Supplementary Material

Cerebrospinal Fluid Panel of Synaptic Proteins in Cerebral Amyloid Angiopathy and Alzheimer's Disease

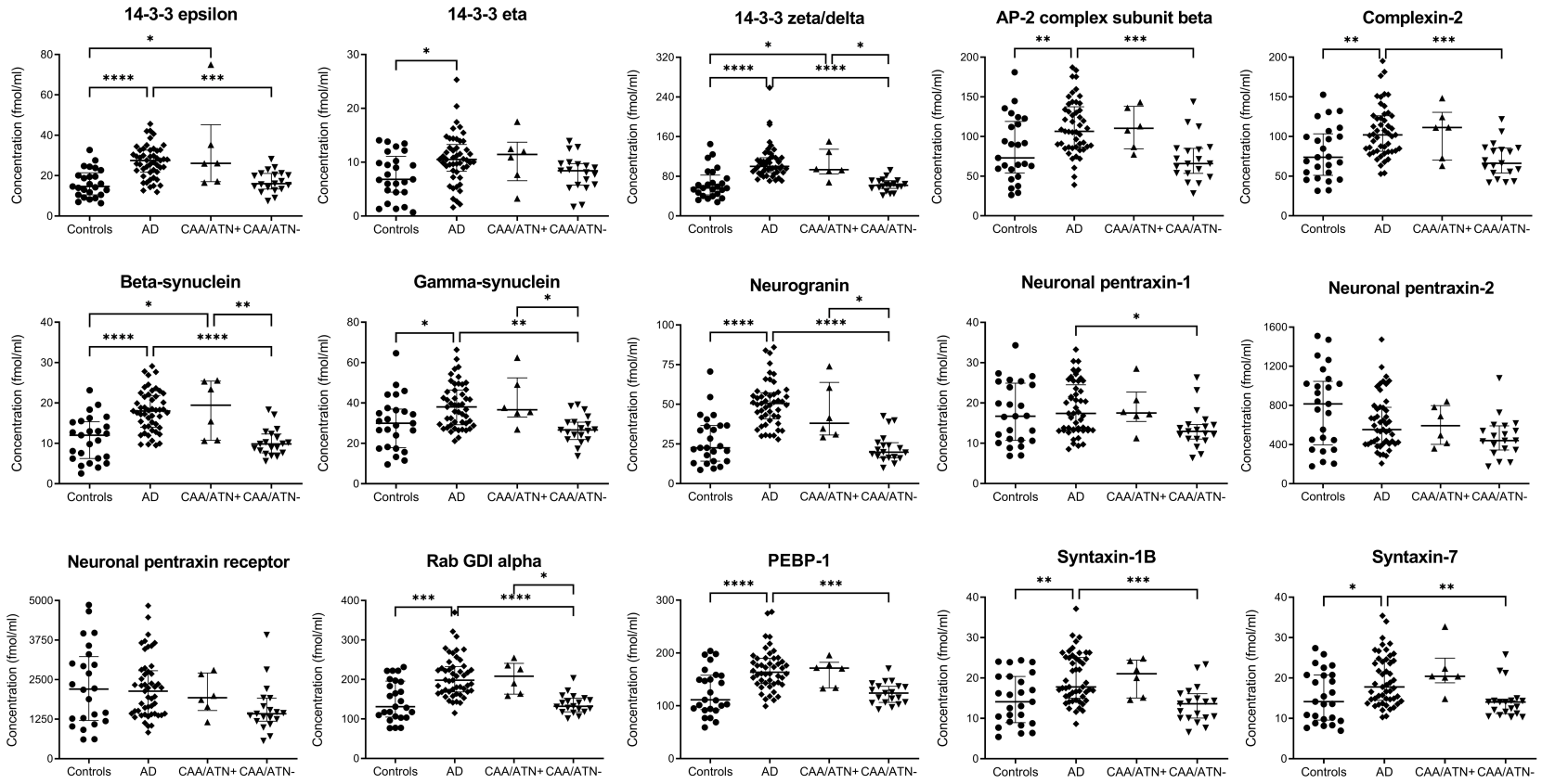
Cerebrospinal fluid collection

Lumbar punctures were performed following standard procedures. Cerebrospinal fluid (CSF) was collected in polypropylene tubes, centrifuged, and aliquoted. Samples were stored in polypropylene tubes at –80°C until analysis.

Ethical statement

Lumbar punctures were performed after obtaining informed consent from all participants or their legal representatives. CSF of cerebral amyloid angiopathy (CAA) patients was collected in the context of a clinical workup (n=2) or two cross-sectional studies investigating new CSF biomarkers for CAA (Cerebral Amyloid Angiopathy: Vascular Imaging and fluid markers of Amyloid deposition [CAVIA; n=3]; BIOmarkers for cogNitive Impairment due to Cerebral amyloid angiopathy study [BIONIC; http://www.radboudumc.nl/BCS; n=20]). All Alzheimer's disease (AD) CSF samples were collected in the context of clinical workup. Most control participants (n=19) underwent a lumbar puncture as routine diagnostic workup to exclude central nervous system involvement of a systemic disease, a central neurological cause for their symptoms or neurological infection or inflammation. Exclusion criteria were neurodegenerative disease, known cognitive impairment, sepsis, a recent stroke (<6 months) or a malignancy in the central nervous system. CSF of four controls was collected as part of the BIONIC study. They were selected based upon the absence of (subjective) memory complaints, a recent (<6 months) stroke or a neurodegenerative disease, and a modified Telephone Interview of Cognitive Status score of ≥35 or a Mini-Mental State Examination score of ≥28. Two controls were patients that underwent thoracoabdominal aortic aneurism repair, for which they had an external lumbar drain, of which CSF was sampled before the operation. They did not have known cognitive impairment or recent (<3 months) stroke of traumatic brain injury. This study was approved by the Medical Ethics Committee Arnhem-Nijmegen (file numbers 2016-3011 (controls and AD patients), 2017-3810 (BIONIC), and 2014-1401 (CAVIA)).

Supplementary Figure 1. Cerebrospinal fluid levels of synaptic proteins in controls, AD, CAA/ATN+, and CAA/ATN-. Concentrations (fmol/ml) were obtained after multiple reaction monitoring analysis of the synaptic proteins.



Statistical comparison was performed with analysis of variance with Bonferroni's post hoc test, or Kruskal-Wallis with Dunn's post hoc test, as appropriate. p values: *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, ****$p \leq 0.0001$. Median values and interquartile range are indicated. AD, Alzheimer's disease; AP-2, activating protein 2; ATN, amyloid/tau/neurodegeneration; CAA, cerebral amyloid angiopathy; GDI, GDP dissociation inhibitor; PEBP-1, phosphatidylethanolamine-binding protein 1.

Supplementary Table 1. Quality control inter- and intra-assay variation for all peptides in the analysis.

Protein	Peptide sequence	Inter-assay CV (%)	Intra-assay CV (%)
14-3-3 epsilon	IISSIEQK	0.6	6.6
14-3-3 eta	AVTELNEPLSNEDR	0.9	11.8
14-3-3 zeta/delta	VVSSIEQK	0.3	6.0
AP-2 complex subunit beta	IQPGNPNYTLSLK	1.0	4.5
Complexin-2	AALEQPCEGSLTRPK	0.3	3.8
Beta-synuclein	EGVVQGVASVAEK	9.7	10.8
Gamma-synuclein	ENVVQSVTSVAEK	4.3	8.3
Neurogranin	KGPGPGGPGGAGVAR	1.6	5.3
Neuronal pentraxin-1	ETVLQQK[#]	0.8	4.4
Neuronal pentraxin-1	CESQSTLDPGAGEAR	6.1	7.8
Neuronal pentraxin-1	LENLEQYSR	2.9	13.2
Neuronal pentraxin-2	VAELEDEK[#]	0.2	4.4
Neuronal pentraxin-2	ETVVQQK	2.6	9.3
Neuronal pentraxin-2	WPVETCEER	0.5	5.0
Neuronal pentraxin receptor	NNYMYAR[#]	4.7	5.0
Neuronal pentraxin receptor	LVEAFGGATK	0.9	7.6
Rab GDI inhibitor	QLICDPSYIPDR	3.0	4.4
PEBP-1	LYEQLSGK[#]	0.1	3.5
PEBP-1	NRPTSISWDGLDSGK	2.0	4.5
Syntaxin-1b	QHSAILAAPNPDEK	6.4	9.0
Syntaxin-7	EFGSLPTTPSEQR	0.6	6.1

AP-2, activating protein 2; CV, coefficient of variation; GDI, GDP dissociation inhibitor; PEBP-1, phosphatidylethanolamine-binding protein 1.

[#]Peptide used in statistical comparisons.

Supplementary Table 2. Cerebrospinal fluid levels of synaptic proteins in controls, AD, and CAA.

Synaptic protein (fmol/ml)	Controls (n = 25)	AD (n = 49)	CAA (n = 25)	*p*
14-3-3 protein epsilon	14.6 [10.5 – 21.4]	27.5 [11.9 – 32.6]	17.3 [14.5 – 23.6]	**<0.0001**[b,#,$]
14-3-3 protein eta	6.82 [4.43 – 11.1]	10.5 [8.32 – 13.3]	8.60 [5.80 – 11.4]	**0.01**[a,$]
14-3-3 protein zeta/delta	56.3 [42.6 – 82.3]	99.9 [87.4 – 118]	64.5 [58.0 – 85.9]	**<0.0001**[b,#,$]
AP-2 complex subunit beta	73.1 [54.1 – 119]	107 [86.7 – 138]	77.4 [56.3 – 110]	**0.0004**[a,#,$]
Complexin-2	73.9 [51.0 – 104]	102 [81.4 – 126]	72.7 [57.1 – 98.7]	**0.0004**[a,#,$]
Beta-synuclein	12.0 [6.25 – 15.4]	18.0 [14.0 – 21.9]	10.2 [8.48 – 14.5]	**<0.0001**[b,#,$]
Gamma-synuclein	30.0 [17.8 – 37.0]	38.0 [29.2 – 46.4]	27.4 [24.1 – 36.2]	**0.001**[b,#,$]
Neurogranin	22.6 [14.1 – 36.6]	50.6 [40.8 – 57.3]	24.0 [16.9 – 36.8]	**<0.0001**[b,#,$]
NPTX1	16.7 [10.7 – 24.9]	17.4 [13.3 – 24.5]	13.8 [11.2 – 17.5]	0.08[b]
NPTX2	816 [397 – 1046]	552 [409 – 780]	488 [359 – 608]	**0.04**[b,&]
NPTXR	2201 [1211 – 3230] (n = 24)	2136 [1423 – 2784]	1472 [1217 – 1957]	0.06[b]
Rab GDI alpha	131 [105 – 196]	198 [168 – 233]	144 [126 – 168]	**<0.0001**[b,#,$]
PEBP-1	111 [92.8 – 158]	164 [142 – 190] (n = 48)	133 [110 – 147]	**<0.0001**[a,#,$]
Syntaxin-1B	14.1 [8.97 – 20.4]	17.8 [15.0 – 25.1]	14.4 [10.3 – 18.9]	**0.0004**[b,#,$]
Syntaxin-7	14.2 [9.58 – 20.8]	17.8 [14.5 – 24.5]	14.3 [11.9 – 20.4]	**0.01**[b,$]

Data are presented as medians and interquartile range. Bold *p* values indicate statistical significance. AD, Alzheimer's disease based on A+T+N+ classification; AP-2, activating protein 2; CAA, cerebral amyloid angiopathy; GDI, GDP dissociation inhibitor; NPTX1, neuronal pentraxin-1; NPTX2, neuronal pentraxin-2; NPTXR, neuronal pentraxin receptor; PEBP-1, phosphatidylethanolamine-binding protein 1.

[a]Analysis of variance with Bonferroni's post hoc test.

[b]Kruskal-Wallis test with Dunn's post hoc test.

[#]Statistically significant for AD versus CAA

[$]Statistically significant for controls versus AD

[&]Statistically significant for controls versus CAA

Supplementary Table 3. Magnetic resonance imaging parameters and cognitive assessment score for CAA/ATN+ and CAA/ATN- patients.

	CAA/ATN+ (n = 6)	**CAA/ATN- (n = 19)**	***p***
MRI parameters			
ICH			0.18[a]
No	5	10	
Yes	1	9	
Lobar CMB			0.15[a]
0	0	1	
1-5	0	8	
6-10	3	5	
11-15	0	2	
16-50	0	1	
≥51	3	2	
EPVS			0.81[a]
<21	1	4	
>20	5	15	
cSS			0.50[a]
Absent	4	8	
Focal	1	3	
Disseminated	1	8	
WMH			0.41[a]
0	0	0	
1	0	4	
2	1	4	
3	5	11	
SVD burden score			0.40[a]
0	0	0	
1	0	0	
2	0	4	
3	1	2	
4	4	6	
5	0	4	
6	1	3	
MoCA	21.8 ± 4.09 (n=5)	23.8 ± 3.53 (n=16)	0.31[b]

MoCA scores are presented as means ± standard deviations.

ATN, amyloid/tau/neurodegeneration; CAA, cerebral amyloid angiopathy; CMB, cortical microbleeds; cSS, cortical superficial siderosis; EPVS, enlarged perivascular spaces; ICH, intracerebral hemorrhage; MoCA, Montreal Cognitive Assessment; MRI, magnetic resonance imaging; SVD, small vessel disease; WMH, white matter hyperintensities.

[a]Chi-square test; [b]Student's t-test.