effect	San Francisco AQP4-/- mice	Mainly et al. 2000; Papadopoulos and Verkman 2005
Cerebral vessels	Evans blue extravasation	Whole brain	No effect	San Francisco AQP4-/- mice	Papadopoulos et al. 2004; Bloch et al. 2005; Feng et al. 2009
Microvessels	Electron microscopy and the horseradish peroxidase extravasation technique	Brain	No effect	San Francisco AQP4-/- mice	Saadoun et al. 2009
Microvessels	Electron microscopy and the horseradish peroxidase	Brain	The integrity and permeability of the BBB were altered	Nanjing AQP4-/- mice	Zhou et al. 2009
Ependyma	Electron microscopy	Brain	The functional properties of BBB and blood-ependyma interfaces were altered	Nanjing AQP4-/- mice	Li et al. 2009
Cerebral vessels	Electron microscopy and Evans blue extravasation	Brain	More severe BBB disruption occurred after ICH	Nanjing AQP4-/- mice	Chu et al. 2012

AQP4 and connexins have been colocalized [68]. Cx30 and Cx42 are the primary hippocampal GJ proteins and, like AQP4, are also concentrated at the perivascular podocyte membranes [69]. In addition to being in close cellular proximity as AQP4, connexins have similar functions to those of AQP4. Katoozi et al. [70] demonstrated raised hippocampal astrocytic gap junctions in the presence of targeted AQP4 deletion. The number of perivascular Cx43-positive GJs doubled upon AQP4 deletion. These findings concur with those of Strohschein et al. [71], suggesting that junctional connexins—and of Cx43 in particular—are upregulated in the absence of AQP4. Furthermore, it was hypothesized Cx43 may be a downstream effector of AQP4 in the mechanism of cerebral edema [72]. Additional exploration of this relationship may grant better insight towards the formulation of ALS therapies targeting the AQP4-Cx43 interactions.

AQP4 suppression augments motor function recovery in ALS in an NGF-dependent manner

Alterations in AQP4 have been correlated to the development of spinal cord edema in animal models of spinal cord injury [73–75]. Preservation of neuronal motor integrity and function has been found to be dependent on AQP4 levels [73–77]. In fact, AQP4 may be intricately involved in motor function recovery, as evidenced by poor locomotor skills mice lacking AQP4 [78]. The improved neurological outcomes in AQP-null mice suggested that a lack of AQP4 improved motor function recovery [77]. It appears that AQP4 manipulation confers differing motor outcomes. The dual roles of AQP4 in vasogenic versus cytogenic edema in CNS have been characterized in several experiments [79]. AQP4 may function differently in different settings of cerebral injury. AQP4 has also been documented to mediate astrocyte migration, which may be another potential mechanism underlying the ability of AQP4 to affect motor function.

Nerve growth factor (NGF) is a component that has demonstrated neuroprotective properties in in vitro and in vivo as well as in humans with neurodegenerative diseases [80]. NGF is secreted in both the peripheral and central nervous systems and is crucial in maintaining neuronal health, growth, and survival. It has been postulated that neurodegenerative disorders stem from a deficiency of such growth factors [81]. Dorsal spinal cord specimens from those with ALS have been found to demonstrate reduced NGF levels [82]. Intriguingly, Chen et al. [83] demonstrated that AQP4 inhibition upregulated NGF and augmented recovery of motor neurons using lentivirus-mediated RNAi suppression of AQP4. This study lays the foundation to future studies exploring the relationship between NGF and AQP4—a promising field that may uncover new targets in the developing novel therapeutic strategies.

The potential and challenges for AQP4 to be an effective drug target in ALS

AQP4 might be a potential target for drug development, since the data from AQP4 knockout mice have implicated AQP4 involvement in a wide range of CNS functions including water accumulation and clearance, neuroinflammation, neuroexcitatory processes, ALS, and various other neurological disorders. However, the most important question is whether effective AQP4-targeted therapeutics could be developed. There are many challenges in the development of small-molecule therapeutics. AQP4 is a member of a family of at least a dozen homologous proteins expressed in humans. Thus, an AQP4-selective therapeutic would be needed, which poses a challenge because of conserved amino acid sequences in the pore region of the various AQP isoforms. In addition to usual pharmacological considerations, an AQP4 therapeutic for ALS or various other neurological diseases would require BBB penetration for effective treatment. Besides, previous efforts have not produced verified small-molecule inhibitors of AQP4, raising questions about its druggability. Perhaps, this may be related to the narrow pore structure of AQP4 that excludes molecules other than water. With regard to AQP4-targeted antibody therapeutics such as aquaporumab [84], the challenge will be in delivery into the CNS at sustained therapeutic concentrations. Although there exist many challenges, advances in screening and computational methods may yield bona fide small-molecule AQP4 inhibitors for testing in experimental animal models and for advancement to the clinic.

Conclusions

This review highlights the potential roles of AQP4 in ALS. Various studies have indicated that impaired function of AQP4 in ALS through AQP4 depolarization or inhibition may be a pivotal contributor of motor neuron degeneration in ALS. Increased AQP4 expression has been reported in ALS. AQP4 depolarization may trigger and propagate neuronal dysfunction in ALS via GLT-1. Additionally, alterations in AQP4 expression in ALS may result in loss of BBB integrity, disrupt K^+ homeostasis, and cause connexin dysregulation—all of which are key components in ALS pathophysiology. Furthermore, AQP4 inhibition augments the recovery of motor function in ALS in a NGF-dependent manner.

Several lines of evidence support a significant role of AQP4 in ALS neurobiology. Defects in the BBB induced by AQP4 deficiency may be a triggering factor for this neurodegenerative disease. Future studies that further clarify the role of AQP4 in ALS as well as highlighting the pattern of AQP4 expression throughout the clinical course of ALS are important for developing potential ALS biomarkers and more definitive treatment modalities against ALS.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical standards This article does not contain any study with human subjects performed by any of the authors.

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