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# The role of aquaporins in the Alzheimer´s disease

# Úloha akvaporinů v Alzheimerově chorobě

Bachelor´s thesis

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## Poděkování

Chtěla bych moc poděkovat své úžasné školitelce Mgr. Janě Turečkové, Ph.D. a jejímu, rovněž skvělému, kolegovi Mgr. Ondřeji Vaňátkovi za jejich cennou pomoc, čas a ochotu, kterou mi při zpracovávání této práce věnovali.

## Prohlášení

Prohlašuji, že jsem závěrečnou práci zpracovala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

V Praze, 4. 5. 2022

Podpis:

## Abstract

Alzheimer's disease (AD) is a progressive neurodegenerative disorder with complex pathophysiology affecting the central nervous system (CNS). In progress of the disease, various pathological changes occur in the brain, leading to neurodegeneration and subsequent impairment of physiological and cognitive functions. Although it is the most common cause of dementia in elderly, currently, there is no effective treatment for AD that that targets its underlying mechanisms. There are different theories as to which process is the key trigger for the development of AD. The generally accepted theory considers increased production of amyloid β (Aβ), its accumulation in the ECS and the formation of amyloid plaques as the main cause of the disease. However, recent studies show that the primary cause of amyloid plaque formation is not increased Aβ production, but rather its impaired clearance through the glymphatic system, the main component of which are aquaporin water channels, specifically aquaporin-4 (AQP4). The goal of this thesis is to provide an overview of the available knowledge on the involvement of aquaporins in AD pathophysiology, with a particular focus on AQP4 and its role in the glymphatic system.

**Key words:** Alzheimer's disease, neurodegeneration, central nervous system, astrocytes, aquaporins, glymphatic system

## Abstrakt

Alzheimerova choroba (AD) je progresivní neurodegenerativní onemocnění s komplexní patofyziologií postihující centrální nervový systém (CNS). V průběhu onemocnění dochází k různým patologickým změnám v mozku, které vedou k neurodegeneraci a následnému narušení fyziologických a kognitivních funkcí. Přestože se jedná o nejčastější příčinu demence u starších osob, v současné době neexistuje účinná léčba AD, která by se cíleně zaměřovala na její základní mechanismy. Existují různé teorie o tom, který proces je klíčovým spouštěčem rozvoje AD. Obecně přijímaná teorie považuje za hlavní příčinu onemocnění zvýšenou produkci amyloidu β (Aβ), jeho akumulaci v ECS a tvorbu amyloidních plaků. Nejnovější studie však ukazují, že primární příčinou tvorby amyloidních plaků není zvýšená produkce Aβ, ale jeho zhoršená klearance prostřednictvím glymfatického systému, jehož hlavní součástí jsou akvaporinové vodní kanály, konkrétně akvaporin-4 (AQP4). Cílem této práce je poskytnout přehled dostupných poznatků o zapojení akvaporinů do patofyziologie AD, se zvláštním zaměřením na AQP4 a jeho roli v glymfatickém systému.

**Klíčová slova:** Alzheimerova choroba, neurodegenerace, centrální nervový systém, astrocyty, akvaporiny, glymfatický systém

## Shortcut list

| | |
|---|---|
| **Aβ** | amyloid-β-peptide |
| **AD** | Alzheimer's disease |
| **APP** | amyloid precursor protein |
| **APOE** | apolipoprotein E protein |
| **APOE-e4** | apolipoprotein E gene allele 4 |
| **AQP1, 4, 9** | aquaporin-1, 4, 9 |
| **BBB** | blood-brain barrier |
| **CSF** | cerebrospinal fluid |
| **ECS** | extracellular space |
| **EOAD** | early-onset Alzheimer's disease |
| **FAD** | familial Alzheimer's disease |
| **GFAP** | glial fibrillary acidic protein |
| **GLT-1** | glutamate transporter 1 |
| **ISF** | interstitial fluid |
| **KO** | knock-out |
| **LTD** | long-term depression |
| **LTP** | long-term potentiation |
| **NFTs** | neurofibrillary tangles |
| **NMDA** | N-methyl-D-aspartate |
| **SAD** | sporadic Alzheimer's disease |
| **PSEN1, 2** | presenilin 1,2 |
| **WT** | wild-type |

# Table of contents

# 1 Introduction

Alzheimer's disease (AD) is a severe neurodegenerative disorder that the World Health Organization considers a major public health concern. It is a progressive disease characterized by slight changes in the brain leading to damage and death of neurons. More neurons are destroyed over time, and more parts of the brain are damaged. Memory, language, and cognitive impairments are often the earliest signs of AD. As the disease progresses, neurodegeneration extends to parts of the brain that are responsible for basic physiological processes like walking and swallowing. Impacted people become incapacitated and bed-bound requiring 24-hour care. Essential bodily functions of the affected person slowly deteriorate, leading to death of the patient. As the population ages and the human lifespan increases, the prevalence of AD is rapidly rising with an economic and social burden.

Despite the overall effort, there is currently no effective cure. Avaliable medications only temporarily reduce AD symptoms but do not affect the underlying causes of the disease. In the last years, the focus has been on aquaporins, mainly on aquaporin-4 (AQP4) for being an integral part of the glymphatic system, which promotes clearance of interstitial solutes, including amyloid-β (Aβ), which is thought to be one of the leading causes, and thus removal of the brain's waste products. Moreover, AQP4 is involved in other pathological changes in AD. Therefore, AQP4 presents a new aspect and approach linked to the pathophysiology of AD. This work aims to summarize the available knowledge about aquaporins and their role in Alzheimer's disease.

# 2 Alzheimer's disease

Alzheimer's disease is a neurodegenerative disorder with complex pathobiology. It is characterized by intracellular and extracellular accumulation of aggregates from hyperphosphorylated tau protein and Aβ. AD is usually accompanied by amnestic mild cognitive impairment, which is characterized by memory loss and often occurs as a prodromal stage of Alzheimer’s dementia (Knopman et al. 2021). Clinical manifestations include decreased cognitive functions, such as worsening to loss of articulation, spatial perception, motor skills, and memory. In addition, AD may cause depression, insomnia, or hallucinations (McKhann et al. 1984). Various methods are used for diagnosis of AD, from psychological tests

and molecular genetic techniques to a neuroradiological examination done by positron emission tomography, computed tomography, single photon emission computed tomography, or magnetic resonance imaging. Imaging methods can display brain atrophy, a valid marker of AD. The AD diagnosis also includes neuropathologic postmortem examination (Frisoni et al. 2010; Khachaturian 1985).

## 2.1 Epidemiology

Alzheimer's disease is the most common cause of dementia in the elderly (Schneider et al. 2009), as it accounts for 60-80 % of cases. There were 46,8 million people in 2015 who have dementia, and it was anticipated growth to 131,5 million people in 2050 worldwide. The vast majority of people who have dementia live in low- or middle- income countries. By 2050, the proportion is predicted to rise to 71 or 72% (Prince et al. 2015; Patterson et al. 2018). The percentage of people with AD is increasing with age. The prevalence is up to 3 % for people aged 65-74 years, 17 % in the 75-84 age group, and 32 % in the group of people aged 85 years and more (Hebert et al. 2013). Incidence in the age group 65-69 years is 2,8 per 1000 person-years, and 56,1 per 1000 person-years in the 90± years group (Kukull et al. 2002). There is no significant difference in the prevalence, incidence, or increased risk of death between men and women with AD (Hebert et al. 2001).

## 2.2 Genetics and risk factors

Two types of AD have been described, late-onset AD (LOAD) and early-onset AD (EOAD). LOAD is responsible for most cases and is defined with an age of onset later than 65 years. EOAD represents 1-6 % of cases, and the age of onset is between 30 to 65 years (Bekris et al. 2010). Genetically based on inheritance, AD occurs in two forms, familial AD (FAD) and sporadic AD (SAD). These forms share the same clinical phenotype, but some studies found there is an earlier age of onset for FAD (Duara et al. 1993). FAD is a rare form accounting for a minority of AD cases, while SAD is more common and is caused by various factors, including genetic predisposition and environmental risk factors. Approximately 61,5 % of EOAD is FAD. EOAD has another form, autosomal dominant form (Campion et al. 1999).

FAD is linked with autosomal dominant mutations in the gene for amyloid precursor protein (APP), presenilin 1 (PSEN1), and presenilin 2 (PSEN2) (Goate et al. 1991; Sherrington

et al. 1995; Levy-Lahad et al. 1995). On the other hand, the sporadic SAD is usually associated with the apolipoprotein E gene (APOE)., specifically with allele 4 (APOE-e4), which substantially impacts the risk, increasing with the number of APOE-e4 alleles (Saunders et al. 1993, Corder et al. 1993).

Many epidemiologic studies agree that age is a significant risk factor for developing AD. Furthermore, head injury, smoking, and exposure to aluminum can increase the risk of AD. Other risk factors associated with AD include immune system dysfunction, infectious diseases, cardiovascular diseases, and cardiovascular risk factors such as high cholesterol, hypertension, or diabetes (Henderson 1988; Breteler 2000; Armstrong 2019).

## 2.3 Pathophysiology

Pathologically from a macroscopic view, the AD brain is associated with atrophy of the hippocampus, neocortex, locus coeruleus, and nucleus basalis (Khachaturian, 1985). Neurofibrillary tangles (**NFTs**), and **Aβ plaques** represent microscopical marks of AD, and they are linked to the events that eventually lead to neuronal death and functional deficiencies (Stelzmann et al. 1995).

**NFTs** contain paired helical filaments composed of phosphorylated tau protein, and their presence correlates with neuronal loss and cognitive impairment. With the duration and severeness of AD, there is an increase in both the amount of NFTs and the loss of neurons. Quantification of NFTs and neurons revealed loss of neurons outnumbered NFTs implying that the loss of neurons directly contributes to cognitive impairment in AD (Lee et al. 1991; Gómez-Isla et al. 1997). Tau is a multifunctional protein, and its dysfunction contributes to neurofibrillary degeneration in neurons, synaptic withdrawal, and neuronal death. Tau protein is mainly expressed in axons in the central nervous system. There are 6 isoforms of tau with 3 or 4 repeat domains that allow tau to interact with microtubules. In a healthy tissue, tau can function as a postsynaptic scaffold protein and affect growth factor signaling. It has a microtubule stabilization function, affects microtubule dynamics, cell signal transduction, and axonal transport (Mietelska-Porowska et al. 2014). However, increased expression leads to its aggregation, phosphorylation and formation of NFTs. The overexpression of tau and subsequent neurodegeneration may result from activation of extrasynaptic N-methyl-D-aspartate (NMDA) receptors (Sun et al. 2016).

APP is cleaved in two pathways, α- and β-secretase pathways; Aβ is a product of APP cleavage in the β-secretase pathway; by β- and γ-secretase (Fig. 1). Amyloid isoforms are mostly 40 or 42 residues long. $A\beta_{40}$ is generally produced by cells in a healthy organism; it is a soluble dominant form that prevents formation of the **Aβ plaques**, whereas $A\beta_{42}$ promotes the plaques and is predominant in AD tissue. APP, PSEN1, and PSEN2 mutations increase the amount of extracellular deposition of Aβ (Shoji et al. 1992; Haass et al. 1992; Younkin 1998; Kim et al. 2007). Aβ levels are regulated by synaptic activity, the circadian cycle, and glymphatic system. Clearance of Aβ is more effective during sleep due to higher activity of the glymphatic system (Cirrito et al. 2005; Lucey et al. 2017; Iliff et al. 2012).

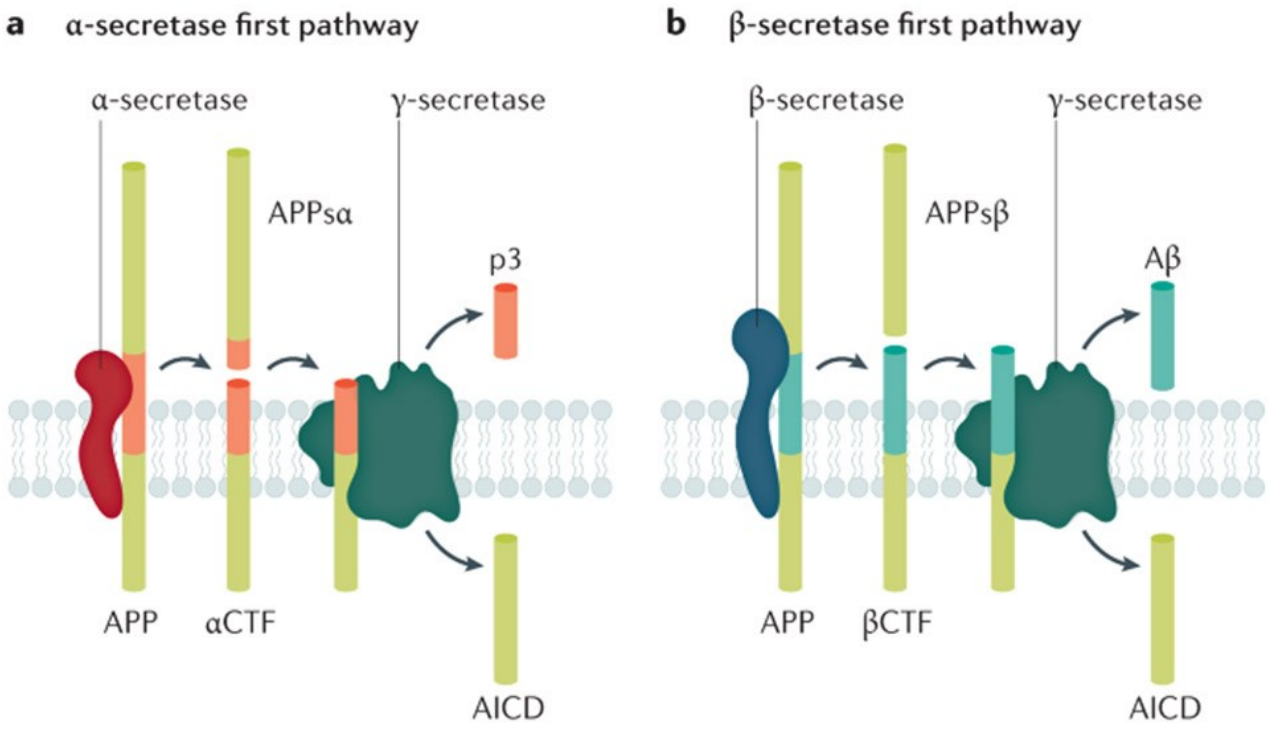


Fig. 1. Schematic representation of amyloid precursor protein (APP) cleavage pathways. Amyloid-β (Aβ) peptides are derived from a transmembrane protein APP, that is localized in neuronal synapses. APP can be cleaved by two pathways, α-secretase pathway or β-secretase pathway, also known as non-amyloidogenic and amyloidogenic pathways, respectively. After its production, Aβ is secreted to the extracellular space as a monomer, where it can aggregate. Its production and release is controlled by synaptic activity. a) In α-secretase pathway APP is first cleaved by α-secretase and generates APPsα and αCTF, preventing Aβ formation. APPsα may modulate synaptic transmission through a GABA receptor. Then, γ-secretase cleaves αCTF to produce an extracellular peptide p3 and an intracellular fragment AICD, with physiological functions. b) In β-secretase pathway APP is first cleaved by β-

secretase and APPsβ and βCTF are produced. βCTF is involved in early endosomal abnormalities in Alzheimer's disease. Subsequently, βCTF is cleaved by γ-secretase which results in formation of Aβ and AICD. Production of Aβ is in equal amounts to AICD and APPsβ and in inverse amounts to p3 and APPsα. AICD- amyloid precursor protein intracellular domain, p3- amyloidβ peptide (Knopman et al. 2021).

Recent findings imply that rather than Aβ plaques, small soluble oligomeric structures interact with receptors in the cell membrane and their structural flexibility and hydrophobic exposure are main factors of their capability to induce cellular dysfunction and neurodegeneration (Benilova et al. 2012). Aβ, in its oligomeric form, binds to several postsynaptic and presynaptic receptors such as α7 nicotinic acetylcholine receptors or N-methyl-D-aspartate (NMDA) receptors (Wang et al. 2000; Dineley et al. 2002; Snyder et al. 2005). Consequently, Aβ oligomers affect cell signaling and may cause celullar dysfunction (Campioni et al. 2010).

Aβ can also bind to neurotrophin p75 receptor, metabotropic glutamate receptors, ephrin type-A4 receptor, and type-B2 receptor; thus, Aβ being able to bind to these several receptors leads to disruption of synaptic plasticity, dendritic spine reduction, and long-term potentiation (LTP). It also results in synaptic depression and memory impairment (Cissé et al. 2011; Li et al. 2009; Renner et al. 2010; Vargas et al. 2014; Yaar et al. 1997).

Aβ and tau mutually support and complement each other toxicity, for instance, by disrupting different components of the oxidative phosphorylation system, leading to disruption of mitochondrial function in primary neurons. Mitochondrial dysfunction has been proposed as a crucial factor in AD development. Lots of mitochondrial functions and dynamics are affected in AD. $Ca^{2+}/Na^{+}$ exchanger in the mitochondria matrix is disrupted, leading to an increase in $Ca^{2+}$ concentration within mitochondria. Other studies have reported disruption of the electron transport chain. It has been shown that fractions of Aβ can increase ROS generation; overproduction of ROS results in increased hyperphosphorylation of tau and β-secretase (Bell et al. 2021; Quintanilla et al. 2012; Rhein et al. 2009).

Aβ metabolism is also affected by astrocytes as they produce Aβ degrading proteases, namely neprilysin, angiotensin-converting enzyme-1, and endothelin-converting enzyme-2. Increased expression of these proteases was observed in adult mouse eGFP-positive astrocytes transplanted into a APdE9 mouse model of AD. However, these astrocytes undergo Aβ-induced

apoptosis (Pihlaja et al. 2011). Generally, following brain ischemia, traumatic injury, or neurodegeneration, astrocytes undergo morphology and function changes, and become activated. This process is known as reactive astrocytosis. Astrocytes in AD pathology show typical signs of activation, which include increased expression of glial fibrillary acidic protein (GFAP). A study using human AD samples presented continuously increased levels of GFAP in astrocytes together with genes encoding other intermediate filaments (Kamphuis et al. 2014). Similar results were obtained by immunohistochemical analysis, which showed increased transcript levels of all GFAP isoforms linked with Braak stage of AD. Additionally, an inversive correlation was shown between rising AD severeness and decreasing levels of excitatory amino acid transporter 2 (EAAT2), also called glutamate transporter 1 (GLT-1; Simpson et al. 2010). Also, in mouse models of AD an increase of all GFAP isoforms was observed (Kamphuis et al. 2012). The involvement of reactive astrocytes in Aβ metabolism was confirmed by deleting genes responsible for functional astrocytosis. Deletion of GFAP and Vimentin in APP/PS1 mice led to a twofold increase in Aβ plaques. The deletion did not influence APP processing or Aβ degrading proteases thus, it selectively impaired the Aβ burden. Moreover, differences in astrocyte morphology were observed between knock-out (KO) mice and wild types (WT). KO astrocytes displayed limited process hypertrophy, no interaction with nearby plaques, and absent processes in areas immediately proximal to the plaques. In contrast, astrocytes from WT mice showed typical signs of activation, exhibited hypertrophied processes that encircled and invaded Aβ plaques. These results indicate that reactive astrocytes negatively influence Aβ plaque formation by interacting directly with plaques (Fig. 2, Kraft et al. 2013).

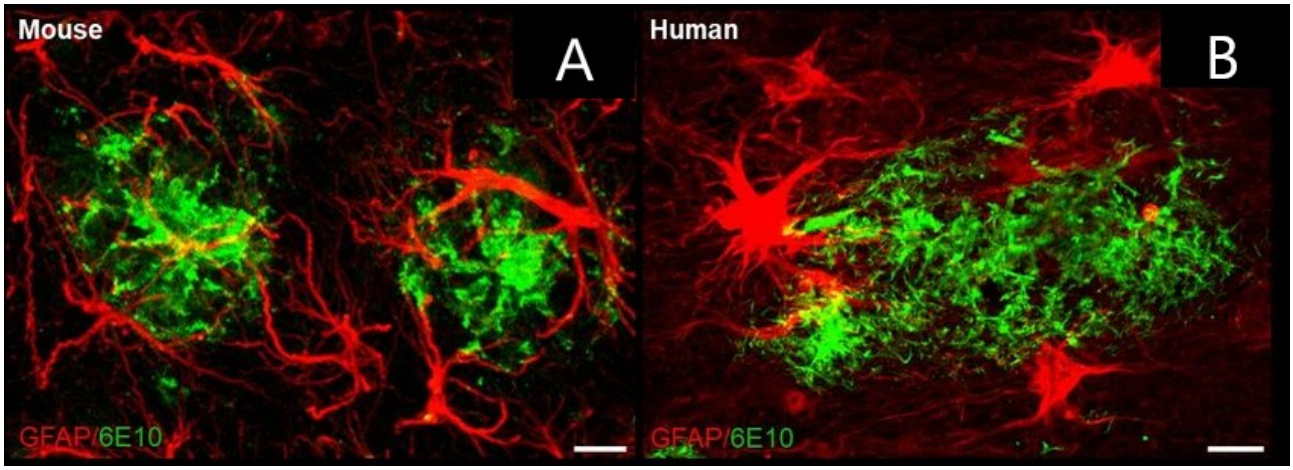


Fig. 2 Image of astrocyte morphologies in healthy and in Alzheimer's disease brains. A) Close interaction of GFAP-positive mouse astrocytes (red) with β-amyloid plaques (6E10, green) in the cortex

of an APP/PS1 mouse. B) GFAP-positive human astrocytes (red) around β-amyloid plaques (6E10, green) in the entorhinal cortex of an Alzheimer´s disease patient brain (Preman et al. 2021).

In addition, phagocytic function of astrocytes was reported as they are able to engulf Aβ after the activation of Aβ-binding receptors (Jones et al. 2013). In the brains of AD patients, plaque and neuron derived Aβ has been shown to accumulate in astrocytes via the phagocytic process of astrocytes. The amount of Aβ internalized by astrocytes positively correlates with the local severity of AD. Some Aβ-overburdened activated astrocytes can go through lysis and generate Aβ plaques. Aβ plaques derived from astrocytes were smaller, had high immunoreactivity to GFAP, and were observed only in areas where surrounding astrocytes had high levels of intracellular $A\beta_{42}$ (Nagele et al. 2003). Adult astrocytes isolated from aged AD mice demonstrated impairment in clearing Aβ as they exhibited 20% reduced Aβ uptake compared to old WT mice. Moreover, old AD astrocytes had less internalized Aβ and, in cultures, stimulated shorter neurites production than old WT cultured astrocytes. Data from this study imply that changes in astrocytes exposed to AD pathology may impair Aβ clearance and speed up neurodegeneration (Iram et al. 2016).

Above that, astrocytes migrate to Aβ plaques as a reaction to released chemokine present in Aβ plaques and subsequently bind to them (Wyss-Coray et al. 2003). Astrocytes produce significantly higher levels of astrocyte-derived enzymes in AD, including β-site APP-cleaving enzyme 1 (BACE-1), soluble $A\beta_{42}$, soluble APP-β -α, glial-derived neurotrophic factor (GDNF), P-T181-tau, and P-S396-tau (Goetzl et al. 2016). Furthermore, under specific inflammatory conditions, astrocytes affect processing of APP, which leads to an increase in Aβ production and accumulation of Aβ plaques (Blasko et al. 2000). Last but not least, a significant involvement of astrocytes in Aβ burden is through the glymphatic system, which mediates clearance of Aβ and requires proper functioning of astrocytic aquaporin 4 (AQP4; Iliff et al. 2012). This topic will be further discussed in a separate chapter.

# 3 Aquaporins

Water transport across the cell membrane is involved in key physiological processes such as maintaining osmotic pressure, generation and absorption of cerebrospinal fluid (CSF), cell volume regulation, and fluid transport across the vascular endothelium and neuropil. Maintaining ion and water homeostasis is also essential for the proper functioning of neurotransmission. On the other hand, membrane water transport also plays a crucial role in the development of cerebral edema, a serious life-threatening complication of some diseases and brain injuries. There are 3 possible mechanisms for water transport across the cell membrane; mere diffusion through the lipid layer, co-transport through ion transporters, and flux through specialized water channels, AQPs (Nielsen et al. 1996; Badaut et al. 2002)

The first report about aquaporins, namely aquaporin-1 (AQP1), and their basic biophysical description originated from discovering a human red blood cell protein that increased water permeability in Xenopus oocytes (Preston et al. 1992). Since then, hundreds of homologous proteins have been identified and structurally studied.

Aquaporins are a family of membrane proteins forming pores and are responsible for water transport. They are localized in the plasma membrane of multiple cells in majority of organs. There are currently 13 AQPs characterized in humans. They are divided into 3 groups: aquaglyceroporins (AQP3, AQP6, AQP7, AQP9, AQP10), aquaporins (AQP1, AQP2, AQP4, AQP5, AQP8), and super aquaporins (AQP11, AQP12A, AQP12B). Aquaglyceroporins can transport glycerol together with water molecules. Some of the AQPs can also participate in transport of ions. AQPs are associated with different physiological roles such as cell proliferation, epidermal hydration, cell migration, or neural activity. Their dysfunction is linked to pathological conditions such as brain swelling, epilepsy, and other diseases. Thus, specific AQPs can serve as biomarkers and serve as potential therapeutic targets (Magouliotis et al. 2020; Verkman 2012).

AQPs are composed of variable-length loops and 6 transmembrane α-helices forming homotetramers in membranes with intracellular amino- and carboxyl- terminals. Tetramer form and packing of helices ensure stability. Tetramer's structure consists of 4 monomers operating as independent pores. A typical Asn-Pro-Ala (NPA) motif is found in pore-forming AQP helices (Fig. 3; Engel et al. 2009; Smith and Agres 1991).

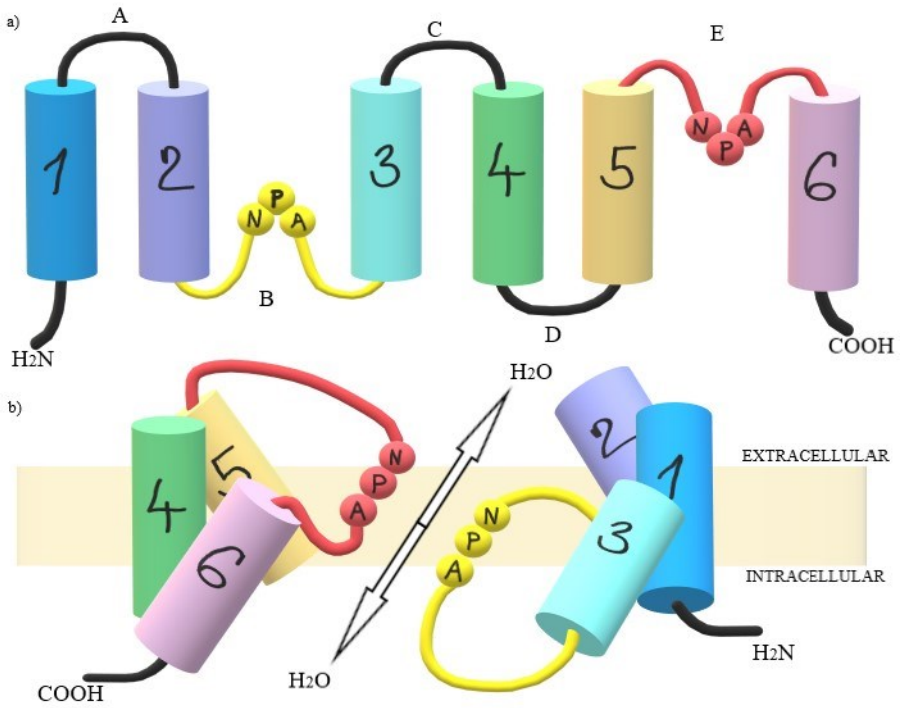


Fig. 3 A schematic representation of AQP1 monomer. a) In the AQP1 monomer, there are 6 membrane helices (1-6) connected by 5 loops (A-E) with amino- and carboxy- terminals. b) In the functional monomer, the hydrophilic loops B and E are bent over into the cavity formed by the helices, forming the water-selective pore that contains the NPA (Asn-Pro-Ala) motifs. The permeation barrier is believed to be formed by the hydrogen bonding capabilities of the polar side groups of the two Asn residues (Zeuthen 2001).

## 3.1 Aquaporins in the central nervous system

Ten aquaporins have been currently described in the brain, AQP1,3-9,11 and 12. The primary attention is on AQP1, AQP4 and AQP9 since they are involved in several types of brain pathophysiology (Shin et al. 2006; Gorelick et al. 2006; Elkjar et al. 2000a; Badaut et al. 2002).

### 3.1.1 AQP1

AQP1 is primarily expressed at the apical membrane in the choroid plexus epithelial cells and is involved in CSF production. In AQP1 KO mice, CSF production was reduced by ~20 % compared to WT mice (Nielsen et al. 1993; Oshio et al. 2005). In addition, AQP1

expression was observed in other brain regions, both in neurons and glial cells where it plays specific roles. AQP1 was also found in cell bodies of significant numbers of small-diameter neurons of both the dorsal root ganglia and the trigeminal ganglia. As is AQP1 expressed in nociceptive neurons, it is likely that it may be involved in pain signaling (Oshio et al. 2006; Shields et al. 2007). Additionally, in non-human primates, AQP1 was found in the processes and perivascular endfeet of astrocytes in the white matter and the glia limitans, as well as in neurons innervating the pial blood vessels (Arciénega et al. 2010). The expression levels also vary in different pathological states and after brain injuries. In astrocytes, AQP1 was also found in the temporal neocortex, especially in the pyramidal cell layers, in people with AD and Parkinson's Disease (Hoshi et al. 2017). Elevated levels of AQP1 were detected in some brain tumors; in astrocytoma cells, AQP1 is present in cell membrane and cytoplasm and in vascular structures of glioblastomas (Saadoun et al. 2002; Endo et al. 1999). After subarachnoid hemorrhage, there is a significant increase in AQP1 expression in astrocytes (Badaut et al. 2003). These changes in expression imply AQP1 involvement in the edema process following injury. Spinal cord injury resulted in AQP1 upregulation in ependymal cells, dorsal horn fibers, neuronal cell bodies, and reactive scar-forming astrocytes (Nesic et al. 2008).

### 3.1.2 AQP1 in Alzheimer’s disease

The expression of AQP1 is increased in reactive astrocytes, implying that AQP1-expressing astrocytes play a role in the brain under pathological conditions. In AD, AQP1 is overexpressed in the cerebral cortex, in the pyramidal cell layers, and in reactive astrocytes with hypertrophy cell bodies and heavily branching processes (Hoshi et al. 2012). Research on an association between Aβ plaques and astrocytic AQP1 expression in AD patients' motor cortex and hippocampus reported that AQP1 was frequently found on top of or close to Aβ plaques. The tissue protein overlay analysis showed that AQP1 interacts with Aβ plaque core. In AD, the amount of AQP1-immunoreactive plaques was increased compared to non-AD. This study demonstrated that AQP1 may interact not only with Aβ but also with a segment of APP (Misawa et al. 2008). A protective function of AQP1 was proposed as the absence of AQP1 increased the BACE1-mediated APP amyloidogenic processing, and the Aβ burden was significantly higher. Presence of AQP1 reduced Aβ production by decreasing APP processing via transfer of AQP1 to the endosomal compartment due to cell stress, where AQP1 suppressed the interaction of APP with BACE1 (Park et al. 2021).

On the other hand, the adverse effects of AQP1 were reported. In a mouse model of AD induced by injection of $A\beta_{1-42}$ solution high AQP1 expression was correlated with. impaired memory and learning function. The hippocampal neuron amount was decreased and unevenly distributed in AD mice. The overexpression of AQP1 also caused a Wnt signaling pathway disruption and neuronal apoptosis, hence impairing cognitive functions. Silencing of AQP1 in AD mice led to an improvement in cognitive functions, protective effects on hippocampal neurons, and activating the Wnt pathway (Yu et al. 2020).

### 3.1.3 AQP9

AQP9 is localized on astrocytes in the white matter, hippocampus, hypothalamus, lateral septum, and in astrocytic processes surrounding the subarachnoid space and ventricles. Increased astrocytic expression of AQP9, mainly in the cortex after ischemia, was reported. It was proposed that AQP9 may be involved in clearance of lactate and glycerol from the extracellular space (ECS; Badaut et al. 2001). AQP9 is also present in cells lining the cerebral ventricles, including ependymal cells and mediobasal hypothalamic tanycytes (Elkjar et al. 2000). AQP9 was also found in the endothelial cells of pial vessels as well as in catecholaminergic neurons; in the adrenergic, noradrenergic and dopaminergic cells. Minor expression was seen in non-catecholaminergic neurons in the paraventricular nucleus of the hypothalamus. Due to its location, a suggestion that AQP9 could participate in CSF transport between the brain parenchyma and the subarachnoid space was made (Badaut et al. 2004). In the non-human primate brain, AQP9 was additionally observed in neurons of primary motor cortex and the insula cortex (Arciénega et al. 2010). It has been reported that silencing of AQP9 on astrocytes cultures using siRNA led to a decrease in glycerol uptake and changes in energy metabolism of astrocytes (Badaut et al. 2012). Similar to AQP1, AQP9 expression also changes in different pathological conditions. Increased AQP9 expression coinciding with a decrease in insulin levels was observed in a rat model of diabetes (Badaut et al. 2008). Upregulation of AQP9 in neurons and astrocytes was also described after permanent cerebral ischemia. Here, it is probably related to the development of cerebral edema and it is also involved in lactate transport (Wei et al. 2015). Finally, AQP9 expression was also increased in astrocytic tumors, where it was positively correlated with pathological grade (Tan et al. 2008).

### 3.1.4 AQP9 in Alzheimer's disease

Although less has been discovered so far about the role of AQP9 in AD compared to AQP1 and AQP4, recent study showed that this aquaglyceroporin is probably also involved in Aβ-induced neurotoxicity during the pathogenesis of AD. Decreased AQP9 mRNA and protein expression has been observed in both hippocampus and cerebral cortex of APPdE9 mouse models and it was accompanied by simultaneous accumulation of APP. Moreover, silencing of AQP9 using siRNA enhanced Aβ-induced toxicity and apoptosis in PC12 cells. (Liu et al. 2018).

### 3.1.5 AQP4

AQP4 is predominant AQP in CNS which is abundantly expressed throughout the most brain structures, and in recent years there has been accumulating pieces of evidence about AQP4 and its association with pathology of AD. In situ hybridization first revealed the presence of AQP4 mRNA in the rat brain in 1994. AQP4 mRNA is widely distributed in the ependymal lining system, glial cells creating the edge of the cerebral cortex and brainstem, hippocampal dentate gyrus, Purkinje cell layer of the cerebellum, and in the vasopressin secretory neurons in supraoptic and paraventricular nuclei of the hypothalamus (Jung et al. 1994). Furthermore, in situ hybridization showed localization of AQP4 in neuronal layers of the CA1-CA3 hippocampal pyramidal cells, neocortex, medial habenular nucleus, and nucleus of the terminal stria. Colocalization with GFAP confirmed the expression of AQP4 mRNA in astrocytes as well (Venero et al. 1999), in which it is localized primarily on endfeet that closely surround blood vessels and on processes contacting neuronal synapses. Astrocytic AQP4 is a key player in a number of important functions that are responsible for maintaining the homeostasis of ions, water and glutamate in the brain, or control of neuronal activity. For this reason, it plays a role in several neurological diseases. Apart from astrocytes, AQP4 expression has been demonstrated to a lesser extent in ependymal cells, where it is mainly localized on the basolateral membranes (Nielsen et al. 1996; Badaut et al. 2000).

Furthermore, it was shown that AQP4 plays a role in astrocyte migration and glial scar formation. Astrocytes of AQP4 deficient mice have different shape and reduced migration capability when compared to WT astrocytes (Auguste et al. 2007). Moreover, the sensory function of the CNS may be impacted by AQP4 dysfunction, as the vast majority of AQP4 KO

mice showed severe impairment in hearing (Li and Verkman 2001) and decreased sense of smell (Lu et al. 2008).

As was mentioned AQP4 is associated with processes that maintain the homeostasis of ions. It was also demonstrated that AQP4 alters levels of ions. AQP4 plays a role in astrocytic $Ca^{2+}$ signaling. $Ca^{2+}$ signaling in astrocytes is triggered by brain edema, and it was reported that AQP4 is required for the induction of astrocytic $Ca^{2+}$ spikes, as in AQP4 KO mice, frequency, amplitude, and duration of $Ca^{2+}$ signals were affected. Additionally, osmotically induced neuroactive ATP release from cultured astrocytes is inhibited when AQP4 is deleted (Thrane et al. 2011). In vivo NADH fluorescence study linked together $K^+$ uptake and oxidation of brain tissue. This research showed evidence of AQP4 affecting oxidation and implies that $K^+$ uptake is lowered in AQP4 KO mice as a result of reduced oxygen transport to the furthest tissue from oxygen source (Thrane et al. 2013).

AQP4 is also linked with the metabolism of basal amino acids and monoamines. Both sexes of AQP4 KOs showed increased glutamine and decreased aspartate levels compared to WTs. Moreover, in females, higher levels of glutamate and serotonin were reported, while in males, a level of dopamine was increased (Fan et al. 2005).

### 3.1.6 AQP4 in Alzheimer's disease

As mentioned above, synaptic plasticity and memory are disrupted in AD. The association between AQP4 dysfunction and the impairment of synaptic plasticity, memory, and cognition has been suggested by studies using AQP4 knock out models. LTP and long-term depression (LTD) were significantly impaired in AQP4 KO mice compared to WT. Moreover, location-specific object memory test revealed a cognitive deficit in KO mice (Skucas et al. 2011). Involvement of AQP4 in spatial learning has been proposed in study employing Morris water maze, which reported deterioration in velocity and decreased motivation of AQP4 KO mice. (Zhang et al. 2013).

Impaired LTP in AQP4 KO mice has been shown to be associated with the downregulation of GLT-1 and increased activation of NMDA receptors due to higher extracellular glutamate concentration. Therefore, it is probably that AQP4 regulates GLT-1 expression and thus plays a role in synaptic plasticity in the amygdala (Li et al. 2012). Accordingly, boosting the expression of GLT-1 with β-lactam antibiotic Ceftriaxone reversed

the LTP induced by AQP4 deficiency (Yang et al. 2013). Changes of glutamate transporter expression levels in astrocytes of AQP4 KO mice have been also analyzed by Zeng and coauthors. It was shown that the lack of AQP4 resulted in reduced astrocytic expression of GLT-1 as well as glutamate uptake, but the expression of glutamate/aspartate transporter (GLAST) remained intact. Therefore, the authors propose that the direct physical interaction between AQP4 and GLT-1, along with their signal transduction pathway and GLT-1 function, is disrupted in AQP4 KO mice (Zeng et al. 2007).

Astrocytic glutamate transport has been also shown to be affected by Aβ. In slices treated with monomeric or oligomeric $A\beta_{1–42}$, mislocalization and internalization of GLT-1 in astrocytes has been observed, resulting in a decreased speed of astrocytic removal of glutamate from the ECS. These data suggest that astrocytic GLT-1 dysfunction may play an important role in the pathogenesis of AD, which subsequently impaired the GLT-1 function (Scimemi et al. 2013)

Additionally, recent studies show that GLT-1 and AQP4 can form structural complexes in the perivascular membrane of astrocytes, suggesting a functional link between water transport and glutamate homeostasis. The relationship between AD pathology and expression changes of AQP4 and GLT-1 has been proposed based on an immunohistochemical study performed in human samples from AD patients. However, the exact mechanism of the functional interaction between these two proteins has not yet been elucidated (Hoshi et al. 2018).

In addition to glutamate uptake, reduced AQP4 expression may affect some other astrocyte functions, such as their ability to phagocytose Aβ. Reduced $A\beta_{1-42}$-induced astrocyte activation, and apoptosis has been observed in AQP4 KO mice. Moreover, this was associated with reduced uptake of Aβ from the ECS due to the reduced expression of low-density lipoprotein receptor related protein-1 (LRP1; Yang et al. 2012).

Disrupted blood-brain barrier (BBB) was revealed in patients with early AD, who had overall BBB widespread leakage throughout the cerebrum, linked to cognitive impairment (van de Haar et al. 2016). Studies using AQP4 KO mice revealed that astrocytic AQP4 might be involved in the maintenance of BBB integrity. Decreased GFAP-immunoreactivity and defects in tight junction integrity were observed in the BBB of AQP4 KO mice compared to controls. Capillaries of AQP4 KO mice were regularly covered by swollen astrocytic endfeet, which

were detected by the reduction or absence of glycogen granules and organelles on electronic micrographs. Consequently, the BBB was hyperpermeable in AQP4 deficient mice (Zhou et al. 2008). Since AQP4 is expressed and functionally altered in Alzheimer's disease, it is likely that it is responsible for the disruption of the BBB in this disease.

As already mentioned, astrocytic AQP4 is involved in the maintenance of ion homeostasis. Above all, it concerns $K^+$ homeostasis, which is crucial for the proper function of neurotransmission. Upregulation of the voltage gated potassium channel Kv3.4 in early stages of AD linked to Aβ pathology have been reported in Tg2576 mouse model. Increased expression of neuronal voltage-gated $K^+$ channels results in altered synaptic activity that may underlie the neurodegeneration in AD (Angulo et al. 2004). The concentration of $K^+$ ions in the ECS may be influenced by astrocytic uptake and buffering. The Astrocytic Kir4.1 channel is mainly responsible for $K^+$ uptake from the ECS. This channel tends to colocalize with AQP4 and astrocytic $K^+$ uptake has been shown to be attenuated in AQP4 KO mice compared to WT, suggesting some form of functional interaction. Delayed $K^+$ uptake from the ECS resulting in higher seizure activity and electrographic threshold has been observed in AQP4 KO mice (Binder et al. 2006; Padmawar et al. 2005).

Moreover, AQP4 is involved in another important process, in clearance of brain products through glymphatic system. Glymphatic system is a system that mediates the exchange of CSF and interstitial fluid (ISF) and thus clearing of substances from the brain interstitium. The mechanism of the glymphatic system and role of AQP4 in it was described for the first time in 2012. It was researched by using two-photon microscopy in mice in vivo, and it was shown that the glymphatic pathway includes 3 separate anatomical parts; periarterial spaces, perivenous spaces, and brain parenchyma. CSF, labeled with fluorescent tracers, entered the brain interstitium along cortical pial arteries. This para-arterial influx was followed by a perivenous efflux of ISF, providing a clearance of fluid and solutes from the brain interstitium. A key role in this system is played by astrocytic water transport and especially AQP4, which is expressed along the perivascular space and facilitates the exchange of CSF and ISF (Iliff et al. 2012).

Disruption of the glymphatic system is one of the causes of the origin and development of some brain diseases. Among others, it is AD, in which dysfunction of the glymphatic system is probably responsible for the accumulation of Aβ in the ECS.

Recent studies show that removal of AQP4 results in a major change in the leaching of substances from the brain. Iliff et al. described a significant reduction (by 70%) of clearance from the brain interstitium in AQP4 KO mice compared to controls. Moreover, they showed that Aβ injected into the brain parenchyma is cleared along the paravenous efflux pathway, and impairment in the glymphatic system in AQP4 KO mice led to a 55 % reduction in clearance (Fig. 4, Iliff et al. 2012).

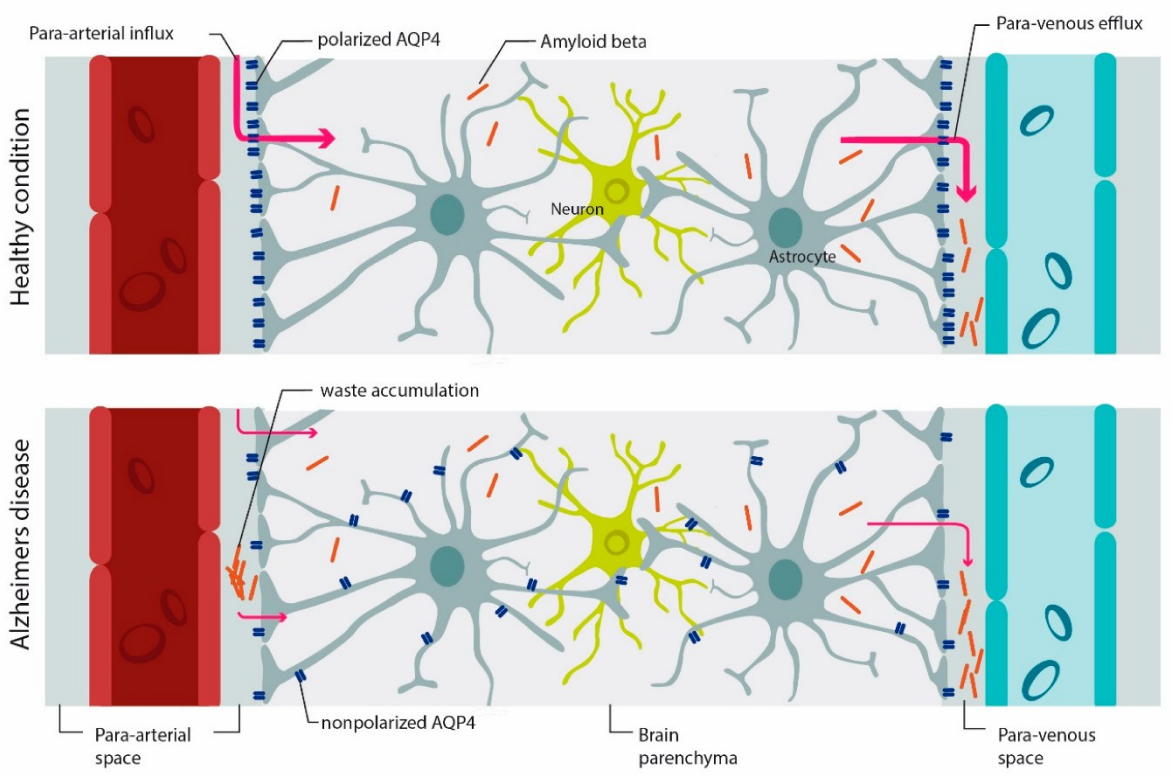


Fig. 4. Model of glymphatic system in a healthy brain and Alzheimer's disease (AD). The glymphatic system mediates the exchange of cerebrospinal fluid (CSF) and interstitial fluid (ISF) and thus allows clearing of substances from the brain interstitium. CSF enters the brain interstitium through the para-arterial system into the brain parenchyma and then into the veins. Labeled as para-arterial influx and para-arterial efflux. Aquaporin-4 (AQP4) facilitates the clearance through the glymphatic system. In the healthy brain, AQP4 expression is accumulated on the astrocyte endfeet, which means that it is polarized, and the CSF moves through the brain with AQP4 involvement. AQP4 polarization decreases with age and even more under pathologic conditions like AD. Resulting in AQP4 depolarization as an increased expression of AQP4 on parenchymal processes, which reduces the glymphatic system's efficiency and contributes to the amyloid-β (Aβ) disrupted clearance (Mader and Brimberg 2019).

Another confirmation that AQP4 is crucial in Aβ clearance was provided by research with AD mouse model APP/PS1. APP/PS1 are double transgenic mice expressing a chimeric

mouse/human amyloid precursor protein and a mutant human presenilin. It was demonstrated that deletion of AQP4 in APP/PS1 mice increased Aβ accumulation in the brain. It did not affect the APP processing and production of Aβ but specifically disrupted the clearance. Long-term deficiency of AQP4 led to a rise of not only Aβ plaque deposits but also a concentration of $A\beta_{40}$ and $A\beta_{42}$. Deletion of AQP4 also subsequently results in worsened cognitive impairments in APP/PS1 mice. Furthermore, astrocyte atrophy was enhanced by AQP4 loss (Xu et al. 2015). The APP/PS1 mouse model was used in another study that confirmed disruption of the glymphatic system in AD pathology. The results showed that the glymphatic system is already impaired in young APP/PSEN1 mice that have not yet developed disease symptoms, giving the early impairment potential to be a biomarker of AD. Glymphatic dysfunction may also be caused by Aβ oligomers and long-term exposure to the $A\beta_{40}$ in the CSF (Peng et al. 2016).

Recent studies show that the glymphatic system impairment may also be the cause of NFTs formation. Reduced clearance of parenchymal tau due to the disrupted AQP4 expression was demonstrated in a mouse model rTg4510 developing tau NFT pathology characteristic for AD. These mice express a repressible form of human tau containing the P301L mutation. Moreover, the pharmacological inhibition of AQP4 or the genetic deletion in AQP4 KO mice led to the significant reduction in tau clearance further implying that AQP4 is crucial for tau clearance from the brain (Harrison et al. 2020).

A connection between the AQP4 and clearance of Aβ was also shown in a study that focused on an impaired glymphatic system in an aging brain. There was significantly decreased perivascular AQP4 polarization in aged brains, which aligned with CSF-ISF exchange impairment compared to young brains. Moreover, clearance of Aβ from the aged brains was reduced by 40 % compared to the young ones (Kress et al. 2014). Evaluation of AQP4 expression and localization was done in a postmortem study of young, aged, and AD individuals, and a link between AQP4, age, and AD pathology was also shown. AQP4 expression increased with aging in all individuals, and AQP4 astrocytic perivascular localization was reduced among AD individuals. Reduced AQP4 localization was associated with increasing age, neurofibrillary, and Aβ pathology. Furthermore, increasing loss of AQP4 localization was also linked with AD progression (Zeppenfeld et al. 2017).

# 4 Conclusion

AD is a severe progressive neurodegenerative disease that is ultimately fatal as it is an irreversible brain disorder that slowly destroys cognition functions, memory, and basic physiological functions. Although this disease has been known for more than a century, its exact causes have not been yet fully elucidated. For this reason, effective treatments have also not yet been discovered to affect the underlying mechanisms of AD, but it instead targets the alleviation of the symptoms and improving quality of life. Therefore, the search for new therapeutic approaches is still the focus of scientific capacities worldwide. Recently, there has been increased focus on AQPs, which participate in several cellular processes, such as synaptic plasticity, glutamate homeostasis, maintaining BBB, or in the Aβ clearance through glymphatic system. They are thought to be potential targets for various neurological diseases, including stroke, CNS injury, edema associated with brain tumors and infection, epilepsy, multiple sclerosis, and AD. However, due to the complex roles of aquaporins in various mechanisms, influencing their function (inhibition/ activation, using AQP-targeted antibody therapeutics) or expression (transcriptional activators/ inhibitors) may prove to be difficult. For example, blocking AQP4 in the early stages of ischemia to reduce brain swelling could produce seizures or other side effects within or outside the CNS. Therefore, it is essential to properly investigate all mechanisms and consider possible side effects in the development of therapeutics specifically targeting AQPs.

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