Supplement

Deep learning pathway-based metabolic code for screening of Alzheimer's disease in postmortem cerebrospinal fluid

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Experimental section

Materials. Analytical standards were obtained from Fujifilm Wako Pure Chemical Co. (Osaka, Japan) included 1-aminoacetone, glycine (Gly), β-alanine (β-Ala), cytosine, uracil, creatinine (CRE), valine (Val), threonine (Thr), taurine (Tau), thymine, adenine, 5-aminolevulinic acid (5-ALA), 4-aminoimidazole, isoleucine (Ile), creatine (Cr), asparagine (Asn), aspartate (Asp), anthranilic acid (AA), tyramine, glutamine (Gln), histidine (His), methionine sulfoxide (MetSO), cysteate, arginine (Arg), citrulline (Cit), tyrosine (Tyr), *L*-NG-monomethyl arginine (NMMA), β-methylamino-alanine (BMAA), selenomethionine (Se-Met), tryptophan (Trp), N^1,N^8-diacetylspermidine (DAc-SPD), cytidine, adenosine, guanosine, guanosine 5'-monophosphate (GMP), adenosine 3'5'-cyclic monophosphate (cAMP), 5-aminoimidazole-4-carboxanmide (AICA), 1,3-diaminopropane (DAP), putrescine (PUT), acetylspermidine (Ac-SPD), inosine, cytidine 5'-monophosphate (CMP), Saccharopine, cystathionine, carnosine (Car), cystine, anserine, N^1, N^{12}-diacetylspermine (DAc-SPM), spermidine (SPD), N^1-acetylspermine (Ac-SPM), and spermine (SPM). Analytical standards were obtained from SigmaAldrich (Buchs, Switzerland) included 1-amino-2-propanol, 4-aminobutanoic acid (GABA), 2-aminoethanesulfinic acid (hypotaurine), hydroxyproline (ProOH), methionine (Met), cysteinesulfinic acid, phenylalanine (Phe), 3-methoxy-tyramine (3-MeO-tyramine), kynuramine, acetylornithine (Ac-Orn), *N*-acetyllysine (Ac-Lys), NG,NG-dimethyl-*L*-arginine (ADMA), kynurenine (Kyn), uridine, ophthalmic acid, histidinol, cystamine, 5-hydroxylysine (LysOH), 1-methylhistidine (1-MeHis), 3-methylhistidine (3-MeHis), 3-hydroxykynurenine (3-KynOH), glutathione (GSH), and S-adenosyl-homocysteine (SAH). Alanine (Ala), serine (Ser), proline (Pro), leucine (Leu), glutamate (Glu), cadaverine (CAD), and lysine (Lys) were obtained from Kanto Chemicals (Tokyo, Japan). Ethanolamine (EA), *N*-methylglycine (sarcosine, Sar), 2-aminobutyric acid (homoalanine), 3-aminoisobutyric acid, 2-aminoisobutyric acid, hypoxanthine, 5-aminovaleric acid (5-Ava), homoserine (Hse), allothreonine (aThr), phenethylamine, N^1-acetylputrescine (Ac-PUT), agmatine (Agm), norleucine (Nle), 2,4-diaminobutyric acid, phosphoethanolamine (EAP), *O*-acetylserine, 2-aminophenol, guanine, *N*-methyl-anthranilate (MeAA), 3-hydroxyanthranilate (3-AAOH), tryptamine, 2-aminoadipate, 3-methoxyanthranilate (3-MeOAA), xanthosine, theanine, serotonin (5-HT), homophenylalanine (Hphe), homocysteate, methionine sulfone (MetSO2), 5-methoxytryptamine (5-MeOT), indolelactate, 5-hydroxy-tryptophan (5-TrpOH), histamine (Hst), ornithine (Orn), *O*-phospho-serine, 2,6-diaminopimelate, homocystine, and norspermidine (NorSPD) were obtained from Tokyo Kasei Co. (Tokyo, Japan). Adenosine 5'-monophosphate (AMP) was obtained from Oriental Yeast Co. (Tokyo, Japan). *N*-Methylhistamine (MeHst) was obtained from Calbiochem (Darmstadt, Germany).

IS standards were labeled with deuterium, ^{13}C, ^{15}N, or a combination of stable isotopes. Arginine-$^{13}C_6$ (Arg-$^{13}C_6$) and 3-hydroxyanthranilate-d_3 (3-AAOH-d_3) were obtained from Fujifilm Wako Pure Chemical Co. (Osaka, Japan). Glycine-$^{13}C_{2,}{}_{15}N_2$, alanine-$^{13}C_3$, ^{15}N, GABA-d_6, serine-$^{13}C_3$, ^{15}N, proline-$^{13}C_5$, ^{15}N, valine-d_8, leucine-d_{10}, asparagine-$^{13}C_4$, $^{15}N_2$, and tyrosine-$^{13}C_9$, ^{15}N were obtained from SigmaAldrich (Buchs, Switzerland). 1,6-Diaminohexane (DAH) was obtained from Kanto Chemicals Co. (Tokyo, Japan). *N*-methylglycine-d_3 (Sar-d_3), threonine-d_2, tryptophan-d_5, methionine-d_4, histidine-d_3, and ornithine-d_7 (Orn-d_7) were obtained from C/D/N Isotopes Co. (Quebec, Canada). Creatine-d_3, creatinine-d_3 (CRE-d_3), glutamine-d_5, glutamate-d_5, aspartate-^{13}C, histamine-d_4, phenylalanine-d_6, guanosine-$^{15}N_5$, lysine-$^{13}C_6$, $^{15}N_2$, citrulline-d_4 (Cit-d_4), kynurenine-d_6 (Kyn-d_6), glutathione-$^{13}C_2$,^{15}N (GSH-$^{13}C_2{}^{15}N$), cystathionine-d_4, spermidine-d_8 (SPD-d_8), and spermine-d_8 (SPM-d_8) were obtained from Cambridge Isotope Laboratories (Andover, USA).

LC-MS/MS condition. The mobile phase consisted of 0.1% FA in water (solvent A) and 0.1% FA in acetonitrile (solvent B) was delivered at a flow rate of 0.4 mL/min. Aliquots (5 μL) of the solution were injected into the column, and the total analytical run time was 10 min. The MS system was equipped with an ESI source operating in the positive or negative ion mode. Nitrogen was used for the nebulization and desolvation (N_2 Supplier Model 24S, Anest Iwata, Yokohama, Japan), and argon was used as the collision gas. The LC linear gradient was as follows: 40% Solvent B at 0 min, 70% Solvent B at 5 min, 98% Solvent B at 7 min, 98% Solvent B at 9 min, 40% Solvent B at 9.1 min, and 40% Solvent B at 11 min with the flow rate of 0.4 mL/min. The injection volume was 5.0 μL. The column temperature was 50°C. The mass

spectrometer was operated with an ESI source in the positive and negative ionization mode. The ESI source conditions included a capillary voltage of 3.0 kV, source temperature of 150°C, and desolvation temperature of 400°C. The cone and desolvation gas flows were 50 L/hr and 800 L/hr, respectively and were obtained from a nitrogen source (N_2 Supplier Model 24S, Anest Iwata Co., Yokohama, Japan). Argon was used as the collision gas and was regulated at 0.15 mL/min. The LH resolution 1, HM resolution 1, ion energy 1, LM resolution 2, HM resolution 2, and ion energy 2 were 9.6, 15.0, 0.5, 15.0, 15.0 and 0.5, respectively. The dwell time was automatically set by the software.

p-CSF sample preparation. Samples (2 mL, each) were gently divided into ~1.0 mL aliquots in collection tubes and stored in a −80°C freezer until analyzed. The *p*-CSF samples (200 μL) were transferred into 2.0 mL microcentrifuge tubes and spiked with 20 μL of the mixed IS solution and 180 μL of acetonitrile. The mixtures were vigorously vortexed for 30 s and centrifuged in a CF15RN centrifuge (Hitachi, Tokyo, Japan) at 15000 rpm (21200 × g) for 5 min at 4°C to remove protein. After centrifugation, 300 μL of each supernatant was subjected to FMOC derivatization as described above. The reaction solution was then evaporated using a centrifugal evaporator. The residues were dissolved in acetonitrile (75 μL) and centrifuged at 15000 rpm for 5 min. The supernatants (50 μL) were dispensed into another tube to remove precipitates and mixed with 50 μL of 0.2% FA in water. The solutions were vortexed, and 5 μL of aliquot was injected for LC-MS/MS assay. Method validation is that the following parameters were investigated: linearity, specificity, selectivity, limit of detection (LOD), limit of quantification (LOQ), and recovery. The use of 33 isotopically labeled IS reagents minimized a loss during *p*-CSF pretreatment and the variability of MS detection. Specificity and selectivity were evaluated using the retention time and MS^2 transition. The LOD and LOQ values of analytes were determined based on the signal-to-noise (*S/N*) ratio. The linearity was evaluated by constructing a calibration curve at least six points over the concentration range of 1 nM - 100 μM plotting the ratios of the standard and I.S. peak area (y) against the concentrations of the adjusted standard concentration (x, nM). For validity of bio-pretreatment, high and low recoveries (%) were calculated according to $F/(F_0 + A) \times 100$. F and F_0 are the analyte concentrations in the spiked and unspiked samples, respectively, and A is the spike concentration. The pooled *p*-CSF samples (20 μL) were added to acetonitrile (160 μL) and the adjusted standard solutions (20 μL). The unspiked and spiked (low and high levels) samples were derivatized, pretreated, and injected into the LC–MS/MS system. The same researcher handled the *p*-CSF samples over a short period of time at room temperature for repeatability.

Data collection and pre-processing for statistical analysis. For MS data processing, MassLynx 4.1 software and TargetLynx 4.1 software (Waters Co., Milford, MA, USA) were used for data acquisition and quantitative analysis. The quantitative data obtained from *p*-CSF samples were reported as the mean ± standard deviation (SD) values. Mean value and the SD were compared by performing Student's t-tests and/or Welch's t-tests. A calculated *p*-value (< 0.05) was selected to indicate statistical significance. The missing values were substituted using the median value of each metabolite in every comparison.

Table S1 The detailed MS/MS parameters of the 121 analytes and 33 I.S.

Analytes	Ionization	Retention time (min)	Precursor ions (*m/z*)	Product ions (*m/z*)	Cone voltage (V)	Collision energy (eV)
EA	Positive	2.70	284	179	15	15
1-Aminoacetone	Positive	3.56	296	179	20	20
Gly	Positive	2.84	298	179	15	20
1-Amino-2-propanol	Positive	3.10	298	179	15	10
β-Ala	Positive	2.98	312	179	15	20
Ala	Positive	3.29	312	179	15	10
Sar	Positive	3.30	312	179	15	15
GABA	Positive	3.18	326	179	20	20
2-Aminoisobutyric acid	Positive	3.57	326	179	20	10
3-Aminoisobutyric acid	Positive	3.39	326	179	20	20
Homoalanine	Positive	3.75	326	179	20	20
Ser	Positive	2.32	328	179	20	20
2-Aminophenol	Positive	5.56	332	179	20	15
Hypotaurine	Positive	2.12	332	179	20	20
Cytosine	Positive	1.96	334	179	30	30

Uracil	Positive	3.28	335	179	20	15
CRE	Positive	1.69	336	179	25	30
Pro	Positive	3.72	338	179	25	20
5-Ava	Positive	3.42	340	179	20	20
Val	Positive	4.34	340	179	25	25
Hse	Positive	2.26	342	179	20	20
aThr	Positive	2.51	342	179	20	20
Thr	Positive	2.71	342	179	20	20
Phenethylamine	Positive	6.26	344	179	15	20
Tau	Negative	1.73	346	124	35	25
AICA	Positive	3.18	349	179	25	15
Thymine	Positive	3.80	349	179	15	20
Ac-PUT	Positive	3.02	353	179	25	25
Agm	Positive	1.71	353	179	50	30
5-ALA	Positive	2.93	354	179	15	30
ProOH	Positive	2.18	354	179	20	25
Leu	Positive	4.98	354	179	25	20
Nle	Positive	5.04	354	179	15	15
Ile	Positive	4.92	354	179	20	15
Cr	Positive	1.41	354	179	40	35
Asn	Positive	1.98	355	179	25	30
Asp	Positive	2.37	356	179	20	20

Adenine	Positive	2.79	358	179	30	25
Hypoxanthine	Positive	2.63	359	179	25	20
AA	Positive	5.91	360	179	15	20
Tyramine	Positive	4.48	360	179	20	15
EAP	Positive	1.08	364	179	20	20
Gln	Positive	1.98	369	179	25	25
O-Acetyl-serine	Positive	3.25	370	179	15	25
Glu	Positive	2.42	370	179	20	20
Met	Positive	4.16	372	179	25	20
MeAA	Positive	4.65	374	179	25	25
Guanine	Positive	2.31	374	179	30	25
3-AAOH	Positive	4.19	376	179	20	25
Cysteine sulfinate	Positive	1.68	376	179	20	25
His	Positive	7.74	379	179	50	25
Tryptamine	Positive	5.82	383	179	30	25
2-Aminoadipate	Positive	2.53	384	179	20	20
Kynuramine	Positive	5.20	387	179	25	30
O-Phospho-serine	Negative	1.89	388	79	35	25
MetSO	Positive	2.03	388	179	35	25
Phe	Positive	4.91	388	179	25	15

Cysteate	Negative	1.53	390	112	40	25
3-MeOAA	Positive	4.02	390	179	25	25
3-MeO-tyramine	Positive	4.65	390	179	20	20
Arg	Positive	1.29	397	179	45	35
Ac-Orn	Positive	2.51	397	179	25	30
Theanine	Positive	2.65	397	179	30	20
Cit	Positive	1.96	398	179	25	15
5-HT	Positive	4.03	399	179	30	30
Hphe	Positive	5.38	402	179	20	20
Homocysteate	Negative	1.43	404	208	40	15
$MetSO_2$	Positive	2.53	404	179	20	30
Tyr	Positive	3.23	404	179	25	20
NMMA	Positive	1.38	411	179	40	35
Ac-Lys	Positive	2.45	411	179	25	25
5-MeOT	Positive	5.44	413	179	30	30
Se-Met	Positive	4.38	420	179	20	25
ADMA	Positive	1.49	425	179	50	35
Trp	Positive	4.54	427	179	30	30
Indolelactate	Positive	5.20	428	179	20	30
Kyn	Positive	4.31	431	179	30	25
5-TrpOH	Positive	2.96	443	179	20	30
DAc-SPD	Positive	2.31	452	179	40	40

Cytidine	Positive	1.38	466	179	25	25
Uridine	Positive	1.89	467	179	20	40
Adenosine	Positive	1.82	490	179	40	35
Inosine	Positive	2.23	491	179	25	30
Saccharopine	Positive	1.30	499	179	40	35
Guanosine	Positive	1.67	506	179	25	25
Xanthosine	Positive	2.42	507	179	25	20
Ophthalmic acid	Positive	2.04	512	179	30	30
DAP	Positive	7.04	519	179	30	35
4-Aminoimidazole	Positive	7.47	528	179	35	20
PUT	Positive	7.09	533	179	25	25
CMP	Positive	1.07	546	112	35	35
CAD	Positive	7.31	547	179	30	25
cAMP	Positive	1.31	552	136	40	35
Hst	Positive	7.10	556	179	40	40
BMAA	Positive	6.49	563	179	35	30
2,4-Diaminobutyric acid	Positive	6.17	563	179	35	35
AMP	Positive	1.06	570	136	45	35

MeHst	Positive	4.46	570	179	40	40
Orn	Positive	6.28	577	179	25	25
GMP	Positive	1.08	586	152	40	15
Histidinol	Positive	6.29	586	179	40	35
Lys	Positive	6.48	591	179	35	35
Cystamine	Positive	7.48	597	179	35	40
LysOH	Positive	5.57	608	179	30	30
1-MeHis	Positive	3.67	614	179	40	35
3-MeHis	Positive	4.03	614	179	40	40
Ac-SPD	Positive	6.42	633	179	45	40
2,6-Diamino-pimelate	Positive	5.54	635	179	35	30
Cystathionine	Positive	5.71	667	179	35	30
3-KynOH	Positive	7.14	669	179	50	30
Car	Positive	5.88	672	179	50	35
Cystine	Positive	5.92	686	179	50	35
Anserine	Positive	3.92	686	179	50	35
Homocystine	Positive	6.19	713	179	40	35
DAc-SPM	Positive	5.47	731	179	45	35
GSH	Positive	5.45	753	179	45	40
NorSPD	Positive	8.24	798	179	40	40
SPD	Positive	8.29	813	179	45	40

SAH	Positive	4.35	830	179	40	40
Ac-SPM	Positive	7.93	912	179	50	35
SPM	Positive	8.92	1092	179	60	50
Gly-$^{13}C_2{}^{15}N_2$	Positive	2.84	301	179	15	20
Sar-d_3	Positive	3.28	315	179	15	15
Ala-$^{13}C_3{}^{15}N$	Positive	3.29	316	179	15	15
GABA-d_6	Positive	3.18	332	179	20	15
Ser-$^{13}C_3{}^{15}N$	Positive	2.32	332	179	20	20
CRE-d_3	Positive	1.65	339	179	25	20
Thr-d_2	Positive	2.71	344	179	20	20
Pro-$^{13}C_5{}^{15}N$	Positive	3.72	344	179	25	25
Val-d_8	Positive	4.34	348	179	25	20
Cr-d_3	Positive	1.35	357	179	40	15
Asp-^{13}C	Positive	2.37	357	179	20	20
Asn-$^{13}C_4{}^{15}N_2$	Positive	1.98	361	179	25	25
Leu-d_{10}	Positive	4.98	364	179	25	10
Gln-d_5	Positive	1.98	374	179	25	25
Glu-d_5	Positive	2.42	375	179	25	25
Met-d_4	Positive	4.16	376	179	25	20
3-AAOH-d_3	Positive	4.19	379	179	20	20
His-d_3	Positive	7.74	382	179	50	15
Phe-d_6	Positive	4.91	396	179	25	20

Cit-d_4	Positive	1.96	402	179	25	20
Arg-$^{13}C_6$	Positive	1.29	403	179	45	35
Tyr-$^{13}C_9{}^{15}N$	Positive	3.23	414	179	25	20
Trp-d_5	Positive	4.54	432	179	30	30
Kyn-d_6	Positive	4.22	437	179	30	25
Guanosine-$^{15}N_5$	Positive	1.64	511	179	25	25
Hst-d_4	Positive	7.10	560	179	40	40
DAH	Positive	7.52	562	179	35	30
Orn-d_7	Positive	6.28	584	179	25	15
Lys-$^{13}C_6{}^{15}N_2$	Positive	6.48	599	179	35	35
Cystathionine-d_4	Positive	5.71	671	179	35	40
GSH-$^{13}C_2{}^{15}N$	Positive	5.45	755	179	45	35
SPD-d_8	Positive	8.29	821	179	45	40
SPM-d_8	Positive	8.92	1100	179	50	25

Table S2 Detailed pathological information about the participants including age, gender, and the severity of SP and NFT progression.
CAA: Cerebral Amyloid Angiopathy, SP: Senile Plague, NFT: Neurofibrillary Tangle, MI: Multiple Infarct

Groups	No.	Age	Sex	SP Braak stage	NFT Braak stage	Pathological diagnosis
Control	Cont_1	71	Female	CERAD: (-)	I	α-synuclein (-), Stroke
	Cont_2	76	Male	CERAD: (-)	I	α-synuclein (-), MI, Bilateral inferior olivary nuclear pseudohypertrophy
	Cont_3	96	Male	CERAD: (-)	II - III	Lewy body (-), MI, Arteriosclerosis
	Cont_4	88	Female	CERAD: A	I	α-synuclein (-), MI
	Cont_5	77	Male	CERAD: A	I	α-synuclein (-), CAA (light), Stroke
	Cont_6	85	Male	CERAD: A	I	CAA (heavy), MI, Cerebellar infarction
	Cont_7	95	Female	CERAD: A	I	α-synuclein (-), CAA (light), MI
	Cont_8	71	Male	CERAD: B	I	Lewy body (-), CAA, Cortical microinfarction
	Cont_9	78	Male	CERAD: B	I	α-synuclein (-), MI, Willis ring arteriosclerosis, TDP-43 (-)
	Cont_10	84	Female	CERAD: B	III - IV	White thin arteriosclerosis
	Cont_11	90	Female	CERAD: B	III	Lewy body (-), Mild compression of the cervical spinal cord
	Cont_12	89	Male	CERAD: B	II	α-synuclein (-), Chronic subdural hygroma
	Cont_13	85	Female	CERAD: B	II	α-synuclein (+), Cerebellar infarction, Hydrocephalus
	Cont_14	88	Female	CERAD: B	II	α-synuclein (+), Tau deposition (heavy), TDP-43 (-)
	Cont_15	90	Female	CERAD: 0	II	α-synuclein (-), Atlantoaxial subluxation
	Cont_16	82	Male	CERAD: B	II	α-synuclein (-), CAA (medium), Arteriosclerosis, Multiple micro tumor
	Cont_17	92	Female	CERAD: A	II	α-synuclein (-), CAA (-), Cerebrovascular disease
	Cont_18	86	Male	CERAD: (-)	I	Lewy body (-), Multiple stroke

	Cont_19	84	Male	CERAD: B	II	α-synuclein (-), CAA (heavy), Vascular dementia
	Cont_20	84	Female	CERAD: (-)	I	CAA (-), Cerebral vascular dementia
	Cont_21	85	Male	CERAD: (-)	II	α-synuclein (-), TDP-43 (-), Central pontine myelinolysis
	Cont_22	86	Male	CERAD: A	II - III	Lewy body (-), CAA (-), MI, Arteriosclerosis,
	Cont_23	85	Female	CERAD: (-)	(-)	Cervical spondylotic myelopathy, Tau deposition, MI
	AD_1	82	Male	CERAD: C	V	CAA, α-synuclein (-)
	AD_2	75	Female	CERAD: C	VI	CAA, α-synuclein (-)
	AD_3	92	Female	CERAD: C	V	Lewy body (-), α-synuclein (+), CAA, Stroke
	AD_4	88	Female	CERAD: C	VI	α-synuclein (-), CAA, TDP-43 (-)
	AD_5	91	Female	CERAD: C	VI	α-synuclein (-), CAA (heavy)
	AD_6	92	Female	CERAD: C	VI	α-synuclein (-), CAA, MI
	AD_7	88	Female	CERAD: C	VI	α-synuclein (-), CAA (heavy), Cervical spine disease
AD	AD_8	88	Female	CERAD: C	V	α-synuclein (-), Arteriolar sclerosis (light)
	AD_9	87	Male	CERAD: B - C	V	CAA, α-synuclein (+)
	AD_10	83	Female	CERAD: C	VI	α-synuclein (+), CAA (heavy), Changes in vascular disorders
	AD_11	88	Male	CERAD: C	IV	CAA (mild), α-synuclein (+), Arteriolar sclerosis (mild)
	AD_12	80	Male	CERAD: C	VI	CAA (medium), α-synuclein (-), Schwannoma
	AD_13	87	Female	CERAD: C	V	Old cerebral infarction, Metastatic brain tumor, Spinal canal stenosis
	AD_14	76	Male	CERAD: C	VI	CAA (medium), α-synuclein (-), Tau deposition (heavy), TDP-43 (-)
	AD_15	87	Female	CERAD: B	VI	Brain atrophy (heavy), Nerve cell dropout, Gliosis

Bottom note: CAA: Cerebral Amyloid Angiopathy, SP: Senile Plague, NFT: Neurofibrillary Tangle, MI: Multiple Infarct

Table S3 Analytical validation of analytes based on W-Met.

Analytes	LOD (nM)	LOQ (nM)	Ranges	r^2	Internal standard
EA	10.0	25.0	50-100000	0.999	Gly-$^{13}C_2{}^{15}N_2$
1-Aminoacetone	10.0	25.0	50-100000	0.995	Gly-$^{13}C_2{}^{15}N_2$
Gly	1.0	10.0	100-100000	1.000	Gly-$^{13}C_2{}^{15}N_2$
1-Amino-2-propanol	1.0	10.0	50-25000	0.999	Gly-$^{13}C_2{}^{15}N_2$
β-Ala	0.1	1.0	100-100000	1.000	Ala-$^{13}C_3{}^{15}N$
Ala	1.0	3.0	100-100000	1.000	Ala-$^{13}C_3{}^{15}N$
Sar	10.0	25.0	100-50000	0.999	Sar-d_3
GABA	10.0	25.0	50-100000	0.999	GABA-d_6
Homoalanine	100.0	250.0	50-37500	1.000	Ala-$^{13}C_3{}^{15}N$
Ser	0.1	1.0	500-100000	1.000	Ser-$^{13}C_3{}^{15}N$
Hypotaurine	500.0	1000.0	1000-25000	0.997	Ser-$^{13}C_3{}^{15}N$
Cytosine	500.0	1000.0	1000-100000	0.998	Ser-$^{13}C_3{}^{15}N$
Uracil	10.0	25.0	100-25000	0.999	His-d_3
CRE	1.0	10.0	250-100000	1.000	CRE-d_3
Pro	1.0	10.0	100-100000	1.000	Pro-$^{13}C_5{}^{15}N$
5-Ava	1.0	5.0	10-7500	0.999	Val-d_8
Val	1.0	10.0	250-100000	0.999	Val-d_8
Hse	1.0	10.0	50-7500	0.990	Ser-$^{13}C_3{}^{15}N$
aThr	1.0	10.0	10-25000	0.999	Thr-d_2

Thr	1.0	10.0	500-100000	1.000	Thr-d_2
Phenethylamine	1.0	10.0	1-1000	0.999	Phe-d_6
Tau	1.0	10.0	100-100000	0.999	Ser-$^{13}C_3{}^{15}N$
Thymine	1.0	5.0	10-5000	0.999	His-d_3
Ac-PUT	1.0	10.0	10-5000	0.999	Orn-d_7
Agm	0.1	1.0	1-100	0.996	Arg-$^{13}C_6$
5-ALA	10.0	50.0	50-37500	0.999	Gly-$^{13}C_2{}^{15}N_2$
ProOH	25.0	50.0	250-50000	0.999	Pro-$^{13}C_5{}^{15}N$
Leu	1.0	10.0	100-100000	1.000	Leu-d_{10}
Nle	1.0	5.0	10-100000	1.000	Leu-d_{10}
Ile	1.0	10.0	1000-100000	0.999	Leu-d_{10}
Cr	100.0	250.0	500-100000	1.000	Cr-d_3
Asn	25.0	50.0	2500-100000	0.999	Asn-$^{13}C_4{}^{15}N_2$
Asp	1.0	3.0	10-25000	1.000	Asp-^{13}C
AA	1.0	5.0	5-1000	1.000	Trp-d_5
Tyramine	5.0	10.0	10-25000	0.999	Tyr-$^{13}C_9{}^{15}N$
EAP	10.0	25.0	100-10000	0.999	Ser-$^{13}C_3{}^{15}N$
Gln	0.5	1.0	50-100000	0.999	Gln-d_5
O-Acetyl-serine	50.0	100.0	500-100000	1.000	Ser-$^{13}C_3{}^{15}N$
Glu	1.0	3.0	500-75000	0.999	Glu-d_5
Met	1.0	10.0	100-100000	1.000	Met-d_4
MeAA	1.0	5.0	500-75000	0.999	3-AAOH-d_3

3-AAOH	250.0	500.0	500-100000	0.999	3-AAOH-d_3
Cysteine sulfinate	1.0	10.0	100-50000	0.999	Ser-$^{13}C_3{}^{15}N$
His	1.0	10.0	50-100000	0.999	His-d_3
Tryptamine	0.1	1.0	1-1000	0.999	Trp-d_5
2-Aminoadipate	10.0	50.0	250-100000	0.999	Glu-d_5
MetSO	25.0	50.0	250-25000	0.999	Met-d_4
Phe	5.0	10.0	1000-50000	0.999	Phe-d_6
Cysteate	25.0	500.0	2500-75000	0.997	Ser-$^{13}C_3{}^{15}N$
3-MeOAA	0.1	1.0	25-75000	1.000	3-AAOH-d_3
3-MeO-tyramine	5.0	10.0	10-10000	0.999	Tyr-$^{13}C_9{}^{15}N$
Arg	1.0	3.0	500-100000	1.000	Arg-$^{13}C_6$
Ac-Orn	1.0	5.0	5-1000	0.999	Orn-d_7
Theanine	1.0	5.0	5-1000	0.995	Glu-d_5
Cit	1.0	3.0	25-100000	1.000	Cit-d_4
5-HT	0.5	1.0	50-100000	0.999	Trp-d_5
Hphe	1.0	5.0	50-2500	0.999	Phe-d_6
$MetSO_2$	5.0	10.0	10-10000	0.999	Met-d_4
Tyr	250.0	500.0	500-100000	1.000	Tyr-$^{13}C_9{}^{15}N$
NMMA	0.5	1.0	500-25000	0.999	Arg-$^{13}C_6$
Ac-Lys	1.0	5.0	1-100	0.999	Lys-$^{13}C_6{}^{15}N_2$
5-MeOT	0.1	0.1	5-1000	0.999	Trp-d_5
Se-Met	25.0	50.0	25-1000	0.999	Met-d_4

ADMA	3.0	5.0	100-25000	0.992	Arg-$^{13}C_6$
Trp	10.0	25.0	500-25000	0.999	Trp-d_5
Indolelactate	50.0	100.0	100-100000	0.999	Trp-d_5
Kyn	1.0	5.0	50-100000	0.999	Kyn-d_6
5-TrpOH	1.0	5.0	10-37500	0.999	Trp-d_5
DAc-SPD	0.5	1.0	1-10000	0.999	SPD-d_8
Cytidine	250.0	500.0	1000-25000	0.999	His-d_3
Uridine	250.0	500.0	500-75000	1.000	His-d_3
DAP	0.5	1.0	25-5000	0.999	DAH
PUT	0.5	1.0	10-5000	0.998	DAH
CAD	0.5	1.0	1-100000	0.999	Orn-d_7
Hst	0.5	1.0	1-5000	0.999	Hst-d_4
MeHst	0.5	1.0	1-37500	0.999	His-d_3
Orn	5.0	10.0	500-50000	0.999	Orn-d_7
Histidinol	5.0	10.0	25-7500	0.999	His-d_3
Lys	1.0	3.0	50-10000	0.999	DAH
Cystamine	5.0	10.0	25-75000	0.997	Ser-$^{13}C_3{}^{15}N$
LysOH	5.0	10.0	2500-100000	0.999	DAH
1-MeHis	1.0	5.0	10-7500	0.999	His-d_3
3-MeHis	100.0	250.0	500-75000	0.998	His-d_3
Ac-SPD	5.0	10.0	10-10000	1.000	SPD-d_8
2,6-Diamino-pimelate	10.0	25.0	50-100000	1.000	DAH
Cystathionine	25.0	50.0	50-10000	0.999	Cystathionine-d_4

3-KynOH	0.5	1.0	1-1000	0.998	Kyn-d_6
Car	0.5	1.0	10-10000	0.997	His-d_3
Cystine	100.0	250.0	100-7500	0.999	Ser-$^{13}C_3{}^{15}N$
Homocystine	50.0	100.0	250-7500	0.999	Ser-$^{13}C_3{}^{15}N$
DAc-SPM	500.0	1000.0	250-100000	0.999	SPM-d_8
GSH	100.0	250.0	25-100000	0.999	GSH-$^{13}C_2{}^{15}N$
NorSPD	1.0	5.0	100-5000	0.999	SPD-d_8
SPD	3.0	5.0	500-100000	1.000	SPD-d_8
SAH	50.0	100.0	100-10000	0.999	Ser-$^{13}C_3{}^{15}N$
Ac-SPM	10.0	25.0	1-10000	0.998	SPM-d_8
SPM	10.0	25.0	10-5000	0.999	SPM-d_8

Table S4 Accuracy and precision for W-Met in *p*-CSF.

Analytes	Spiked levels (nM)		Accuracy (%) [RSD (%)]	
	Low levels	High levels	Low levels	High levels
EA	5000	10000	103.4 [8.6]	105.5 [6.2]
Gly	5000	10000	96.0 [2.3]	97.8 [1.8]
1-Amino-2-propanol	50	100	109.0 [24.4]	106.4 [6.3]
β-Ala	1000	5000	115.5 [3.3]	101.6 [3.3]
Ala	5000	10000	97.4 [2.4]	96.4 [2.1]
Sar	5000	10000	98.1 [1.9]	91.2 [2.1]
GABA	5000	10000	96.1 [3.5]	94.5 [4.6]
Homoalanine	5000	10000	95.8 [3.3]	97.6 [2.2]
Ser	5000	10000	94.2 [2.9]	98.5 [3.4]
Hypotaurine	500	1000	100.8 [14.2]	96.0 [16.6]
Cytosine	500	1000	121.3 [8.0]	72.1 [10.2]
CRE	5000	10000	101.9 [5.8]	98.1 [3.1]
Pro	5000	10000	101.9 [1.8]	101.0 [2.7]
5-Ava	50	100	97.2 [2.8]	107.8 [3.7]
Val	5000	10000	93.5 [5.0]	95.0 [4.0]
Hse	100	500	97.4 [12.9]	91.8 [11.3]
Thr	5000	10000	98.3 [3.3]	95.0 [2.6]
Phenethylamine	500	1000	90.3 [7.2]	84.6 [5.7]
Tau	5000	10000	102.4 [4.7]	96.8 [1.3]
Ac-PUT	50	100	103.6 [4.1]	102.7 [4.7]
Agm	10	50	111.6 [1.8]	109.4 [16.5]
5-ALA	100	500	97.4 [7.3]	113.4 [4.1]
ProOH	1000	5000	98.3 [4.0]	91.7 [0.96]
Leu	5000	10000	109.2 [4.2]	108.6 [3.9]
Ile	5000	10000	89.3 [5.0]	94.7 [5.2]
Cr	5000	10000	93.1 [3.6]	98.1 [3.1]
Asn	5000	10000	97.9 [5.4]	98.8 [6.6]
Asp	1000	5000	99.1 [7.5]	96.0 [1.5]
Adenine	500	1000	102.3 [18.7]	105.4 [17.6]
AA	10	50	92.2 [16.4]	78.8 [5.5]
Tyramine	10	50	101.6 [17.2]	103.9 [17.3]
EAP	5000	10000	71.0 [17.0]	95.2 [8.8]

Gln	5000	10000	101.4 [2.7]	99.4 [2.1]
O-Acetyl-serine	1000	5000	105.1 [2.8]	90.0 [4.1]
Glu	5000	10000	90.5 [2.2]	94.7 [3.6]
Met	5000	10000	95.0 [6.5]	94.5 [2.5]
Guanine	1000	5000	92.5 [4.9]	85.7 [5.7]
3-AAOH	500	1000	82.0 [18.6]	107.2 [5.4]
Cysteine sulfinate	1000	5000	104.5 [4.3]	100.5 [4.4]
His	5000	10000	95.5 [2.2]	95.2 [1.9]
Tryptamine	10	50	90.6 [7.2]	97.8 [1.6]
2-Aminoadipate	5000	10000	103.3 [3.5]	95.4 [4.3]
O-Phospho-serine	5000	10000	21.5 [15.5]	13.8 [24.5]
MetSO	1000	5000	87.2 [2.4]	83.7 [2.9]
Phe	5000	10000	92.2 [3.9]	87.6 [1.7]
Cysteate	5000	10000	45.7 [8.2]	44.9 [37.8]
3-MeOAA	10	50	77.0 [7.8]	64.9 [7.1]
Arg	5000	10000	103.2 [3.0]	102.4 [1.3]
Cit	1000	10000	95.9 [3.5]	92.2 [5.0]
5-HT	50	100	90.2 [15.9]	82.8 [9.6]
Hphe	5000	10000	88.0 [6.3]	85.2 [3.9]
Tyr	5000	10000	95.5 [4.1]	91.6 [3.8]
NMMA	1000	5000	107.3 [3.5]	108.9 [5.8]
Ac-Lys	50	100	85.3 [11.8]	93.6 [12.6]
5-MeOT	10	50	85.7 [5.6]	84.5 [4.3]
ADMA	5000	10000	101.4 [5.4]	101.5 [3.6]
Trp	5000	10000	93.3 [4.4]	98.3 [6.3]
Indolelactate	500	1000	98.1 [11.8]	101.4 [7.0]
Kyn	500	1000	100.4 [5.6]	95.6 [2.1]
5-TrpOH	10	50	77.0 [11.8]	60.3 [9.3]
DAc-SPD	500	1000	108.9 [3.6]	102.3 [2.9]
Cytidine	5000	10000	107.3 [16.1]	107.3 [12.0]
Adenosine	5000	10000	71.9 [3.2]	78.7 [7.9]
Guanosine	5000	10000	78.2 [8.0]	76.8 [2.9]
Ophthalmic acid	5000	10000	64.9 [20.6]	106.3 [5.72]
DAP	1000	5000	101.4 [3.7]	89.7 [5.3]
PUT	500	1000	101.9 [2.2]	89.3 [2.0]

CAD	10	50	94.7 [5.5]	99.4 [1.5]
Hst	10	50	94.5 [3.8]	90.4 [8.8]
MeHst	5000	10000	85.1 [4.3]	105.7 [5.1]
Orn	5000	10000	94.1 [1.1]	92.6 [1.9]
Histidinol	5000	10000	92.2 [5.9]	104.0 [1.4]
Lys	1000	5000	99.8 [0.95]	92.8 [2.7]
LysOH	10	50	100.3 [10.4]	92.7 [7.6]
3-MeHis	5000	10000	84.3 [14.5]	104.1 [1.9]
Ac-SPD	500	1000	99.7 [2.3]	103.1 [1.7]
2,6-Diamino-pimelate	10000	50000	113.8 [19.5]	82.5 [18.8]
Cystathionine	5000	10000	103.8 [1.4]	98.9 [3.6]
3-KynOH	10	100	90.9 [9.0]	90.9 [4.9]
Car	50	100	84.9 [5.3]	89.8 [9.2]
Cystine	5000	10000	109.1 [2.0]	105.7 [2.3]
Anserine	5000	10000	108.5 [6.3]	84.7 [2.4]
Homocystine	5000	10000	68.6 [14.1]	90.8 [10.0]
DAc-SPM	5000	10000	101.8 [4.1]	102.0 [6.8]
GSH	5000	10000	96.4 [5.3]	92.0 [4.0]
SPD	5000	10000	102.0 [7.2]	98.5 [10.6]
SAH	100	1000	98.5 [10.6]	65.2 [12.7]
Ac-SPM	500	1000	96.2 [7.0]	98.8 [9.1]
SPM	500	1000	100.2 [6.3]	96.7 [3.6]

n=6

Table S5 Detection levels of analytes in *p*-CSF from AD and control subjects.

Analytes	Control	AD
EA	101.1 ± 74.4	98.9 ± 84.1
1-Aminoacetone	0.426 ± 0.277	0.438 ± 0.205
Gly	65.8 ± 64.1	62.2 ± 67.4
1-Amino-2-propanol	0.210 ± 0.118	0.244 ± 0.225
β-Ala	5.62 ± 5.31	5.15 ± 4.98
Ala	312.7 ± 158.2	270.0 ± 156.1
Sar	N.D.	N.D.
GABA	20.6 ± 19.3	21.9 ± 30.7
2-Aminoisobutyric acid	N.D.	N.D.
3-Aminoisobutyric acid	0.801 ± 0.684	1.44 ± 1.89
Homoalanine	5.85 ± 3.67	4.27 ± 3.25
Ser	332.2 ± 139.6	295.9 ± 123.0
2-Aminophenol	N.D.	N.D.
Hypotaurine	3.99 ± 1.81	3.37 ± 2.12
Cytosine	N.D.	N.D.
Uracil	1.96 ± 1.74	2.35 ± 1.24
CRE	100.8 ± 59.6	87.4 ± 43.9
Pro	16.9 ± 15.9	13.6 ± 11.9
5-Ava	0.064 ± 0.036	0.039 ± 0.031
Val	21.3 ± 8.74	21.8 ± 12.2
Hse	0.491 ± 0.214	0.477 ± 0.392
aThr	0.667 ± 0.553	0.865 ± 0.546
Thr	78.9 ± 30.2	90.5 ± 41.9
Phenethylamine	N.D.	N.D.
Tau	20.9 ± 14.1	18.3 ± 18.0
AICA	0.063 ± 0.010	0.053 ± 0.010
Thymine	0.412 ± 0.018	0.421 ± 0.024
Ac-PUT	0.171 ± 0.081	0.105 ± 0.045
Agm	0.016 ± 0.014	0.006 ± 0.004
5-ALA	N.D.	N.D.
ProOH	3.03 ± 2.76	1.85 ± 1.58
Leu	81.7 ± 42.0	72.3 ± 41.8
Nle	N.D.	N.D.

Ile	16.6 ± 11.5	13.3 ± 8.57
Cr	112.1 ± 51.7	97.2 ± 28.5
Asn	107.1 ± 54.7	96.9 ± 63.7
Asp	14.1 ± 16.7	13.0 ± 24.5
Adenine	4.22 ± 2.57	2.41 ± 1.63
Hypoxanthine	58.4 ± 16.7	58.3 ± 16.7
AA	0.035 ± 0.037	0.021 ± 0.009
Tyramine	N.D.	N.D.
EAP	0.210 ± 0.082	0.083 ± 0.021
Gln	593.4 ± 183.1	586.6 ± 216.8
O-Acetyl-serine	N.D.	N.D.
Glu	704.6 ± 723.4	642.4 ± 782.1
Met	9.71 ± 5.56	9.18 ± 5.31
MeAA	0.028 ± 0.027	0.008 ± 0.006
Guanine	N.D.	N.D.
3-AAOH	N.D.	N.D.
Cysteine sulfinate	2.76 ± 1.07	2.80 ± 1.26
His	8.53 ± 2.74	8.38 ± 3.98
Tryptamine	0.016 ± 0.006	0.006 ± 0.002
2-Aminoadipate	11.7 ± 19.1	6.40 ± 6.48
Kynuramine	0.592 ± 0.705	0.068 ± 0.060
O-Phospho-serine	N.D.	N.D.
MetSO	1.74 ± 0.799	1.72 ± 0.865
Phe	28.8 ± 8.36	31.4 ± 11.9
Cysteate	N.D.	N.D.
3-MeOAA	0.014 ± 0.016	0.009 ± 0.008
3-MeO-tyramine	0.318 ± 0.215	0.250 ± 0.161
Arg	33.4 ± 11.7	34.3 ± 11.1
Ac-Orn	N.D.	N.D.
Theanine	0.079 ± 0.069	0.066 ± 0.088
Cit	4.18 ± 3.13	3.25 ± 2.18
5-HT	0.040 ± 0.026	0.021 ± 0.014
Hphe	0.014 ± 0.007	0.012 ± 0.006
Homocysteate	N.D.	N.D.

$MetSO_2$	0.189 ± 0.308	0.145 ± 0.181
Tyr	34.2 ± 18.8	23.4 ± 12.3
NMMA	1.85 ± 1.36	1.63 ± 0.849
Ac-Lys	0.082 ± 0.035	0.066 ± 0.036
5-MeOT	0.012 ± 0.006	0.003 ± 0.0009
Se-Met	N.D.	N.D.
ADMA	15.4 ± 10.4	12.9 ± 9.01
Trp	132.3 ± 41.3	97.3 ± 35.6
Indolelactate	0.268 ± 0.211	0.215 ± 0.208
Kyn	1.39 ± 0.947	0.675 ± 0.439
5-TrpOH	0.036 ± 0.011	0.020 ± 0.004
DAc-SPD	0.149 ± 0.085	0.110 ± 0.061
Cytidine	N.D.	N.D.
Uridine	6.64 ± 2.35	6.10 ± 3.13
Adenosine	N.D.	N.D.
Inosine	18.7 ± 12.6	16.2 ± 11.0
Saccharopine	N.D.	N.D.
Guanosine	N.D.	N.D.
Xanthosine	N.D.	N.D.
Ophthalmic acid	N.D.	N.D.
DAP	2.30 ± 0.948	3.08 ± 0.931
4-Aminoimidazole	0.042 ± 0.018	0.030 ± 0.007
PUT	1.98 ± 1.37	1.00 ± 1.20
CMP	N.D.	N.D.
CAD	0.089 ± 0.153	0.030 ± 0.051
cAMP	N.D.	N.D.
Hst	0.017 ± 0.007	0.010 ± 0.007
BMAA	0.089 ± 0.066	0.065 ± 0.044
2,4-Diaminobutyric acid	0.011 ± 0.004	0.003 ± 0.001
AMP	N.D.	N.D.
MeHst	4.30 ± 4.78	1.65 ± 1.60
Orn	10.9 ± 3.88	8.93 ± 3.53
GMP	N.D.	N.D.
Histidinol	0.065 ± 0.028	0.027 ± 0.003

Lys	14.7 ± 2.92	15.0 ± 3.78
Cystamine	0.259 ± 0.159	0.159 ± 0.089
LysOH	0.206 ± 0.038	0.206 ± 0.059
1-MeHis	1.21 ± 1.44	0.448 ± 0.310
3-MeHis	9.49 ± 8.82	7.41 ± 6.83
Ac-SPD	0.644 ± 0.445	0.438 ± 0.336
2,6-Diamino-pimelate	N.D.	N.D.
Cystathionine	12.9 ± 14.4	10.4 ± 9.00
3-KynOH	N.D.	N.D.
Car	0.086 ± 0.096	0.118 ± 0.305
Cystine	194.7 ± 107.7	115.7 ± 87.1
Anserine	N.D.	N.D.
Homocystine	N.D.	N.D.
DAc-SPM	17.4 ± 22.8	18.1 ± 47.9
GSH	35.1 ± 73.0	17.0 ± 15.9
NorSPD	0.024 ± 0.009	0.019 ± 0.003
SPD	15.9 ± 16.6	10.8 ± 7.97
SAH	0.919 ± 0.854	0.882 ± 0.710
Ac-SPM	0.083 ± 0.055	0.072 ± 0.028
SPM	1.58 ± 1.12	1.42 ± 1.51