Cerebrospinal fluid level of Aquaporin4: a new window on glymphatic system involvement in neurodegenerative disease?

Andrea Arighi, MD

Andrea Di Cristofori, Chiara Fenoglio, Stefano Borsa, Marianna D'Anca, Giorgio Giulio Fumagalli, Marco Locatelli, Giorgio Carrabba, Anna Margherita Pietroboni, Laura Ghezzi, Tiziana Carandini, Annalisa Colombi, Marta Scarioni, Milena Alessandra De Riz, Maria Serpente, Paolo Maria Rampini, Elio Scarpini, Daniela Galimberti

FONDAZIONE IRCCS CA' GRANDA
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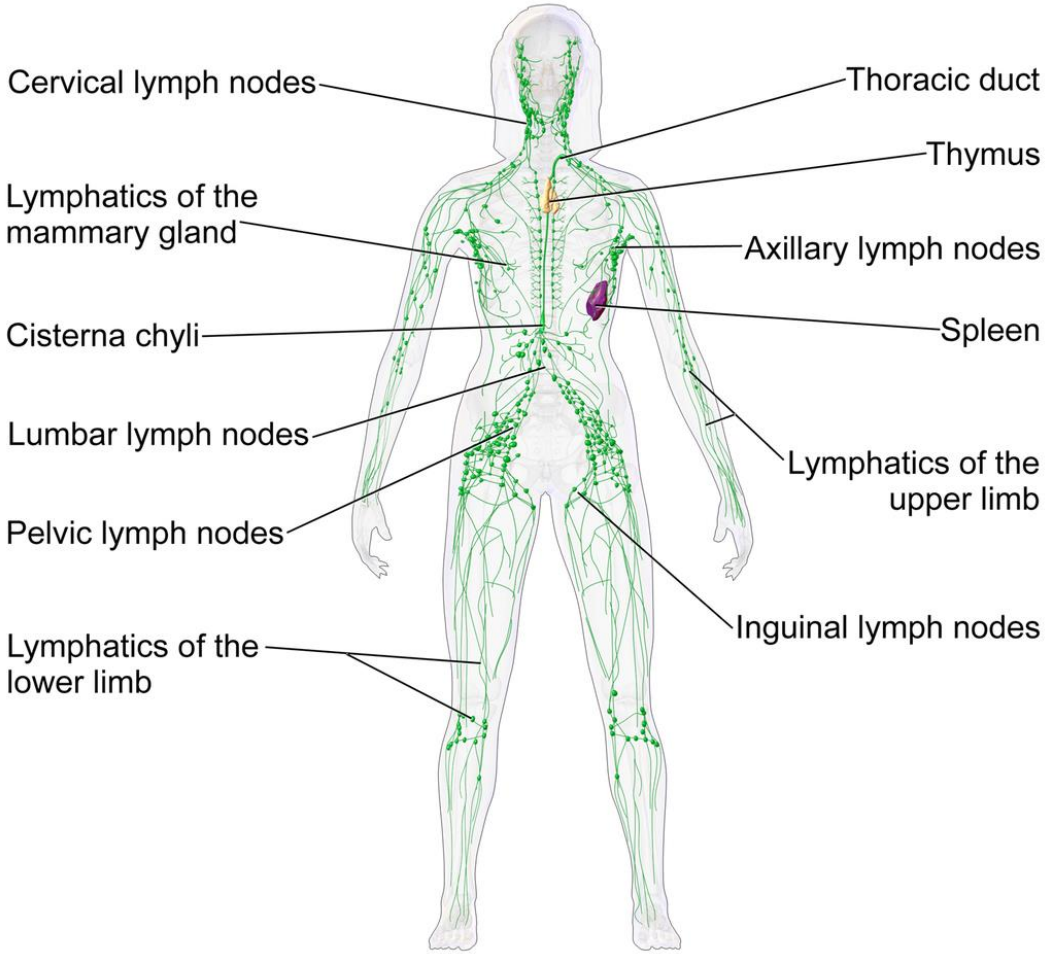
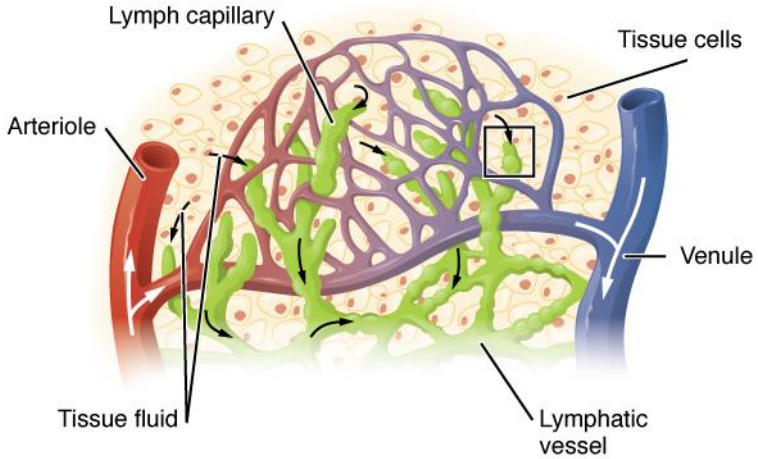
Lymphatic system

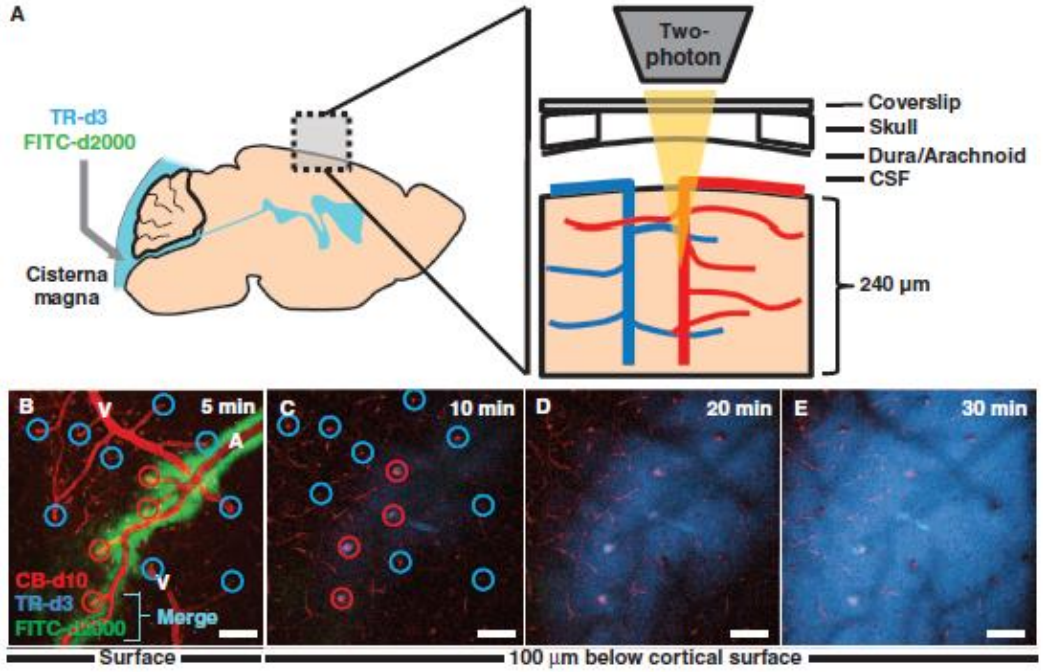




CEREBROSPINAL FLUID CIRCULATION

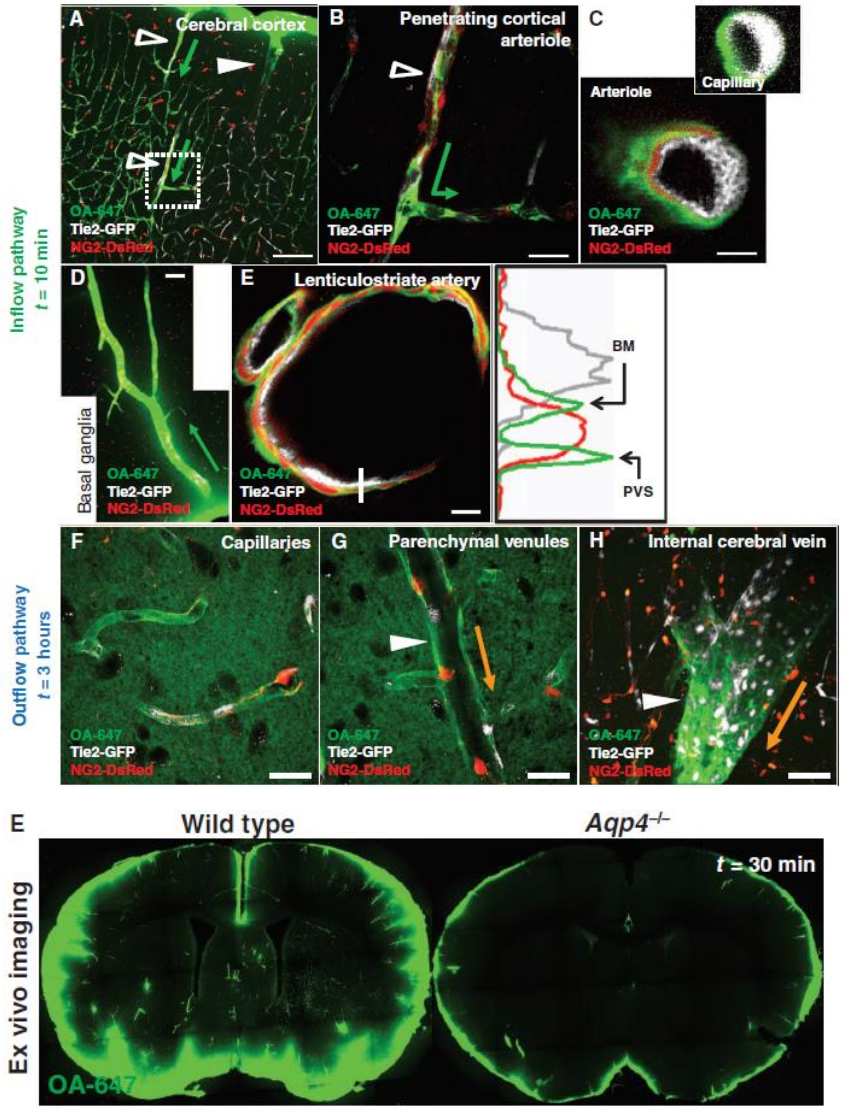
A Paravascular Pathway Facilitates CSF Flow Through the Brain Parenchyma and the Clearance of Interstitial Solutes, Including Amyloid β

Jeffrey J. Iliff,[1]* Minghuan Wang,[1,2] Yonghong Liao,[1] Benjamin A. Plogg,[1] Weiguo Peng,[1] Georg A. Gundersen,[3,4] Helene Benveniste,[5,6] G. Edward Vates,[1] Rashid Deane,[1] Steven A. Goldman,[1,7] Erlend A. Nagelhus,[3,4] Maiken Nedergaard[1]*

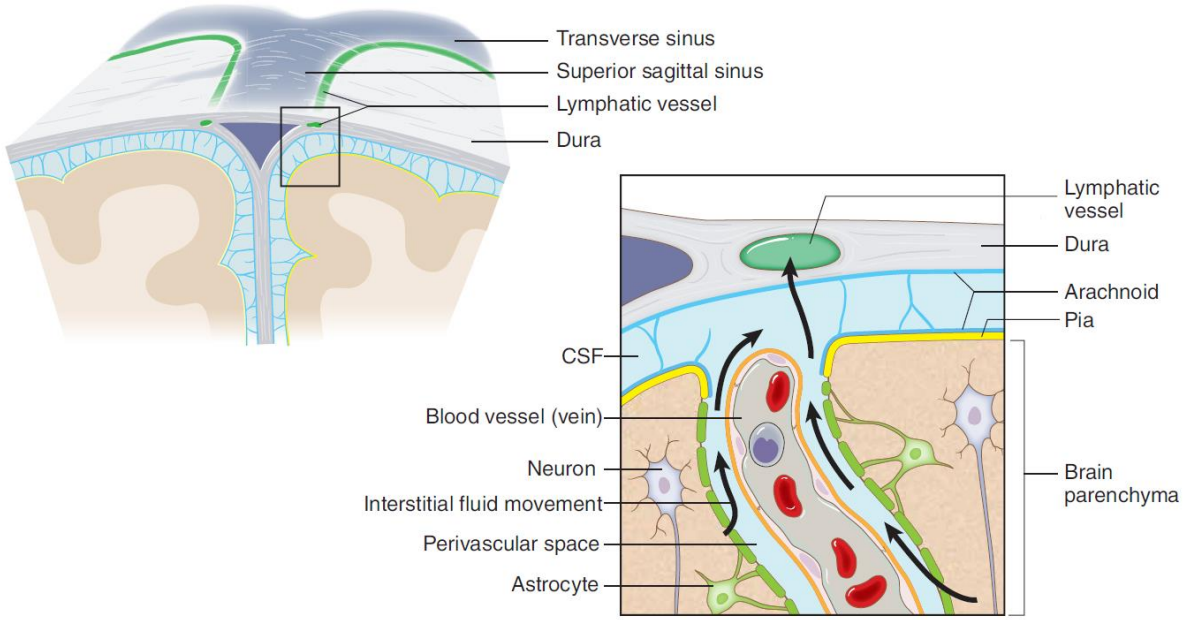


On the basis of in vivo two-photon imaging of small fluorescent tracers, we showed that CSF enters the parenchyma along paravascular spaces that surround penetrating arteries and that brain interstitial fluid is cleared along paravenous drainage pathways.

Animals lacking the water channel aquaporin-4 (AQP4) in astrocytes exhibit slowed CSF influx through this system and a ~70% reduction in interstitial solute clearance, suggesting that the bulk fluid flow between these anatomical influx and efflux routes is supported by astrocytic water transport.



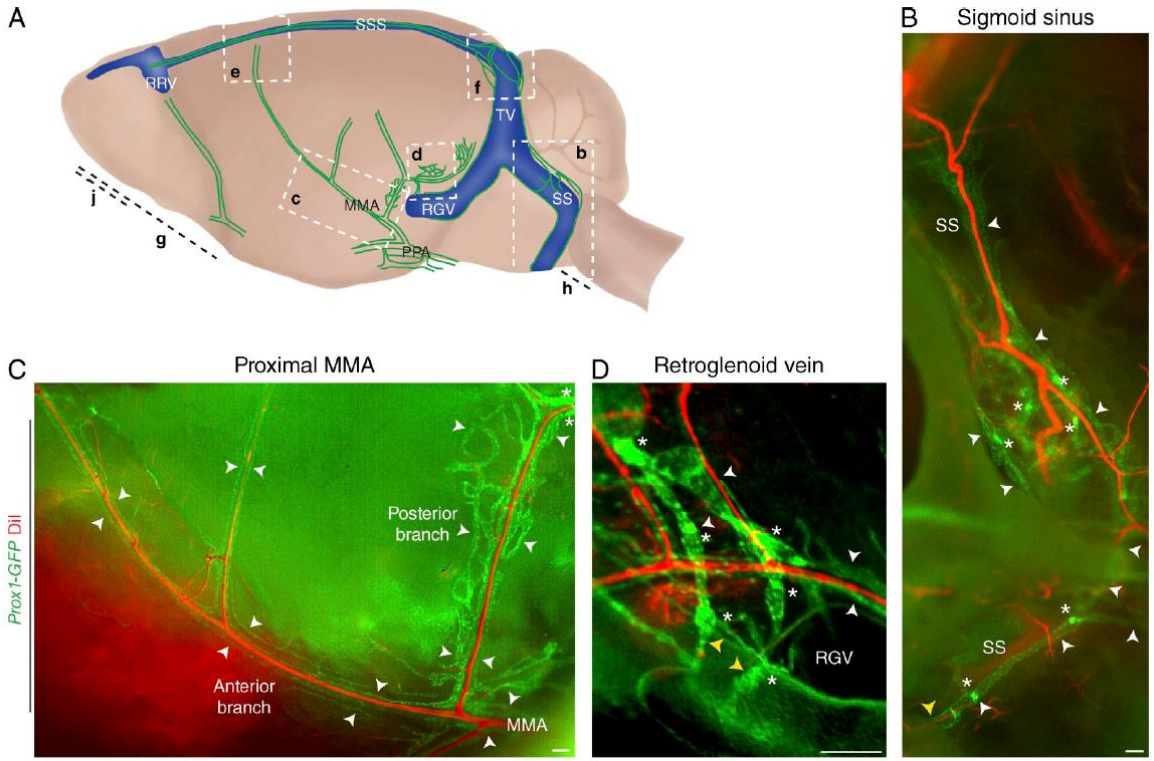
JJ Iliff, 2012



A Louveau, 2015

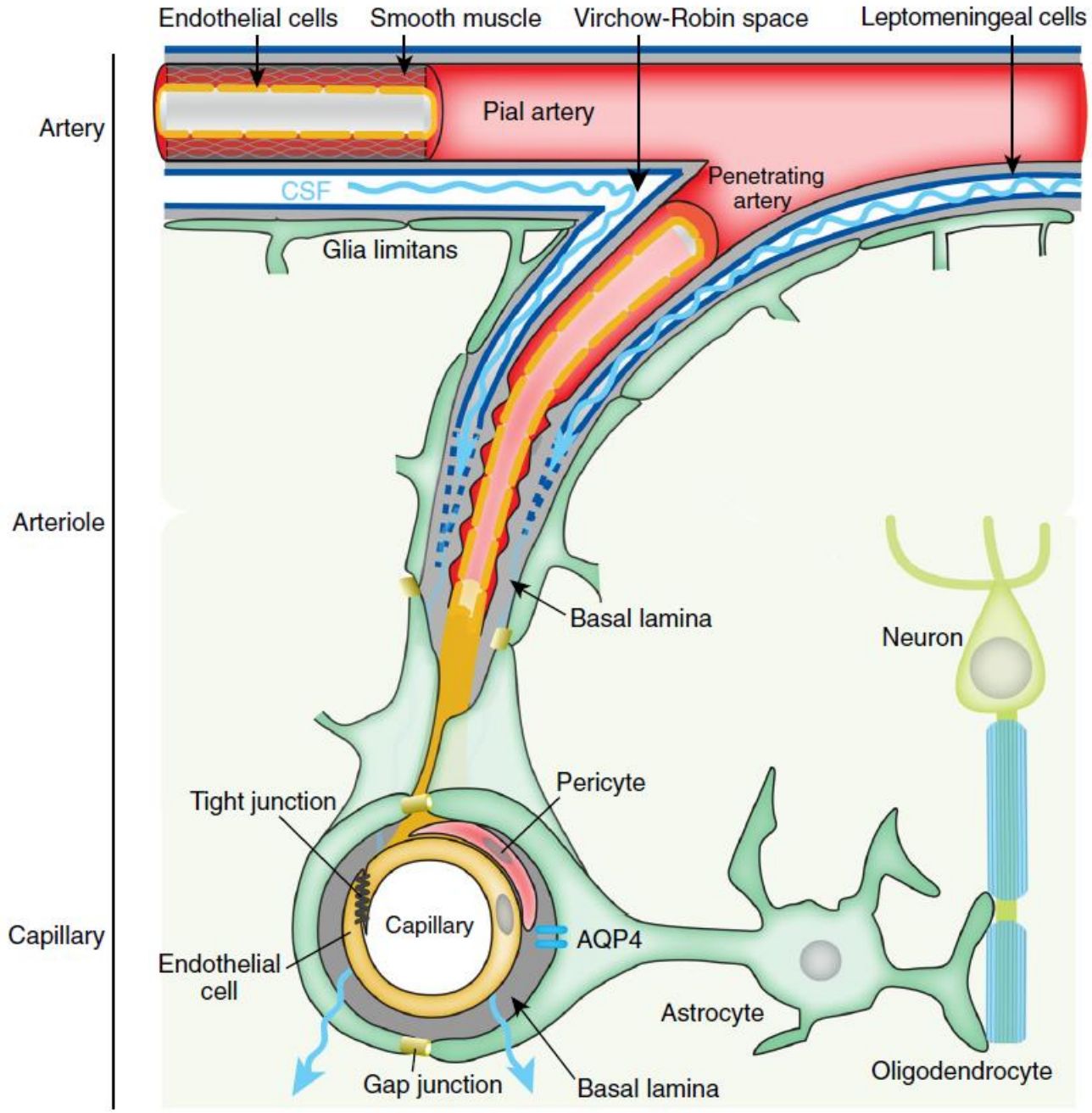
A dural lymphatic vascular system that drains brain interstitial fluid and macromolecules

Aleksanteri Aspelund,[1,2] Salli Antila,[1,2] Steven T. Proulx,[3] Tine Veronica Karlsen,[4] Sinem Karaman,[3] Michael Detmar,[3] Helge Wiig,[4] and Kari Alitalo[1,2]



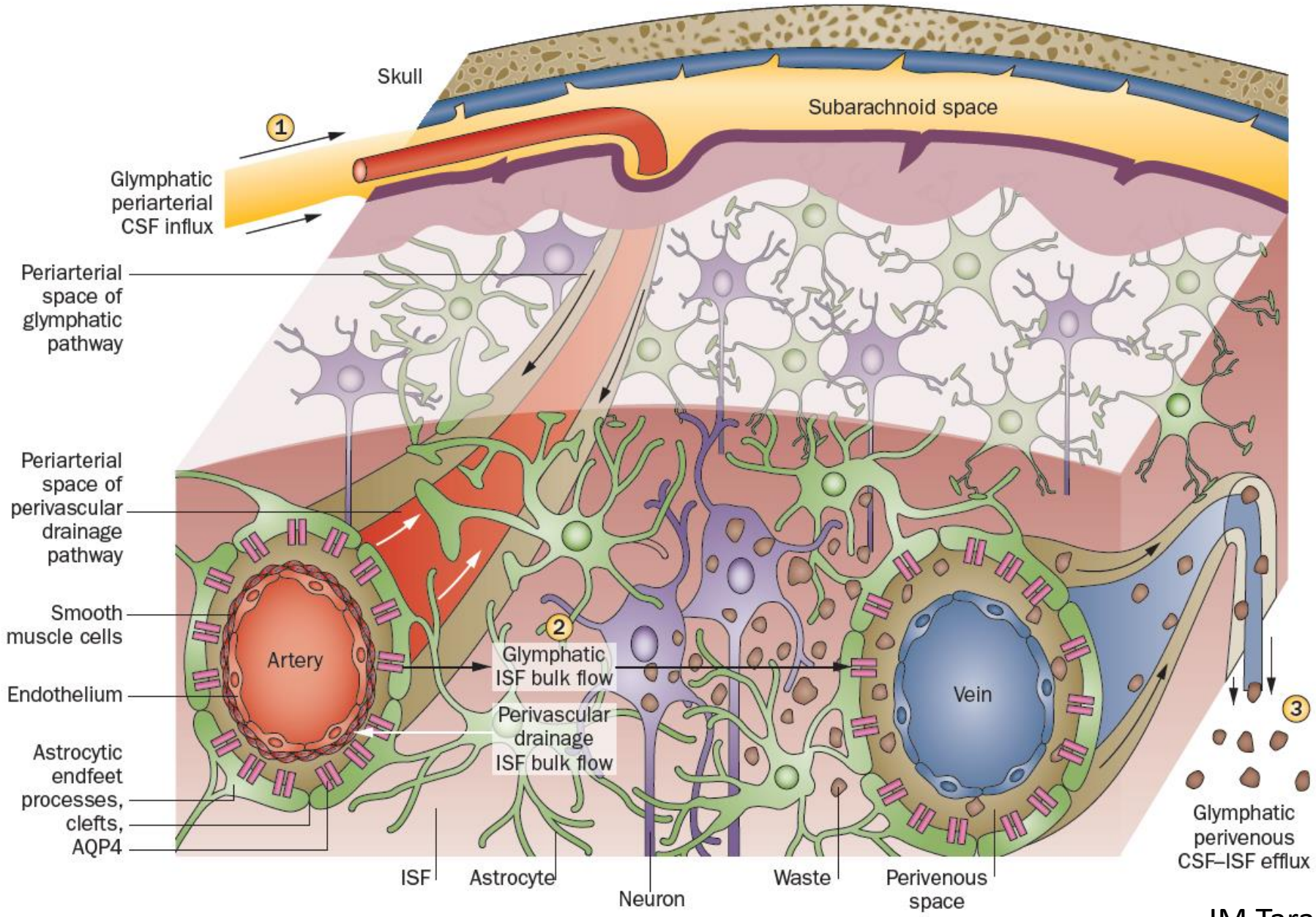
A Aspelund, 2015

The neurovascular unit



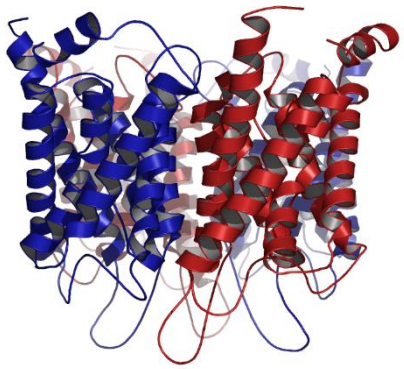
NA Jessen, 2015

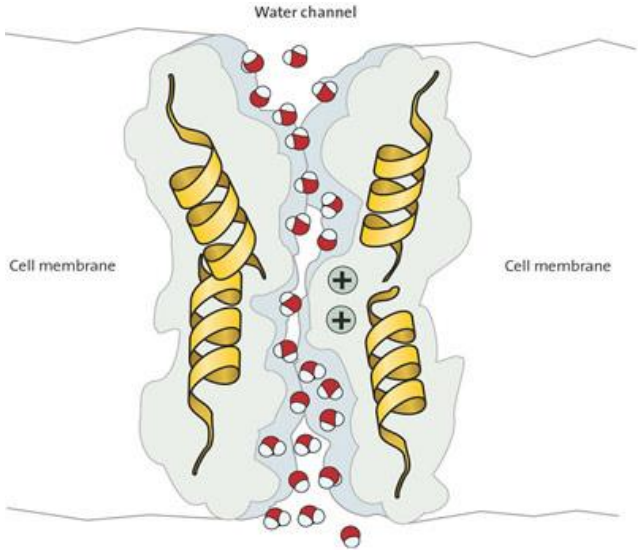
The glymphatic (glial+lymphatic) **system**



JM Tarasoff-Conway, 2015

Aquaporins





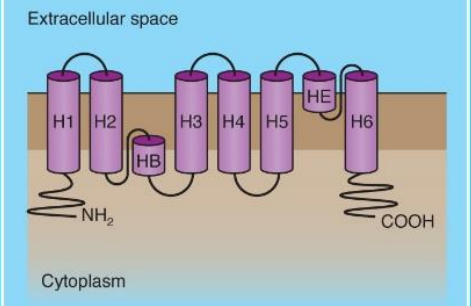
Aquaporins also called water channels, are integral membrane proteins from a larger family of major intrinsic proteins that form pores in the membrane of biological cells, mainly facilitating transport of water between cells

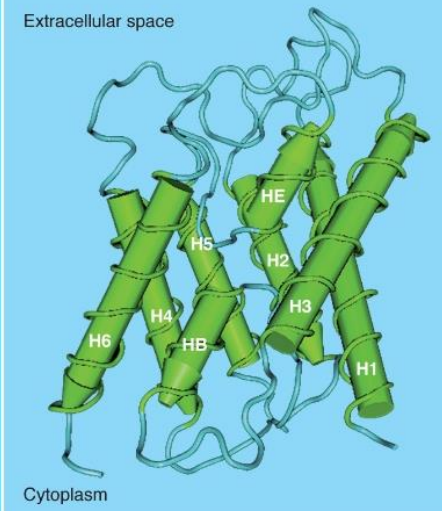
The 2003 Nobel Prize in Chemistry was awarded jointly to Peter Agre for the discovery of aquaporins

Aquaporin structure

1. Topology and structure

AQP monomers contain helical domains surrounding a narrow aqueous pore. Monomers assemble to form tetramers in the membrane.

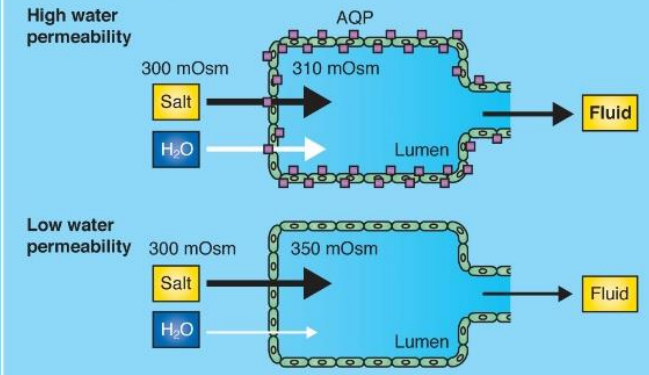




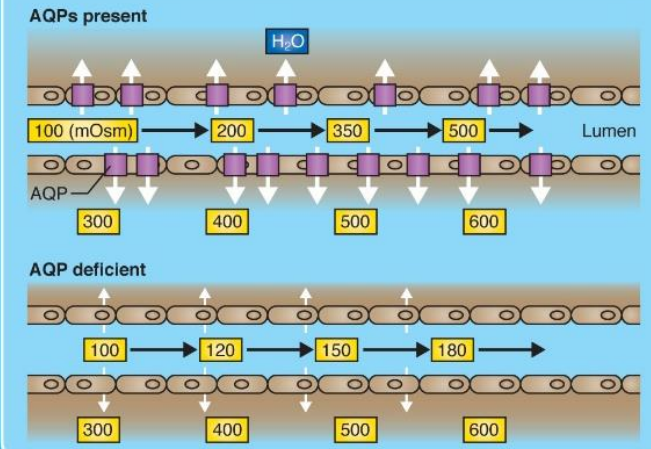
Water-selective aquaporins

2. Fluid secretion

AQPs increase transepithelial water transport in response to osmotic gradients.

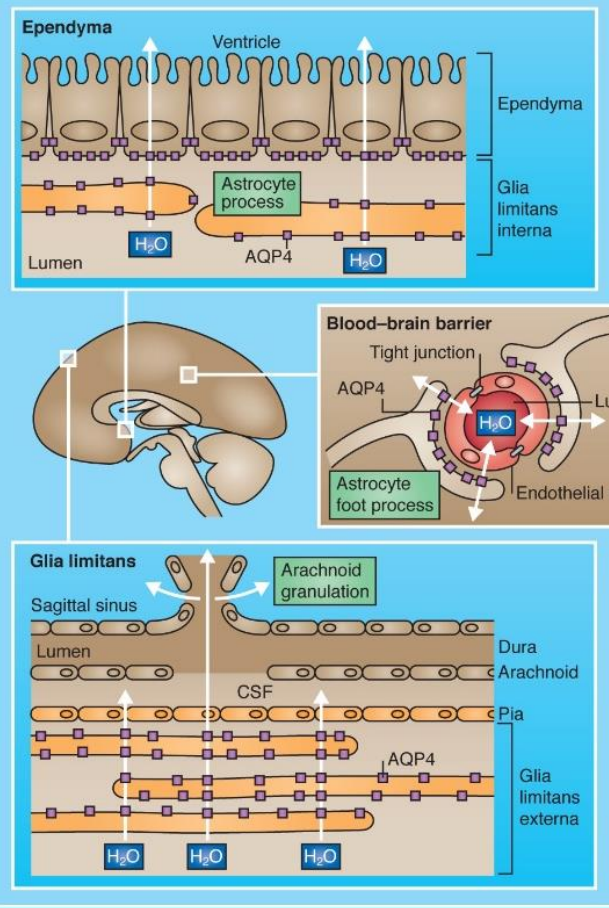


3. Kidney tubule fluid absorption



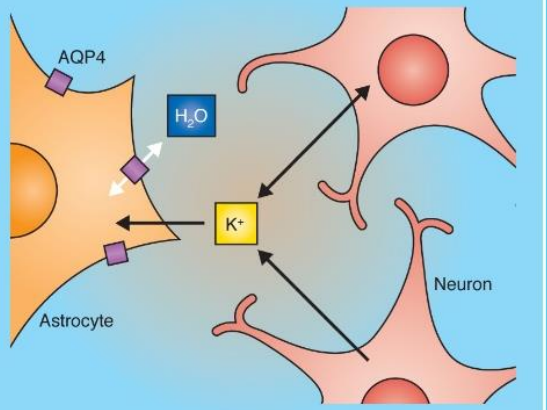
4. Brain water balance

AQP4 facilitates water movement into and out of the brain across brain–fluid barriers at locations indicated.



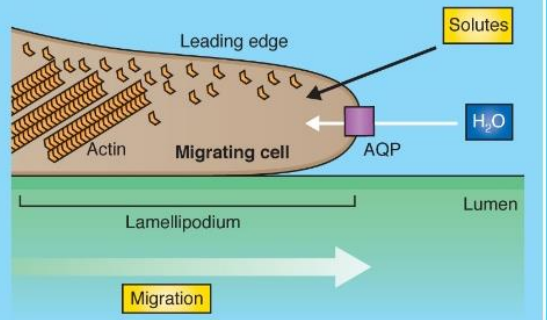
5. Neural AQP functions – a model

AQP4 allows rapid water uptake from the ECS after neuroexcitation, maintaining the driving force for K^+ uptake.



6. AQPs in cell migration – a model

This model shows AQP-facilitated entry of water at the leading edge of a migrating cell.



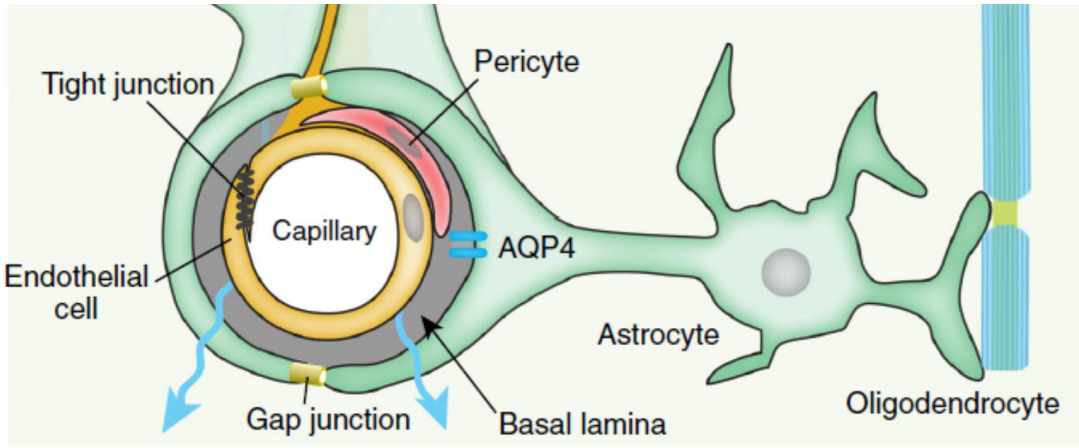
from Journal of Cell Science, 2011

Table 1 | **Mammalian aquaporins**

Aquaporin	Size	Permeability	Distribution
Aqp0	26 kD, 263 aa	Water (low)	Lens epithelium
Aqp1	28 kD , 269 aa	Water	Kidney, capillary endothelia (except brain), red blood cells, cornea, choroid plexus
Aqp2	29 kD, 271 aa	Water	Kidney collecting duct cells (intracellular and apical membranes)
Aqp3	31 kD, 292 aa	Water, glycerol, urea	Kidney, colon
Aqp4	M1: 32 kD, 301 aa M23: 34 kD, 323 aa	Water (Hg^{++} insensitive)	CNS, skeletal muscle, lung, kidney, inner ear, gastric parietal cells
Aqp5	29 kD, 265 aa	Water	Lung, salivary glands, lacrimal glands, trachea, cornea
Aqp6	28–30 kD, 276 aa	Water (low), anions (HNO_3^-; high)	Intracellular vesicles in kidney intercalated cells, proximal tubules
Aqp7	26 kD, 269 aa	Water, glycerol	Adipose tissue, testis, kidney
Aqp8	27 kD, 269 aa	Water	Testis, liver, pancreas
Aqp9	32 kD, 342 aa	Water, glycerol, urea	Liver, testis, brain
Aqp10	28 kD, 301 aa	Water (low), glycerol, urea	Small intestine

aa, amino acid; Aqp, aquaporin; CNS, central nervous system; M1/M23, two different isoforms of Aqp4.

Mahmood Amiry-Moghaddam, 2003



In the central nervous system, there are five members of the AQP family, AQP1, AQP4, AQP7, AQP9, and AQP11.
Of the five expressed AQPs, only **AQP1** and **AQP4** are expressed in abundance, with **AQP4** showing the highest expression pattern of any other member

Filippidis, 2011

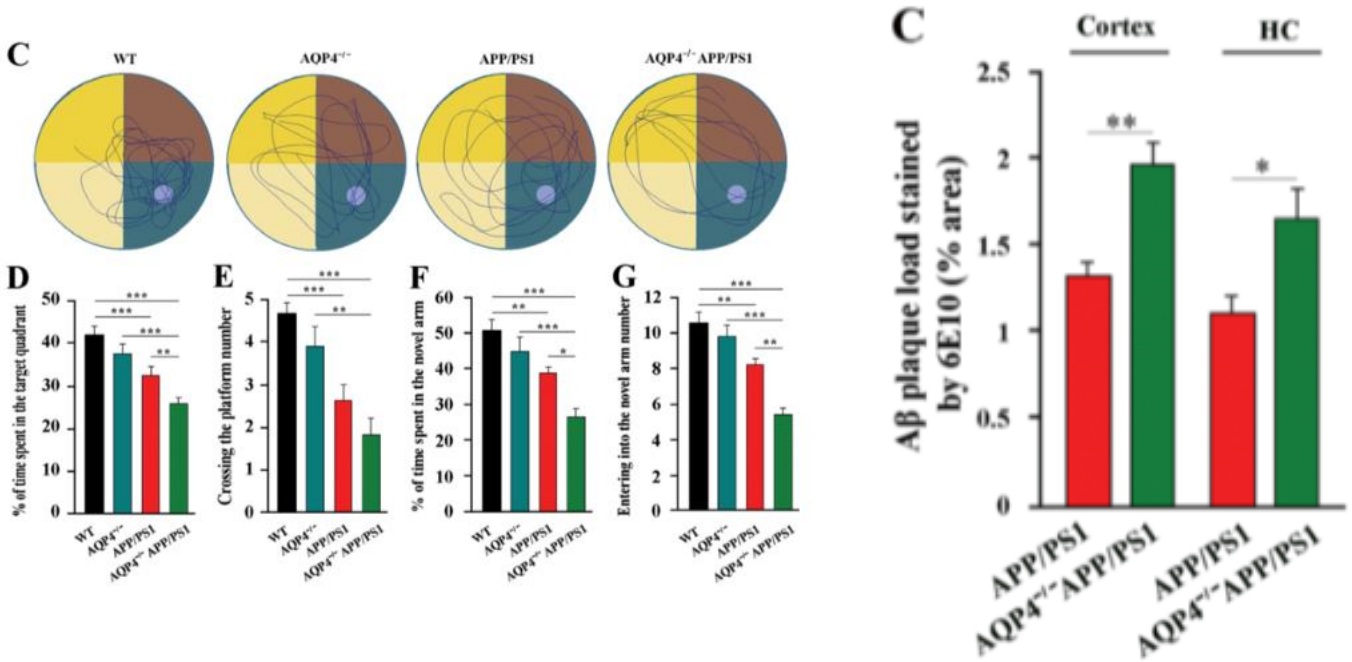
Xu et al. Molecular Neurodegeneration (2015) 10:58
DOI 10.1186/s13024-015-0056-1

MOLECULAR NEURODEGENERATION

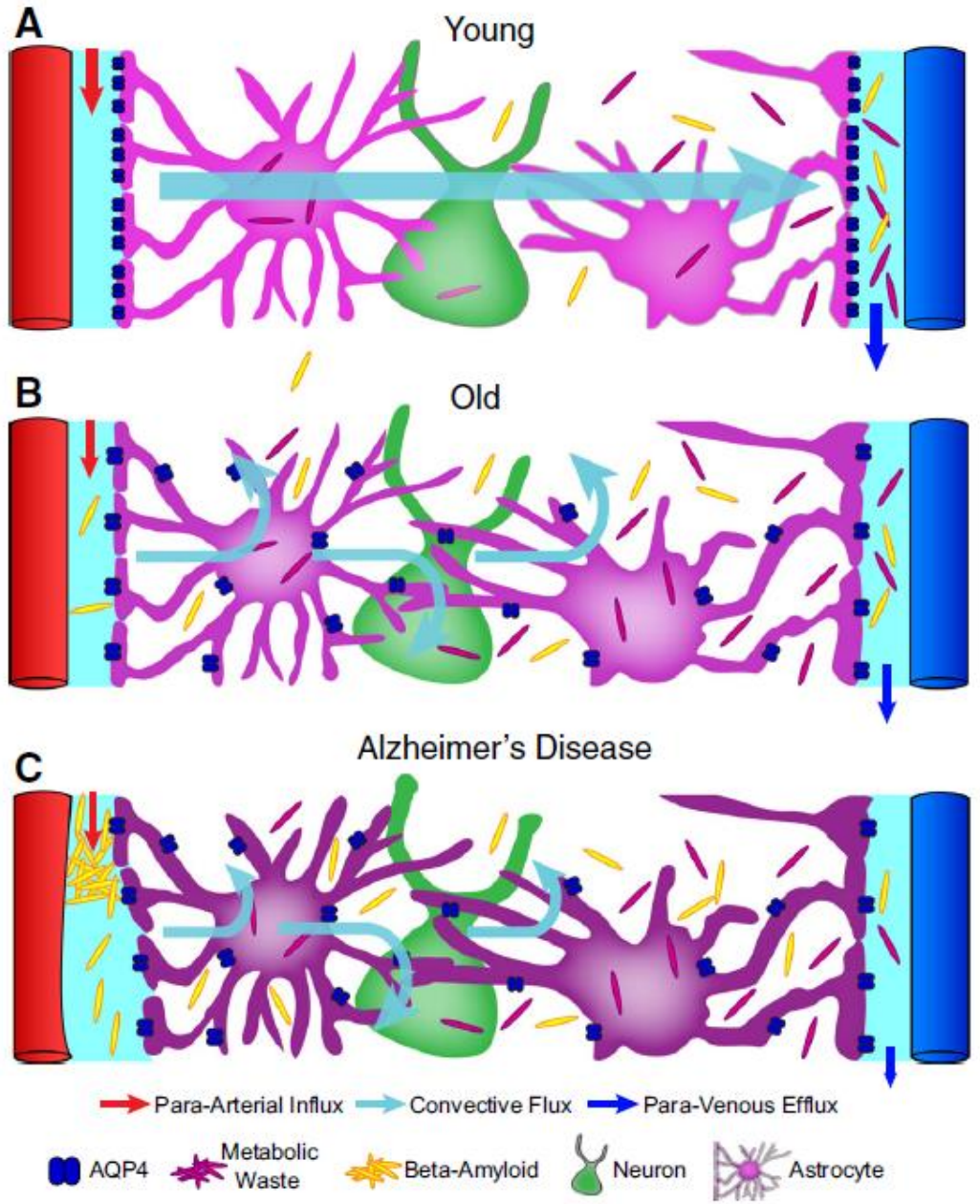
RESEARCH ARTICLE Open Access

Deletion of aquaporin-4 in APP/PS1 mice exacerbates brain Aβ accumulation and memory deficits

Zhiqiang Xu[1†], Na Xiao[1†], Yali Chen[1†], Huang Huang[1], Charles Marshall[2], Junying Gao[1], Zhiyou Cai[3], Ting Wu[4], Gang Hu[1] and Ming Xiao[1*]



Xu Z et al, 2015

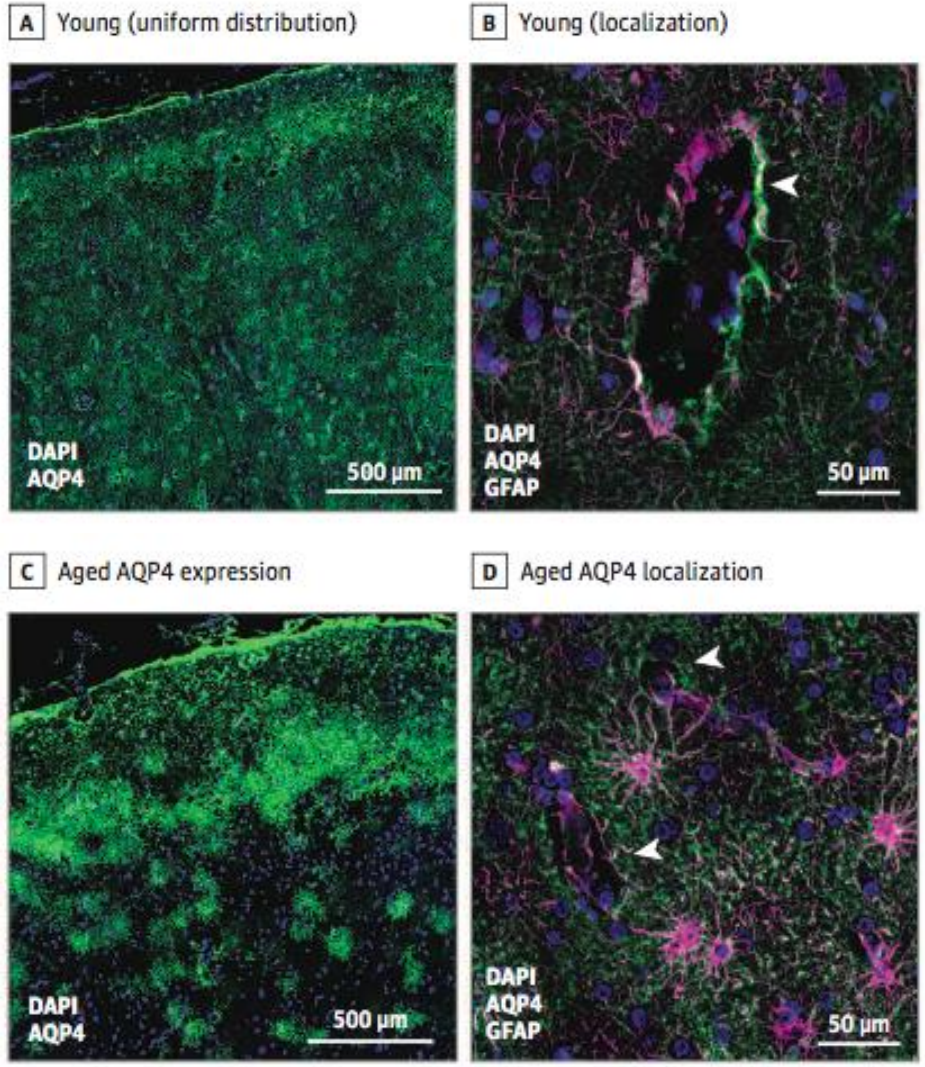


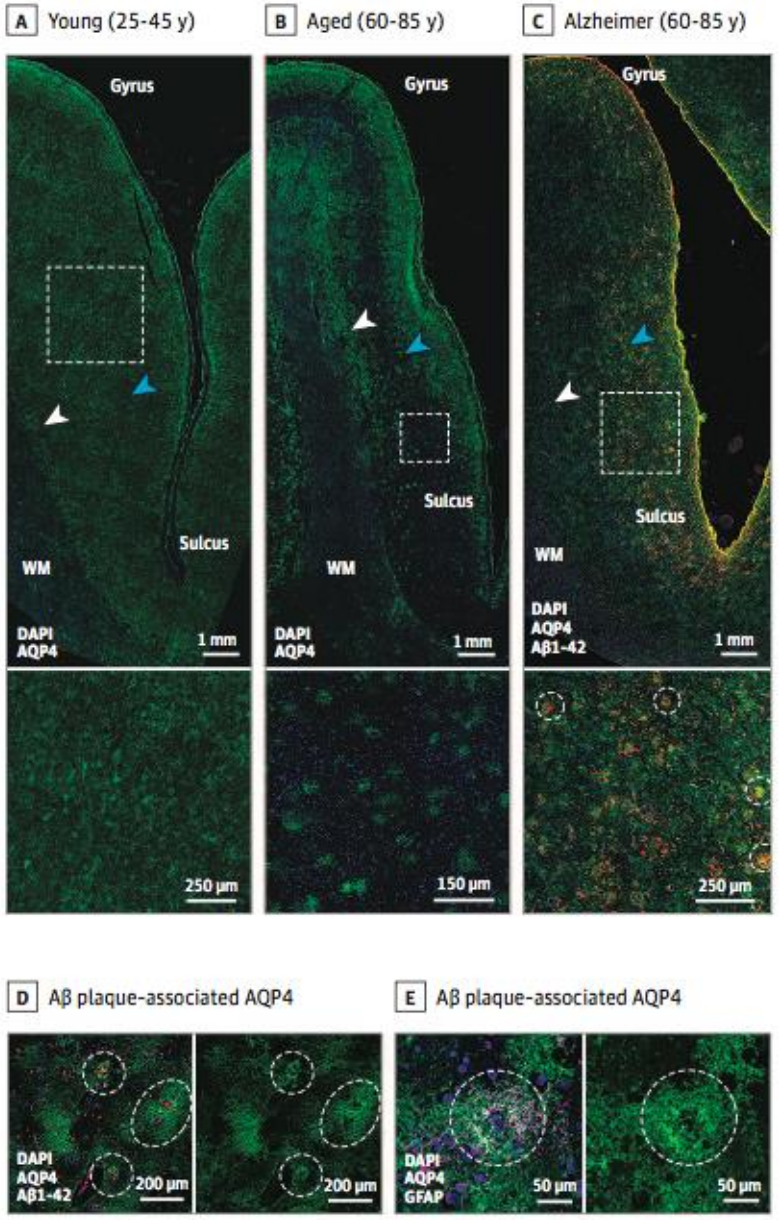
NA Jessen, 2015

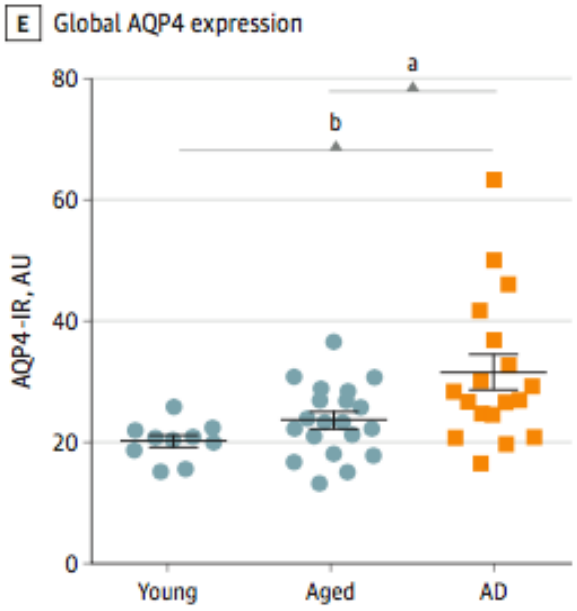
JAMA Neurology | Original Investigation

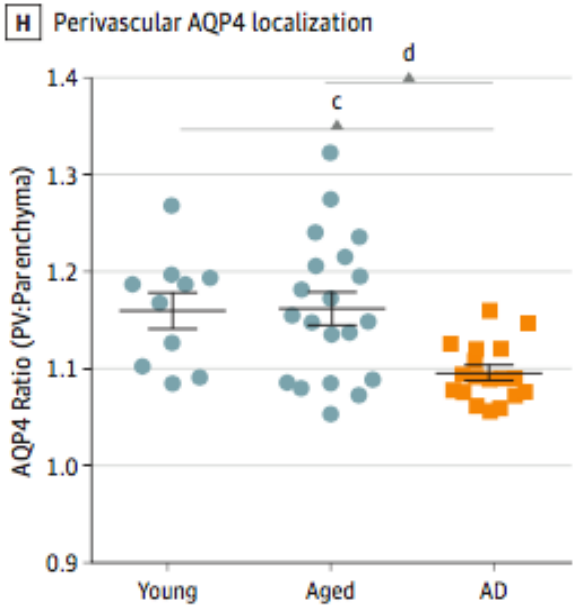
Association of Perivascular Localization of Aquaporin-4 With Cognition and Alzheimer Disease in Aging Brains

Douglas M. Zeppenfeld, BS; Matthew Simon, BS; J. Douglas Haswell, BS; Daryl D'Abreo; Charles Murchison, MS; Joseph F. Quinn, MD; Marjorie R. Grafe, MD, PhD; Randall L. Woltjer, MD, PhD; Jeffrey Kaye, MD; Jeffrey J. Iliff, PhD









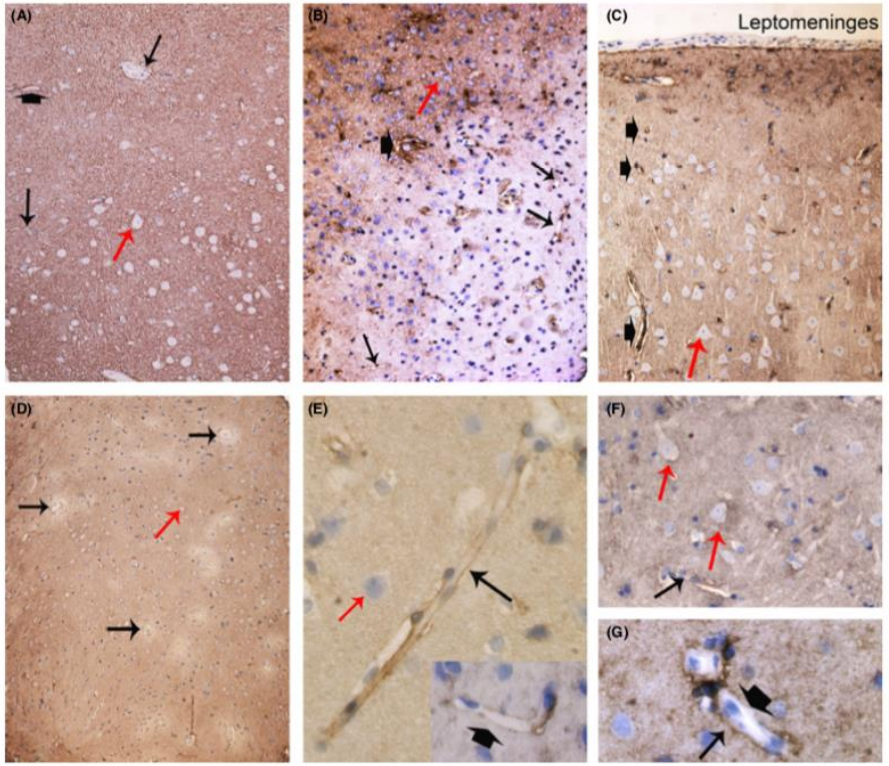
DM Zeppenfeld, 2017

Neuropathology and Applied Neurobiology (2017) doi: 10.1111/nan.12420

Astrogliosis and impaired aquaporin-4 and dystrophin systems in idiopathic normal pressure hydrocephalus

P. K. Eide*,† and H.-A. Hansson‡

*Department of Neurosurgery, Oslo University Hospital – Rikshospitalet, †Faculty of Medicine, University of Oslo, Oslo, Norway and ‡Institute of Biomedicine, University of Gothenburg, Göteborg, Sweden



PK Eide et al, 2017

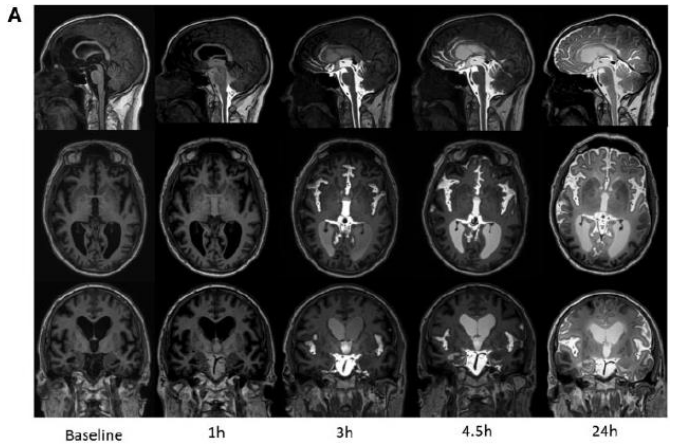
doi:10.1093/brain/awx191 BRAIN 2017: 140; 2691–2705 | 2691

BRAIN
A JOURNAL OF NEUROLOGY

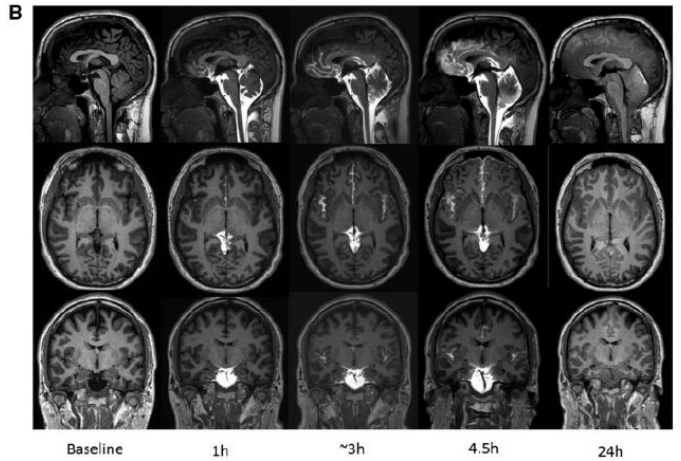
Glymphatic MRI in idiopathic normal pressure hydrocephalus

Geir Ringstad,[1,2] Svein Are Sirirud Vatnehol[3] and Per Kristian Eide[2,4]

NPH



Reference subjects



G Ringstad et al, 2017

Materials and methods

	Controls	AD	NPH
Number	9	11	10
Gender (M:F)	6:3	5:6	6:4
Age (years)	68.2 ± 7.7	71.8 ± 5.5	73.4 ± 6.0
MMSE	28.3/30 ± 1.5	25.4/30 ± 4.1	25.3/30 ± 4.8
GDS	1.2 ± 0.4	2.3 ± 0.8	1.9 ± 0.7

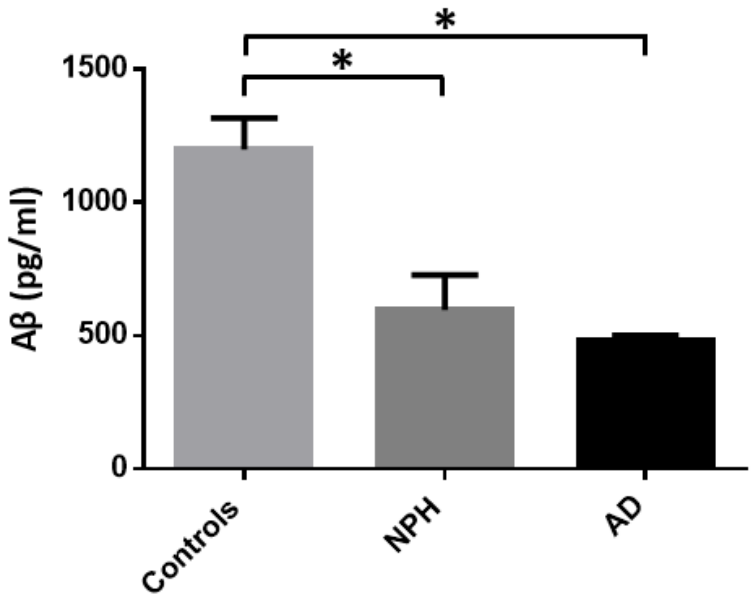
Diagnostic criteria
- AD → IWG2
- NPH →clinico-radiological presentation + tap-test

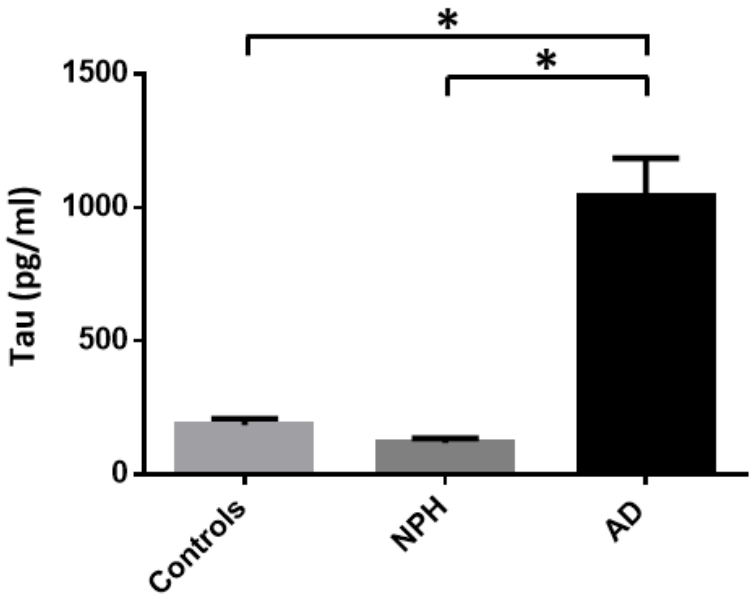
CSF

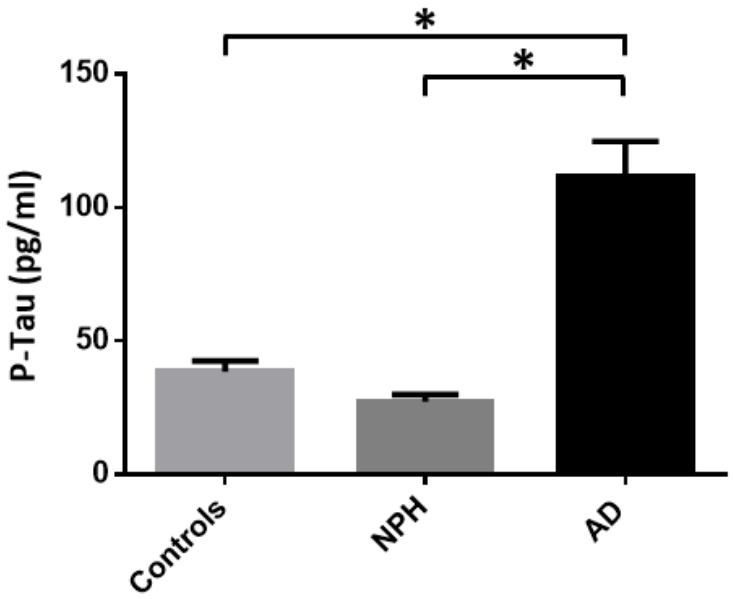
- Beta-Amyloid (Aβ) → ELISA kit (Fujirebio, Ghent, Belgium)
- Total Tau (Tau) → ELISA kit (Fujirebio, Ghent, Belgium)
- Phospho Tau (P-Tau) → ELISA kit (Fujirebio, Ghent, Belgium)
- Aquaporin4 (AQP4) → ELISA kit (Abbexa, UK)

Results and discussion

	Control		NPH		AD		Kruskal Wallis test	Intergroup comparison		
	Mean	SD	Mean	SD	Mean	SD		Con vs NPH	Con vs AD	NPH vs AD
Amyloid-β	1197.89	356.73	596.90	414.13	479.73	72.99	**0.0005**	**<0.05**	**<0.05**	>0.05
Total Tau	186.22	70.58	118.20	55.49	1043.36	472.08	**<0.0001**	>0.05	**<0.05**	**<0.05**
Phospho Tau	38.56	12.20	27.30	8.33	111.73	43.50	**<0.0001**	>0.05	**<0.05**	**<0.05**
Aquaporin 4	1.17	0.17	1.07	0.17	1.02	0.10	**0.0405**	>0.05	**<0.05**	>0.05







Cerebrospinal Fluid Amyloid-β 42, Total Tau and Phosphorylated Tau are Low in Patients with Normal Pressure Hydrocephalus: Analogies and Differences with Alzheimer's Disease

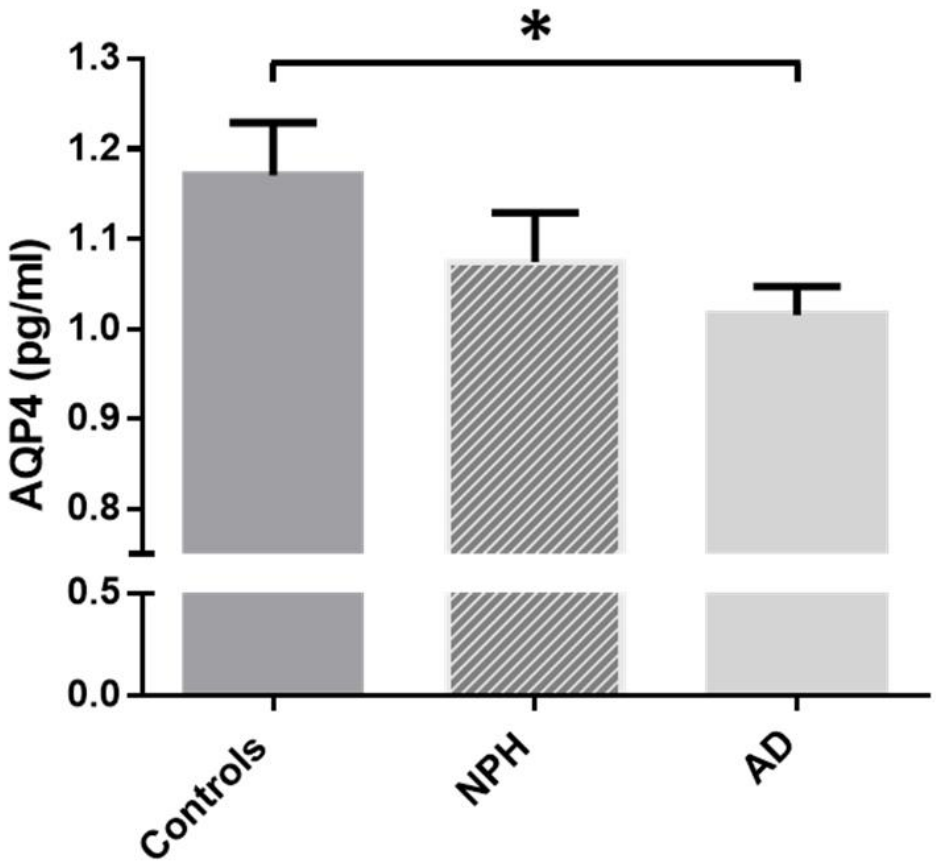
Roberto Santangelo[a,*], Giordano Cecchetti[a], Maria Paola Bernasconi[a], Rosalinda Cardamone[a], Alessandra Barbieri[a], Patrizia Pinto[b], Gabriella Passerini[c], Francesco Scomazzoni[d], Giancarlo Comi[a] and Giuseppe Magnani[a]

R Santangelo, 2017

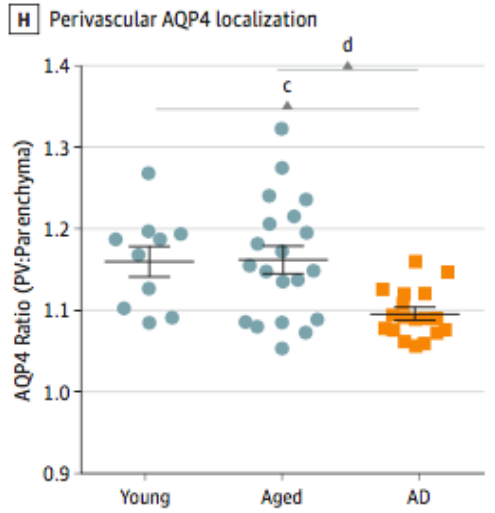
Revisiting the Cerebrospinal Fluid Biomarker Profile in Idiopathic Normal Pressure Hydrocephalus: The Bologna Pro-Hydro Study

Samir Abu-Rumeileh[a], Giulia Giannini[a], Barbara Polischi[b], Luca Albini-Riccioli[b], David Milletti[b], Federico Oppi[b], Michelangelo Stanzani-Maserati[b], Sabina Capellari[a,b], Paolo Mantovani[b], Giorgio Palandri[b], Pietro Cortelli[a,b], Sabina Cevoli[b] and Piero Parchi[b,c,*]

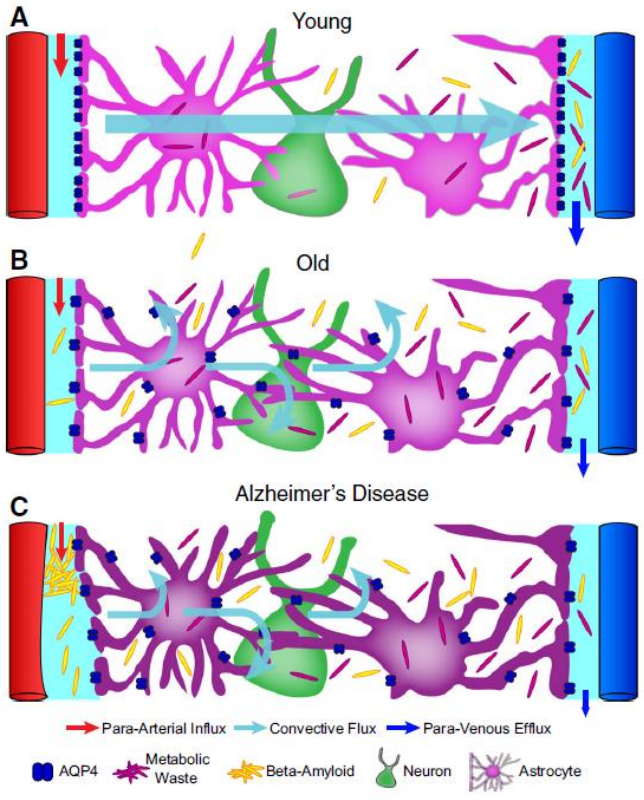
S Abu-Rumeileh, in press

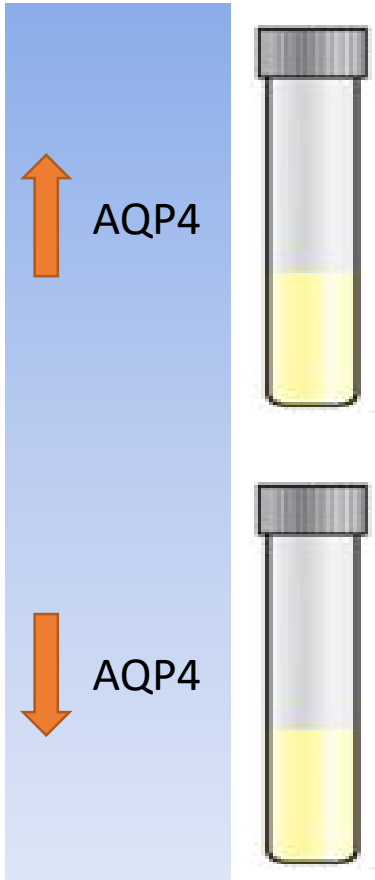


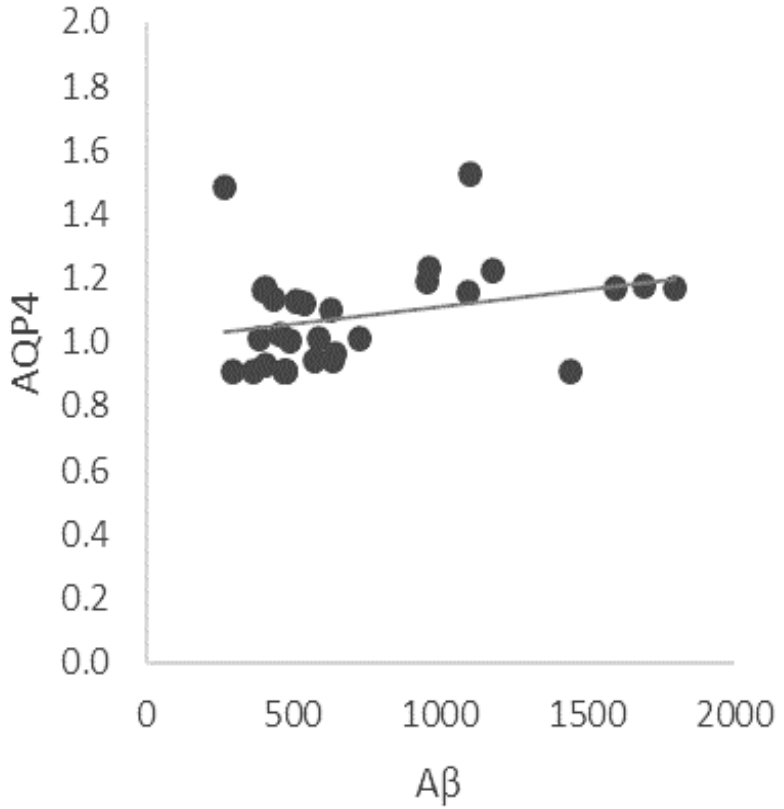
Column graphs showing mean and standard error of the mean (SEM) of Aquaporin4 (AQP4) cerebrospinal fluid values in controls, in patients with normal pressure hydrocephalus (NPH) and in patients with Alzheimer's disease (AD).
AD patients compared to controls showed a significant decrease of AQP4 in CSF ($p<0.05$, with correction for multiple comparison).



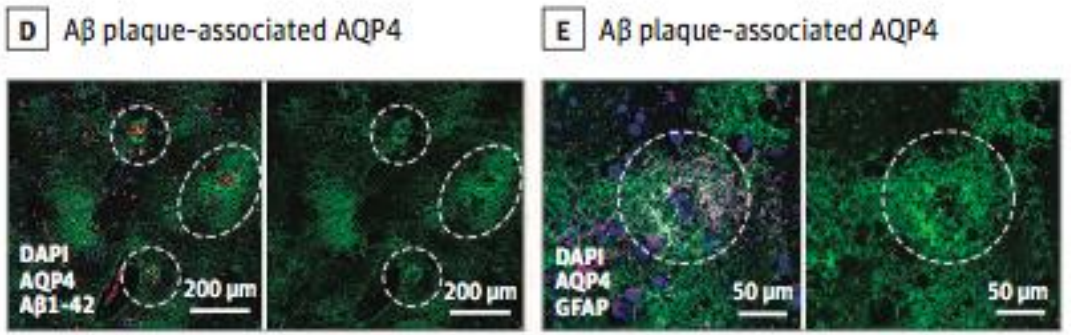
DM Zeppenfeld, 2017



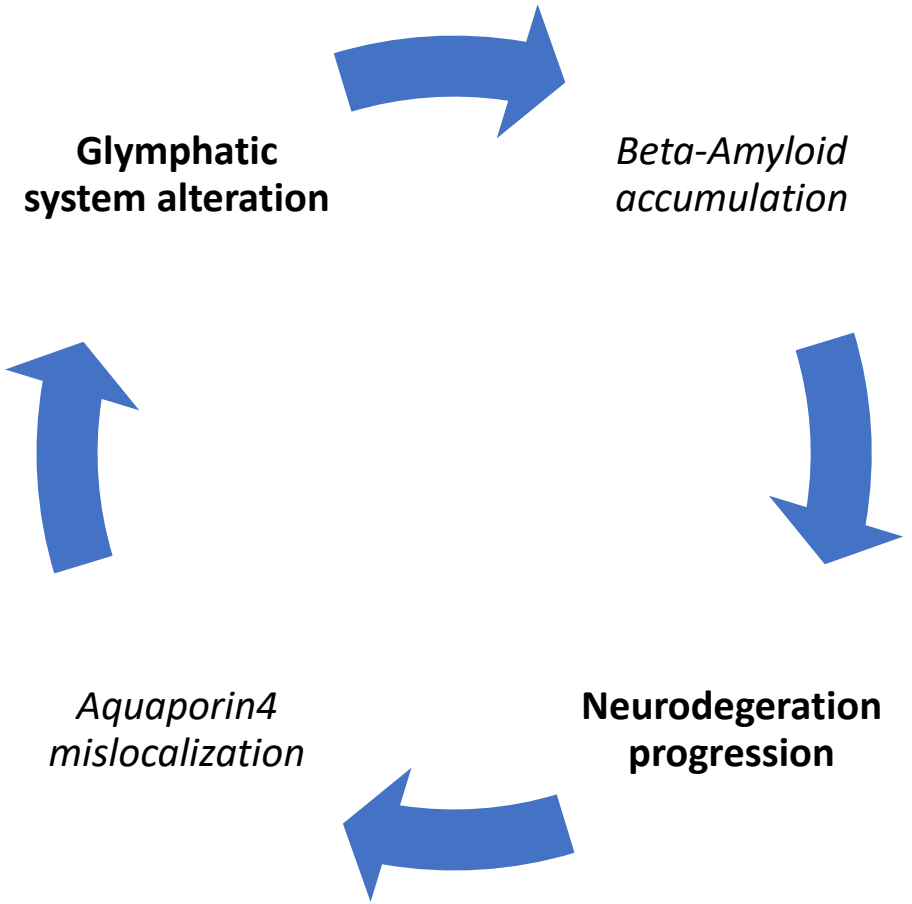




Correlation between Aquaporin4 (AQP4) values and Amyloid-β (Aβ) levels in cerebrospinal fluid
Spearman's correlation coefficient 0.373, p=0.042



DM Zeppenfeld, 2017



- **Reduced levels of AQP4 in AD**

→ explained by the mislocalization of AQP4 related with the loss of perivascular AQP4

[Rasmussen MK et al, 2018; Zeppenfeld DM et al, 2017]

- **AQP4 reduction trend in NPH**

→ explained by reduced AQP4 expression in astrocytic endfeet *[Eide PK and Hansson HA, 2018]*

- **Correlation between Aβ and AQP4 levels in CSF**

→ explained by correlation between Aβ deposition and glymphatic system dysfunction

[Zeppenfeld DM et al, 2017]

The low levels of AQP4 in both AD and NPH patients and the correlation with Aβ levels may be **the link between these two neurodegenerative diseases** *[Golomb J et al, 2000; Santangelo R et al, 2017; Abu-Rumeileh S eta al, 2019]*

The major limitation of the present study is the **small number of participants**, although very well characterized by using CSF biomarkers in AD group and tap-test in NPH group


The Future
NEXT EXIT

Population

Larger groups of patients and controls and including groups with other neurodegenerative disease (AD, FTD, LBD → 150)

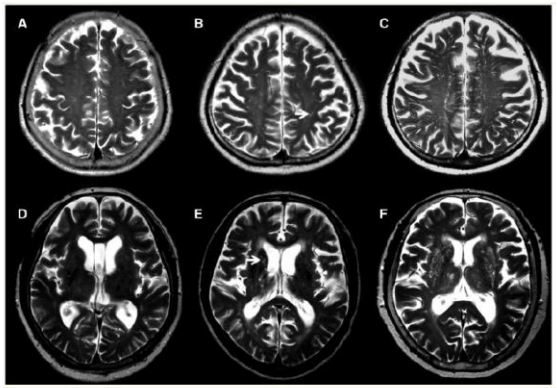
A new biomarker????

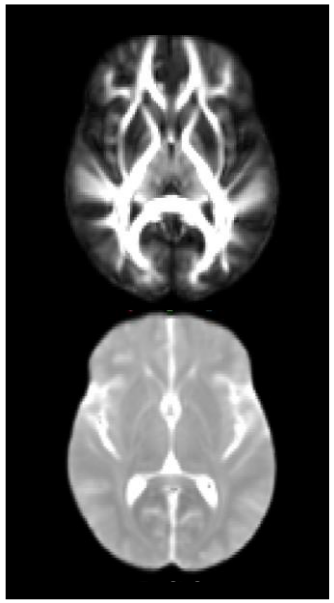
Neuroimaging

- Perivascular space
- Microstructural damage (DTI → MD and FA maps)

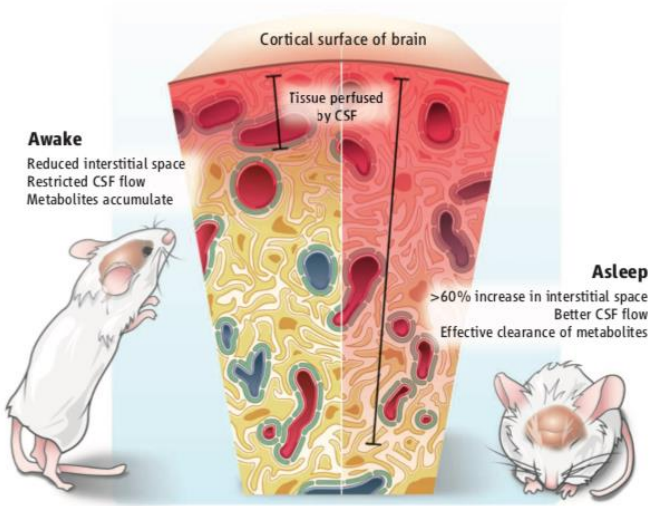
MRI-visible perivascular space location is associated with Alzheimer's disease independently of amyloid burden

Gargi Banerjee,[1] Hee Jin Kim,[2,3] Zoe Fox,[4] H. Rolf Jäger,[5] Duncan Wilson,[1] Andreas Charidimou,[1] Han Kyu Na,[2] Duk L. Na,[2,3] Sang Won Seo[2,3] and David J. Werring[1]





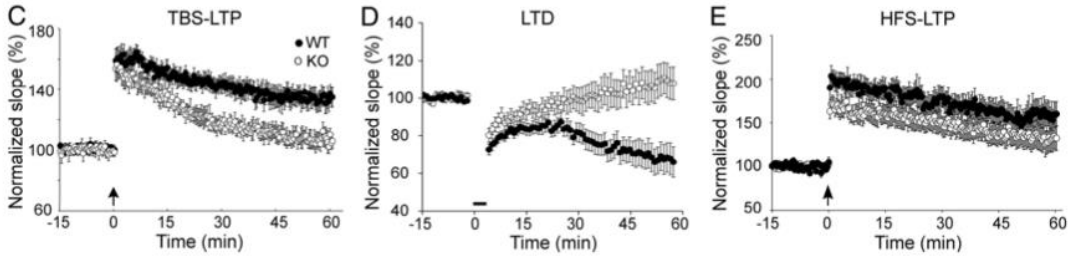
Sleep



TMS

Impairment of Select Forms of Spatial Memory and Neurotrophin-Dependent Synaptic Plasticity by Deletion of Glial Aquaporin-4

Vanessa A. Skucas,[1] Ian B. Mathews,[1] Jianmin Yang,[2] Qi Cheng,[1] Andrew Treister,[3] Aine M. Duffy,[1] Alan S. Verkman,[4] Barbara L. Hempstead,[2] Marcelo A. Wood,[3] Devin K. Binder,[5] and Helen E. Scharfman[1,6]



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UOC Neurochirurgia

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