Received: 1 February 2023 | Accepted: 2 March 2023

DOI: 10.1002/cdt3.64

SHORT COMMUNICATION

Chronic Diseases and Translational Medicine

Alignment of human aquaporin 4 and ß-amyloid proteins may indicate involvement of ß-amyloid in brain water homeostasis and prevention of brain edema

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KEYWORDS
alignment, brain, edema, protein

Edited by Yi Cui

The amyloid hypothesis states that the buildup of ß-amyloid in the brain is the main factor for Alzheimer's disease (AD) pathogenesis. An imbalance between ß-amyloid production and ß-amyloid clearance causes the advanced stages of the disease, including the development of neurofibrillary tangles containing tau protein.[1]

Many medications that aim to reduce ß-amyloid in AD are not clinically effective. FDA has approved aducanumab, one of four anti-ß-amyloid antibodies that have been demonstrated to mediate the removal of amyloid plaque from the brains of AD patients. FDA accepted the decrease of amyloid plaque as a surrogate endpoint for aducanumab. But there is intense disagreement over the justification for approval and the scope of the clinical benefit provided by antiamyloid antibodies.[2]

One side effect of the antibodies is brain edema, effusion, and hemorrhages, so called amyloid-related imaging abnormalities (ARIA). ARIA occurs in aged squirrel monkeys as well as in humans.[3]

Lecanemab, an antiamyloid monoclonal antibody, was associated with edema or effusions in 12.4% of subjects, including three fatal brain hemorrhages; the placebo group had 1.7% brain edema.[4–9] In the case of donanemab, another anti-amyloid monoclonal antibody, if edema or effusion occurred with the first three doses of the drug, the dosage was not increased.[10]

A serious clinical condition, brain edema is defined by a pathological swelling of the brain tissue brought on by an increase in the water content of the brain. In humans[11] and in a mouse model, APOE isoform affects neurological prognosis following intracerebral hemorrhage. Poor functional outcome and more cerebral edema are linked to APOE4.[12] Three SNPs of the ABCC8 gene, rs2283261, rs3819521, and rs2283258, are significantly associated with brain edema, measured by increased intracranial pressure and CT imaging. Haptoglobin type, Hp2 versus Hp1, may also influence brain edema.[13]

Aquaporins, a family of water channel proteins that have been found in animals, may provide an explanation for AD brain edema. Aquaporin-4 (AQP4), the most significant form of aquaporin in the central nervous system, mediates water homeostasis in healthy and pathological settings, such as severe brain injury.[13,14]

Because brain edema has occurred during clinical trials of most anti-amyloid antibodies, we hypothesize that ß-amyloid might be an important element in brain water homeostasis. Removing ß-amyloid could cause brain edema and bleeding in some AD patients. To investigate this idea, we analyzed structures of aquaporin-4 and ß-amyloid from the RCSB protein data bank.

To help identify the brain regions where anti-amyloid antibodies may act, we used the Allen Brain Atlas and the Human Protein Atlas to examine AQP4 and APP (amyloid ß precursor protein) RNA expression in the brain.[15,16]

We then examined two RCSB Protein Data Bank molecules:

- 2D57: Double layered 2D crystal structure of Aquaporin-4 (AQP4M23) at 3.2 Å resolution by electron crystallography.[17]
- 1 × 45: Solution structure of the first PDZ domain of ß-amyloid A4 precursor protein-binding family A, member 1. The PDZ domain is a common structural domain of 80–90 amino acids found in the signaling proteins of bacteria, yeast, plants, viruses, and animals.

The protein structures were superimposed and aligned on PYMOL v 2.5.0 with the Super command, which super aligns two protein selections. Super does a sequence-independent structure-based dynamic programming alignment (unlike the align command) followed by a series of refinement cycles intended to improve the fit by eliminating pairing with high relative variability. The Super command is more reliable than the *align* command for proteins with low sequence similarity.

AQP4 expression (Allen Brain Atlas) is shown in Figure 1. AQP4 RNA is strongly and broadly expressed in a variety of brain areas, including the hippocampal and parahippocampal regions where AD originates.

Figure 2 shows AQP4 RNA expression diagrammatically. AQP4 RNA is strongly and broadly expressed in a variety of brain areas in both the human and mouse (not shown). The donor was a 24-year-old Male Black or African American (Allen Brain Atlas).

Amyloid precursor protein (APP) RNA expression is in Figure 3. Like AQP4, APP is strongly expressed throughout the brain (Human Protein Atlas).

Pymol performed five cycles of calculations on 29 aligned atoms of aquaporin-4 and ß-amyloid proteins, with a final root mean square deviation of atomic positions (RMSD) of 0.300 Å for 21 atoms (Figure 4). Pymol automatically determines the optimum number of cycles to calculate. Lower values of RMSD indicate that alignment is validated with higher accuracy. RMSD values of 1 Å or less indicate very good alignment. The two aligned molecules aquaporin-4 and ß-amyloid are shown in Figure 5. The 21-atom alignment is excellent. The arrow indicates isoleucine 77 of ß-amyloid overlying valine 162 in exon 3 of aquaporin 4.

Alignments are a powerful way to compare related protein sequences. They can be used to record a variety

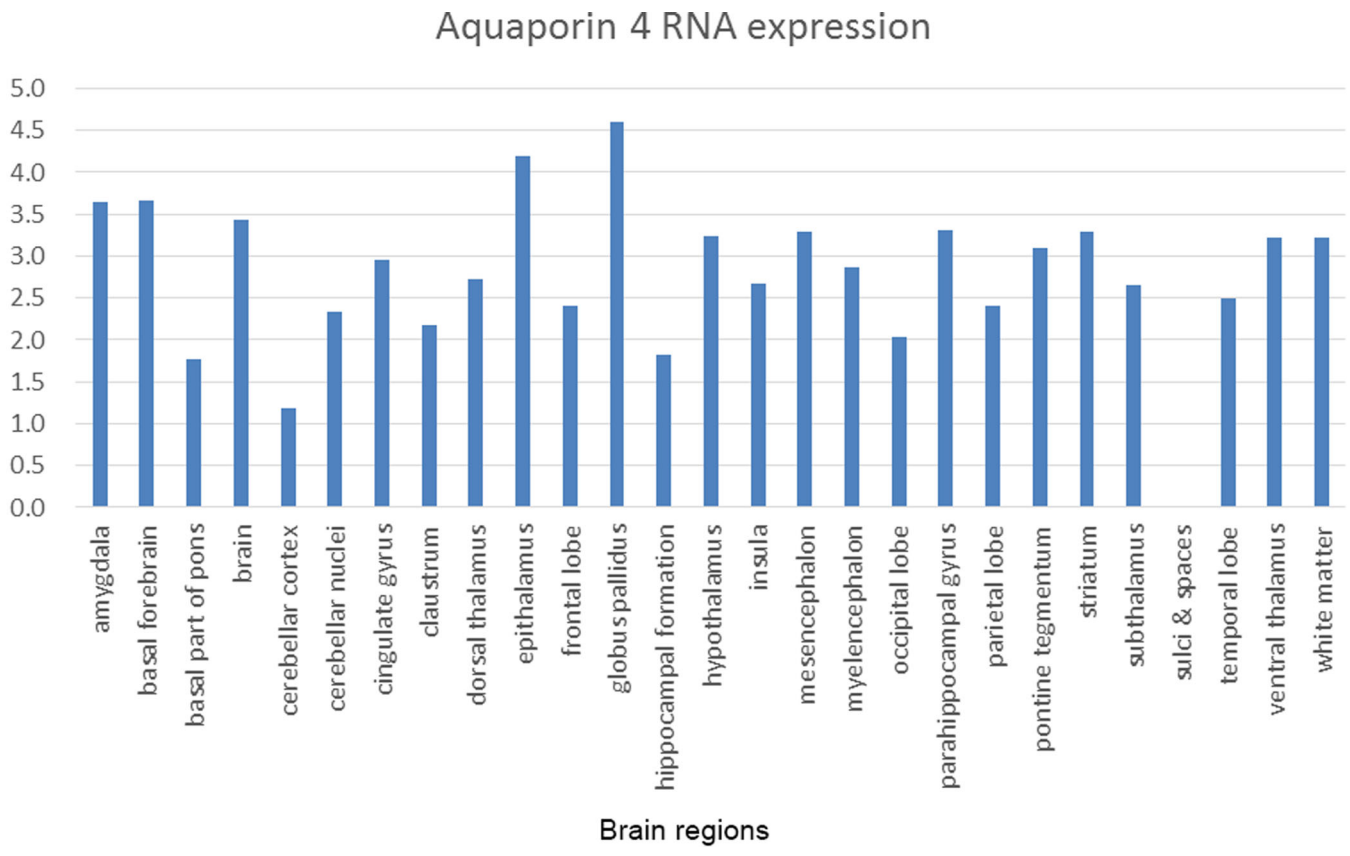

FIGURE 1 AQP4 RNA human brain expression, data normalized. A_23_P107565 probe. AQP4 RNA is strongly and broadly expressed in a variety of brain areas, including the hippocampal and parahippocampal regions, where Alzheimer's disease (AD) originates. Data from Allen Brain Atlas.

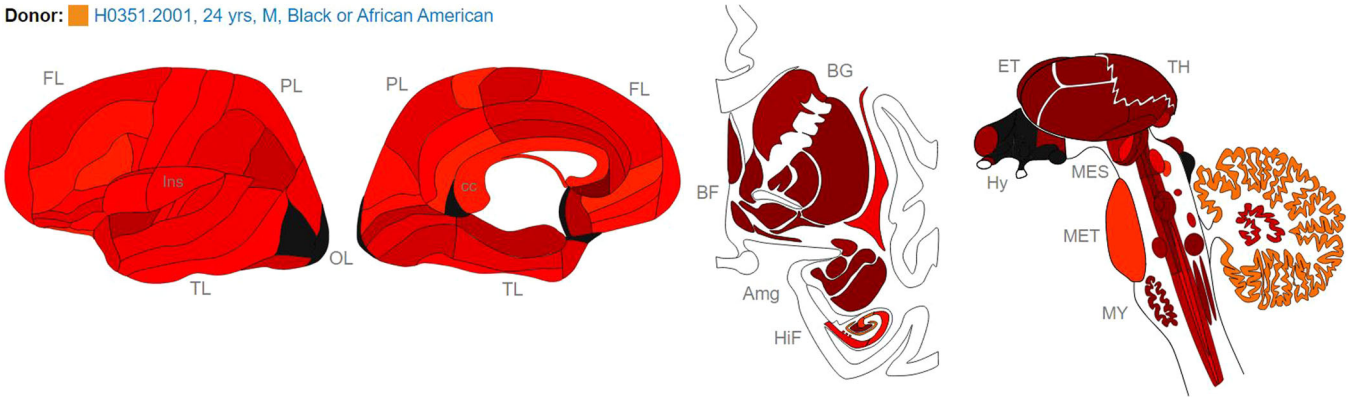

FIGURE 2 AQP4 RNA expression. AQP4 RNA is strongly and broadly expressed in a variety of brain areas in both the human and mouse (not shown). The donor was a 24-year-old Male Black or African American. Allen Brain Atlas http://human.brain-map.org/microarray/gene/show/358.

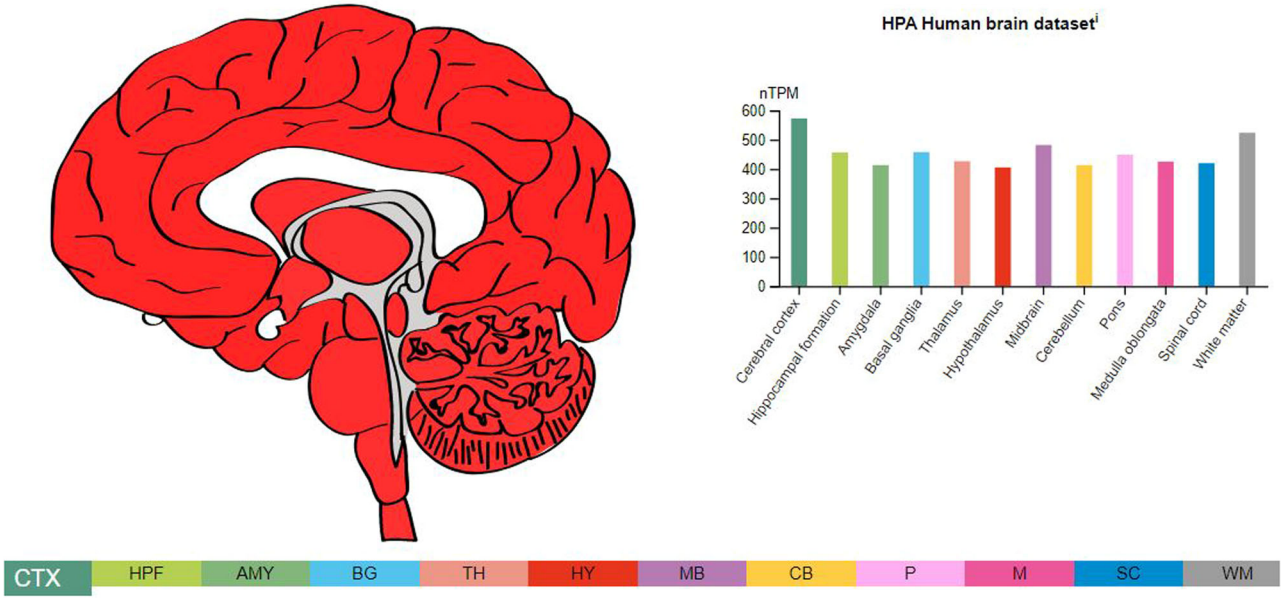

FIGURE 3 Amyloid precursor protein (APP) RNA expression. Like AQP4, APP is strongly expressed throughout the brain. Human Protein Atlas https://www.proteinatlas.org/ENSG00000142192-APP/brain.

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ExecutiveAlign: 29 atoms aligned.
ExecutiveRMS: 2 atoms rejected during cycle 1 (RMSD=0.71).
ExecutiveRMS: 2 atoms rejected during cycle 2 (RMSD=0.56).
ExecutiveRMS: 1 atoms rejected during cycle 3 (RMSD=0.45).
ExecutiveRMS: 1 atoms rejected during cycle 4 (RMSD=0.40).
ExecutiveRMS: 2 atoms rejected during cycle 5 (RMSD=0.36).
Executive: RMSD =    0.300 (21 to 21 atoms)
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FIGURE 4 Pymol performed five cycles of calculations on 29 aligned atoms of aquaporin-4 and ß-amyloid proteins, with a final root mean square deviation of atomic positions (RMSD) of 0.300 Å for 21 atoms.

of information about matched sequences, such as shared structural function or common evolutionary ancestry. Over the past few decades, protein sequence alignment analyses have become an essential stage in bioinformatics analytic research. Numerous protein databases with information on protein families were created using sequence alignments.[18] Our analysis indicates that AQP4 and ß-amyloid may have shared functions, including maintenance of brain water homeostasis and prevention of brain edema. The similarities in brain expression of AQP4 and APP (Figures 2 and 3) reinforce this conclusion.

The most widespread CNS aquaporin channel, AQP4, is frequently seen in the astrocytic end feet. AQP4 RNA is strongly and broadly expressed in a variety of brain areas in both the human and mouse. Additionally, the entire mouse brain exhibits significant AQP4 intensity and broad immunolabelling of astrocyte end-feet, with this pattern representing the vasculature and capillary walls.

AQP4 variants may be a risk factor for AD vasogenic edema. A direct result of tight junction breakdown between

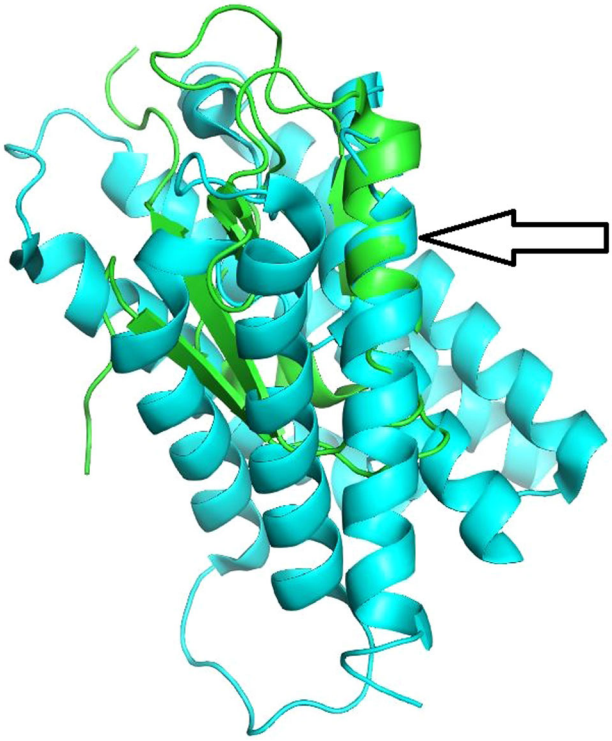

FIGURE 5 Aligned alpha helices of ß-amyloid (green) and aquaporin4 (blue). Arrow indicates isoleucine 77 of ß-amyloid overlying valine 162 in exon 3 of aquaporin 4.

vascular endothelial cells, vasogenic edema develops because of a disturbance of blood–brain barrier integrity. The extracellular compartment of the brain enlarges because fluid and proteins from the vasculature penetrate the interstitial space. Vasogenic edema results in increased intracranial pressure, decreased cerebral blood flow, brain herniation, and ultimately death. Vasogenic edema can follow trauma, arterial hypertension, tumor-released vasoactive substances, or endothelium-damaging substances, for example, arachidonic acid, excitatory neurotransmitters, eicosanoids, bradykinin, histamine, and free radicals.[13,14] Vasogenic edema is a common side effect of anti-amyloid AD drugs[19] and may be a sign that amyloid is being cleared from the brain.[20]

One study of AQP4 exon 4 did not find mutations. But in another, seven tag single nucleotide polymorphisms (SNPs) were detected along the AQP4 gene region in a study that examined clinical, neuroimaging, and genetic data from 363 traumatic brain injury patients. A tag SNP is a SNP in a region of the genome with high linkage disequilibrium, part of a group of SNPs called a haplotype. Two tag SNPs, rs3763043, associated with schizophrenia,[21] and rs3875089, associated with intracerebral hemorrhage,[22] were connected to poor clinical outcomes as assessed 6 months after traumatic brain injury.[23]

Our finding that AQP4 aligns closely with ß-amyloid may indicate that ß-amyloid, like AQP4, might be important in maintaining brain water homeostasis and preventing brain edema. ß-amyloid structure has been highly conserved throughout mammalian evolution, indicating one or more vital functions. For example, ß-amyloid is antimicrobial and may be an inherited defense against herpes simplex type 1.[24]

The results of the current study have two notable implications: (1) Screening for AQP4 polymorphisms SNPs rs3763043, rs3875089, and APOE4 isoform before antiamyloid AD treatment could identify patients at high risk of brain edema and hemorrhage. Screening for ABCC8 polymorphisms and haptoglobin form could be of value as well. (2) Screening for the same in children could detect those with increased vulnerability to traumatic brain injury in certain sports: football, hockey, basketball, and baseball.

APOE2, APOE4, AQP4, and antiamyloid antibodies are not the only substances associated with AD vasogenic edema. The Alzheimer's drug, avagacestat, a small molecule gamma-secretase inhibitor that reduces ß-amyloid levels, also caused vasogenic edema.[25] Moreover, asymptomatic vasogenic edema has been found in AD patients who have received no treatment at all.[19] Therefore, focal, localized vasogenic edema may be part of the AD pathologic process.

Since APOE and ABCC8 genes are associated with cerebral edema, it would be worthwhile to evaluate the alignment and other similarities of these protein structures with AQP4.

We conclude that ß-amyloid may be involved in brain water homeostasis and protect against vasogenic brain edema. Removing ß-amyloid from AD patients may promote vasogenic brain edema and bleeding. Screening for AQP4 and ABCC8 polymorphisms, APOE2 and APOE4 isoforms, and haptoglobin form could identify patients at high risk of brain edema and hemorrhage from anti-amyloid treatment. Further studies are warranted.

Preprint posted https://doi.org/10.21203/rs.3.rs-2350250/v1

AUTHOR CONTRIBUTIONS

Dr. Steven Lehrer and Dr. Peter H. Rheinstein contributed equally to this work.

ACKNOWLEDGMENTS

None.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

All data from publicly available sources.

ETHICS STATEMENT

Not applicable, all data from publicly available sources.

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How to cite this article: Lehrer S, Rheinstein PH. Alignment of human aquaporin 4 and ß-amyloid proteins may indicate involvement of ß-amyloid in brain water homeostasis and prevention of brain edema. *Chronic Dis Transl Med*. 2023;9:177-181. doi:10.1002/cdt3.64