

Development of an antibody-free mass spectrometry method for synaptic vesicle glycoprotein 2A quantification in CSF



UNIVERSITY OF
GOTHENBURG

Mathias Sauer¹, Alexandra DiFilippo², Christopher van Dyck², Ryan O'Dell², Henrik Zetterberg^{1,3,4}, Adam Mecca², Nicholas J. Ashton^{1,5,6}, Johan Gobom¹

(1) Institute of Neuroscience and Physiology, the Sahlgrenska Academy at the University of Gothenburg, Gothenburg, Sweden, (2) Alzheimer's Disease Research Unit, Department of Psychiatry, Yale School of Medicine, New Haven, CT, USA, (3) Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden, (4) Department of Medicine, School of Medicine and Public Health, University of Wisconsin–Madison, Madison, WI, USA, (5) Banner Alzheimer's Institute and University of Arizona, Phoenix, AZ, USA, (6) Banner Sun Health Research Institute, Sun City, AZ 85351, USA

Background

- Synaptic dysfunction and degeneration is a hallmark of several neurodegenerative disorders, including **Alzheimer's disease (AD)**.
- In AD, there is a stronger association between synaptic degeneration and cognitive decline than amyloid- β plaque pathology [1].
- **Synaptic vesicle glycoprotein 2A (SV2A)** is a well-established marker of synaptic density due to its continuous expression throughout the brain.
- Positron emission tomography (PET) studies have demonstrated **reductions in synaptic density** in neurodegenerative disorders, including Alzheimer's disease (AD), compared with cognitively unimpaired (CU) older adults [2].



Develop an **anti-body free mass spectrometer (MS) method** for quantifying SV2A in cerebrospinal fluid (CSF). Measure and evaluate its performance in **correlation to SV2A PET** in a cohort containing AD and CU individuals.

Method

SV2A was quantified using an anti-body free MS method. All CSF samples were measured in singlicates (50 μ L). Samples were spiked with a stable isotope labeled heavy standard prior to sample preparation. Samples underwent protein processing, tryptic digestion, and peptide cleanup before analysis on an Orbitrap Lumos MS (Thermo Fisher Scientific). Three N-terminal peptides (49-67, 124-136, and 146-157) were used to create a SV2A MS composite.

Clinical cohort

Thirty participants were included (21 AD, 9 CU). Demographic and clinical characteristics are shown in Table 1.

Characteristic	Overall N = 30 ¹	CU N = 9 ¹	AD N = 21 ¹	p-value ²
Age	73 (11)	77 (8)	69 (8)	0.057
Sex				0.2
female	13 (43%)	2 (22%)	11 (52%)	
male	17 (57%)	7 (78%)	10 (48%)	
YOE	16.00 (4.00)	18.00 (2.00)	16.00 (2.00)	0.14
MMSE	24.5 (5.5)	30.0 (1.0)	23.0 (3.0)	<0.001
CDR	3.75 (5.00)	0.00 (0.00)	5.00 (1.50)	<0.001
Ab42/40	0.046 (0.033)	0.085 (0.010)	0.041 (0.016)	<0.001
pTau181	84 (70)	33 (20)	103 (50)	<0.001
tTau	550 (335)	324 (148)	652 (303)	<0.001
NfL	1,424 (751)	1,230 (615)	1,508 (1,136)	0.074
GAP43	3,817 (2,284)	2,625 (1,126)	4,491 (1,788)	0.002
Neurogranin	240 (151)	153 (48)	265 (89)	0.006
SYT1	21 (13)	19 (12)	25 (13)	0.14

¹ Median (IQR); n (%)

² Kruskal-Wallis rank sum test; Fisher's exact test

Table 1. Baseline demographic, clinical, and CSF biomarker characteristics of CU and AD participants. Values are reported as median (IQR) or n (%).

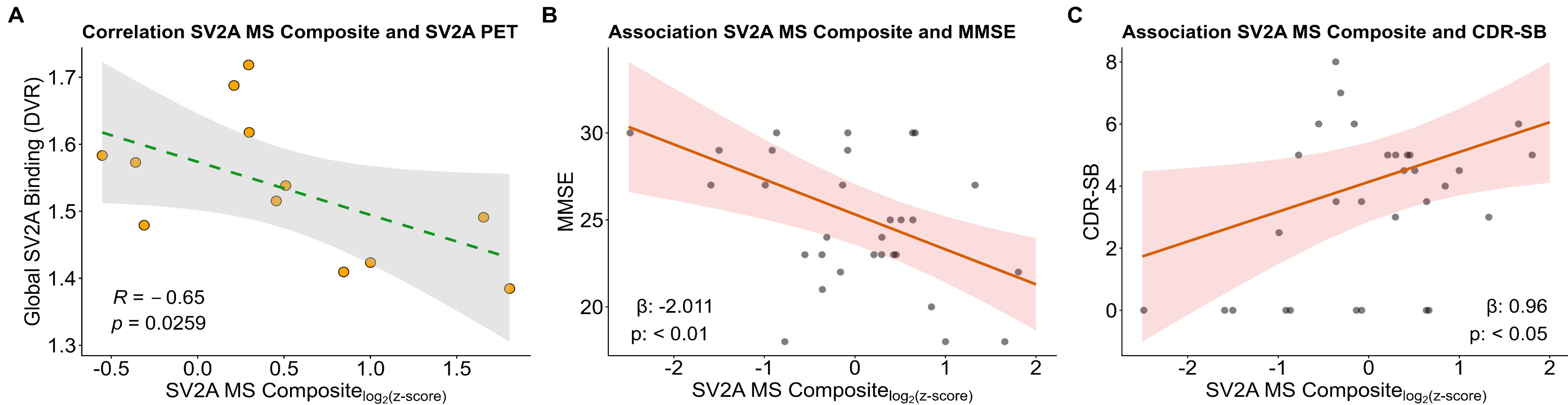
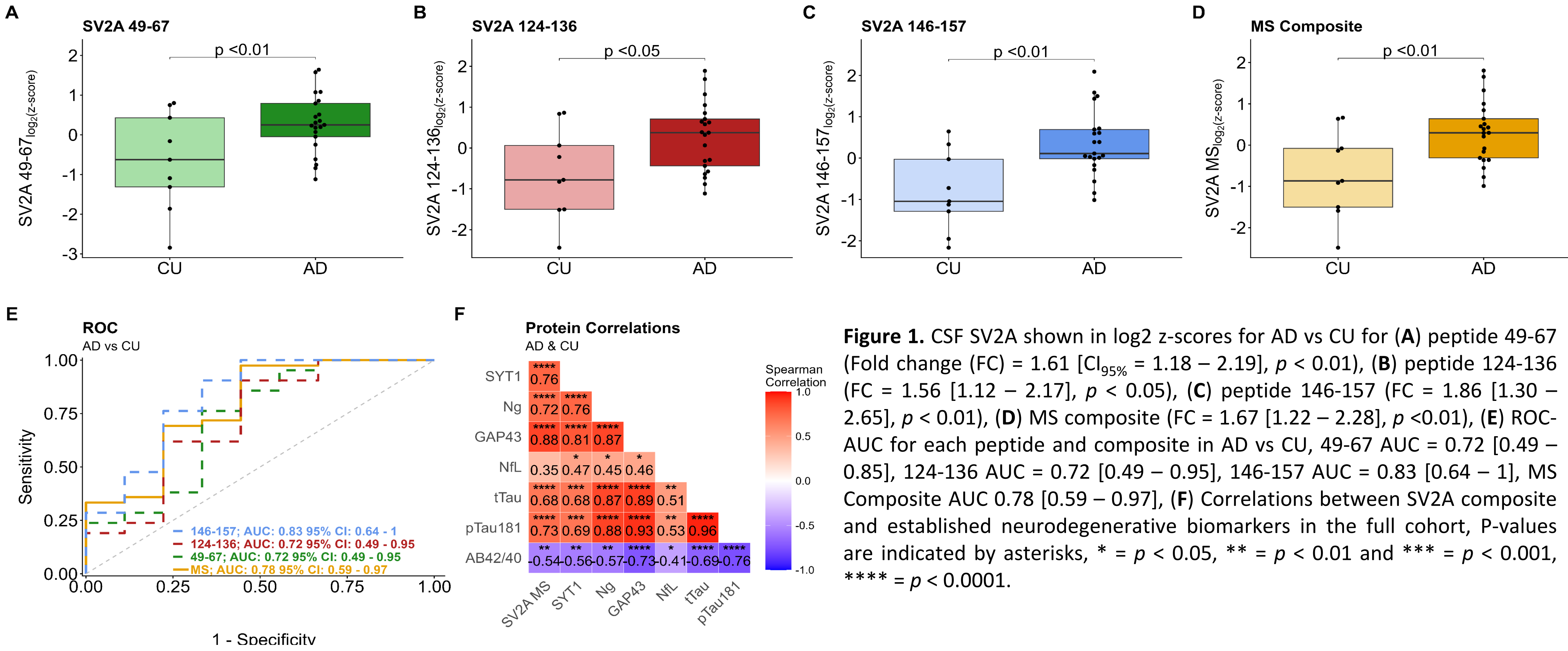
References

1. Terry RD, Masliah E, Salmon DP, et al. Physical basis of cognitive alterations in Alzheimer's disease: Synapse loss is the major correlate of cognitive impairment. *Annals of Neurology*. 1991;30(4):572-580. doi:10.1002/ana.410300410
2. Chen M, Mecca AP, Naganawa M, et al. Assessing Synaptic Density in Alzheimer Disease With Synaptic Vesicle Glycoprotein 2A Positron Emission Tomographic Imaging. *JAMA Neurol*. 2018;75(10):1215–1224. doi:10.1001/jamaneurol.2018.1836

Results

SV2A Log2 transformed

Adjusted for age, sex, and education



Conclusion

- We developed an antibody-free MS method for quantification of SV2A in CSF.
- **CSF SV2A concentrations were significantly increased in AD** compared with CU individuals.
- CSF SV2A showed strong correlations with established biomarkers of neurodegeneration.
- **CSF SV2A correlated significantly with global SV2A binding** measured by the established SV2A PET tracer [¹¹C]UCB-J.
- Higher CSF SV2A levels were significantly associated with worse cognitive and clinical performance, reflected by lower MMSE scores and higher CDR-SB scores.

