D-serine levels in Alzheimer's disease: Implications for novel biomarker development

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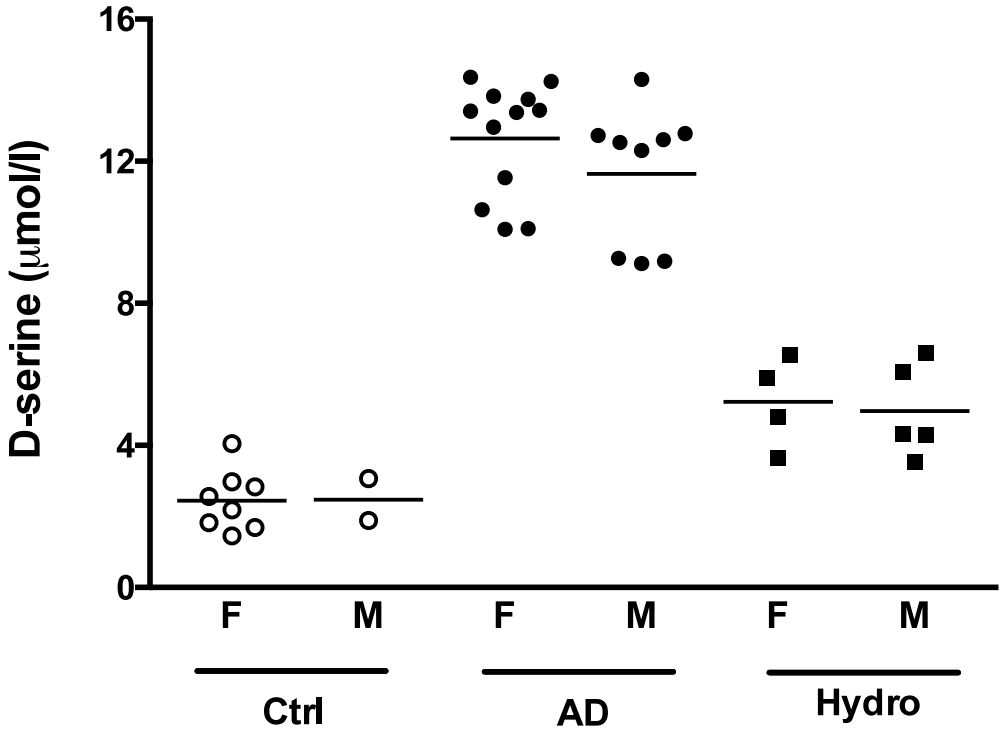
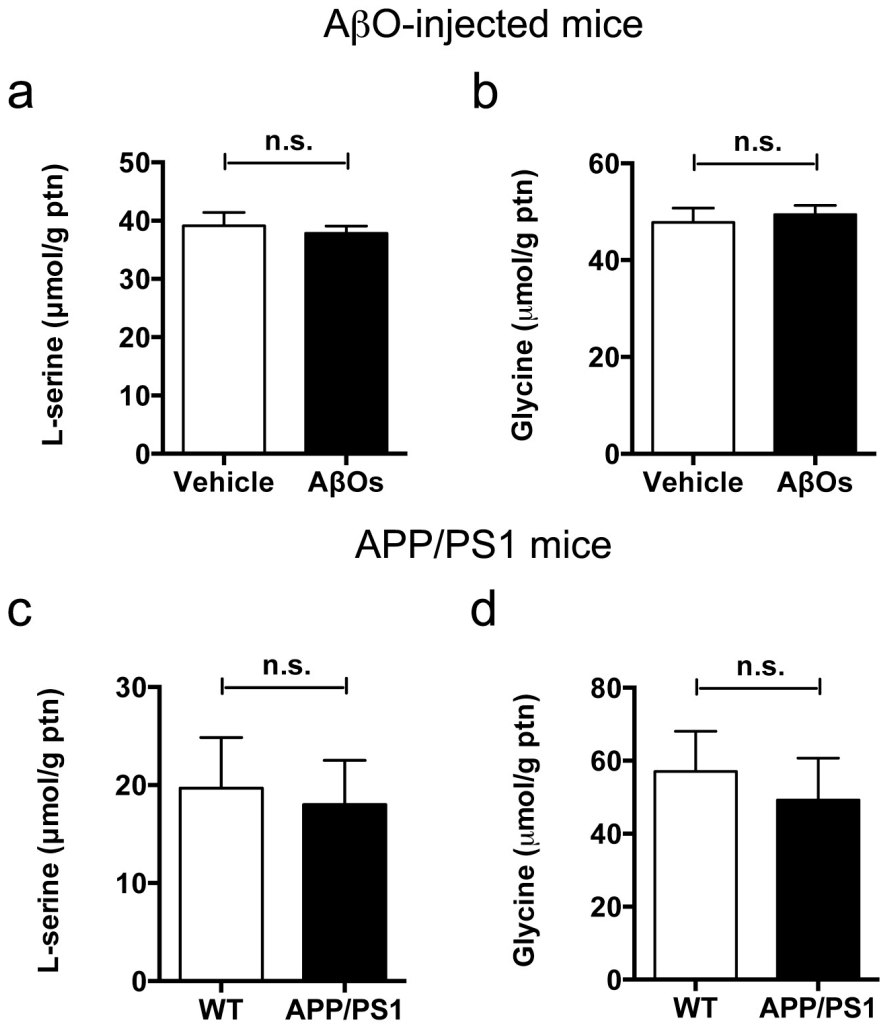
Supplemental Information



Supplementary Figure 1 – Similar CSF D-serine levels in males and females across diagnostic groups. Horizontal lines represent mean values for each group (Ctrl: healthy controls; AD: Alzheimer's disease; Hydro: hydrocephalus). Data points show individual values. Statistical significance was evaluated by one-way ANOVA followed by Bonferroni adjustment for selected groups comparing females (F) *versus* males (M) in each diagnostic group.



Supplementary Figure 2 – Hippocampal L-serine and glycine levels *in vivo*. (a,b) AβOs did not alter L-serine (a) or glycine (b) levels in the hippocampi of mice that received a single intracerebroventricular injection of AβOs (10 pmol, or 45 ng). Amino acid levels were measured 8 days post-injection (n= 10 per group). (c,d) Thirteen to fourteen month-old APPSwe/PS1ΔE9 (APP/PS1) transgenic mice did not show increased hippocampal levels of L-serine (c) or glycine (d) compared to wild-type (WT) mice (n= 8 per group). Amino acid levels were measured by HPLC and its values were corrected by total protein (ptn) content in the analyzed samples. n.s. non-significant in comparison to controls (Student's t-test). Results are presented as means ± SEM.

Supplementary Table 1 – Characteristics of individual subjects in *postmortem* analysis

ID/Age (years)/Sex	CDR	PMI (hours)	Neuropathological diagnosis	Comorbidity	Smoking	Brain region studied
C1/65/M	0	10,85	Control	MDD	smoker	H, PC, OC
C2/74/M	0	10,82	Control	CD	smoker	H, PC
C3/95/M	0	15,33	Control	SAH, HF	smoker	H, PC, OC
C4/89/M	0	14,17	Control	SAH, DM, CD, HF, S	smoker	H, PC, OC
C5/82/F	0	14,83	Control	SAH	nonsmoker	H, PC, OC
C6/81/M	0	9	Control	CD, MDD	smoker	OC
C7/61/F	0	14,66	Control	Absent	nonsmoker	OC
C8/75/F	0	12,58	Control	SAH, DM, CD	nonsmoker	OC
C9/59/F	0	14	Control	HF, S	smoker	OC
C10/64/F	0	21,5	Control	SAH	nonsmoker	H
C11/69/M	0	16,5	Control	SAH, DM	smoker	H
C12/83/M	0	15,92	Control	S	smoker	H
A1/69/M	1	7,43	AD	SAH, DM,CD	smoker	H, PC, OC
A2/85/M	1	14,3	AD + AGD	SAH, DM, CD	smoker	H
A3/82/F	2	13,5	AD	SAH, S	nonsmoker	H, PC, OC
A4/72/M	2	7,78	AD	SAH, DM, S	smoker	H, PC, OC
A5/81/F	2	13,88	AD	DAH, DM, CD, S	nonsmoker	H, OC
A6/83/F	3	10,77	AD	Absent	nonsmoker	H, PC, OC
A7/77/F	3	10,83	AD	Absent	nonsmoker	H, PC, OC
A8/80/F	3	8,33	AD	SAH, CD, D	smoker	OC
A9/84/M	3	12,33	AD	SAH, S	smoker	H, PC, OC
A10/80/F	3	8,08	AD + AGD	SAH, S	smoker	OC
A11/83/F	3	12,05	AD	Absent	nonsmoker	H, PC, OC
A12/84/F	3	12,92	AD	SAH	nonsmoker	H
A13/82/M	3	15,33	AD	DM, CD	smoker	H
A14/81/F	3	10,67	AD	Absent	nonsmoker	H
A15/86/F	3	17,08	AD	SAH, DM, S	nonsmoker	H
A16/87/F	3	17,72	AD	Absent	nonsmoker	H
A17/80/M	3	18,6	AD	HF	nonsmoker	H

M, Male; F, Female; PMI, *Postmortem* interval; AD, Alzheimer's disease; AGD, Argyrophilic Grain Disease; SAH, Systemic Arterial Hypertension; DM, Diabetes Mellitus; CD, Coronary Disease; MDD, Major Depressive Disorder; HF, Heart Failure; S, Stroke; D, Dyslipidemia; H, Hippocampus; PC, Parietal Cortex; OC, Occipital Cortex.

Supplementary Table 2 – Characteristics of individual subjects in CSF analysis

Patient No/ Age (years)/ Sex	CDR	Disease duration (years)	Education (years)	MMSE	Aβ42 (pg/ml)	p-tau$_{181}$ (pg/ml)	t-tau (pg/ml)	IATI	Currently Medicated	Clinical diagnosis
C1/62/F	0	N.A.	16	27	933	47	159	2,18	N.A.	Control
C2/77/M	0	N.A.	6	27	679	39,5	154	1,61	N.A.	Control
C3/65/M	0	N.A.	9	29	855	75	294	1,46	N.A.	Control
C4/76/M	0	N.A.	2	26	669	29,9	106	1,83	N.A.	Control
C5/67/F	0	N.A.	16	28	1152	51,3	231	2,25	N.A.	Control
C6/73/F	0	N.A.	11	27	671	14,1	132	1,70	N.A.	Control
C7/71/F	0	N.A.	6	26	480	15,1	56	1,57	N.A.	Control
C8/72/F	0	N.A.	4	27	687	18,4	108	1,87	N.A.	Control
C9/81/F	0	N.A.	7	25	1138	51,9	219	2,28	N.A.	Control
C10/63/F	0	N.A.	2	29	1169	41	161	2,72	N.A.	Control
A1/64/F	2	4.2	0	4	328	36,7	229	0,64	C	AD
A2/78/M	2	8.2	4	10	280	N.D.	N.D.	N.D.	R	AD
A3/61/F	3	1.5	4	9	332	53,1	272	0,59	No	AD
A4/80/M	2	7.5	12	18	493	85	608	0,51	R	AD
A5/65/M	3	3	16	9	289	33,7	120	0,76	Mem and Ris	AD
A6/79/F	2	3.5	8	14	264	34,3	141	0,65	R and T	AD
A7/72/F	3	9	0	3	N.D.	77,3	393	N.D.	R, Ris, B and Cl	AD
A8/68/M	2	4	4	11	486	14,4	132	1,23	D	AD
A9/83/F	1	5	5	22	459	52,5	161	1,07	R	AD
A10/68/M	2	3	2	14	198	81,4	575	0,22	R, Ris and Cl	AD
A11/77/F	2	3.5	4	11	412	15,5	N.D.	N.D.	R and Mem	AD
A12/71/M	2	1.5	2	16	700	55,4	429	0,94	No	AD
A13/82/F	2	1.8	0	17	462	43,3	359	0,70	D	AD
A14/59/F	1	7	16	23	351	69,1	587	0,38	R, Mem and Ris	AD
A15/80/F	2	3	0	15	376	21,9	602	0,40	D and Ris	AD
A16/75/M	1	3	1	23	631	38,4	245	1,19	Mem and Cl	AD
A17/61/F	2	1.2	4	13	504	58,2	390	0,72	Ris	AD
A18/70/M	3	2	4	3	311	32	629	0,32	R, Mem, E and Ris	AD
A19/78/F	1	1.5	3	16	635	N.D.	160	1,48	Mem and R	AD
A20/58/M	3	3.5	8	3	397	34,1	265	0,72	D and Mir	AD
A21/85/F	2	1.5	4	13	506	46,2	317	0,82	D, Ris and Cl	AD
D1/63/F	0	N.A.	4	26	1123	51,9	214	2,28	Fl and Cl	Depression
D2/74/F	0.5	N.A.	4	25	464	60,4	257	0,85	Desv	Depression
D3/65/F	0.5	N.A.	1	25	625	15,5	141	1,54	P	Depression
D4/77/F	0	N.A.	8	26	1100	39,5	159	2,57	Bus and Cl	Depression
D5/63/F	0.5	N.A.	0	24	783	70,1	322	1,26	S	Depression
D6/74/F	0.5	N.A.	4	24	1063	70,5	276	1,88	Fl	Depression
D7/64/F	0.5	N.A.	1	26	689	37	156	1,62	V and Cl	Depression
D8/73/F	0.5	N.A.	0	19	870	88,5	N.D.	N.D.	No	Depression
D9/75/F	0.5	N.A.	2	25	1085	68,4	471	1,36	C	Depression
H1/71/M	0	2	8	27	350	15	98	0,98	N.A.	Hydrocephalus
H2/81/F	0	1	10	28	706	38	112	1,90	N.A.	Hydrocephalus
H3/68/F	0	3	12	29	355	14	56	1,16	N.A.	Hydrocephalus
H4/78/M	0	2	12	25	551	33	97	1,55	N.A.	Hydrocephalus
H5/74/F	0	4	2	29	727	32	93	2,08	N.A.	Hydrocephalus
H6/66/M	0	3	0	29	863	15	69	2,68	N.A.	Hydrocephalus
H7/66/M	0	0.5	0	28	389	35	99	1,09	N.A.	Hydrocephalus
H8/80/F	0	1	8	25	302	54	111	0,81	N.A.	Hydrocephalus
H9/87/M	0	2	16	25	697	15	97	1,97	N.A.	Hydrocephalus

CDR, Clinical Dementia Rating; MMSE, Mini Mental State Examination; F, Female; M, Male; N.A., Not Applicable; N.D. Not determined; C, Citalopram; R, Rivastigmine; Mem, Memantine, Ris, Risperidone; T, Trazodone; B, Biperiden; Cl, Clonazepam; D, Donepezil; E, Escitalopram; Mir, Mirtazapine; Fl, Fluoxetine; Desv, Desvenlafaxine; P, Paroxetine, Bus, Buspirone; S, Sertraline; V, Venlafaxine; AD, Alzheimer's disease.

Supplementary Table 3 – Medication use does not affect CSF D-serine levels in Alzheimer's disease patients

Medication	D-Serine (μmol/l)		
	Not users	Users	t-test (p value)
Rivastigmine	12.5 (1.84) (N=11)	11.9 (1.74) (N=10)	0.80 (0.43)
Memantine	12.1 (1.82) (N=15)	12.5 (1.80) (N=6)	-0.45 (0.65)
Risperidone	12.4 (1.71) (N=13)	11.9 (1.94) (N=8)	0.71 (0.48)
Clonazepam	12.4 (1.76) (N=17)	11.4 (1.89) (N=4)	0.97 (0.34)
Donepezil	12.1 (1.78) (N=16)	12.6 (1.91) (N=5)	-0.50 (0.62)

Values are presented as means (standard deviation). Statistical significance was assessed by unpaired t-test.

Supplementary Table 4 – Sensitivity and specificity obtained using different IATI and IATI/D-ser cutoffs.

IATI Cutoff	*Sensitivity (%)*	*Specificity (%)*
0.8391	96,30	72,22
0.8961	92,59	72,22
0.9611	92,59	77,78
1.026	88,89	77,78
1.079	88,89	83,33
1.125	85,19	83,33
1.176	81,48	83,33
IATI/D-ser Cutoff	*Sensitivity (%)*	*Specificity (%)*
0.1005	96,30	83,33
0.1083	96,30	88,89
0.1217	96,30	94,44
0.1408	96,30	100,0
0.1680	92,59	100,0
0.1889	88,89	100,0
0.1935	85,19	100,0