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

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

Aleksanteri Aspelund,1,2 Salli Antila,1,3 Steven T. Proulx,1 Tine Veronika Karlsen,4 Sinem Karaman,3 Michael Detmar,3 Helge Wiig,4 and Kari Alitalo1,2

A Louveau, 2015

A Aspelund, 2015
The neurovascular unit
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
Aquaporins (AQPs) are transmembrane proteins that facilitate the movement of water across cell membranes. They consist of six transmembrane helices, each with a hydrophilic domain in the middle that forms a water-conducting channel.

Water-selective aquaporins
2. Fluid secretion
Aquaporins increase transcellular water transport in response to osmotic gradients.

3. Kidney tubule fluid absorption
Aquaporins (AQPs) facilitate water absorption in the kidneys. In the presence of AQPs, water is absorbed from the tubule lumen into the interstitial space.

4. Brain water balance
Aquaporin-4 (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. AQP4 in cell migration – a model
This model shows AQP4 facilitated entry of water at the leading edge of a migrating cell.

from Journal of Cell Science, 2011
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.
Deletion of aquaporin-4 in APP/PS1 mice exacerbates brain Aβ accumulation and memory deficits

Zhiqiang Xu1, Na Xiao1, Yali Chen1, Huang Huang1, Charles Marshall2, Junying Gao1, Zhiyou Cai3, Ting Wu4, Gang Hu5 and Ming Xiao1

Xu Z et al, 2015
Association of Perivascular Localization of Aquaporin-4 With Cognition and Alzheimer Disease in Aging Brains

A Young (uniform distribution)  B Young (localization)

Aged AQP4 expression  Aged AQP4 localization

Ab plaque-associated AQP4  Ab plaque-associated AQP4

Global AQP4 expression

Perivascular AQP4 localization

AQP4 Ratio (PV2/2x cereb)
Astrogliosis and impaired aquaporin-4 and dystrophin systems in idiopathic normal pressure hydrocephalus

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Glymphatic MRI in idiopathic normal pressure hydrocephalus

Geir Ringstad,1,2 Svein Are Sirirud Vatnehol3 and Per Kristian Eide3,4

NPH

Reference subjects

PK Eide et al, 2017

G Ringstad et al, 2017
Materials and methods
<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>AD</th>
<th>NPH</th>
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</thead>
<tbody>
<tr>
<td><strong>Number</strong></td>
<td>9</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td><strong>Gender (M:F)</strong></td>
<td>6:3</td>
<td>5:6</td>
<td>6:4</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>68.2 ± 7.7</td>
<td>71.8 ± 5.5</td>
<td>73.4 ± 6.0</td>
</tr>
<tr>
<td><strong>MMSE</strong></td>
<td>28.3/30 ± 1.5</td>
<td>25.4/30 ± 4.1</td>
<td>25.3/30 ± 4.8</td>
</tr>
<tr>
<td><strong>GDS</strong></td>
<td>1.2 ± 0.4</td>
<td>2.3 ± 0.8</td>
<td>1.9 ± 0.7</td>
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</tbody>
</table>

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

- 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
<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>NPH</th>
<th>AD</th>
<th>Kruskal Wallis test</th>
<th>Intergroup comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
<td>Con vs NPH</td>
</tr>
<tr>
<td><strong>Amyloid-β</strong></td>
<td>1197.89</td>
<td>356.73</td>
<td>596.90</td>
<td>414.13</td>
<td>*0.0005</td>
</tr>
<tr>
<td><strong>Total Tau</strong></td>
<td>186.22</td>
<td>70.58</td>
<td>118.20</td>
<td>55.49</td>
<td>*&lt;0.0001</td>
</tr>
<tr>
<td><strong>Phospho Tau</strong></td>
<td>38.56</td>
<td>12.20</td>
<td>27.30</td>
<td>8.33</td>
<td>*&lt;0.0001</td>
</tr>
<tr>
<td><strong>Aquaporin 4</strong></td>
<td>1.17</td>
<td>0.17</td>
<td>1.07</td>
<td>0.17</td>
<td>*0.0405</td>
</tr>
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</table>

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

R Santangelo, 2017

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

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

Sleep

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

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