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

Emma van den Berg[a,1], Johanna Nilsson[b,1], Iris Kersten[a], Gunnar Brinkmalm[b,c], Anna M. de Kort[a], Catharina J.M. Klijn[a], Floris H.B.M. Schreuder[a], Lieke Jäkel[a], Johan Gobom[b,c], Erik Portelius[c], Henrik Zetterberg[b,c,d,e,f], Ann Brinkmalm[b,c], Kaj Blennow[b,c], H. Bea Kuiperij[a] and Marcel M. Verbeek[a,g,*]

[a]*Department of Neurology, Donders Institute for Brain, Cognition and Behaviour, Radboud University Medical Center, Nijmegen, The Netherlands*
[b]*Institute of Neuroscience and Physiology, the Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden*
[c]*Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden*
[d]*UK Dementia Research Institute at UCL, London, United Kingdom*
[e]*Department of Neurodegenerative Disease, UCL Institute of Neurology, London, United Kingdom*
[f]*Hong Kong Center for Neurodegenerative Diseases, Hong Kong, China*
[g]*Department of Laboratory Medicine, Radboud University Medical Center, Nijmegen, The Netherlands*

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Abstract.
Background: Alzheimer's disease (AD) and cerebral amyloid angiopathy (CAA) share pathogenic pathways related to amyloid-β deposition. Whereas AD is known to affect synaptic function, such an association for CAA remains yet unknown.
Objective: We therefore aimed to investigate synaptic dysfunction in CAA.
Methods: Multiple reaction monitoring mass spectrometry was used to quantify cerebrospinal fluid (CSF) concentrations of 15 synaptic proteins in CAA and AD patients, and age- and sex-matched cognitively unimpaired controls.
Results: We included 25 patients with CAA, 49 patients with AD, and 25 controls. Only neuronal pentraxin-2 levels were decreased in the CSF of CAA patients compared with controls ($p = 0.04$). CSF concentrations of 12 other synaptic proteins were all increased in AD compared with CAA or controls (all $p \leq 0.01$) and were unchanged between CAA and controls. Synaptic protein concentrations in the subgroup of CAA patients positive for AD biomarkers (CAA/ATN+; $n = 6$) were similar to AD patients, while levels in CAA/ATN- ($n = 19$) were comparable with those in controls. A regression model including all synaptic proteins differentiated CAA from AD at high accuracy levels (area under the curve 0.987).
Conclusion: In contrast to AD, synaptic CSF biomarkers were found to be largely unchanged in CAA. Moreover, concomitant AD pathology in CAA is associated with abnormal synaptic protein levels. Impaired synaptic function in AD was confirmed in this independent cohort. Our findings support an apparent differential involvement of synaptic dysfunction in CAA and AD and may reflect distinct pathological mechanisms.

Keywords: Alzheimer's disease, biomarkers, cerebral amyloid angiopathy, cerebrospinal fluid, synaptic pathology

[1]These authors contributed equally to this work.

*Correspondence to: Marcel M. Verbeek, Department of Neurology, Radboud University Medical Center, 830 TML, P.O. Box 9101, 6500 HB, Nijmegen, The Netherlands. E-mail: marcel.verbeek@radboudumc.nl.

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INTRODUCTION

Deposition of abnormally folded amyloid-β (Aβ) peptides is a common pathologic mechanism in both Alzheimer's disease (AD) and cerebral amyloid angiopathy (CAA). In AD, Aβ deposits as plaques in the brain parenchyma, whereas in CAA, Aβ aggregates are found within cortical and leptomeningeal blood vessel walls [1, 2]. Involvement of the same protein fits the high comorbidity of moderate-to-severe CAA observed in almost 50% of AD patients [3]. Symptoms of cognitive impairment and dementia are central to both diseases.

Cognitive dysfunction correlates strongly with synaptotoxicity in AD [2, 4], where synaptic loss is recognized as one of the earliest detectable events in AD pathogenesis. Elevated levels of synaptotagmin-1, growth-associated protein 43, synaptosomal-associated protein 25, and neurogranin have repeatably been demonstrated in the cerebrospinal fluid (CSF) of AD patients [5–7]. Similarly, increased levels of synaptic proteins involved in vesicular transport and synaptic stability have been observed in patients with mild cognitive impairment, particularly those who progressed to AD [8]. A meta-analysis confirmed widespread synaptic loss in AD, with endosomal pathways, vesicular assembly mechanisms, glutamate receptors, and axonal transport being primarily affected [9]. Liquid chromatography-mass spectrometry (LC-MS) methods demonstrated that CSF levels of beta- and gamma-synuclein, neurogranin, phosphatidylethanolamine-binding protein-1 (PEBP-1), 14–3–3 proteins, and neuronal pentraxins levels were altered in AD compared with healthy controls [10], suggesting these proteins may serve as synaptic biomarkers for AD.

In contrast to AD, possible synaptic dysfunction in CAA remains understudied. Vascular amyloid might inflict synaptic degeneration, since a mouse model of non-Aβ Danish CAA demonstrated impaired inhibitory synaptic pathways, and increased tau hyperphosphorylation and misfolding [11, 12]. In contrast, it has previously been demonstrated that CSF levels of the synaptic protein neurogranin are similar in controls and patients with CAA [13]. Studying synaptic dysfunction in CAA may elucidate underlying mechanisms leading to cognitive decline in CAA and reveal yet unknown interactions or differences with AD pathophysiology.

We therefore aimed to investigate synaptic dysfunction in CAA by employing the analysis of a synaptic protein panel in CSF in cohorts including patients with clinical CAA and AD, and controls. Furthermore, we aimed to explore the relation of synaptic protein CSF levels to cerebrovascular imaging markers and cognitive decline in CAA.

MATERIALS AND METHODS

Cohorts

We included CSF samples from 25 patients with probable CAA, 49 patients with AD, and 25 control participants from the Radboud University Medical Center (Radboudumc, Nijmegen, the Netherlands; Table). CSF was collected via lumbar puncture according to a standardized protocol. See the Supplementary Material for details on CSF sample collection and ethical statements for all study participants.

Probable CAA diagnosis was obtained via magnetic resonance imaging (MRI) analysis based on the modified Boston criteria [14]. Cognitive function was assessed using the Montreal Cognitive Assessment (MoCA) in 21 of the CAA patients [15]. AD patients had a positive amyloid/pathological tau/neurodegeneration (ATN) biomarker profile [16, 17], as defined by CSF $A\beta_{42} < 659$ pg/ml (A+), phosphorylated $tau_{181} > 64$ pg/ml (T+), and total tau > 400 pg/ml (N+) quantified by automated immunoassays using a Lumipulse apparatus (Fujirebio, Ghent, Belgium). Details on the selection of control participants are provided in the Supplementary Material. Age- and sex-matched control participants were cognitively unimpaired. Information on CAA imaging markers was available neither for AD patients, nor for controls.

Magnetic resonance imaging

All CAA patients underwent an MRI scan of the brain. Of those, twenty participants underwent a 3.0 Tesla MRI scan (Siemens Magnetom Prisma, Siemens Healthineers, Erlangen, Germany) using a 32-channel head coil. Participants were examined using a comprehensive protocol, and we analyzed the 3D multi-echo gradient echo T2*-weighted sequence (voxel size $0.8 \times 0.8 \times 0.8$ mm), the 3D T2-weighted sequence (voxel size $0.8 \times 0.8 \times 0.8$ mm), and 3D fluid-attenuated inversion recovery (FLAIR) sequence (voxel size $0.8 \times 0.8 \times 0.8$ mm). Magnitude and phase data from the multi-echo gradient sequence was processed to a susceptibility-weighted imaging (SWI) using the Contrast-weighted,

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Laplace-unwrapped, bipolar multi-Echo, ASPIRE-combined, homogeneous, improved Resolution SWI (CLEAR-SWI) method [18]. The remaining five participants underwent a 3.0 Tesla MRI using different Tesla systems with varying protocols in either the Radboud University Medical Center (Radboudumc, Nijmegen, the Netherlands) or referral hospitals, which at least included T2*-weighted images or SWI sequence images, FLAIR and T2 sequences.

The imaging markers that were assessed included lobar cerebral microbleeds (CMB), enlarged perivascular spaces (EPVS) in the centrum semi-ovale, cortical superficial siderosis (cSS), and white matter hyperintensities (WMH). CMBs within the parenchyma followed consensus criteria as small, rounded, well-defined hypointense lesions of 2–10 mm in size [19]. Number of lobar CMB was categorized for statistical comparisons (0; 1–5; 6–10; 11–15; 16–50; ≥ 51). EPVS were characterized as fluid-filled spaces in the centrum semi-ovale that follow the typical course of a vessel as it goes through grey or white matter, with signal intensity similar to CSF on all sequences [20]. EPVS were dichotomously scored as a low (≤ 20) or high incidence (≥ 21) [21]. cSS was characterized as linear residues of chronic blood products in the superficial cerebral cortical layers showing a distinctive gyriform pattern of low signal on blood-sensitive images [22]. cSS was scored as either absent, focal (≤ 3 sulci) or disseminated (≥ 4 sulci) [23]. Deep and periventricular WMH were ordinally scored using the Fazekas scale, ranging from 0–3 (none, punctuate, early confluent, confluent) [24].

The total burden of small vessel disease (SVD) ordinal score was computed based on the individual scores obtained from all four abovementioned parameters, ranging from 0–6 [21]. One point was granted for 2–5 lobar CMBs, and two points if ≥ 5 CMBs. One point was granted for a high incidence (≥ 21) of EPVS. One point was granted for focal cSS, and two points if disseminated cSS. One point was granted for either (early) confluent deep WMH (i.e., the region between juxtacortical and ventricular areas; Fazekas score ≥ 2), or irregular periventricular WMH spreading out into deep white matter (Fazekas score 3).

LC-MS/MS analysis

The panel of synaptic biomarkers for simultaneous quantification included 14–3–3 epsilon, 14–3–3 eta, 14–3–3 zeta/delta, activating protein-2 (AP-2) complex subunit beta, complexin-2, beta-synuclein, gamma-synuclein, neurogranin, neuronal pentraxin-1 (NPTX1), neuronal pentraxin-2 (NPTX2), neuronal pentraxin receptor (NPTXR), rab GDP dissociation inhibitor (GDI) alpha, PEBP-1, syntaxin-1B, and syntaxin-7. The applied methodology has been described in detail elsewhere [10]. See Supplementary Table 1 for all analyzed peptides. In brief, 100 μL CSF was mixed with stable isotope labeled peptide standards (internal standard), followed by sample preparation in a consecutive four-step process consisting of reduction, alkylation, tryptic digestion, and purification by solid-phase extraction. For multiple reaction monitoring MS quantitation, a micro-high-performance LC-MS system (6495 Triple Quadrupole LC/MS system, Agilent Technologies, Santa Clara, CA, USA), equipped with a Hypersil Gold reversed phase column (dim. 100×2.1 mm, particle size 1.9 μm, Thermo Fisher Scientific, Waltham, MA, USA) was used. Pooled CSF samples were used as quality control and injected at regular intervals to monitor assay performance over time and to assess inter- and intra-assay variation.

Data processing and statistical analyses

Skyline version 20.1 (MacCoss Lab, University of Washington, USA) was used for chromatographic spectra peak assessment and adjustment. Ratio of the total peak areas for each peptide and corresponding internal standard, multiplied by the amount of standard added per μL CSF, was used as relative peptide concentration for each peptide.

Data was analyzed using GraphPad Prism software version 9.0.0 (GraphPad Software, Inc., San Diego, CA, USA). Shapiro-Wilk tests were used to analyze data normality. Parametric data were analyzed with a Student's *t*-test or analysis of variance with Bonferroni's *post hoc* test. Non-parametric data were analyzed with a Kruskal-Wallis with Dunn's *post hoc* test. Categorical variables were analyzed with a Chi-square test. For proteins with multiple quantified peptides, the peptide with the lowest coefficients of variation (CV) was used for statistical analysis (Supplementary Table 1). Multiple logistic regression and receiver operating curve analyses were used to analyze diagnostic accuracy in differentiating CAA from AD including all synaptic proteins. Spearman rank correlations were used to evaluate associations between MoCA score and imaging markers with synaptic protein concentrations in

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Table 1
Cohort demographics

	Controls	AD	CAA	*p*
Demographics				
Number of patients	25	49	25	
Age (y)	71.9 ± 7.3	69.6 ± 7.2	73.2 ± 6.6	0.10[a]
Sex, M/F (% male)	12/13 (48%)	22/27 (45%)	13/12 (52%)	0.84[b]
Aβ/tau levels (pg/ml)				
$A\beta_{40}$	10,876 [6,419–14,737]	9,837 [7,574–11,727]	7,530 [6,125–8,391]	**0.002**[c,#,&]
$A\beta_{42}$	641 [530–1,116]	419 [307–482]	346 [288–410]	**< 0.0001**[c,$,&]
t-tau	256 [177–387]	856 [726–952]	391 [272–537]	**< 0.0001**[c,#,$]
p-tau_{181}	32.8 [25.8–51.6]	132 [115–148]	47.2 [34.6–61.8]	**< 0.0001**[c,#,$]

Age is presented as means ± standard deviations. Aβ and tau levels are presented as medians [interquartile range]. Bold *p* values indicate statistical significance. Aβ, amyloid-β peptide; AD, Alzheimer's disease; CAA, cerebral amyloid angiopathy; F, female; M, male; p-tau181, phosphorylated tau; t-tau, total tau. [a]Analysis of variance with Bonferroni's *post hoc* test. [b]Chi-square test. [c]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

CAA patients. *p* values ≤ 0.05 were considered significant.

To explore the association of synaptic dysfunction with the presence of AD pathology, we performed a subgroup analysis where CAA patients were subdivided into having a positive (CAA/ATN+) or negative (CAA/ATN-) ATN biomarker status.

RESULTS

The intra-assay CV was < 10% and the inter-assay CV < 14% for all peptides (Supplementary Table 1).

Synaptic protein differences

NPTX2 levels were decreased in CAA compared with controls ($p = 0.04$), but not in AD (Fig. 1 and Supplementary Table 2). All other synaptic proteins were present at similar concentrations in CAA and controls.

Twelve synaptic proteins displayed higher levels in AD compared with controls ($p < 0.01$ for syntaxin-7, gamma-synuclein, and 14–3–3 eta; $p < 0.001$ for syntaxin-1B, complexin-2, AP-2 complex subunit beta; $p < 0.0001$ for beta-synuclein, rab GDI alpha, PEBP-1, neurogranin, 14–3–3 epsilon and zeta/delta). Compared to CAA, levels in AD were also higher for gamma-synuclein ($p < 0.01$), syntaxin-1B, complexin-2, AP-2 complex subunit beta (all $p < 0.001$), beta-synuclein, rab GDI alpha, PEBP-1, neurogranin, 14–3–3 epsilon and zeta/delta (all $p < 0.0001$). Levels of NPTX1 and NPTXR were similar for all groups. All synaptic proteins combined differentiated CAA from AD with an area under the curve of 0.987 (95% confidence interval: 0.97–1.00, $p < 0.0001$).

Correlations with MoCA and imaging markers in CAA

Both NPTX2 ($r_s = 0.64$, $p = 0.002$) and NPTXR ($r_s = 0.49$, $p = 0.03$) correlated with MoCA score in CAA (Fig. 2). None of the imaging parameters correlated with any of the synaptic proteins.

Exploratory analysis including CAA/ATN+and CAA/ATN-

When we stratified CAA patients according to their ATN biomarker status, six CAA patients were AD biomarker positive (CAA/ATN+) and nineteen were negative (CAA/ATN-). The exploratory analysis showed that compared with CAA/ATN-, levels in AD were increased for NPTX1 ($p < 0.05$), gamma-synuclein, syntaxin-7 (both $p < 0.01$), 14–3–3 epsilon, AP-2 complex subunit beta, complexin-2, PEBP-1, syntaxin-1B (all $p < 0.001$), 14–3–3 zeta/delta, beta-synuclein, neurogranin, and rab GDI alpha (all $p < 0.0001$; Supplementary Figure 1). Five synaptic proteins displayed increased levels in CAA/ATN+compared with CAA/ATN- ($p < 0.05$ for 14–3–3 zeta/delta, gamma-synuclein, neurogranin, and rab GDI alpha; $p < 0.01$ for beta-synuclein).

Levels of 14–3–3 epsilon and zeta/delta, and beta-synuclein were increased in CAA/ATN+compared with controls (all $p < 0.05$). All synaptic proteins were present at similar concentrations in CAA/ATN- and controls, and in CAA/ATN+ and AD.

MoCA scores and cerebrovascular imaging markers did not differ between CAA/ATN+ and CAA/ATN- (Supplementary Table 3).

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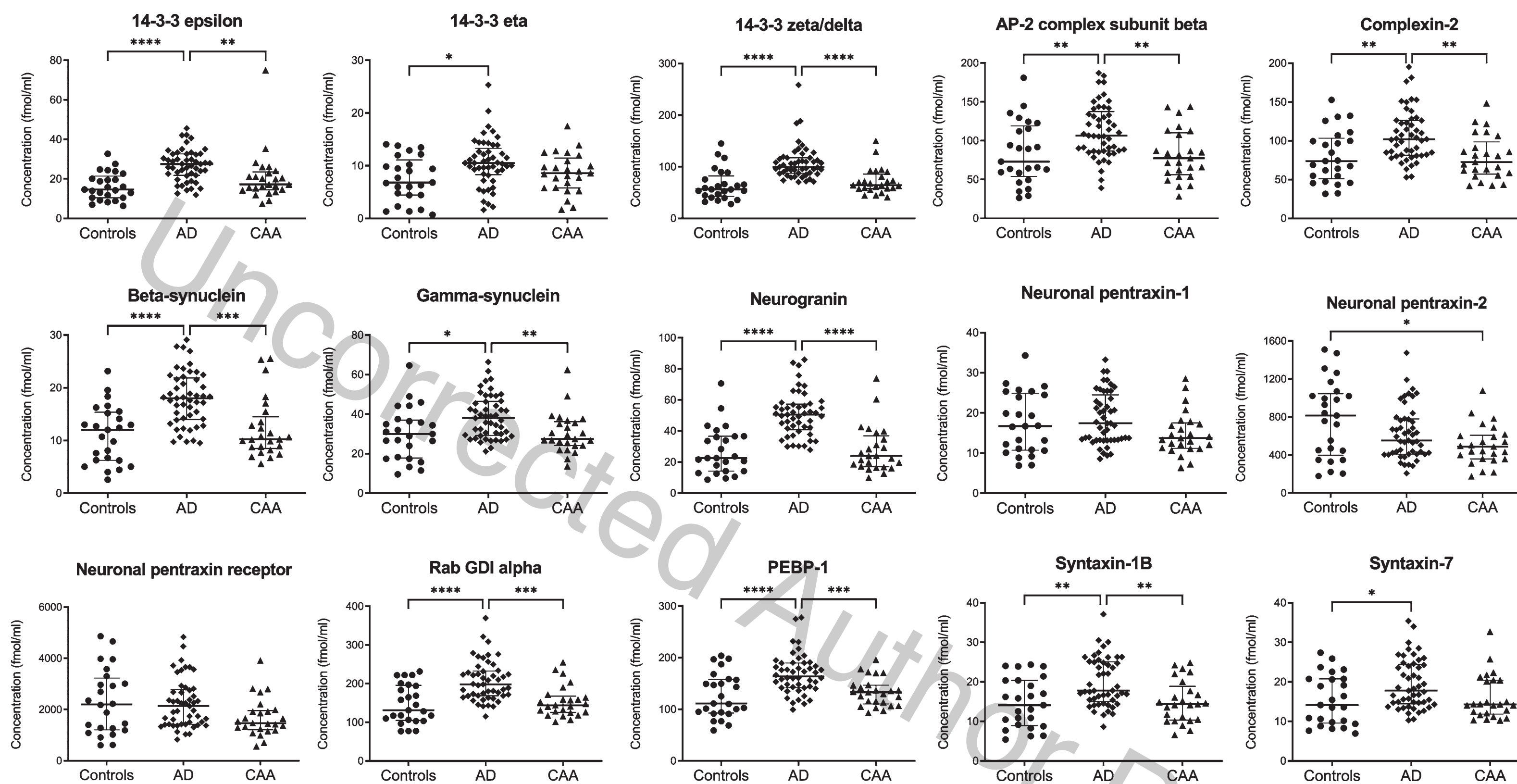

Fig. 1. Cerebrospinal fluid levels of synaptic proteins in controls, AD, and CAA. Concentrations (fmol/ml) were obtained after multiple reaction monitoring analysis of the synaptic proteins. Statistical comparison was performed with analysis of variance with Bonferroni's *post hoc* test, or Kruskal-Wallis 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; CAA, cerebral amyloid angiopathy; GDI, GDP dissociation inhibitor; PEBP-1, phosphatidylethanolamine-binding protein 1.

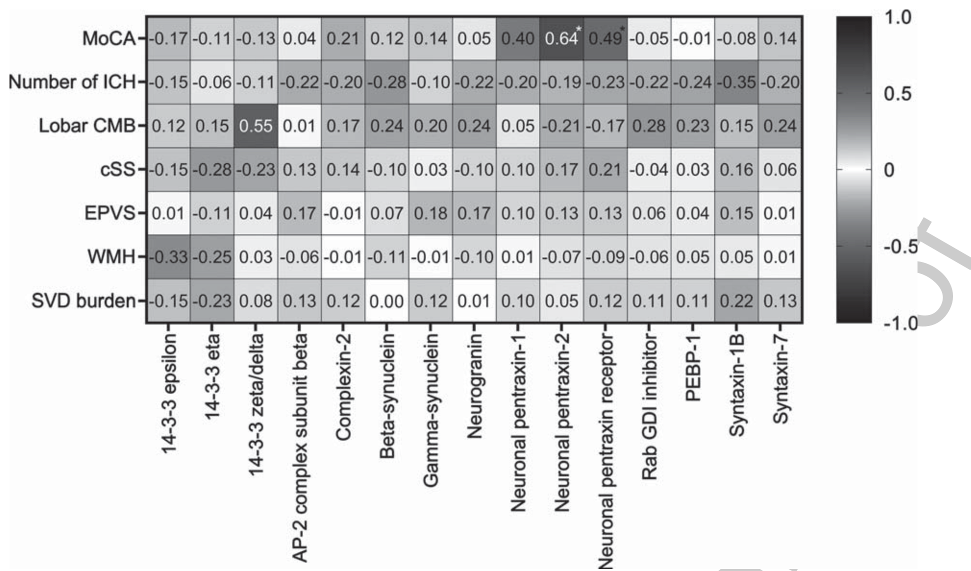

Fig. 2. Correlations of synaptic proteins with MoCA score and cerebrovascular imaging markers in CAA patients. Spearman rank correlation coefficients are displayed. MoCA was available in a subset ($n = 21$) of CAA patients. Asterix indicates a significant p value. AP-2, activating protein 2; CMB, cerebral microbleeds; cSS, cortical superficial siderosis; EPVS, enlarged perivascular spaces; GDI, GDP dissociation inhibitor; ICH, intracerebral hemorrhages; MoCA, Montreal Cognitive Assessment; PEBP-1, phosphatidylethanolamine-binding protein 1; SVD, small vessel disease; WMH, white matter hyperintensities.

DISCUSSION

In this study, we demonstrated a decrease of NPTX2 concentrations in the CSF of CAA patients, but other synaptic protein levels were unchanged in CAA. Moreover, we were able to confirm previous findings of synaptic degeneration in AD in our cohort. The synaptic panel differentiated CAA from AD at high accuracy levels. Finally, the exploratory analysis regarding CAA subgroups showed that CAA/ATN+ patients have a synaptic protein profile resembling that of AD patients, whereas CAA/ATN- were similar to controls.

Several synaptic proteins displayed higher CSF levels in AD as compared with both CAA and controls, coinciding with previous observations. Synapse loss is one of the main neurodegenerative mechanisms in AD, preceding indicators of neuronal death [25] and presenting during the preclinical disease stage [8]. Since synaptotoxicity and cognitive decline correlate well [2, 4], synaptic protein CSF levels may serve as indicators of disease severity in AD. In contrast to this synaptotoxicity in AD driven by parenchymal Aβ, growing evidence suggests that neurodegeneration induced by vascular Aβ in CAA more prominently presents as ischemic brain injury and vascular integrity loss, leading to progressive atrophy and cognitive decline [1, 26]. Thus, unchanged synaptic proteins levels in CAA might reflect mechanistic differences in the pathological pathways of AD and CAA. The observed similar synaptic protein concentrations in CAA/ATN- and controls underline the lack of synaptic loss in CAA in the absence of AD pathology.

Decreased levels of NPTXs secreted into the synaptic cleft, such as NPTX2, could reflect a different mechanisms of synaptic dysfunction in patients with CAA, in the form of inducing short-term or long-term depression by interacting with AMPA receptors to modulate synaptic plasticity [27, 28]. NPTX2 CSF levels correlate the best with cognitive status compared with other synaptic markers [10, 29]. Decreased NPTX2 CSF levels were previously reported in AD, however differentially expressed pentraxins have also been associated with other neurodegenerative diseases, including multiple sclerosis, frontotemporal dementia, and Parkinson's disease [27, 29]. Moreover, NPTX2 levels have been associated with processes like neuroinflammatory responses [30] and blood-brain barrier dysfunction [31]. Although NPTX2 levels correlated with MoCA scores in CAA, it is questionable whether the lowered NPTX2 levels in CAA display disease-specific synaptotoxicity, or rather reflect universal neurodegeneration. Additionally, synaptic protein levels of CAA/ATN+ resembled that of AD patients, indi-

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cating that AD pathology may be driving synaptic dysfunction. As opposed to NPTXs, all other synaptic proteins included in the current study are located at presynaptic and postsynaptic terminals, and not secreted into the synaptic cleft [10]. Unchanged concentrations of these synaptic proteins might consequently point to more conserved synapses in CAA, in contrast to what is commonly seen in AD. Of all currently investigated synaptic proteins, only neurogranin was previously studied in patients with CAA [13], yielding CSF concentrations similar to controls like in our study, further corroborating our hypothesis on more conserved synapses in CAA than AD.

Our results should be considered in light of several limitations. The relatively small sample sizes may lead to a limited study power; however, we could confirm previously reported results of altered levels in AD compared with controls. The *APOE* $\varepsilon 4$ allele is a known major risk factor for the development of both vascular and parenchymal Aβ deposits [2], but *APOE* genotype status was not available to include as confounder. Moreover, AD diagnosis was solely based on ATN-biomarker status, without knowledge of clinical phenotype. Finally, general cognitive assessment scores and MRI data were only available for patients with CAA. A major strength includes using a robust and validated LC-MS quantification method, with high sensitivity and selectivity for the targeted synaptic proteins.

To conclude, our findings show that synaptic functioning may be more conserved in CAA compared with AD. CSF levels of synaptic markers could serve as biomarkers of synaptic pathology in AD. Our findings support a possible differential involvement of synaptic dysfunction in CAA and AD, which is particularly pronounced in the presence of AD pathology. However, since CSF levels are an indirect reflection of pathological processes occurring in the central nervous system, neuropathology studies assessing regional differences will aid in verifying the lack of synaptotoxicity in CAA. Furthermore, longitudinal studies including cognitive assessments are warranted to examine the association of synaptic dysfunction with disease severity.

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CONFLICT OF INTEREST

HZ has served at scientific advisory boards and/or as a consultant for Abbvie, Alector, Annexon, Artery Therapeutics, AZTherapies, CogRx, Denali, Eisai, Nervgen, Pinteon Therapeutics, Red Abbey Labs, Passage Bio, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave, has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure, Biogen, and Roche, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program. KB has served as a consultant, at advisory boards, or at data monitoring committees for Abcam, Axon, Biogen, JOMDD/Shimadzu. Julius Clinical, Lilly, MagQu, Novartis, Roche Diagnostics, and Siemens Healthineers, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program. MMV serves as a consultant for Vico. The other authors declare no conflict of interest.

DATA AVAILABILITY

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

SUPPLEMENTARY MATERIAL

The supplementary material is available in the electronic version of this article: https://dx.doi.org/10.3233/JAD-220977.

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