Decoding perineuronal net glycan sulfation patterns in the Alzheimer's disease brain

Aric F. Logsdon, PhD[1,2#]; Kendra L. Francis, MD[3,4#]; Nicole E. Richardson, PhD[3]; Shannon J. Hu, BSc[3]; Chelsea L. Faber, PhD[3]; Bao Anh Phan, BSc[3]; Vy Nguyen, BSc[5]; Naly Setthavongsack, BSc[5]; William A. Banks, MD[1,2]; Randy L. Woltjer, MD, PhD[5]; C. Dirk Keene, MD, PhD[6]; Caitlin S. Latimer, MD, PhD[6]; Michael W. Schwartz, MD[3]; Jarrad M. Scarlett, MD, PhD[3,4†]; Kimberly M. Alonge, PhD[3†*]

[#]Equal first author contribution

[†]Equal senior author contribution

[1]Geriatric Research Education and Clinical Center (GRECC), Veterans Affairs Puget Sound Health Care System, University of Washington, Seattle, WA, USA.

[2]Division of Gerontology and Geriatric Medicine, Department of Medicine, University of Washington, Seattle, WA, USA.

[3]University of Washington Medicine Diabetes Institute, University of Washington, Seattle, WA, USA.

[4]Department of Pediatric Gastroenterology and Hepatology, Seattle Children's Hospital, Seattle, WA, USA.

[5]Department of Pathology, Oregon Health & Science University, Portland, OR, USA.

[6]Department of Laboratory Medicine and Pathology, University of Washington, Seattle, WA, USA.

List of Materials:

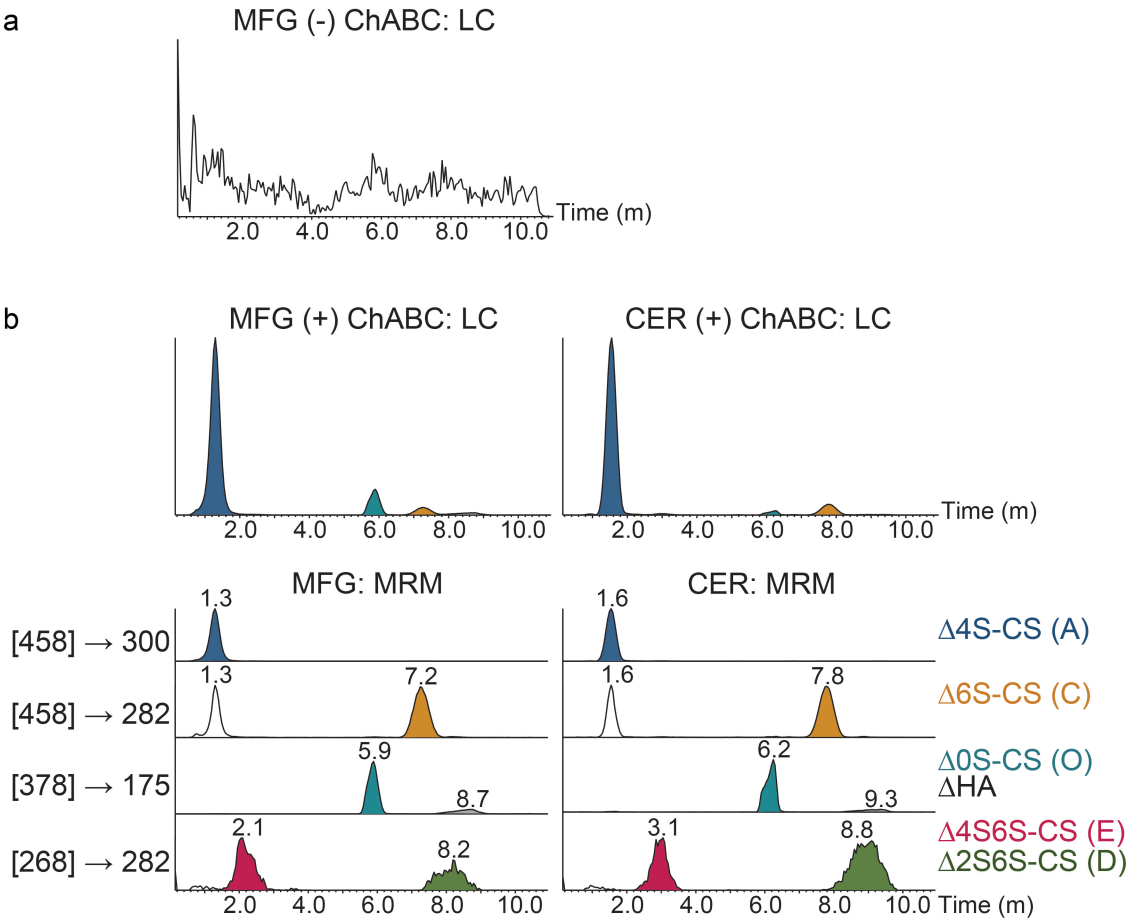


Figure S1. Detection of CS isomers isolated from the MFG and CER by LC-MS/MS and MRM. 30 µm-thick formalin-fixed human middle frontal gyrus (MFG) and cerebellar cortex (CER) tissues were treated with (**a**) 50 mM ammonium bicarbonate (vehicle) or (**b**) 500 mU/mL of Chondroitinase ABC (ChABC) in 50 mM ammonium bicarbonate for 24 h at 37°C. Chromatogram of vehicle treatment alone (a) failed to release ΔCS from the fixed tissue compared to ChABC treatment (b). The extracted CS disaccharides are identified using the following MRM channels: ΔA (4S), *m/z* 458 > 300; ΔC (6S), *m/z* 458 > 282; ΔD (2S6S) and ΔE (4S6S), *m/z* 268 > 282; ΔO (0S), *m/z* 378 > 175. The release of hyaluronan (ΔHA) by ChABC is also observed in the *m/z* 378 > 175 channel. LC, liquid chromatography; MRM, multiple reaction monitoring.

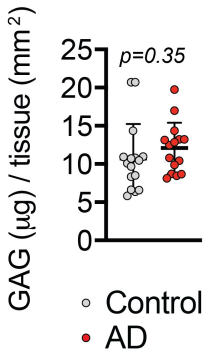


Figure S2. Total CS-GAG abundance is not altered between AD and non-demented control subjects. Total CS-GAGs extracted from ChABC-treated middle frontal gyrus tissue were quantified using cetylpyridinium chloride (CPC) analysis and normalized to total tissue area (mm^2). (*n* = 15-16 subjects per group; mean ± SD). Data were analyzed using Student's *t*-test (unpaired, two-sided).

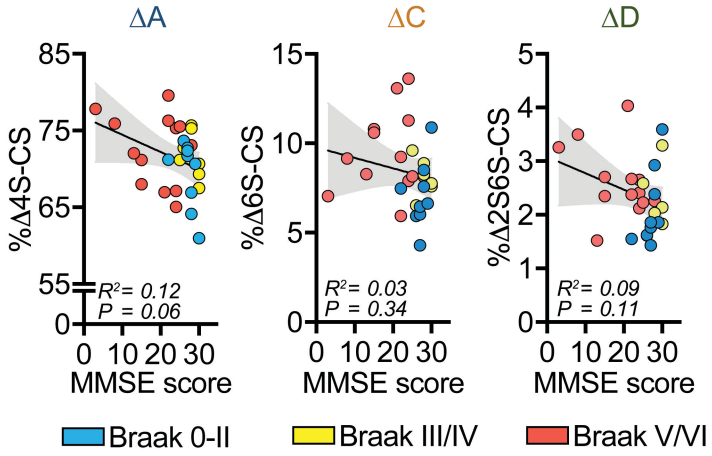


Figure S3. MMSE cognitive scoring does not associate with changes in the relative abundance of MFG matrix ΔA, ΔC, or ΔD. No significant association was observed between MMSE cognitive scoring and changes in the relative abundance of ΔA, ΔC, or ΔD isolated from the middle frontal gyrus (MFG). (n = 29 subjects). Data were analyzed using a linear regression with the 95% confidence bands shown in gray. MMSE, Mini-Mental State Exam; CS, chondroitin sulfate.

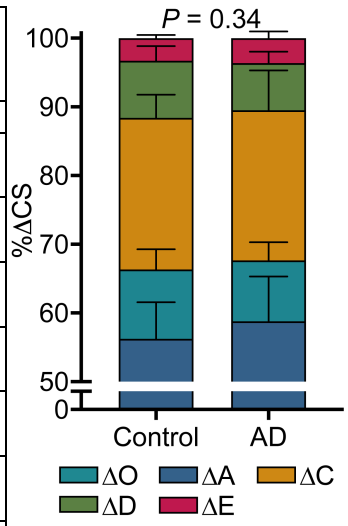
Table S1. Expanded characteristics of patient data.

	Age (years)	Sex (M,F)	PMI (hours)	Braak Stage	CERAD	MMSE Score	Year Collected	Study	Cohort Status
Non-demented Individuals									
ND-1	70	F	7.0	0	0	27	2017	ACT	Included
ND-2	81	M	4.5	I	0	22	2002	ACT	Included
ND-3	89	M	5.0	I	0	28	2013	ACT	Included
ND-4	79	F	2.1	II	0	26	2006	ACT	Included
ND-5	92	F	5.2	II	0	30	2007	ACT	Included
ND-6	86	M	5.7	II	0	27	2007	ACT	Included
ND-7	84	M	4.5	II	0	28	2013	ACT	Included
ND-8	88	F	3.2	II	0	27	2014	ACT	Included
ND-9	84	M	3.9	II	0	29	2016	ACT	Included
ND-10	77	F	6.0	III	0	30	2006	ADRC	Included
ND-11	92	F	6.9	III	0	30	2008	ACT	Included
ND-12	88	F	3.5	III	0	28	2009	ACT	Included
ND-13	91	M	5.0	III	0	28	2011	ACT	Included
ND-14	86	M	3.3	III	0	26	2013	ACT	Included
ND-15	87	M	3.7	III	0	25	2014	ACT	Included
ND-16	90	F	4.5	III	0	30	2015	ACT	Included
Demented (AD) Individuals									
D-1	81	M	5.0	V	3	n/a	2003	ADRC	Included
D-2	86	M	2.7	V	3	24	2010	ADRC	Included
D-3	90	F	3.9	V	3	22	2011	ACT	Included
D-4	85	M	4.0	V	3	25	2013	ACT	Included
D-5	89	M	80.75	V	3	n/a	2010	ADRC	*Excluded*
D-6	91	M	47.3	VI	3	n/a	2006	ADRC	Included
D-7	89	M	5.5	VI	3	24	2007	SLS	Included
D-8	80	F	7.9	VI	3	24	2008	ADRC	Included
D-9	91	M	5.8	VI	3	15	2008	ADRC	Included
D-10	92	F	3.2	VI	3	21	2011	ACT	Included
D-11	86	M	4.3	VI	3	28	2012	ACT	Included
D-12	88	F	3.3	VI	3	15	2012	ACT	Included
D-13	88	F	6.0	VI	3	22	2014	ACT	Included
D-14	70	F	5.0	VI	3	3	2015	ADRC	Included
D-15	85	M	3.5	VI	3	13	2016	ADRC	Included
D-16	77	F	3.3	VI	3	8	2018	ADRC	Included

Abbreviations: AD, Alzheimer's disease; ND, non-demented; D, demented; PMI, postmortem interval; MMSE, Mini-Mental State Examination; M, male; F, female; n/a, not available; ADRC, UW Alzheimer's Disease Research Center; ACT, Adult Changes in Thought Study; SLS, Seattle Longitudinal Study.

Table S2. Relative percentages of CS isomers from the cerebellar cortex.

	Non-demented (n=16)	Demented (AD) (n=15)	Difference of means	95% CI of difference	P_{adj}
%CS (SD)					
ΔA (4S)	56.24 (5.32)	58.79 (6.52)	2.55	-3.38 to 8.47	0.76
ΔC (6S)	22.03 (3.40)	21.86 (5.78)	-0.17	-4.99 to 4.65	>0.99
ΔE (4S6S)	3.29 (0.47)	3.60 (1.00)	0.31	-0.50 to 1.11	0.82
ΔD (2S6S)	8.32 (2.16)	6.87 (1.62)	-1.44	-3.33 to 0.44	0.20
ΔA (4S)	56.24 (5.32)	58.79 (6.52)	2.55	-3.38 to 8.47	0.76
ΔO (0S)	10.11 (2.94)	8.87 (2.63)	-1.24	-3.99 to 1.51	0.72
Avg # S Per ΔCS	1.02 (0.04)	1.02 (0.03)	<0.00	-0.02 to 0.03	0.93



Abbreviations: AD, Alzheimer's disease; SD, standard deviation; CS, chondroitin sulfate. Stats: mixed-effects, repeated measures two-way ANOVA with matched CS isomers and Šidák's multiple comparisons. Stacked bars are represented with SD.

Table S3. Intraregional differences in middle frontal gyrus CS-GAG sulfation patterns.

	ΔO (0S)	ΔA (4S)	ΔC (6S)	ΔD (2S6S)	ΔE (4S6S)
MFG: A01	10.1 ± 0.2	79.1 ± 0.3	7.3 ± 0.1	2.3 ± <0.1	1.2 ± 0.2
MFG: A14	10.8 ± 0.1	78.9 ± 0.2	7.2 ± <0.1	2.1 ± <0.1	1.1 ± 0.2

Data are relative mean percentage ± SD. Samples were run in technical triplicate. MFG, middle frontal gyrus. A01 and A14, two separate samples from the same MFG region of the same patient.