Received: 27 October 2022 | Revised: 29 December 2022 | Accepted: 4 January 2023

DOI: 10.1002/alz.13042

Alzheimer's & Dementia®
THE JOURNAL OF THE ALZHEIMER'S ASSOCIATION

RESEARCH ARTICLE

SCRN1: A cerebrospinal fluid biomarker correlating with tau in Alzheimer's disease

Sophia Weiner[1] | **Mathias Sauer**[2] | **Gunnar Brinkmalm**[1] | **Julius Constantinescu**[3] | **Radu Constantinescu**[3] | **Bárbara Fernandes Gomes**[1] | **Bruno Becker**[2] | **Bengt Nellgård**[4] | **Keti Dalla**[4] | **Douglas Galasko**[5] | **Henrik Zetterberg**[1,2,6,7,8] | **Kaj Blennow**[1,2] | **Johan Gobom**[1,2]

[1]Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, University of Gothenburg, Mölndal, Sweden

[2]Clinical Neurochemistry Laboratory, Institute of Neuroscience and Physiology, Sahlgrenska University Hospital, Mölndal, Sweden

[3]Department of Neurology, Institute of Neuroscience and Physiology, The Sahlgrenska Academy at the University of Gothenburg, Gothenburg, Sweden

[4]Department of Anesthesiology and Intensive Care Medicine, Institute of Clinical Sciences, Sahlgrenska University Hospital, Mölndal, Sweden

[5]Department of Neurosciences, University of California, San Diego, La Jolla, California, USA

[6]Department of Neurodegenerative Disease, UCL Institute of Neurology, London, UK

[7]UK Dementia Research Institute, London, UK

[8]Hong Kong Center for Neurodegenerative Diseases, Hong Kong, China

Correspondence
Sophia Weiner, Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, The Sahlgrenska Academy, University of Gothenburg, Mölndal S-431 80, Sweden.
Email: sophia.weiner@gu.se

Funding information
Swedish Research Council, Grant/Award Number: #2017-00915; the Alzheimer Drug Discovery Foundation (ADDF), USA, Grant/Award Number: #RDAPB-201809-2016615; the Swedish Alzheimer Foundation, Grant/Award Numbers: #AF-930351, #AF-939721, #AF-968270, #FO2017-0243, #ALZ2022-0006, #ALFGBG-715986, #ALFGBG-965240; the European Union Joint Program for Neurodegenerative Disorders, Grant/Award Number: JPND2019-466-236; the National Institute of Health (NIH), USA, Grant/Award Number: #1R01AG068398-01; the Alzheimer's Association 2021 Zenith Award, Grant/Award Number: ZEN-21-848495; the Swedish Research Council, Grant/Award Number: #2018-02532; the European Union's

Abstract

INTRODUCTION: Secernin-1 (SCRN1) is a neuronal protein that co-localizes with neurofibrillary tangles in Alzheimer's disease (AD), but not with tau inclusions in corticobasal degeneration (CBD), progressive supranuclear palsy (PSP), or Pick's disease.

METHODS: We measured SCRN1 concentration in cerebrospinal fluid (CSF) using a novel mass spectrometric parallel reaction monitoring method in three clinical cohorts comprising patients with neurochemically characterized AD ($n = 25$) and controls ($n = 28$), clinically diagnosed Parkinson's disease (PD; $n = 38$), multiple system atrophy (MSA; $n = 31$), PSP ($n = 20$), CBD ($n = 8$), healthy controls ($n = 37$), and neuropathology-confirmed AD ($n = 47$).

RESULTS: CSF SCRN1 was significantly increased in AD ($P < 0.01$, fold change = 1.4) compared to controls (receiver operating characteristic area under the curve = 0.78) but not in CBD, PSP, PD, or MSA. CSF SCRN1 positively correlated with CSF total tau ($R = 0.78$, $P = 1.1 \times 10^{-13}$), phosphorylated tau_{181} ($R = 0.64$, $P = 3.2 \times 10^{-8}$), and Braak stage and negatively correlated with Mini-Mental State Examination score.

DISCUSSION: CSF SCRN1 is a candidate biomarker of AD, reflecting tau pathology.

Horizon Europe research and innovation program, Grant/Award Number: No 101053962; Swedish State Support for Clinical Research, Grant/Award Number: #ALFGBG-71320; the Alzheimer Drug Discovery Foundation (ADDF), USA, Grant/Award Number: #201809-2016862; the AD Strategic Fund and the Alzheimer's Association, Grant/Award Numbers: #ADSF-21-831376-C, #ADSF-21-831381-C, #ADSF-21-831377-C; the Bluefield Project, the Olav Thon Foundation, the Erling-Persson Family Foundation, Stiftelsen för Gamla Tjänarinnor, Hjärnfonden, Sweden, Grant/Award Number: #FO2022-0270; European Union's Horizon 2020 research and innovation program, Grant/Award Number: No 860197; the European Union Joint Programme – Neurodegenerative Disease Research, Grant/Award Number: JPND2021-00694; the UK Dementia Research Institute, Grant/Award Number: UKDRI-1003; Alzheimerfonden, Grant/Award Number: AF-930934; Stiftelsen för Gamla tjänarinnor

KEYWORDS
Alzheimer's disease, biomarkers, cerebrospinal fluid, mass spectrometry, Parkinsonian syndromes, secernin-1, tauopathies

HIGHLIGHTS

- We developed a parallel reaction monitoring assay to measure secernin-1 (SCRN1) in cerebrospinal fluid (CSF).
- CSF SCRN1 was increased in Alzheimer's disease compared to healthy controls.
- CSF SCRN1 remained unchanged in Parkinson's disease, multiple system atrophy, progressive supranuclear palsy, or corticobasal degeneration compared to controls.
- CSF SCRN1 correlated strongly with CSF phosphorylated tau and total tau.
- CSF SCRN1 increased across Braak stages and negatively correlated with Mini-Mental State Examination score.

1 | BACKGROUND

Alzheimer's disease (AD) is the most common neurodegenerative disease, accounting for up to 70% of all dementia cases.[1] On a pathophysiological level, AD is characterized by two hallmark neuropathologies: extracellular plaques composed of amyloid beta (Aβ) peptides, and intracellular protein inclusions consisting of hyperphosphorylated tau, referred to as neurofibrillary tangles (NFTs).[2] Under physiological conditions, tau is associated with the microtubules of neurons, stabilizing them, and aiding in axonal transport. In AD, tau becomes phosphorylated at multiple sites, causing the protein to detach from the microtubules and inducing conformational changes that lead to its aggregation into NFTs.[3]

Tau inclusions are also implicated in several other neurodegenerative disorders such as corticobasal degeneration (CBD) and progressive supranuclear palsy (PSP), which are collectively termed tauopathies.[4] Tau exists in six isoforms, differing by the number of repeats in the microtubule-binding domain (3R or 4R), as well as by the presence or absence of an N-terminal insert.[5] The different tauopathies may vary in their relative abundance of 3R and 4R in the NFTs, with AD exhibiting a mixture of 3R and 4R, CBD and PSP containing solely 4R, and Pick's disease (PiD) harboring 3R tau only.[6–8] Studies suggest that the ratio of splice variants determines the conformation of phosphorylated tau (p-tau) in the aggregates, which may affect its interactions with surrounding proteins to mediate toxic effects of p-tau and influence the development of different tauopathies.[9]

Secernin-1 (SCRN1) is a cytosolic protein mainly expressed in the brain but also in other tissue types.[10–12] Its physiological functions are poorly understood, but a recent study suggested an involvement of SCRN1 in endoplasmic reticulum (ER) remodeling and synaptic vesicle recycling at presynaptic sites.[13] Using mass spectrometry (MS)-based proteomics, SCRN1 has been identified as an amyloid plaque–associated protein, and subsequent immunohistochemistry (IHC) studies have shown that it is present in plaque-associated dystrophic neurites.[14] In addition, co-immunoprecipitation studies indicated that SCRN1 was able to pull down p-tau in AD cortex samples further supporting an interaction of SCRN1 with p-tau.[15] This is further supported by two studies in which SCRN1 was (1) co-immunoprecipitated with tau and A$\beta_{1\text{-}42}$ in human AD hippocampal brain aggregates[16] and (2) identified as a component of the phosphorylated tau interactome in human AD brain via affinity-purification MS.[17] Recently, quantification of SCRN1 by IHC demonstrated that it accumulates in NFTs of AD patients, both in early and late stages of the disease, as well as in Down syndrome patients, and patients with progressive age-related tauopathy (PART), but not in CBD, PSP or PiD, suggesting that SCRN1 is specifically associated with tau pathology in AD.[15]

We herein present the first clinical study to test if SCRN1 in cerebrospinal fluid (CSF) is a biomarker of AD. We developed a method to quantify SCRN1 in CSF using targeted high-resolution MS. To evaluate the potential of SCRN1 as a biomarker to distinguish AD from other tauopathies, we analyzed three cohorts, comprising patients with the tauopathies AD, CBD, and PSP, as well as other neurodegenerative disorders: multiple system atrophy (MSA) and Parkinson's disease (PD).

2 | METHODS

2.1 | Materials

Stable isotope labeled peptide standard corresponding to a tryptic peptide located between amino acid 99 and 114 in SCRN1 (EPAAEIEALLGMDLV[R($^{13}C_6{}^{15}N_4$)]) was purchased from Thermo

Fisher. The tryptic peptide was selected based on results of a previous explorative tandem mass tag proteomics study conducted in our lab.[18] First, the standard (30 nmol) was dissolved in 300 μL 20% acetonitrile (ACN) and aliquots of 1 nmol were lyophilized with a SpeedVac vacuum concentrator. Aliquots (1 nmol) were then reconstituted in 100 μL 20% ACN, further aliquoted into 100 pmol aliquots, and stored at −20°C.

2.2 | Discovery cohort

Individual CSF samples (n = 53) as well as pool CSF samples used in the discovery cohort and to evaluate measurement reproducibility, respectively, were obtained from the neurochemistry laboratory at Sahlgrenska University Hospital, Mölndal, Sweden. AD core biomarkers were measured using a chemiluminescent enzyme-immunoassay (CLEIA) on the LUMIPULSE® G1200 platform (Fujirebio Europe). The following cut-off values were applied for neurochemical AD classification: $A\beta_{1\text{-}42}$ < 620 pg/mL, p-tau_{181} > 61 pg/mL, and total (t)-tau > 440 pg/mL.

2.3 | Gothenburg cohort

The Gothenburg disease cohort consisted of patients with Parkinsonian disorders (n = 97), which were diagnosed and treated at the Department of Neurology, Movement Disorders Unit, Sahlgrenska University Hospital, Gothenburg, Sweden, between January 1, 1999 and December 31, 2016. The clinical diagnoses of the entire cohort were confirmed by retrospectively assessing medical records. Furthermore, relevant clinical, laboratory, and demographic data were extracted and analyzed. Information gathered from the medical records was used to assign a research diagnosis for each patient in accordance with established research diagnostic criteria. Thirty-eight patients were diagnosed with established/probable PD according to the Movement Disorder Society (MDS) clinical diagnostic criteria for PD,[19] 31 with probable/possible MSA according to Gilman's criteria,[20] 20 with probable/possible/suggestive PSP according to the MDS criteria for clinical diagnosis of PSP,[21] and 8 with probable/possible CBD according to Armstrong et al.'s criteria.[22]

Healthy controls (n = 37) consisted of orthopedic patients who underwent spinal anesthesia as part of their surgery of the lower limb, between January 1, 2018 and December 31, 2020. CSF was collected during the spinal anesthesia procedure, immediately before the injection of the anesthetic drug. The anesthesiologist evaluated if the healthy controls suffered from neurological disorders (including dementia). If they did, they were excluded.

2.4 | US cohort

Participants were volunteers enrolled in the University of California San Diego Shiley–Marcos Alzheimer's Disease Research Center who underwent longitudinal annual assessments, were followed until death, and agreed to brain examination at autopsy. Participants received a comprehensive annual evaluation, including medical, neurological, and psychiatric history; mental status testing; ratings of functional and global impairment; neuropsychological testing; neurological examination; and structural brain imaging with magnetic resonance imaging at the baseline visit, which has been described in detail.[23] For each participant, all information was used at a consensus conference to determine an overall cognitive diagnosis (normal, mild cognitive impairment [MCI; diagnosed following standard criteria], or dementia). In participants with MCI or dementia, one or more etiological diagnoses were assigned, following research criteria (e.g., AD, dementia with Lewy bodies [DLB], PD with dementia, frontotemporal degeneration [FTD], other dementias). Participants who consented underwent a research lumbar puncture (LP) at their initial evaluation and in some instances a repeat LP 3 to 4 years later. For this study, we selected CSF samples from subjects who had provided CSF and later came to autopsy, with a detailed neuropathological evaluation of their brains.

An autopsy was performed using a standardized protocol.[24] Brains were divided sagittally and the left hemibrain was fixed in 10% buffered formalin while the right hemibrain was sectioned coronally and frozen at −80°C. Sectioning and staining (including immunostaining) followed a standard protocol, in accordance with the National Institute on Aging–Alzheimer's Association (NIA-AA) 2012 guidelines.[25] Neuritic plaque density was estimated using methods recommended by the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) and Braak stage for NFT pathology was determined. For more recent

RESEARCH IN CONTEXT

1. **Systematic Review**: In recent studies, secernin-1 (SCRN1) has been found to specifically co-localize with neurofibrillary tangles (NFTs) in Alzheimer's disease (AD) patients, but not with tau inclusions in other tauopathies, suggesting it may have potential as an AD-specific biomarker.
2. **Interpretation**: We developed a mass spectrometric method based on parallel reaction monitoring, to measure SCRN1 in cerebrospinal fluid (CSF) and applied it in a study of AD patients, and patients across the spectrum of Parkinsonian syndromes, as well as healthy controls. We found that SCRN1 is increased in AD compared to controls and remains unchanged in patients with Parkinsonian syndromes. Further, it correlates strongly with CSF total tau and phosphorylated tau.
3. **Future Directions**: The identification of SCRN1 in CSF as a promising biomarker candidate of AD merits further characterization of SCRN1 concentration changes in other neurodegenerative disorders, as well as preclinical and early AD.

cases, the pathological diagnosis of AD was made using NIA-AA consensus criteria for the *post mortem* diagnosis of AD, wherein Thal phase 4 to 5 (A3), Braak stage V to VI (B3), and moderate to severe neuritic plaque density (C2/3) corresponds to high AD neuropathologic change.[25] Non-AD pathology was assessed using immunostaining for α-synuclein and TAR DNA-binding protein 43, assessment of macro- and microvascular pathological changes and other pathologies, as described.[23]

2.5 | Preparation of CSF samples

CSF samples (100 μL) were spiked with 4 fmol heavy peptide standard dissolved in 20% ACN. Cysteine disulfides were reduced by the addition of 34 μL of 24.2 mM Tris-(2-carboxyethyl)-phosphine in 5% sodium deoxycholate (DOC), 0.5 M triethylammonium bicarbonate (TEAB), and incubation for 1 hour at 55°C. After the samples had cooled to room temperature, 9 μL 200 mM iodoacetamide was added and the samples were incubated for 30 minutes in the dark to alkylate cysteine residues. Trypsin (0.8 μg; Promega) was added, and proteins were digested overnight at 37°C. DOC was precipitated by the addition of 4 μL 10% trifluoroacetic acid (TFA) and 30 μL 1 M hydrochloric acid (HCl) to the samples. The samples were centrifuged at 30,000 × g for 15 minutes at 4°C and the supernatants were transferred onto an OASIS HLB prime 96-well solid phase extraction plate (Waters) for desalting. The solid phase extraction plate wells were washed twice with 200 μL 5% methanol and eluted with 200 μL 80% ACN, 0.1% TFA into a deep-well polypropylene plate (VWR). Samples were then dried using a SpeedVac vacuum concentrator.

2.6 | MS analysis

Samples were dissolved in 100 μL 0.1% bovine serum albumin, 0.05% TFA and 25 μL were analyzed with a nano-LC (Ultimate RSLC Nano, Thermo Scientific) equipped with a C_{18} trap column (PepMap Acclaim 300 μm mm × 5 mm, Thermo Scientific) as well as a C_{18} separation column (PepMap Acclaim 75 μm × 500 mm, Thermo Scientific), coupled to a Fusion Tribrid Orbitrap mass spectrometer (Thermo Scientific), fitted with an Easy Spray ion source. Mobile phases included loading buffer: 0.05% TFA; Buffer A: 0.1% formic acid (FA); Buffer B: 84% ACN, 0.1% FA. A 65-minute gradient of 0 minutes, B 4%; 50 minutes, B 35%; 55 minutes, B 100%; 60 minutes, B 100%; 61 minutes B 4% was used. The mass spectrometer was operated in the positive ion mode. A full MS scan was acquired (R = 120 k, automatic gain control [AGC] target = custom, max injection time = 110 ms) followed by a targeted MS/MS scan (R = 240 k, AGC target = 2000%, max injection time = 502 ms, high energy collision detection = 25%, isolation window = 1.6 *m/z*). Prior to the analysis of clinical cohort samples, assay repeatability was evaluated by injecting a series of quality control (QC) samples (n = 4). Additionally, QC samples were analyzed regularly during runs to monitor assay performance. Figure S1 in supporting information displays typical chromatographic peaks of the transitions used for quantification while Table S1 in supporting information shows relevant peptide-specific parameters for the peptides targeted in our parallel reaction monitoring (PRM) method. Method reproducibility was assessed by preparing and analyzing four aliquots of the same CSF pool in a single analysis run, resulting in a coefficient of variation (CV) of 4.0%.

2.7 | Data processing and statistical analysis

Peak quantification was performed with Skyline version 21.2.0.425 (MacCoss Lab Software). All peaks of peptide transitions used for quantification were visually inspected and peak boundaries manually adjusted if required (Figure S1). Upon inspection, it was ensured that the sum transition peak intensities exceeded the instrument's lower limit of detection estimated at around 4 x 10^3. For the determination of SCRN1 peptide concentration, a one-point calibration was used. Relative peptide abundances were calculated by dividing the sum of all transition peak areas of the measured peptide by the sum of all transition peak areas of the corresponding isotopically labeled internal standard.

Demographic characteristics were assessed by Kruskal–Wallis test for continuous variables and chi-square goodness of fit test for categorical variables. Group-wise comparisons were performed using analysis of covariance, adjusting for the covariates sex and age. Differences in protein abundance across Braak stages were evaluated with Student t test and correlation analyses were performed using Spearman's rank-order correlation. To assess the biomarker's discriminatory ability, a receiver operating characteristic curve (ROC) was calculated yielding an area under the curve (AUC) value. Both data visualization and statistical analyses were conducted with R software (version 4.1.2).

3 | RESULTS

3.1 | CSF SCRN1 in AD and other neurodegenerative disorders

We first analyzed the discovery cohort (Table 1A) comprising patients with neurochemically characterized AD (n = 25) and neurochemical controls (n = 28). SCRN1 was found to be increased by 41% in AD compared to controls ($P < 0.01$; Figure 1A).

To verify that SCRN1 is increased in AD, we proceeded to measure CSF samples of pathology-confirmed AD patients from the US cohort (n = 17; Table 1C). In addition, to test its specificity for AD, we analyzed patients across the spectrum of Parkinsonian syndromes (PD, n = 38; MSA, n = 31, PSP, n = 20; CBD, n = 8) and controls (n = 37) from the Gothenburg cohort (Table 1B). Importantly, in contrast to the US cohort, patient diagnosis in the Gothenburg cohort was solely based on clinical evaluation without *post mortem* pathological confirmation. Scatterplots of SCRN1 in both cohorts are shown in Figure 1B. SCRN1 was significantly increased by 43% in pathology-confirmed AD

TABLE 1 Demographic characteristics of (A) discovery cohort, (B) Gothenburg cohort, and (C) US cohort. Corresponding *P*-values were calculated using chi-square goodness of fit test or Kruskal–Wallis for categorical and continuous variables, respectively. Continuous variables are displayed as "median [Q1, Q3]."

A.	Discovery cohort		
	Neurochemical control	Neurochemical AD	*P*-value
n	28	25	
Sex			0.0696
Female	11 (39.3%)	17 (68.0%)	
Male	17 (60.7%)	8 (32.0%)	
Age (years)	69.5 [63.0, 76.0]	73.0 [70.0, 76.0]	0.28
CSF $A\beta_{1\text{-}42}$ (pg/mL)	811 [649, 989]	455 [405, 510]	
CSF p-tau (pg/mL)	28.5 [24.0, 43.3]	112 [87.0, 154]	
CSF t-tau (pg/mL)	254 [193, 322]	760 [614, 949]	
CSF SCRN1 (fmol/mL)	35.4 [28.3, 43.4]	47.6 [43.8, 67.5]	

B.	Gothenburg cohort					
	control	PD	MSA	PSP	CBD	*P*-value
n	37	38	31	20	8	
Sex						0.00998
Female	19 (51.4%)	9 (23.7%)	15 (48.4%)	12 (60.0%)	5 (62.5%)	
Male	11 (29.7%)	27 (71.1%)	12 (38.7%)	7 (35.0%)	3 (37.5%)	
Age (years)	76.5 [65.3, 80.8]	66.5 [62.8, 70.3]	63.0 [60.5, 68.0]	74.0 [67.5, 74.5]	72.0 [69.0, 73.3]	<0.001
CSF $A\beta_{1\text{-}42}$/ $A\beta_{1\text{-}40}$	0.981 [0.929, 1.03]	0.958 [0.869, 1.02]	0.997 [0.910, 1.03]	0.984 [0.932, 1.02]	0.978 [0.895, 1.03]	
CSF p-tau_{181} (pg/mL)	31.6 [28.7, 36.5]	28.7 [21.6, 34.5]	23.8 [17.9, 29.2]	31.2 [24.2, 36.3]	30.3 [21.2, 34.3]	
CSF t-tau (pg/mL)	261 [217, 300]	226 [188, 286]	250 [184, 338]	261 [209, 334]	262 [183, 279]	
CSF SCRN1 (fmol/mL)	32.7 [26.4, 41.9]	37.1 [29.4, 43.1]	37.3 [29.3, 44.6]	38.8 [29.0, 47.2]	35.1 [32.3, 39.9]	

C.	US cohort			
	Pathology-confirmed control	Pathology-confirmed AD	Pathology-confirmed AD & comorbidities	P-value
n	3	17	37	
Sex				0.942
Female	1 (33.3%)	7 (41.2%)	16 (43.2%)	
Male	2 (66.7%)	10 (58.8%)	21 (56.8%)	
Age at lumbar puncture (years)	80.0 [73.5, 81.0]	77.0 [71.0, 83.0]	74.0 [67.0, 81.0]	0.532
CSF $A\beta_{1\text{-}42}$/ $A\beta_{1\text{-}40}$	0.0939 [0.0932, 0.0969]	0.0548 [0.0454, 0.0606]	0.0494 [0.0400, 0.0602]	
CSF p-tau_{181} (pg/mL)	37.2 [30.7, 39.1]	83.8 [51.5, 111]	87.3 [53.0, 123]	
CSF t-tau (pg/mL)	269 [222, 298]	418 [285, 615]	547 [340, 730]	
CSF SCRN1 (fmol/mL)	36.4 [35.6, 41.5]	45.0 [38.6, 48.4]	49.0 [37.1, 57.0]	

Abbreviations: Aβ, amyloid beta; AD, Alzheimer's disease; CBD, corticobasal degeneration; CSF, cerebrospinal fluid; MSA, multiple system atrophy; PD, Parkinson's disease; PSP, progressive supranuclear palsy; p-tau, phosphorylated tau; SCRN1, secernin-1; t-tau, total tau.

compared to controls ($P < 0.01$), corroborating the results obtained in the discovery cohort. Interestingly, however, SCRN1 concentrations did not differ from the control group in PD, MSA, CBD, and PSP (Figure 1B, Table S2 in supporting information).

3.2 | Correlation with core AD biomarkers

To evaluate whether CSF SCRN1 is associated with tau pathology in AD as a recent IHC study suggests,[15,17] we performed correlation

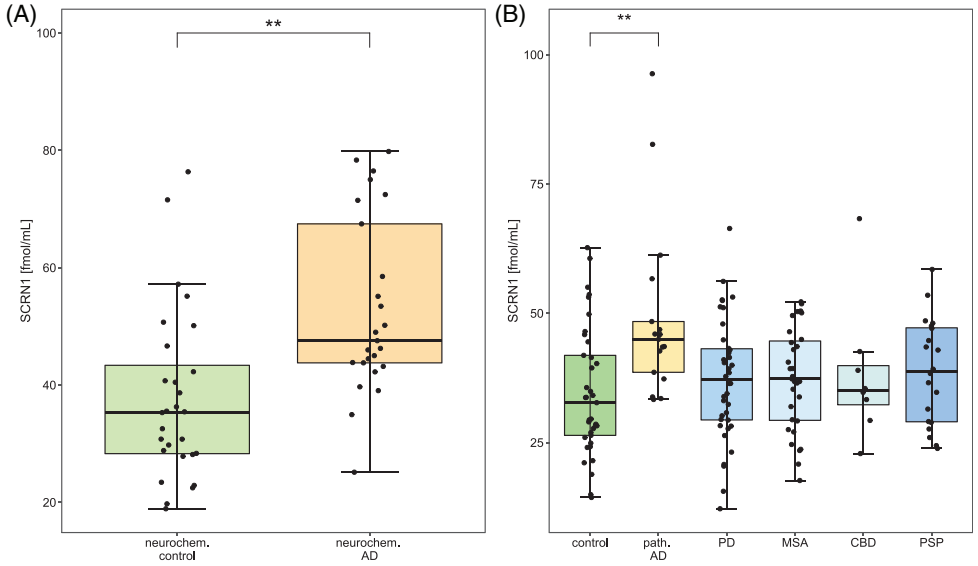

FIGURE 1 CSF SCRN1 concentrations in controls, AD, and across the spectrum of Parkinsonian syndromes. CSF concentrations of SCRN1 measured in (A) the discovery cohort comprising patients with neurochemically characterized AD (neurochem. AD, $n = 25$) and controls (neurochem. controls, $n = 28$) and (B) the US cohort comprising patients with pathology-confirmed AD (path. AD, $n = 17$) as well as the Gothenburg cohort including patients with idiopathic PD ($n = 38$), MSA ($n = 31$), CBD ($n = 8$), PSP ($n = 20$), and controls ($n = 37$). "**" = $P < 0.01$. AD, Alzheimer's disease; CBD, corticobasal degeneration; CSF, cerebrospinal fluid; MSA, multiple system atrophy; PD, Parkinson's disease; PSP, progressive supranuclear palsy; SCRN1, secernin-1.

analyses of SCRN1 with both CSF p-tau (Figure 2A) and CSF t-tau (Figure 2B) in patients from the US cohort with pathology-confirmed AD ($n = 17$) and pathology-confirmed AD and other comorbidities in the spectrum of neurodegenerative diseases ($n = 37$). SCRN1 correlated strongly with p-tau ($R = 0.64$, $P = 3.2 \times 10^{-08}$) as well as t-tau ($R = 0.78$, $P = 1.1 \times 10^{-13}$), and less strongly and negatively with the $A\beta_{1\text{-}42}/A\beta_{1\text{-}40}$ ratio (Figure 2C; $R = -0.45$, $P = 2.8 \times 10^{-04}$) in AD patients pointing to an association of SCRN1 with tau.

3.3 | Correlation with tau pathology

To further evaluate a possible association of CSF SCRN1 with tau pathology, we examined how the concentration of the protein changed across Braak stages in the US cohort (Figure 3A). SCRN1 gradually increased from Braak stages 1 and 2 over stages 3 and 4 by 8% and reached its highest concentration at stages 5 and 6 with a 27% increase compared to stages 1 and 2 ($P < 0.05$), reflecting the growing extent of tau pathology in the brain. In comparison, the increase of CSF p-tau and t-tau concentrations across Braak stages (Figure 3B,C) is more pronounced than that of SCRN1, potentially reflecting the fact that Braak stages directly mirror the degree of tau pathology itself.

3.4 | Correlation with symptomatic progression

To assess whether SCRN1 reflects clinical disease progression, we performed correlation analyses with Mini-Mental State Examination (MMSE) scores in pathology-confirmed AD cases, including patients with comorbidities, as well as healthy controls (Figure 4A). SCRN1 correlated negatively with MMSE score ($R = -0.27$, $P < 0.05$), suggesting that it may be a marker of cognitive decline. Notably, in the US cohort, the correlation of SCRN1 with MMSE score was stronger than the correlation of CSF p-tau with MMSE score (Figure 4B), which failed to reach statistical significance ($R = -0.24$, $P = 0.084$). The correlation of CSF t-tau with MMSE score ($R = -0.38$, $Pp < 0.01$), however, appeared to be the strongest out of all three candidates (Figure 4C). Summarizing the results, we found a strong correlation of both CSF p-tau and t-tau with SCRN1 in AD patients as well as a moderate correlation of SCRN1 with the CSF $A\beta_{1\text{-}42}/A\beta_{1\text{-}40}$-ratio. In addition, SCRN1 concentration appeared to increase across Braak stages and was weakly associated with cognitive dysfunction.

3.5 | Biomarker performance

Last, we investigated the ability of CSF SCRN1 to distinguish healthy controls from AD patients using ROC curve analysis (Figure 5). SCRN1 discriminated pathology-confirmed AD patients (US cohort, $n = 17$) from healthy controls (Gothenburg cohort, $n = 37$) with an AUC of 78%. Additionally, we tested how well SCRN1 could differentiate pathology-confirmed AD patients from patients with clinical parkinsonism (PD, MSA, CBD, PSP; $n = 97$), resulting in an AUC of 71%. In summary, CSF SCRN1 displays a relatively high discriminatory ability in distinguishing AD patients from healthy controls and a slightly weaker ability in discriminating patients with Parkinsonian syndromes from AD.

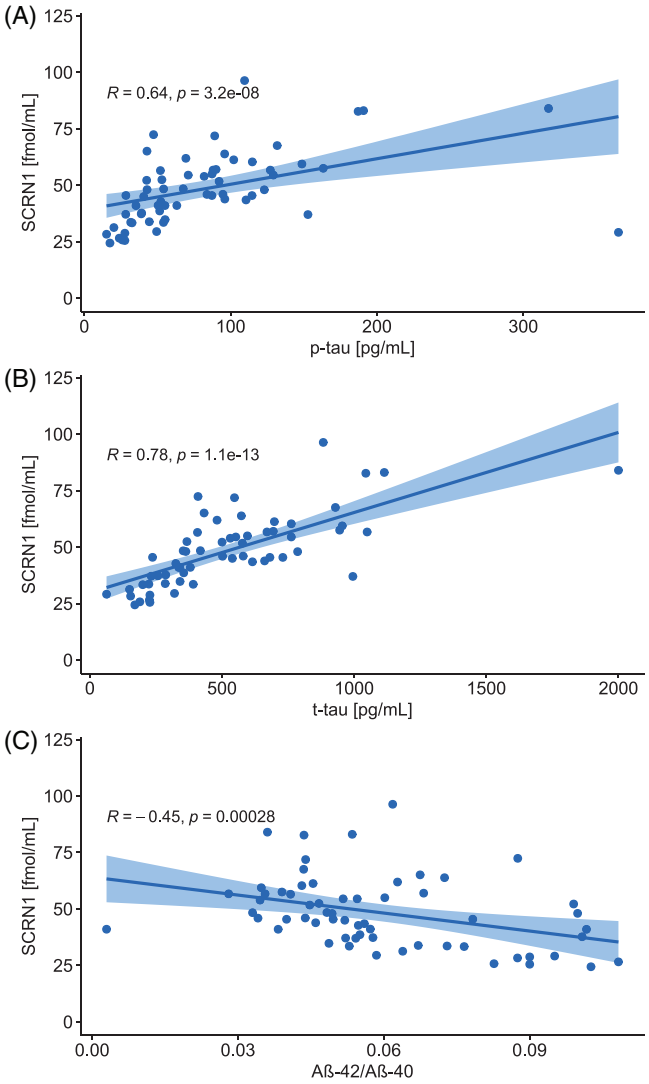

FIGURE 2 CSF SCRN1 correlation with core CSF AD biomarkers. Spearman rank-order correlation of CSF SCRN1 concentration with (A) CSF p-tau, (B) CSF t-tau, and (C) CSF $A\beta_{1\text{-}42}/A\beta_{1\text{-}40}$ ratio in pathology-confirmed AD ($n = 17$) patients and patients with pathology-confirmed AD and comorbidities ($n = 37$) from the US cohort. Aβ, amyloid beta; AD, Alzheimer's disease; CSF, cerebrospinal fluid; p-tau, phosphorylated tau; SCRN1, secernin-1; t-tau, total tau.

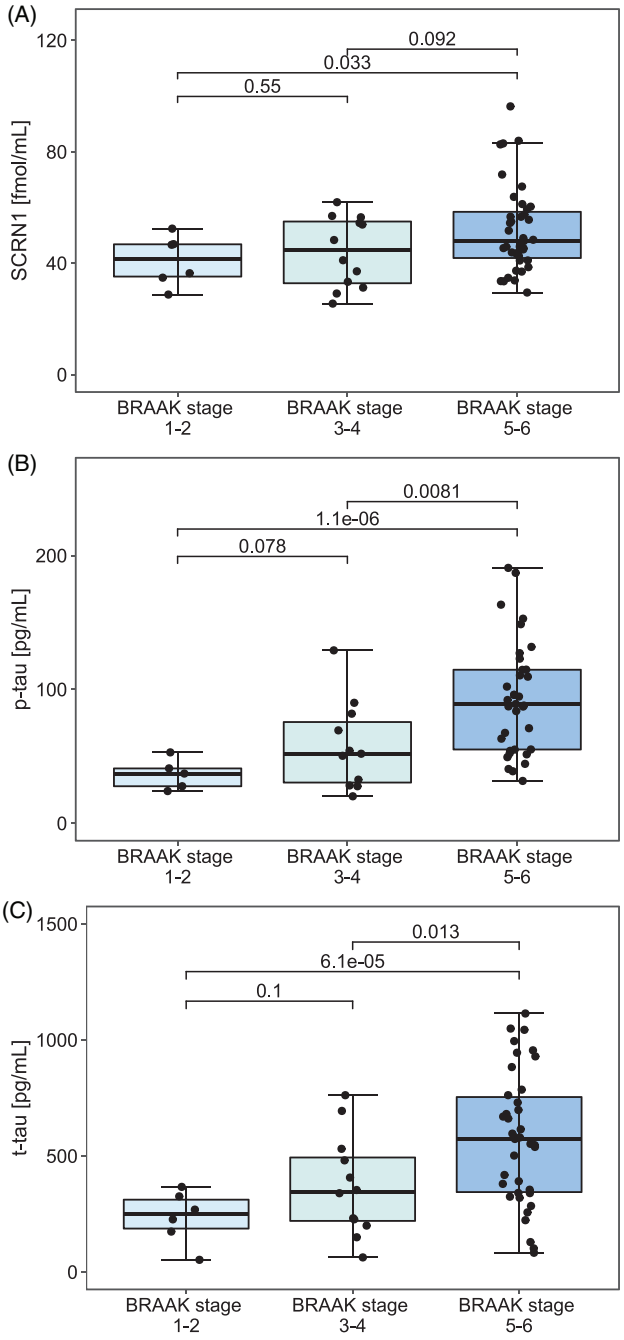

FIGURE 3 CSF SCRN1, p-tau, and t-tau concentrations across Braak stages. CSF concentration of (A) SCRN1, (B) p-tau, and (C) t-tau across the Braak stages 1 and 2, 3 and 4, and 5 and 6 in pathology-confirmed AD ($n = 17$) patients and patients with pathology-confirmed AD and comorbidities ($n = 37$) from the US cohort. *P*-values were obtained with Student *t* test. AD, Alzheimer's disease; CSF, cerebrospinal fluid; p-tau, phosphorylated tau; SCRN1, secernin-1; t-tau, total tau.

4 | DISCUSSION

Here we present the first clinical study showing that SCRN1 in CSF is a new candidate biomarker of AD, with specificity to distinguish between AD and the other tauopathies, CBD and PSP, and which is not altered in other neurodegenerative diseases, including MSA and PD. SCRN1 showed a strong positive correlation with both CSF p-tau and t-tau, corroborating studies that have demonstrated that SCRN1 is associated with tau pathology. Our findings are also in concert with a previous IHC study of the human brain showing that SCRN1 is preferentially associated with NFTs in AD but not in other tauopathies (PSP, CBD).[15] Furthermore, CSF SCRN1 correlated with Braak stage as well as cognitive function, albeit weakly, suggesting its possible utility as a disease progression marker.

ROC curve analysis indicated good performance of SCRN1 to distinguish between AD and controls, with a fold change of ≈40% between the groups. Similarly, CSF SCRN1 was able to discriminate patients

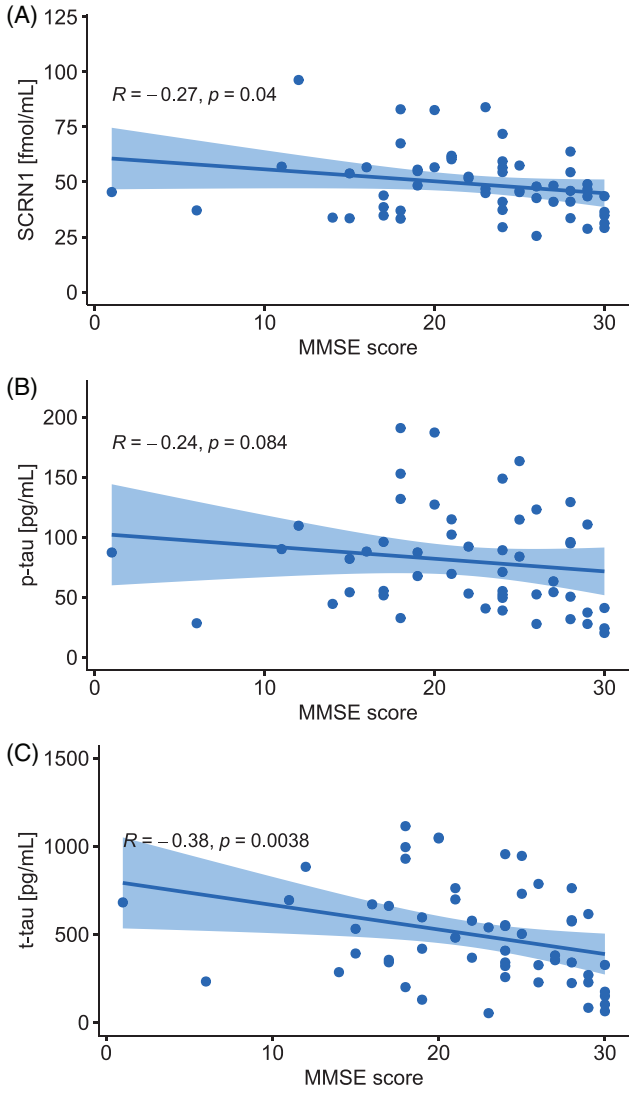

FIGURE 4 Correlation of CSF SCRN1, p-tau, and t-tau with cognition. Spearman rank-order correlation of MMSE score with (A) CSF SCRN1, (B) CSF p-tau, and (C) CSF t-tau in pathology-confirmed AD ($n = 17$) patients, patients with pathology-confirmed AD and comorbidities ($n = 37$), and pathology-confirmed controls ($n = 3$) from the US cohort. AD, Alzheimer's disease; CSF, cerebrospinal fluid; MMSE, Mini-Mental State Examination; p-tau, phosphorylated tau; SCRN1, secernin-1; t-tau, total tau.

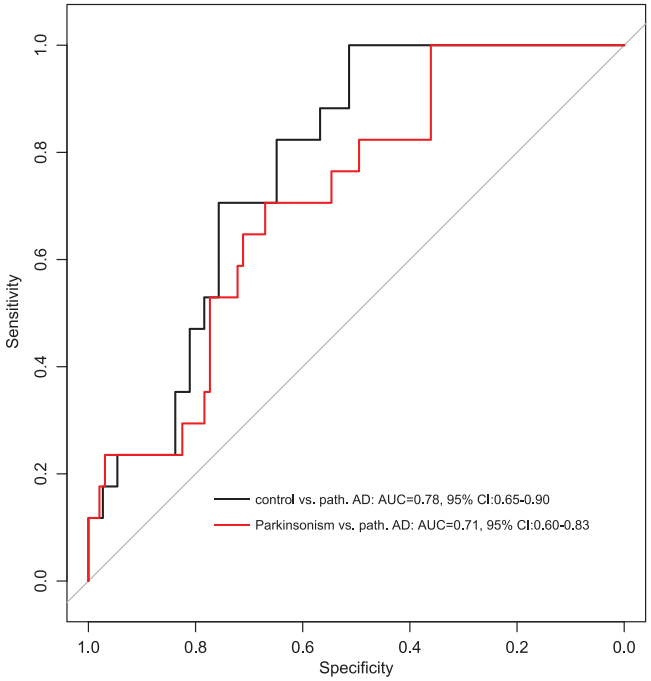

FIGURE 5 Biomarker performance of CSF SCRN1. Receiver operating characteristic (ROC) curves calculated for controls versus pathology-confirmed AD (path. AD, $n = 17$) and parkinsonism patients ($n = 37$) versus path. AD for SCRN1 in CSF. AD, Alzheimer's disease; AUC, area under the curve; CI, confidence interval; CSF, cerebrospinal fluid.

across the spectrum of Parkinsonian syndromes from AD patients. These properties suggest that SCRN1 may have diagnostic value in a clinical setting.

Because SCRN1 has been proposed to be involved in synaptic vesicle recycling[13] and considering that synaptic loss is one of the most prominent features of AD,[26] a potential role of SCRN1 as a marker of synaptic dysfunction in AD could be hypothesized. As it is only increased in the CSF of AD patients and remains unchanged in other neurodegenerative diseases studied herein, it may be implicated in processes of synaptic degeneration specific to AD. Interestingly, recent studies have suggested that tau, besides its role in microtubule stabilization, may have an impact on synaptic vesicle recycling in AD.[27] Combining results of previous studies hinting at a potential interaction of SCRN1 with tau[15–17] and the strong correlation of SCRN1 with tau shown in this study, one could speculate that SCRN1, together with tau, may be involved in pathogenic processes in AD affecting synapses and dystrophic neurites containing aggregated p-tau. More direct binding experiments of SCRN1 with tau could shed light on the potential interplay of both proteins. In addition, further studies are needed to characterize SCRN1 processing as well as post-translational modifications and their potential involvement in disease.

A strength of this study is that it includes pathology-confirmed AD cases, permitting a direct correlation of SCRN1 with tau pathology. Furthermore, the described PRM method involves simple and low-cost sample preparation that can be performed in the 96-well plate format, making it scalable to further larger clinical studies. A limitation is the relatively low number of study subjects for some disease groups, which is attributable to the low prevalence of some of the studied diseases but also to the frequent occurrence of comorbidities in AD patients. Further, patient diagnosis in the Gothenburg cohort lacked pathological confirmation limiting the claim of AD specificity of SCRN1 to clinically defined patient groups.

5 | CONCLUSION

In conclusion, we have shown that SCRN1 in CSF holds potential as both diagnostic and prognostic marker of AD. Further, our results suggest that it is strongly associated with tau pathology in AD patients.

Future work should be directed at confirming present results in another clinical cohort but also at measuring CSF SCRN1 in neurodegenerative diseases that were not included in the current study, such as FTD and DLB. This could give invaluable insights into the role of SCRN1 in other diseases and potentially strengthen the claim for AD specificity. Because SCRN1 has been shown to correlate with disease progression, it would be of great interest to measure its levels across the cognitive trajectory of AD (subjective cognitive impairment, MCI, AD) and investigate its potential as an early marker of AD in pre-clinical AD patients. Finally, we hope that SCRN1 can complement other established AD biomarkers in the assessment of clinical diagnoses and disease progression.

AUTHOR CONTRIBUTIONS

Johan Gobom designed the study; Mathias Sauer, Johan Gobom, and Sophia Weiner performed experiments; Gunnar Brinkmalm, Bruno Becker, and Bárbara Fernandes Gomes contributed to discussions and assisted with experiments; Julius Constantinescu, Radu Constantinescu, Bengt Nellgård, Keti Dalla, and Douglas Galasko provided the patient samples; Johan Gobom and Sophia Weiner analyzed data and wrote the manuscript; Henrik Zetterberg, Kaj Blennow, and Johan Gobom supervised all studies. All authors read and approved the final manuscript.

ACKNOWLEDGMENTS

Kaj Blennow is supported by the Swedish Research Council (#2017-00915), the Alzheimer Drug Discovery Foundation (ADDF), USA (#RDAPB-201809-2016615), the Swedish Alzheimer Foundation (#AF-930351, #AF-939721, and #AF-968270), Hjärnfonden, Sweden (#FO2017-0243 and #ALZ2022-0006), the Swedish state under the agreement between the Swedish government and the County Councils, the ALF-agreement (#ALFGBG-715986 and #ALFGBG-965240), the European Union Joint Program for Neurodegenerative Disorders (JPND2019-466-236), the National Institute of Health (NIH), USA (grant #1R01AG068398-01), and the Alzheimer's Association 2021 Zenith Award (ZEN-21-848495). Henrik Zetterberg is a Wallenberg Scholar supported by grants from the Swedish Research Council (#2018-02532), the European Union's Horizon Europe research and innovation program under grant agreement No 101053962, Swedish State Support for Clinical Research (#ALFGBG-71320), the Alzheimer Drug Discovery Foundation (ADDF), USA (#201809-2016862), the AD Strategic Fund and the Alzheimer's Association (#ADSF-21-831376-C, #ADSF-21-831381-C, and #ADSF-21-831377-C), the Bluefield Project, the Olav Thon Foundation, the Erling-Persson Family Foundation, Stiftelsen för Gamla Tjänarinnor, Hjärnfonden, Sweden (#FO2022-0270), the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement No 860197 (MIRIADE), the European Union Joint Programme – Neurodegenerative Disease Research (JPND2021-00694), and the UK Dementia Research Institute at UCL (UKDRI-1003). Johan Gobom is supported by Alzheimerfonden (AF-930934) and Stiftelsen för Gamla tjänarinnor. Sophia Weiner is supported by Stiftelsen för Gamla tjänarinnor. Bárbara Fernandes Gomes is supported by the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement No 860197 (MIRIADE).

CONFLICT OF INTEREST STATEMENT

Kaj Blennow has served as a consultant, on advisory boards, or on data monitoring committees for Abcam, Axon, BioArctic, Biogen, JOMDD/Shimadzu, Julius Clinical, Lilly, MagQu, Novartis, Ono Pharma, Pharmatrophix, Prothena, 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, outside the work presented in this paper. Henrik Zetterberg has served on scientific advisory boards and/or as a consultant for Abbvie, Acumen, Alector, ALZPath, Annexon, Apellis, Artery Therapeutics, AZTherapies, CogRx, Denali, Eisai, Nervgen, Novo Nordisk, Passage Bio, Pinteon Therapeutics, Red Abbey Labs, reMYND, 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 (outside submitted work). All other authors have nothing to disclose. Author disclosures are available in the supporting information.

REFERENCES

1. Winblad B, Amouyel P, Andrieu S, et al. Defeating Alzheimer's disease and other dementias: a priority for European science and society. *Lancet Neurol*. 2016;15:455-532. doi: 10.1016/S1474-4422(16)00062-4
2. Trejo-Lopez JA, Yachnis AT, Prokop S. Neuropathology of Alzheimer's disease. *Neurotherapeutics*. 2022;19:173-185. doi: 10.1007/s13311-021-01146-y
3. Muralidar S, Ambi SV, Sekaran S, Thirumalai D, Palaniappan B. Role of tau protein in Alzheimer's disease: the prime pathological player. *Int J Biol Macromol*. 2020;163:1599-1617. doi: 10.1016/j.ijbiomac.2020.07.327
4. Kovacs GG. Chapter 25 - tauopathies. In: Kovacs GG, Alafuzoff I, eds. *Handbook of Clinical Neurology*. Elsevier; 2018:355-368. doi: 10.1016/B978-0-12-802395-2.00025-0
5. Sergeant N, Delacourte A, Buée L. Tau protein as a differential biomarker of tauopathies. *Biochim Biophys Acta Mol Basis Dis*. 2005;1739:179-197. doi: 10.1016/j.bbadis.2004.06.020
6. Delacourte A, Sergeant N, Wattez A, Gauvreau D, Robitaille Y. Vulnerable neuronal subsets in Alzheimer's and Pick's disease are distinguished by their tau isoform distribution and phosphorylation. *Ann Neurol*. 1998;43:193-204. doi: 10.1002/ana.410430209
7. Sergeant N, Wattez A, Delacourte A. Neurofibrillary degeneration in progressive supranuclear palsy and corticobasal degeneration: tau pathologies with exclusively "exon 10" isoforms. *J Neurochem*. 1999;72:1243-1249. doi: 10.1046/j.1471-4159.1999.0721243.x
8. Gibbons GS, Banks RA, Kim B, et al. Detection of Alzheimer disease (AD)-specific tau pathology in AD and NonAD tauopathies by immunohistochemistry with novel conformation-selective tau antibodies. *J Neuropathol Exp Neurol*. 2018;77:216-228. doi: 10.1093/jnen/nly010
9. Vaquer-Alicea J, Diamond MI, Joachimiak LA. Tau strains shape disease. *Acta Neuropathol*. 2021;142:57-71. doi: 10.1007/s00401-021-02301-7
10. Tissue expression of SCRN1 - summary - the human protein atlas n.d. Accessed February 18, 2022. https://www.proteinatlas.org/ENSG00000136193-SCRN1/tissue

11. Uhlén M, Fagerberg L, Hallström BM, et al. Proteomics. Tissue-based map of the human proteome. *Science*. 2015;347:1260419. doi: 10.1126/science.1260419
12. Fagerberg L, Hallström BM, Oksvold P, et al. Analysis of the human tissue-specific expression by genome-wide integration of transcriptomics and antibody-based proteomics. *Mol Cell Proteomics*. 2014;13:397-406. doi: 10.1074/mcp.M113.035600
13. Lindhout FW, Cao Y, Kevenaar JT, et al. VAP-SCRN1 interaction regulates dynamic endoplasmic reticulum remodeling and presynaptic function. *EMBO J*. 2019;38:e101345. doi: 10.15252/embj.2018101345
14. Drummond E, Nayak S, Faustin A, et al. Proteomic differences in amyloid plaques in rapidly progressive and sporadic Alzheimer's disease. *Acta Neuropathol*. 2017;133:933-954. doi: 10.1007/s00401-017-1691-0
15. Pires G, McElligott S, Drusinsky S, et al. Secernin-1 is a novel phosphorylated tau binding protein that accumulates in Alzheimer's disease and not in other tauopathies. *Acta Neuropathol Commun*. 2019;7:195. doi: 10.1186/s40478-019-0848-6
16. Ayyadevara S, Balasubramaniam M, Parcon PA, et al. Proteins that mediate protein aggregation and cytotoxicity distinguish Alzheimer's hippocampus from normal controls. *Aging Cell*. 2016;15:924-939. doi: 10.1111/acel.12501
17. Drummond E, Pires G, MacMurray C, et al. Phosphorylated tau interactome in the human Alzheimer's disease brain. *Brain*. 2020;143:2803-2817. doi: 10.1093/brain/awaa223
18. Tijms BM, Gobom J, Reus L, et al. Pathophysiological subtypes of Alzheimer's disease based on cerebrospinal fluid proteomics. *Brain*. 2020;143:3776-3792. doi: 10.1093/brain/awaa325
19. Postuma RB, Berg D, Stern M, et al. MDS clinical diagnostic criteria for Parkinson's disease. *Mov Disord*. 2015;30:1591-1601. doi: 10.1002/mds.26424
20. Gilman S, Wenning GK, Low PA, et al. Second consensus statement on the diagnosis of multiple system atrophy. *Neurology*. 2008;71:670-676. doi: 10.1212/01.wnl.0000324625.00404.15
21. Höglinger GU, Respondek G, Stamelou M, et al. Clinical diagnosis of progressive supranuclear palsy: the movement disorder society criteria. *Mov Disord*. 2017;32:853-864. doi: 10.1002/mds.26987
22. Armstrong MJ, Litvan I, Lang AE, et al. Criteria for the diagnosis of corticobasal degeneration. *Neurology*. 2013;80:496-503. doi: 10.1212/WNL.0b013e31827f0fd1
23. Smirnov DS, Ashton NJ, Blennow K, et al. Plasma biomarkers for Alzheimer's disease in relation to neuropathology and cognitive change. *Acta Neuropathol*. 2022;143:487-503. doi: 10.1007/s00401-022-02408-5
24. Smirnov DS, Galasko D, Hansen LA, Edland SD, Brewer JB, Salmon DP. Trajectories of cognitive decline differ in hippocampal sclerosis and Alzheimer's disease. *Neurobiol Aging*. 2019;75:169-177. doi: 10.1016/j.neurobiolaging.2018.11.015
25. Montine TJ, Phelps CH, Beach TG, et al. National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease: a practical approach. *Acta Neuropathol*. 2012;123:1-11. doi: 10.1007/s00401-011-0910-3
26. Arendt T. Synaptic degeneration in Alzheimer's disease. *Acta Neuropathol*. 2009;118:167-179. doi: 10.1007/s00401-009-0536-x
27. Robbins M, Clayton E, Kaminski Schierle GS. Synaptic tau: a pathological or physiological phenomenon? *Acta Neuropathol Commun*. 2021;9:149. doi: 10.1186/s40478-021-01246-y

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Weiner S, Sauer M, Brinkmalm G, et al. SCRN1: A cerebrospinal fluid biomarker correlating with tau in Alzheimer's disease. *Alzheimer's Dement*. 2023;1-10. https://doi.org/10.1002/alz.13042