Electronic Supplementary Material (ESI) for Molecular BioSystems.

Multi−platform Mass Spectrometry Analysis of the CSF and Plasma Metabolomes of Rigorously Matched Amyotrophic Lateral Sclerosis, Parkinson's Disease and Control Subjects

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Supplementary Tables

Table S1. Overview of the 72 matched (22 ALS +22 PD +28 control) patients.

#	Match #	Sample biobank #	Diagnostic group	Sex	Age of patient *(years)*	Age of sample *(months)*	Age of patient (match group) *(years)*	Age of sample (match group) *(months)*
1	1	NOC 6809	PD	f	54	61		
2	1	SOD 4069	ALS	m	51	62		
3	1	NOC 6896	Control: dizziness	f	54	59		
4	1	NOC 6986	Control: tension headache	f	54	58	53.3 ± 1.3	60 ± 1.6
5	2	NOC 6822	PD	f	71	61		
6	2	SOD 4049	ALS	f	71	62		
7	2	NOC 7335	Control: leg weakness	f	71	51	71 ± 0	58 ± 5
8	3	NOC 6006	PD	f	57	77		
9	3	SOD 3622	ALS	f	56	78		
10	3	NOC 6105	Control: tension headache	f	57	75	56.7 ± 0.5	76.7 ± 1.2
11	4	NOC 9838	PD	m	58	11		
12	4	SOD 6736	ALS	m	56	12		
13	4	NOC 9563	Control: gait disturbance	m	59	15	57.7 ± 1.2	12.7 ± 1.7
14	5	NOC 7824	PD	m	64	42		
15	5	SOD 5388	ALS	m	63	43		
16	5	NOC 7667	Control: pain	m	63	46	63.3 ± 0.5	43.7 ± 1.7
17	6	NOC 6948	PD	m	74	58		
18	6	SOD 4581	ALS	m	74	58		
19	6	NOC 6836	Control: headache	m	73	61	73.7 ± 0.5	59 ± 1.4
20	7	NOC 6669	PD	m	59	64		
21	7	SOD 4036	ALS	m	59	63		
22	7	NOC 6750	Control: tension headache	m	58	63	58.7 ± 0.5	63.3 ± 0.5
23	8	NOC 6659	PD	m	61	64		
24	8	SOD 4023	ALS	m	64	64		
25	8	NOC 6289	Control: headache	m	61	71		
26	8	NOC 6678	Control: CIDP*	m	61	64	61.8 ± 1.3	65.8 ± 3

27	9	NOC 10063	PD	f	73	7		
28	9	NOC 10066	ALS	f	74	7		
29	9	NOC 10157	Control: pain	f	72	5	73 ± 0.8	6.3 ± 0.9
30	10	NOC 9858	PD	f	62	11		
31	10	NOC 9882	ALS	f	63	10		
32	10	NOC 9850	Control: myokymia	f	61	11		
33	10	NOC 9485	Control: headache	f	61	16	61.8 ± 0.8	12 ± 2.3
34	11	NOC 9793	PD	f	66	12		
35	11	NOC 9924	ALS	f	67	10		
36	11	NOC 9798	Control: ataxia	f	66	12	66.3 ± 0.5	11.3 ± 0.9
37	12	NOC 9365	PD	f	74	17		
38	12	NOC 9445	ALS	f	73	17		
39	12	NOC 9160	Control: balance distubance	f	73	21	73.3 ± 0.5	18.3 ± 1.9
40	13	NOC 9833	PD	m	65	11		
41	13	NOC 9750	ALS	m	66	12		
42	13	NOC 9474	Control: polyneuropathy	m	64	17	65 ± 0.8	13.3 ± 2.6
43	14	NOC 9288	PD	m	67	18		
44	14	NOC 9309	ALS	m	66	18		
45	14	NOC 8941	Control: Kennedy syndrome	m	66	24		
46	14	NOC 9590	Control: ataxia	m	66	15	66.3 ± 0.4	18.8 ± 3.3
47	15	NOC 9299	PD	m	60	18		
48	15	NOC 9225	ALS	m	62	19		
49	15	NOC 9231	Control: AIDP**	m	60	19	60.7 ± 0.9	18.7 ± 0.5
50	16	NOC 9117	PD	m	55	22		
51	16	NOC 9170	ALS	m	56	21		
52	16	NOC 8972	Control: hemangioma	m	55	24	55.3 ± 0.5	22.3 ± 1.2
53	17	NOC 9017	PD	m	60	23		
54	17	NOC 9143	ALS	m	62	21		
55	17	NOC 8946	Control: monocular reduced vision	m	60	24	60.7 ± 0.9	22.7 ± 1.2
56	18	NOC 8966	PD	m	65	24		
57	18	NOC 8953	ALS	m	66	24		

58	18	NOC 8843	Control: radiculopathy	m	65	26		
59	18	NOC 8996	Control: focal epilepsy	m	65	24	65.3 ± 0.4	24.5 ± 0.9
60	19	NOC 8868	PD	m	69	25		
61	19	NOC 8977	ALS	m	71	24		
62	19	NOC 8956	Control: lumbal spinal stenosis	m	71	24	70.3 ± 0.9	24.3 ± 0.5
63	20	NOC 8789	PD	m	72	27		
64	20	NOC 8906	ALS	m	73	25		
65	20	NOC 9199	Control: AIDP**	m	76	20	73.7 ± 1.7	24 ± 2.9
66	21	NOC 8884	PD	m	63	25		
67	21	NOC 8842	ALS	m	63	26		
68	21	NOC 8556	Control: stroke	m	63	30		
69	21	NOC 8993	Control: AIDP**	m	63	24	63 ± 0	26.3 ± 2.3
70	22	NOC 8377	PD	m	65	34		
71	22	NOC 8496	ALS	m	65	31		
72	22	NOC 8679	Control: tension headache	m	65	29	65 ± 0	31.3 ± 2.1
Total							**0.8**	**2.1**

*CIDP = chronic inflammatory demyelinating polyneuropathy. ** AIDP = acute inflammatory demyelinating polyneuropathy.

Table S2. Overview of the OPLS-EP models including comparison versus the corresponding OPLS-DA model in CSF and plasma. R2Y (explained variation in Y) and Q2 (predicted variation in Y) are shown. Selected variables corresponds to significant variables in the model of all variables according to the Jack-knife confidence interval (95%) of the weight (w), computed from all seven rounds of cross validation.

		OPLS-EP (CSF)	**OPLS-EP (CSF)**	**OPLS-DA (CSF)**	**OPLS-DA (CSF)**	**OPLS-EP (PLASMA)**	**OPLS-EP (PLASMA)**	**OPLS-DA (PLASMA)**	**OPLS-DA (PLASMA)**
		R2Y	**Q2**	**R2Y**	**Q2**	**R2Y**	**Q2**	**R2Y**	**Q2**
ALS vs. matched control	all variables	0.96	0.22	0.57	−0.05	0.78	0.25	0.88	0.07
ALS vs. matched control	selected variables	0.59	0.50	0.40*	0.29*	0.87	0.70	0.71*	0.53*
PD vs. matched control	all variables	0.996	0.29	0.80	−0.32	0.70	0.04	0.77	−0.30
PD vs. matched control	selected variables	0.69	0.36	0.22*	0.17*	0.79	0.71	0.59*	0.48*
ALS vs. matched PD	all variables	0.76	−0.46	0.79	−0.35	0.92	0.23	0.72	−0.30
ALS vs. matched PD	selected variables	0.74	0.51	0.56*	0.41*	0.75	0.66	0.60*	0.49*

* Values shown for comparative reasons. Model was based on variables optimized from the OPLS-EP models.

Table S3. OPLS-EP and univariate significant metabolites between ALS vs matched controls in CSF.

Metabolite	**HMDB**	**Analysis platform**	**RSD**	**p-value**
β-Methylglucopyranoside	HMDB29965	GC/MS	N/A	3.3 x10-3
Carnitine	HMDB00062	LC/MS ESI+	5.1	6.1 x10-4
Decenedioic acid	HMDB00603	LC/MS ESI+	10.4	6.2 x10-4
Creatine	HMDB00064	LC/MS ESI+	10.0	3.3 x10-3
Isoleucine	HMDB00172	LC/MS AA	4.2	0.03
Sorbitol	HMDB00247	GC/MS	N/A	0.01
Ornithine	HMDB00214	LC/MS AA	4.9	0.05
α-Hydroxybutyrate	HMDB00008	GC/MS	N/A	9.0 x10-5
Saccharic acid 1,4-lactone	N/A	GC/MS	N/A	0.05
Ketoleucine	HMDB00695	LC/MS ESI-	8.5	0.01
Galactitol	HMDB00107	GC/MS	N/A	0.05
Glucose	HMDB00122	GC/MS	N/A	0.01

Allothreonine	HMDB04041	GC/MS	N/A	0.01
Acetyl-carnitine	HMDB13334	LC/MS ESI+	19.3	0.05
Pentonic acid-1,4-lactone	N/A	GC/MS	N/A	0.01
Xylose	HMDB00098	LC/MS ESI-	23.7	6.2 x10-4
Aminoadipic acid	HMDB00510	LC/MS ESI-	7.7	0.05
Acetylalanine	HMDB00766	LC/MS ESI-	8.6	0.01
Xylitol	HMDB02917	GC/MS	N/A	0.05
Arabitol	HMDB00568	GC/MS	N/A	0.05
Creatinine	HMDB00562	LC/MS ESI+	7.8	0.03
Succinyladenosine	HMDB00912	LC/MS ESI+	10.7	0.01
Ribitol	HMDB00508	GC/MS	N/A	0.05
Uracil	HMDB00300	GC/MS	N/A	3.3 x10-3

Table S4. OPLS-EP and univariate significant metabolites between ALS vs matched controls in plasma.

Metabolite	**HMDB**	**Analysis platform**	**RSD**	**p-value**
Ribonic acid	HMDB00867	LC/MS ESI-	32.9	0.01
PGA2	HMDB02752	LC/MS ESI-	6.9	6.1 x10-4
Homovanillic acid	HMDB00118	LC/MS ESI-	4.1	0.05
Hypoxanthine	HMDB00157	LC/MS ESI-	13.2	6.1 x10-4
Cysteamine	HMDB02991	LC/MS ESI-	22.0	0.02
ADMA	HMDB01539	GC/MS	39.8	3.3 x10-3
Deoxycholic acid	HMDB00626	GC/MS	30.5	3.3 x10-3
PC(40:6)	N/A	LC/MS ESI-	38.1	0.05
Hydroxyphenyl-proponate	N/A	LC/MS ESI-	9.1	3.3 x10-3
Hydroxyhippuric acid	HMDB06116	LC/MS ESI-	6.9	0.05
Decenedioic acid	HMDB00603	LC/MS ESI+	10.3	0.01
Valine	HMDB00883	GC/MS	5.5	0.01
D-Galactono-1,4-lactone	HMDB02541	GC/MS	33.6	0.01

Hydroxyindolacetic acid	HMDB01855	LC/MS ESI+	2.7	0.01
Flavone	HMDB03075	LC/MS ESI+	28.9	0.05
Homocysteine	HMDB00742	LC/MS AA	1.5	1.0 x10-5
Tripeptide ValPheLeu	N/A	LC/MS ESI-	18.0	0.01
Citric acid	HMDB00094	GC/MS	8.0	9.0 x10-5
PC (36:3)	N/A	LC/MS ESI+	24.1	3.3 x10-3
Ketolauric acid/oxododecanoic acid	HMDB10727	LC/MS ESI-	8.7	0.01
Dihydroxy-octodecenoic acid	N/A	LC/MS ESI-	19.0	0.01
Methionine	HMDB00696	LC/MS AA	1.7	0.01
Hexadecanedioc acid	N/A	LC/MS ESI-	22.5	0.05
Adipic acid	HMDB00448	GC/MS	84.9	0.01
Traumatic acid	HMDB00933	LC/MS ESI+	17.2	0.01
Isoleucine	HMDB00172	LC/MS AA	4.7	9.0 x10-5
Formylkynurenine	HMDB60485	LC/MS ESI+	5.9	0.05
Isoquinoline	HMDB34244	LC/MS ESI+	7.0	3.3 x10-3
Erythritol	HMDB02994	GC/MS	19.3	6.1 x10-4
Tripeptide TrpPheGly	N/A	LC/MS ESI+	9.1	3.3 x10-3
Proline	HMDB00162	GC/MS	7.0	3.3 x10-3
AMP	HMDB00045	GC/MS	19.1	9.0 x10-5
Oxoquinoline	N/A	LC/MS ESI+	10.7	6.1 x10-4
Tryptophan	HMDB00929	LC/MS ESI+	3.5	6.1 x10-4
Indole	HMDB00738	LC/MS ESI+	16.1	6.1 x10-4

Table S5. OPLS-EP and univariate significant metabolites between PD vs matched controls in CSF.

Metabolite	**HMDB**	**Analysis platform**	**RSD**	**p-value**

Tetracosanoic acid	HMDB02003	LC/MS ESI+	16.5	0.01
Ascorbic acid	HMDB00044	LC/MS ESI-	39.5	0.01
Sorbose	HMDB00247	GC/MS	N/A	0.05
Octadecanol	HMDB02350	GC/MS	N/A	0.01
Histidine	HMDB00177	LC/MS AA	12.3	0.01
Glycerol-3-phosphate	HMDB00126	GC/MS	N/A	0.05
Ornithine	HMDB00214	LC/MS AA	4.9	0.02
Glutamine	HMDB00641	LC/MS AA	3.4	0.05
Benzoic acid	HMDB01870	GC/MS	N/A	0.01
Lysine	HMDB00182	LC/MS AA	3.5	0.03
Ketoleucine	HMDB00695	LC/MS ESI-	8.5	3.3 x10-3
Isoleucine	HMDB00172	LC/MS AA	4.2	0.02
Saccharic acid 1,4-lactone	N/A	GC/MS	N/A	3.3 x10-3
Threonine	HMDB00167	LC/MS AA	2.9	0.01
Mannitol	HMDB00765	GC/MS	N/A	0.01
Galactitol	HMDB00107	GC/MS	N/A	3.3 x10-3
Pyroglutamic acid	HMDB00267	GC/MS	N/A	0.02
Sorbitol	HMDB00247	GC/MS	N/A	0.01
Alanine	HMDB00161	LC/MS AA	4.5	1.1 x10-3

Table S6. OPLS-EP and univariate significant metabolites between PD vs matched controls in plasma.

Metabolite	**HMDB**	**Analysis platform**	**RSD**	**p-value**
PC (36:4)	N/A	LC/MS ESI+	24.1	0.02
Arabinose	HMDB00646	GC/MS	25.1	0.01
Oxalate	HMDB02329	GC/MS	68.7	0.05
Pyruvate	HMDB00243	GC/MS	8.0	3.3 x10-3
4-hydroxyphenylacetic acid	HMDB00020	GC/MS	30.7	0.05
Tripeptide LysLysTrp	N/A	LC/MS ESI-	24.4	0.01
Isoquinonoline N-oxide	N/A	LC/MS ESI-	21.2	3.3 x10-

				3
Threonine	HMDB00167	GC/MS	6.9	0.05
α-Ketoglutarate	HMDB00208	GC/MS	12.5	0.01
Indole	HMDB00738	LC/MS ESI+	16.1	0.01
Threitol	HMDB04136	GC/MS	35.0	0.01
Dioxo-octodecanoic acid	N/A	LC/MS ESI-	15.2	0.01
Proline	HMDB00162	GC/MS	7.0	0.05
Fucose	HMDB00174	GC/MS	9.6	3.3 x10-3
Alanine	HMDB00161	LC/MS AA	1.1	9.0 x10-5
Cysteine	HMDB00574	LC/MS AA	2.9	6.1 x10-4
Urate	HMDB00289	GC/MS	6.8	0.01
Aconitate	HMDB00072	GC/MS	16.2	6.1 x10-4
Maltotriose	HMDB01262	GC/MS	30.9	6.1 x10-4
Tripeptide LysHisMet	N/A	LC/MS ESI-	10.6	0.01

Table S7. OPLS-EP and univariate significant metabolites between ALS vs matched PD in CSF.

Metabolite	**HMDB**	**Analysis platform**	**RSD**	**p-value**
Uracil	HMDB00300	GC/MS	N/A	0.01
Inosine	HMDB00195	LC/MS ESI+	9.6	0.03
Ammonia	HMDB00051	LC/MS AA	12.1	0.05
Carnitine	HMDB00062	LC/MS ESI+	5.1	0.04
β-Hydroxybutyrate	HMDB00357	LC/MS ESI-	15.9	0.05
α-Hydroxybutyrate	HMDB00008	GC/MS	N/A	0.02

Table S8. OPLS-EP and univariate significant metabolites between ALS vs matched PD in plasma.

Metabolite	**HMDB**	**Analysis platform**	**RSD**	**p-value**
Dihydroxybenzoic acid	N/A	LC/MS ESI-	27.9	0.01
Hippurate	HMDB00714	LC/MS ESI+	5.7	0.05
Taurine	HMDB00251	LC/MS AA	3.3	0.01
Hypoxanthine	HMDB00157	LC/MS ESI-	13.2	0.05
Erythronic acid	HMDB00613	GC/MS	8.4	0.03
Adenine	HMDB00034	LC/MS ESI+	26.1	0.05
LysoPC(14:0)	N/A	LC/MS ESI-	17.9	0.05
Pregnenolone sulfate	HMDB00774	LC/MS ESI-	16.2	0.04
Xanthine	HMDB00292	LC/MS ESI-	18.9	0.03
O-phosphoetanolamine	HMDB00224	GC/MS	46.5	0.05
Lactate	HMDB00190	GC/MS	10.5	0.03
α-Hydroxybutyrate	HMDB00008	GC/MS	6.8	0.01
Carnitine	HMDB00062	LC/MS ESI+	14.7	3.3 x10-3

Supplementary Figures

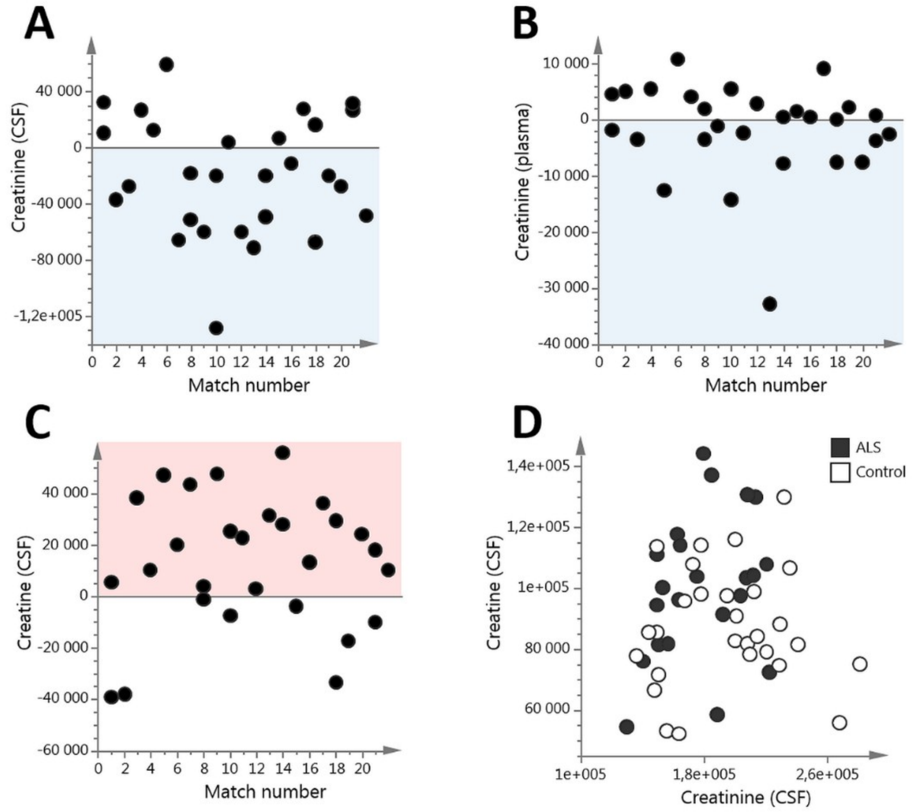

Figure S1. Subset of ALS exhibit altered creatine and creatinine profiles. A) ALS normalized for matched controls plotted versus difference in creatinine in CSF. ‘Expected’ values are below zero (blue). B) ALS normalized for matched controls plotted versus difference in creatinine in plasma. ‘Expected’ values are below zero (blue). C) ALS normalized for matched controls plotted versus difference in creatine in CSF. ‘Expected’ values are above zero (red). D) Creatinine (CSF) vs. creatine (CSF) for controls and ALS (metabolites were not quantified, therefore the levels are the integration of the area of the peaks from chromatography *i.e.* relative quantification).