VAMP-2 Is a Surrogate Cerebrospinal Fluid Marker of Alzheimer-related Cognitive Impairment in Adults With Down Syndrome

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Research

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Abstract

Background. There is an urgent need for objective markers of Alzheimer's disease (AD)-related cognitive impairment in people with Down syndrome (DS) to improve diagnosis, monitor disease progression and assess response to disease-modifying therapies. Previously, GluA4 and Neuronal Pentraxin 2 (NPTX2) showed limited potential as cerebrospinal fluid (CSF) markers of cognitive impairment in adults with DS. Here we compare the CSF profile of a panel of synaptic proteins (Calsyntenin-1, Neuroligin-2, Neurexin-2A, Neurexin-3A, Syntaxin-1B, Thy-1, VAMP-2) to that of NPTX2 and GluA4 in a large cohort of subjects with DS across the preclinical and clinical AD continuum and explore their correlation with cognitive impairment.

Methods. We quantified the synaptic panel proteins by selected reaction monitoring in CSF from 20 non-trisomic cognitively normal controls (mean age 44) and 80 adults with DS grouped according to clinical AD diagnosis (asymptomatic, prodromal AD or AD dementia). We used regression analyses to determine CSF changes across the AD continuum and explored correlations with age, global cognitive performance (CAMCOG), episodic memory (modified cued-recall test; mCRT) and CSF AD biomarkers, CSF $A\beta_{42:40}$ ratio, CSF $A\beta_{1\text{-}42}$ and CSF p-tau. P-values were adjusted for multiple testing.

Results. In adults with DS, VAMP-2 was the only synaptic protein to correlate with episodic memory (delayed recall *adj.p*=.04) and age (*adj.p*=.0008) and was the best correlate of CSF $A\beta_{42:40}$ (*adj.p*=.0001) and CSF p-tau (*adj.p*<.0001). Compared to controls, mean VAMP-2 levels were lower in asymptomatic adults with DS only (*adj.p*=.02). CSF levels of Neurexin-3A, Thy-1, Neurexin-2A, Calysntenin-1, Neuroligin-2, GluA4 and Syntaxin-1B all strongly correlated with NPTX2 (*p*<.0001), which was the only synaptic protein to show reduced CSF levels in DS at all AD stages compared to controls (*adj.p*<.002).

Conclusion. CSF VAMP-2 represents a promising objective surrogate marker of cognitive failure in DS. VAMP-2 also has potential for use in AD clinical trials as a measure of synapse engagement and therapeutic response that does not directly measure the drug target (typically Aβ and tau).

Introduction

Alzheimer's disease (AD) is the leading cause of death in adults with Down syndrome (DS), with a cumulative incidence that exceeds 90% in the seventh decade (1–4). Current standard cerebrospinal fluid (CSF) markers for AD in the DS population are restricted to surrogate markers of amyloidosis ($A\beta_{42:40}$ ratio, $A\beta_{1-42}$) and tau-mediated neurodegeneration (p-tau) and neurofilament light chain combined with neuropsychological assessment. However neuropsychological assessment can be confounded by substantial inter-individual variation in intellectual disability. Therefore, there is an urgent need for objective markers of AD-related cognitive impairment in people with DS to improve diagnosis, monitor disease progression and assess response to disease-modifying therapies.

Synapse loss is an early event in AD (5) and one of the best pathological correlates of cognitive dysfunction (6–9). As such, synaptic proteins that show AD-associated changes in biofluids are rapidly gaining attention as potential surrogate markers of AD-related synapse loss and may be informative markers of early AD-related cognitive dysfunction in adults with DS.

Neuronal Pentraxin-2 (NPTX2), a protein involved in inhibitory circuit dysfunction (10) is a promising biofluid surrogate marker of inhibitory circuit dysfunction and cognitive decline in sporadic AD (11–13), vascular dementia (14), genetic frontotemporal dementia (15) and Lewy body dementia (16). We recently reported low CSF NPTX2 concentrations in adults with DS across the AD continuum, which correlated with cortical atrophy and reduced glucose metabolism. However, CSF NPTX2 levels did not correlate with measures of cognitive decline in our DS cohort (17). In the same study, we also evaluated the glutamatergic receptor, GluA4, and found no association with cognitive measures.

The aim of this study was to evaluate a comprehensive panel of alternative synaptic proteins (Calsyntenin-1, Neuroligin-2, Neurexin-2A, Neurexin-3A, Syntaxin-1B, Thy-1, VAMP-2) as surrogate markers of early AD-related cognitive decline in non-trisomic cognitively normal controls (n = 20) and a large cohort of adults with DS (n = 80) from across the preclinical and clinical AD continuum, exploring their relationship to cognitive performance. The panel comprises 8 proteins that were shown to be specifically expressed at the synapse in human frontal cortex postmortem tissue and show CSF alterations that precede clinical symptoms and markers of neurodegeneration in sporadic AD (18). We also compare the CSF profile of the synaptic panel proteins in adults with DS to that of previously published data on NPTX2 and GluA4 in the same cohort (17).

Material And Methods

Objectives

The primary objective of this study was to evaluate a comprehensive panel of synaptic proteins as surrogate markers of early AD-related cognitive decline in adults with DS from across the preclinical and clinical AD continuum, specifically exploring their relationship to cognitive performance and AD biomarkers.

Study design

This is a single-centre, cross-sectional study of CSF levels of synaptic markers in adults with DS, sporadic AD patients and cognitively normal controls. The study (IIBSP-BMS-2018-103) was approved by the local ethics committee (Comité Ètic d'Investigació Clínica, Fundació de Gestió Sanitària de l'Hospital de la Santa Creu i Sant Pau) and was conducted in accordance with the Declaration of Helsinki. All participants gave their written informed consent to participate in the study. Non-trisomic controls were selected from the Sant Pau Initiative in Neurodegeneration (SPIN) cohort, a prospective longitudinal cohort at Hospital Sant Pau, Barcelona, Spain (19). Adults with DS were selected from the Down Alzheimer Barcelona Neuroimaging Initiative (DABNI), a prospective longitudinal cohort, linked to a

population-based health plan in Catalonia, Spain, led by the Fundació Catalana Síndrome de Down and Hospital de Sant Pau (20). Inclusion criteria for controls required the absence of a cognitive or neurological disorders and normal CSF core AD biomarker ($A\beta_{1-42}$, $A\beta_{42/40}$ ratio, t-tau, p-tau) concentrations using our validated cut-offs for sporadic AD (21). For adults with DS, inclusion criteria for participation in the study required that all participants were over 18 years of age. Where consent was given, participants received a comprehensive neurological and neuropsychological evaluation (22) and underwent a lumbar puncture to assess CSF biomarkers (20). As in previous studies (4, 20), participants with DS were classified by neurologists and neuropsychologists, blind to biomarker data in a consensus meeting into asymptomatic AD (aDS), prodromal AD (pDS) and AD dementia (dDS) according to previously published criteria (20).

Neuropsychological assessment

The level of intellectual disability in adults with DS was categorized according to the Diagnostic and Statistical Manual of Mental Disorders (DSM), Fifth Edition as mild, moderate, severe, or profound intellectual disability, based on caregivers' reports of the individuals' best-ever level of functioning and the Kaufmann Brief Intelligence Test (KBIT) (23). As previously described (20, 22), neurological and neuropsychological examination of the full range of cognitive impairment included a semi-structured health questionnaire (Cambridge Examination for Mental Disorders of Older People with Down Syndrome and others with intellectual disabilities [CAMDEX-DS]) (24) and a neuropsychological battery including the Cambridge Cognition Examination (CAMCOG) adapted for intellectual disabilities in DS participants and was restricted to those with mild and moderate intellectual disability. The Spanish version of the cued recall test modified for use in people with intellectual disability (mCRT) (25) was used to evaluate episodic memory as previously described (26). The total mCRT scores for immediate recall were calculated as free recall score + cued recall score.

CSF collection, biomarker assessment

CSF samples were collected following international consensus recommendations (27) as previously described (28). Samples had been previously stored at -80°C and had not been thawed prior to analysis. Commercially available fully-automated immunoassays were used to determine levels of CSF $A\beta_{1-42,}$ $A\beta_{1-40}$, total tau and p-tau at threonine residue 181 (Lumipulse $A\beta_{1-42}$, $A\beta_{1-40}$, total tau G, p-tau 181, Fujirebio-Europe) (21).

Targeted Liquid Chromatography Mass Spectrometry (LC-SRM)

We monitored a set of 20 proteotypic peptides corresponding to 8 panel proteins identified in our previous study (Calsyntenin-1, GluA2, Neurexin-2A, Neurexin-3A, Neuroligin-2, Syntaxin-1B, Thy-1 and VAMP-2) by Selected Reaction Monitoring (SRM) as previously described (18). Briefly, we digested individual CSF samples overnight and spiked isotopically-labeled peptides (Pepotech SRM custom peptides, grade 2, Thermo Fisher Scientific) into each sample. We analyzed an equivalent of 5µl of each sample in a randomized order over a 120-min gradient (0-35% ACN+0.1% FA) in SRM mode using a triple quadrupole-

Qtrap mass spectrometer (5500 QTrap, Sciex, Massachussetts) coupled to a nano-LC chromatography column (300 µl/min, 25-cm C18 column, 75 µm I.d., 2 µm particle size). We ran BSA technical controls between each sample. We used isotopically-labeled peptides as internal standards. We visualized and analyzed transitions using Skyline 3.5 as previously described (18). Injection of a pool of all the samples over the duration of the mass spectrometric measurements and monitoring the peak area of the standard peptides was used to evaluate the stability of the peptides over the course of the experiment and resulted in the exclusion of GluA2 from the study. We processed the SRM transitions using the dataProcess function of MSstats v3.5 package in R (29) and removed transitions with between-run interference (betweenRunInterferenceScore<0.8). Censored missing values were samples with log base-2 endogenous log2 intensities under the cut-off designated by the MSstats package (8.49). We used the EqualizeMedians function to normalize the transitions and Tukey's Median Polish to generate a summarized value of transitions for each protein.

Statistical Analysis

All statistical analyses were performed in R version 3.4.3 (30). Extreme outliers were excluded using the 3 × interquartile range rule. Group comparisons were performed using χ^2 test, t-test or linear regression and we used Pearson's coefficients to assess correlations. Where residuals deviated from a Gaussian distribution (Shapiro-Wilk p<0.05), tests were performed on square root transformed values. When comparing the association of multiple synaptic proteins, p-values were adjusted for multiple testing using the Benjamini-Hochberg method.

Results

Demographics

The **Table** shows the demographic and clinical data for the participants included in the study, which included 20 controls and 80 adults with DS from across the AD continuum (40 aDS, 19 pDS and 21 dDS). The mean age-at-analysis across the whole study was 44.5 years (standard deviation; SD = 11.2). Compared to controls, the mean age was comparable in pDS (+ 5 years, $p = .20$) and dDS (+ 5 years, $p = .13$) but lower in aDS (-12 years, $p < .0001$). The male:female proportion was comparable across clinical groups ($p = .45$). The level of intellectual disability in the adults with DS was classified as either mild/moderate (78% of cases) or severe/profound (22% of cases), a proportion that was comparable across clinical groups ($p = .37$). Cognitive tests were restricted to individuals with mild or moderate intellectual disability. As would be expected, cognitive scores were sequentially lower in pDS (CAMCOG; -11, $p = .02$, mCRT immediate; -15, $p < .0001$, mCRT delayed; -5, $p < .0001$) and dDS (CAMCOG − 21 $p < .0001$, mCRT immediate; -20. $p < .0001$, mCRT delayed; -7, $p < .0001$) compared to aDS. As previously reported (20), the mean $A\beta_{42:40}$ ratio (all $p < .0001$) was lower in all DS groups compared to controls, while mean CSF p-tau and t-tau levels were higher in pDS and dDS compared to controls (all $p < .0001$).

CSF VAMP-2 levels show a distinct profile to other synaptic proteins in adults with DS

We first sought to determine the degree of correlation between CSF levels of the 7 synaptic panel proteins as well as with previously published data for CSF NPTX2 and GluA4. Figure 1 shows that in adults with DS, synaptic proteins, including Neurexin-3A, Thy-1, Neurexin-2A, Calysntenin-1, Neuroligin-2, GluA4 and Syntaxin-1B, were all correlated (pair-wise $r = .74$ to .93, $p < .0001$). They also all correlated with NPTX2 (pair-wise $r = .54$ to .79, $p < .0001$). VAMP-2 showed the weakest correlation with all other proteins ($r = .35$ to.63, $p < .0001$). In controls, all proteins showed weaker pair-wise correlations than in the DS group, although NPTX2, Neurexin-3A, Thy-1, Neurexin-2A, Calysntenin-1, Neuroligin-2, GluA4 and Syntaxin-1B were moderately correlated in at least one pair-wise combination (pair-wise $r = .45$ to .84, $p < .04$). VAMP-2 did not correlate with any other protein in controls (pair-wise $r = -.31$ to .34, $p > .15$). We took VAMP-2 forward for further analyses due to its relative independence from NPTX2.

Table. *Demographics and clinical data for study participants.*

	Controls	aDS	pDS	dDS
N	**20**	**40**	**19**	**21**
Age-at-analysis, years	**47** (11, 24–64)	**35** (9, 22–57)[b]	**52** (4, 45–60)	**52** (5, 42–62)
% Female	**60%**	**40%**	**42%**	**38%**
% Mild or moderate ID	**0%**	**83%**	**79%**	**67%**
CAMCOG score[a]	**NA**	**80/107** (11, 55–96, n = 31)	**70/107** (13.8, 41–92, n = 11)[c]	**59/107** (13.9, 39–87, n = 10)[c]
mCRT score (immediate)[a]	**NA**	**35/36** (1.5, 30–36, n = 30)	**20/36** (11.2, 0–36, n = 12)[c]	**15/36** (7.9, 0–32, n = 11)[c]
mCRT score (delayed)[a]	**NA**	**12/12** (0.9, 8–12, n = 31)	**6/12** (3.8, 0–12, n = 13)[c]	**4/12** (3.3, 0–12, n = 11)[c]
CSF $A\beta_{42:40}$ ratio	**0.11** (0.01, 0.08–0.12)	**0.09** (0.02, 0.04–0.12)[b]	**0.05** (0.01, 0.03–0.08)[b]	**0.05** (0.01, 0.04–0.08)[b]
CSF p-tau pg/ml	**36** (8, 22–54)	**35** (24, 10–122)	**145** (86, 22–304)[b]	**158** (82, 31–323)[b]
CSF t-tau pg/ml	**243** (57, 167–366)	**295** (166, 86–671)	**936** (658, 118–2565) [b]	**959** (500, 212–1988) [b]

Mean values (standard deviation, range) are given for each variable across clinical groups. NA; not available. [a]In individuals with mild/moderate intellectual disability (ID) only. [b]p<0.05 compared to controls. [c]p<0.05 compared to aDS.

CSF VAMP2 changes over the course of AD and with age in adults with DS

Figure 2a shows that mean CSF VAMP-2 SRM intensities were lower in individuals with DS compared to controls (.84-fold, $p = .04$). Mean CSF VAMP-2 intensities were lower in the aDS group compared to controls (.73-fold, $p = .005$) and compared to the symptomatic group (pDS and dDS combined; .67-fold, $p = .01$). CSF VAMP-2 intensities were comparable to controls in pDS (.98-fold, $p = .26$) and dDS (.93-fold, $p = .47$). This relative increase in CSF VAMP-2 at late AD stages in adults with DS is supported by Fig. 2b, which shows that CSF VAMP-2 directly correlated with age in DS ($r = .43$, $p < .0001$). Conversely, CSF VAMP-2 inversely correlated with age in controls (r=-.51, $p = .02$). The control and DS regression lines for VAMP-2 were non-overlapping at the earliest age included in the study (22 years old) and did not intercept until the age of 42. Figure 2c shows the correlation between CSF VAMP-2 and CSF biomarkers of brain amyloid and tau pathology in adults with DS. VAMP-2 inversely correlated with the $A\beta_{42:40}$ ratio (r=-.47, $p < .0001$) and directly correlated with p-tau, ($r = .56$, $p < .0001$). To determine whether low CSF VAMP-2 is related to AD biomarker positivity in asymptomatic DS, we compared CSF VAMP-2 SRM intensities in the aDS group stratified by positivity for CSF $A\beta_{1-42}$ using our validated in-house cut-offs for sporadic AD. Compared to controls, CSF VAMP-2 SRM intensities were lower in individuals positive for CSF $A\beta_{1-42}$ (0.67-fold, p = .009) but not in individuals negative for CSF $A\beta_{1-42}$ (0.78-fold, p = .30). Thus, low CSF VAMP-2 is related to AD biomarker positivity and changes over the course of AD and with age in adults with DS.

CSF VAMP2 is associated with cognitive performance in adults with DS

Figure 3 shows the relationship between CSF VAMP-2 and measures of intellectual and cognitive impairment in adults with DS. Mean CSF VAMP-2 SRM intensities were comparable across individuals with, mild, moderate and severe intellectual disability (Fig. 3a; $p = .76$) and were not associated with K-bit score (Fig. 3b; $r = .21$, $p = .16$). While Fig. 3c shows a similar regression line for VAMP-2 with CAMCOG and mCRT scores, correlation analyses showed that VAMP-2 SRM intensities were associated with immediate ($r = -.35$, $p = .01$) and delayed ($r = -.38$, $p = .005$) recall in the mCRT test but not with CAMCOG scores ($r = -.25$, $p = .07$). However, linear regression analysis including level of intellectual disability as a covariate, showed that both intellectual disability (t= -3.90, $p = .0003$) and VAMP-2 (t= -2.02, $p = .05$) were associated with CAMCOG score (model $adj.r^2 = .25$, F = 9.7, $p = .0003$), while VAMP-2 (t= -2.20, $p = .03$) but not intellectual disability (t= -0.89, $p = .38$) was associated with immediate recall in the mCRT test. We observed a similar association with delayed recall (VAMP-2; t= -2.81, $p = .007$, intellectual disability t= -1.11, $p = .27$). Therefore, VAMP-2 was associated with both CAMCOG (when controlling for level of intellectual disability) and mCRT score in adults with DS.

Compared to other synaptic proteins, VAMP-2 is the best correlate of cognitive performance, age and AD biomarkers in adults with DS

Finally, we compared these findings for VAMP-2 to the other synaptic panel proteins and to NPTX2 and applied a strict adjustment of p-values to account for multiple testing. The correlation of VAMP-2 with immediate recall ($adj.p = .09$) and association with CAMCOG ($adj.p = .44$) did not survive adjustment for multiple testing. Variables associated with at least one synaptic protein ($adj.p < .05$) in DS are shown in

Fig. 4. VAMP-2 was the only protein to correlate with mCRT delayed recall (*adj.p* = .04) and age (*adj.p* = .0008) and was the best correlate of CSF $A\beta_{42:40}$ (*adj.p* = .0001) and CSF p-tau (*adj.p* < .0001). On the other hand, NPTX2 was the best correlate of CSF $A\beta_{1-42}$ (r = .58, *adj.p* < .0001), showed the greatest fold-change across all AD stages (0.34 to 0.55-fold, adj.*p* < .002) and was the only synaptic protein to show changes in pDS (0.47-fold, adj.*p* = .002).

Discussion

Here we report a comprehensive evaluation of synaptic proteins in CSF from adults with DS across the whole clinical continuum of AD. We show that of the 9 synaptic proteins evaluated, VAMP-2 is the only correlate of cognitive performance and age in this relatively understudied population. We also show that while mean CSF VAMP-2 levels were lower in asymptomatic adults with DS compared to cognitively normal controls, mean VAMP-2 levels were elevated at advanced AD stages. Increased CSF VAMP-2 correlated with low CSF $A\beta_{42/40,}$ increased CSF p-tau and worse cognitive performance. Thus, changes in CSF VAMP-2 are closely related to CSF AD biomarkers and cognitive measures in adults with DS.

In controls, CSF VAMP-2 levels decreased with age and when compared across similar ages, VAMP-2 levels were lower in adults with DS compared to controls from the earliest age included in the study (22 years old) and did not converge until the age of 42. This finding suggests a distinct CSF profile of VAMP-2, and potentially a different mechanism of synaptic pruning, between healthy aging and the presence of AD pathology and/or triplication of chromosome 21. It is possible that the relatively lower CSF VAMP-2 levels in younger adults with DS compared to controls is a result of reduced VAMP-2 expression from birth. However, several lines of evidence suggest that CSF VAMP-2 levels change as a function of AD as opposed to intellectual disability; (a) CSF VAMP-2 was comparable to controls in adults with DS negative for the amyloid marker, (b) the findings reported here for this genetically determined form of AD replicate the nonlinear CSF profile of the 8 synaptic panel proteins previously reported across disease stages in sporadic AD (18) and (c) CSF VAMP-2 did not correlate with K-bit score and was comparable between individuals classified as having mild, moderate, severe or profound intellectual impairment but did correlate with age, AD biomarkers and episodic memory performance. Based on these findings, we propose that low VAMP-2 levels in these individuals may at least partially reflect changes related to the preclinical phase of AD, similar to that previously report in sporadic AD where CSF VAMP-2 levels were nominally reduced in preclinical AD and significantly elevated in prodromal and dementia stages compared to cognitively normal controls (18).

We have previously evaluated NPTX2 as a synaptic marker in the same cohort reported here (17). Similar to VAMP-2, CSF NPTX2 levels were lower in DS compared to controls, albeit that NPTX2 was reduced at all AD stages. In fact, here we report that compared to the other 8 synaptic proteins, NPTX2 was the only protein to be reduced at all AD stages compared to controls. However, unlike VAMP-2, CSF NPTX2 did not correlate with cognition or age. The distinct expression and function of these two proteins could explain their distinct CSF profiles in adults with DS. VAMP-2 is expressed at the human cortical synapse with increased enrichment compared not only to the other 7 synaptic panel proteins evaluated here but also to

the widely-used pre-synaptic marker, synaptophysin (18). This high synapse specificity supports other studies that have shown that VAMP-2 is predominantly found at glutamatergic synapses (31) as part of the synaptic exocytosis core vesicular complex (32) where it is necessary for regulating the releasable pool of glutamate at the pre-synapse (33) and is also critical for post-synaptic trafficking of glutamate receptor subunits, particularly in the CA1 region of the hippocampus (34). Reduced VAMP-2 brain expression has been reported in AD (35). NPTX2 is specifically expressed by pyramidal neurons where it mediates activity-dependent strengthening of pyramidal neuron excitatory synapses on GABAergic parvalbumin interneurons (10). Therefore, both VAMP-2 and NPTX2 are specifically expressed at distinct populations of synapses where they have distinct functions that are critical for synaptic transmission. We therefore propose that CSF levels of these 2 synaptic proteins may reflect degeneration of distinct synapse populations. While, NPTX2 remains a promising surrogate marker of inhibitory circuit dysfunction in AD, DS and other neurodegenerative diseases, VAMP-2 may be a better surrogate marker of cognitive performance in adults with DS.

A previous study reported that CSF NPTX2 correlates well CSF levels of two other synaptic proteins, SNAP-25 and neurogranin in sporadic AD CSF (12). In this study, we report that, with the exception of VAMP-2, CSF levels of synaptic proteins were highly inter-correlated in adults with DS and that VAMP-2 was the only synaptic protein not to correlate with at least one other synaptic protein in controls.

The novelty of VAMP-2 is that it was the only synaptic protein evaluated here to correlate with age (a surrogate measure of disease progression in DS) and mCRT in the DS population. Similar to our previous study, we found that intellectual disability had a greater impact on CAMCOG score than on mCRT (22) such that the association of VAMP-2 with mCRT score was evident without the need to control for level of intellectual disability. The mCRT test is a version of the CRT modified for use in DS and discriminates well between DS adults with and without dementia (26). The CRT test is considered a clinical marker of episodic memory disorders due to medial temporal damage, especially in the CA1 field of the hippocampus (36), which is consistent with the functional role of VAMP-2 at CA1 synapses (34). It should be noted that while tau markers remain the best CSF correlates of cognition in this population, as tau is fast becoming a common drug target in AD clinical trials, an alternative surrogate measure of cognitive performance that does not directly measure the target levels would be a useful addition to the biomarker arsenal to monitor synapse engagement and therapeutic response.

Study Limitations

While DS and autosomal dominant cohorts with available CSF are scarce, further replication of the findings reported here in independent genetic AD and DS cohorts would be valuable. A limitation of this study is the cross-sectional design. Longitudinal studies are needed to fully establish the prognostic value of VAMP-2 in DS cohorts.

Conclusion

VAMP-2 represents a new addition to the DS biomarker arsenal that may be used to detect and monitor AD-related synaptic dysfunction and cognitive decline, independent of intellectual disability, in this relatively under-studied population. VAMP-2 also has potential for use in AD clinical trials as an alternative surrogate measure of cognitive performance that does not directly measure the drug target (typically Aβ and tau). This work opens the door to future studies exploring the prognostic capacity of CSF VAMP-2 in adults with DS and sporadic AD.

Abbreviations

AD; Alzheimer's disease

aDS; asymptomatic Alzheimer's disease in Down syndrome

CAMCOG; Cambridge Cognition Examination

CSF; cerebrospinal fluid

DABNI; Down Alzheimer Barcelona Neuroimaging Initiative

dDS; Dementia due to Alzheimer's disease in Down syndrome

DS; Down syndrome

DSM; Diagnostic and Statistical Manual of Mental Disorders

KBIT; Kaufmann Brief Intelligence Test

ID; intellectual disability

mCRT; Modified cued recall test

NPTX2; Neuronal Pentraxin-2

pDS; prodromal Alzheimer's disease in Down syndrome

SD; standard deviation

SPIN; Sant Pau Initiative in Neurodegeneration

SRM; Selected Reaction Monitoring

VAMP-2; Vesicle-associated membrane protein-2

Declarations

Availability of data and materials

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

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Author contributions

OB, MC-I, LV, SF, BB, JP, IB, MA, SV, RN-L, AB, MFI, MX, DX, MQ-A, SS and DA contributed to the acquisition and analysis of data. AL, DA, JC, JF, RB, PW drafted a significant portion of the manuscript and figures. All authors read and approved the final manuscript.

Ethics declarations

Ethics approval and consent to participate

The study (IIBSP-BMS-2018-103) was approved by the local ethics committee (Comité Ètic d’Investigació Clínica, Fundació de Gestió Sanitària de l’Hospital de la Santa Creu i Sant Pau) and was conducted in accordance with the Declaration of Helsinki. All participants gave their written informed consent to participate in the study.

Consent for publication

Not applicable.

Competing interests

Drs Belbin, Lleó, Fortea and Alcolea declare a filed patent application (WO2019175379 A1 Markers of synaptopathy in neurodegenerative disease) that includes the 9 synaptic proteins evaluated in this study. D.A. participated in advisory boards from Fujirebio-Europe and Roche Diagnostics and received speaker honoraria from Fujirebio-Europe, Nutricia and from Krka Farmacéutica S.L. J.F participated in advisory boards from AC-Inmune and was consultant to Novartis. MX, DX and PW are co-founders of CogNext.

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Figures

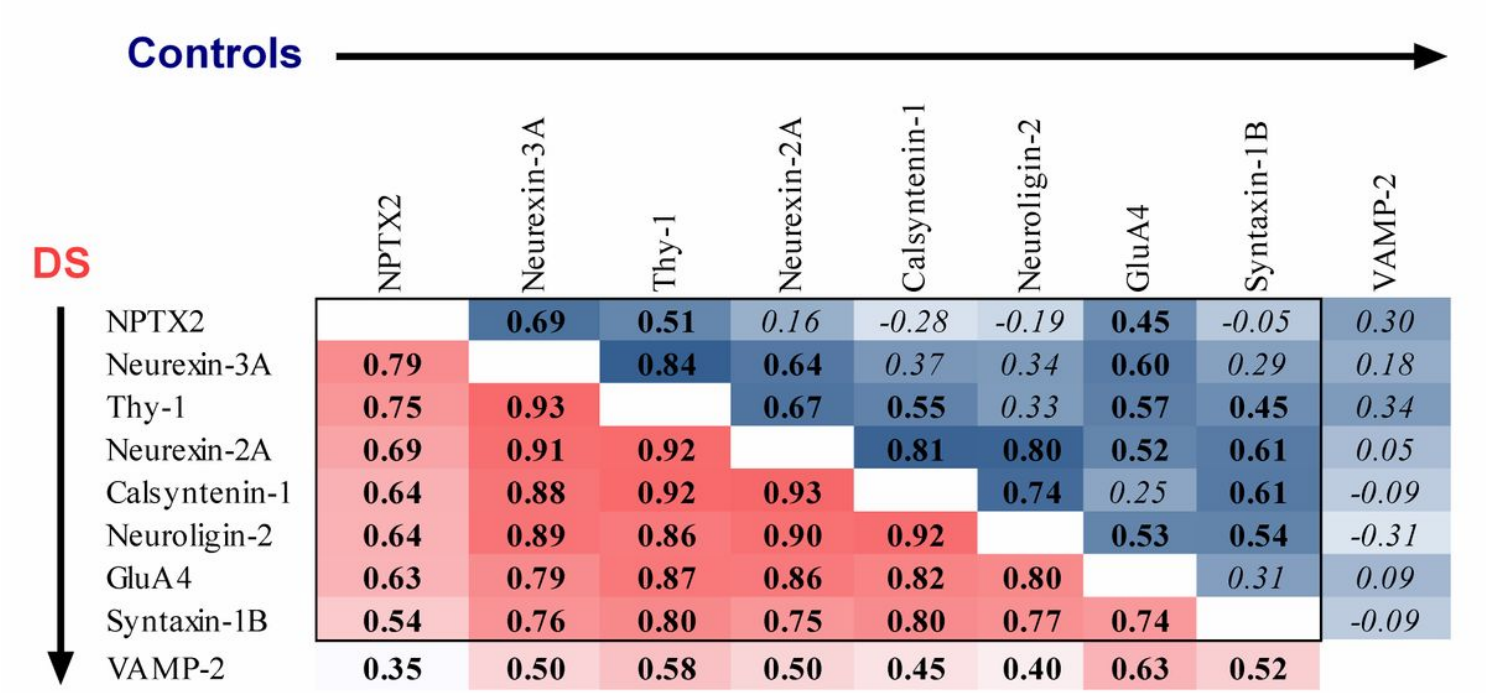

Figure 1

Pair-wise correlation coefficients of CSF levels of 9 synaptic proteins in DS and controls. r coefficients resulting from statistical tests performed in the DS group (red) and controls (blue) for the 8 synaptic panel proteins and NPTX2 are shown. Degree of shading is relative to size of r coefficients, which are shown in bold where p<.05 and italicized where p>.05. NPTX2 and GluA4 data for these samples are published in (17).

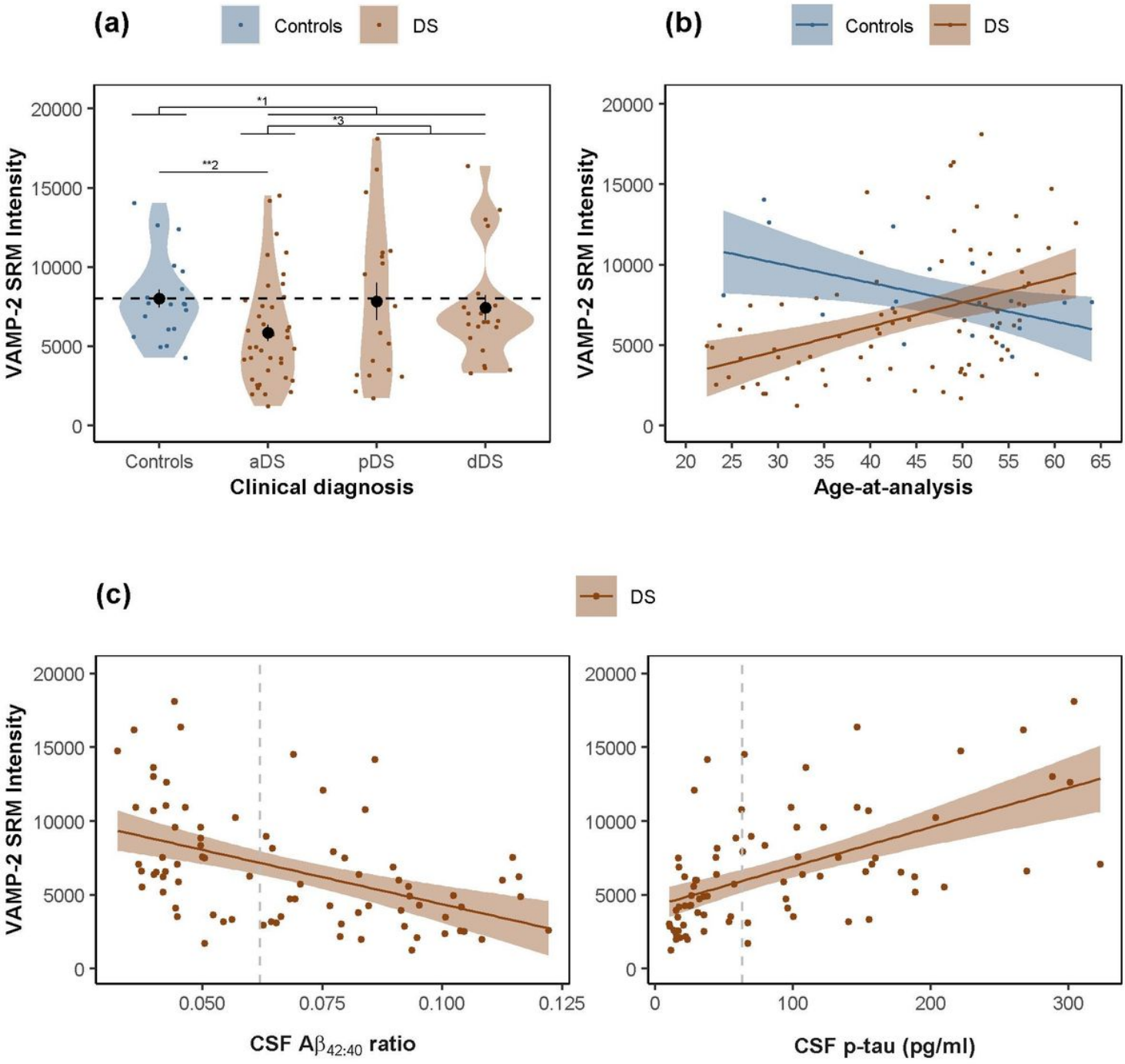

Figure 2

CSF levels of VAMP-2 in non-trisomic controls and DS. (a) Violin plots show the distribution of SRM intensities for VAMP-2 quantified in CSF for non-trisomic cognitively normal subjects (controls) and adults with DS across AD stages; asymptomatic AD (aDS), prodromal AD (pDS) or AD dementia (dDS). The horizontal dotted line represents the mean value in controls. *p<.05, **p<.01 for linear regression using square root transformed log2-transformed VAMP-2 levels in 1 DS vs controls, 2 aDS vs Controls and 3 pDS/dDS vs aDS. (b) Age-at-analysis (years) is plotted against VAMP-2 SRM intensities in controls

and adults with DS. (c) AD CSF biomarkers; Aβ42:40 ratio, Aβ1-42 and p-tau are plotted against VAMP-2 SRM intensities in adults with DS. Linear regression lines in (b) and (c) are shown for each group (see legends). Shaded areas represent standard error of the regression lines. The vertical dotted lines in (c) represent the validated cut-offs for biomarker positivity in sporadic AD.

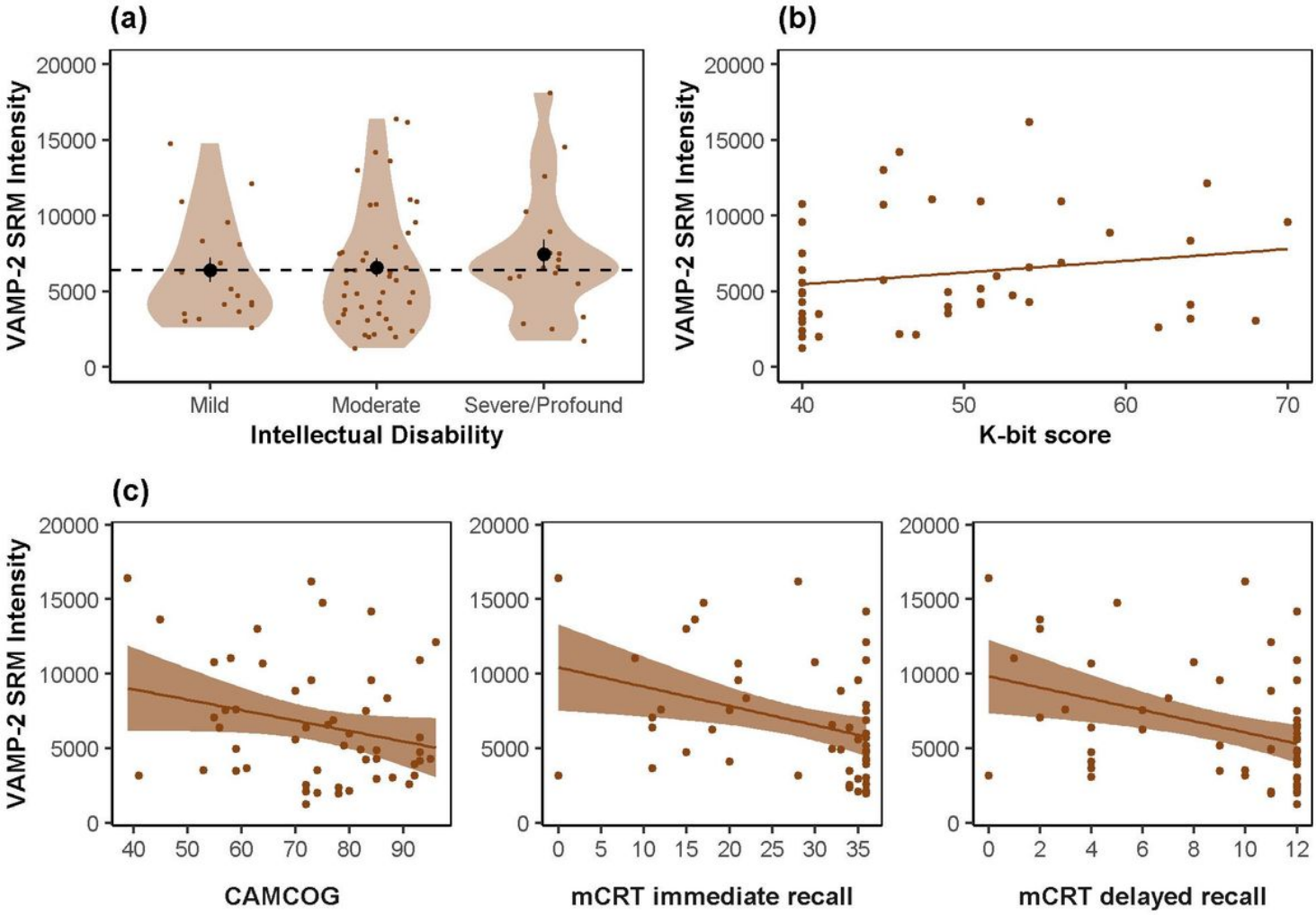

Figure 3

Relationship between CSF VAMP-2 and measures of intellectual impairment and cognitive performance in DS. (a) Violin plots show the distribution of CSF VAMP-2 SRM intensities in adults with DS grouped according to degree of intellectual disability. Circles represent mean intensities and error bars represent standard error of the mean. The horizontal dotted line represents the mean value in individuals with mild intellectual disability. VAMP-2 SRM intensities in adults with DS are plotted against quantitative measures of intellectual disability (b) and cognitive performance (c). Linear regression lines are shown for all models and standard error of the regression lines are shown as shaded region, where p<.05.

Assesmment	Statistic	VAMP-2	Syntaxin-1B	GluA4	Neuroligin-2	Neurexin-3A	Thy-1	Neurexin-2A	Calsyntenin-1	NPTX2
mCRTd	r	**-0.38**	*-0.16*	*-0.19*	*-0.23*	*-0.23*	*-0.15*	*-0.20*	*-0.09*	*0.08*
Age	r	**0.43**	*0.24*	*0.23*	*0.22*	*0.18*	*0.17*	*0.09*	*0.06*	*-0.09*
CSF $A\beta_{42:40}$	r	**-0.47**	**-0.29**	*-0.22*	*-0.13*	*-0.16*	*-0.18*	*-0.11*	*-0.04*	*0.06*
CSF p-tau	r	**0.56**	**0.45**	**0.32**	**0.30**	**0.34**	**0.30**	**0.30**	**0.23**	*0.07*
CSF $A\beta_{1-42}$	r	*-0.01*	*0.18*	**0.32**	**0.34**	**0.38**	**0.40**	**0.44**	**0.50**	**0.58**
aDS vs Ctrl	FC	**0.83**	*0.91*	0.88	*0.89*	**0.83**	**0.84**	**0.84**	**0.85**	**0.60**
dDS vs Ctrl	FC	*0.95*	*0.92*	*0.86*	*0.87*	**0.81**	**0.80**	**0.79**	**0.79**	**0.30**
pDS vs Ctrl	FC	*0.95*	*1.00*	*0.97*	*0.95*	*0.89*	*0.91*	*0.89*	*0.86*	**0.50**

Figure 4

Comparison of CSF profile of 9 synaptic proteins in adults with DS. Assessment of the 9 synaptic panel proteins and their association (r co-efficients) with delayed recall in the mCRT test (mCRTd), age, CSF Aβ42:40, CSF p-tau and CSF Aβ1-42 and the fold-change (FC) compared to controls in adults with DS across clinical groups. Degree of shading is relative to r coefficients or FC. Values are shown in bold where Benjamini-Hochberg adjusted p<.05 and italicized where adjusted p>.05. Quantification of NPTX2 and GluA4 and FC for NPTX2 have been published previously in (17).