Supplementary

Appendix 1 Acquisition of NMR spectra

All spectra were acquired on a Bruker Avance III DRX 600 spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany) operating at a frequency of 1H of 600.13 MHz and equipped with a QXI 5 mm quadruple resonance probe $^{1}H/^{13}C/^{15}N/^{31}Q$. A SampleJet thermostatted sampling robot was used to handle the measuring tubes, and inputs and outputs of the spectrometers. The sample temperature inside the equipment was controlled using a BCU Xtreme cooling unit. Magnetic field uniformity was initially achieved by manual adjustment of the homogeneity coils using a 1D experiment with water presaturation in interactive mode. For the remaining measurements, field homogeneity was maintained using an automatic correction routine (TopShim) implemented in the equipment software. The nominal temperature of the sample during the measurements was kept constant at 310 K. For all samples, a 3 min single-pulse water presaturation experiment was run with 256 repetitions, 65,000 points, 2 s relaxation time and 3.94 sec acquisition time. Water presaturation was run for 1 s throughout the recycling time for solvent signal suppression. The spectral width for all spectra was set to 8,000 Hz for the ^{1}H. Before applying the Fourier transform to the data, the FID (free signal decay) was multiplied by a 0.3 Hz linewidth exponential function.

We confirmed metabolite identification by combining chemical shift and multiciplicity information from 1D-1H-NMR spectra with 2D NMR (COSY and HSQC) correlations and comparison against reference spectra in the Chenomx NMY Suite database (version 8.6). Key metabolites, including carnosine, were additionally verified by spiking representastive samples with commercial standards, wich reproduced identical resonance patterns.

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Table S1 Average metabolite levels (in ppm) between different groups (cases with and without biochemical recurrence)

Metabolite	Sample total	With recurrence	No recurrence	P
2-furoylglycine	4.93±5.07	4.50±3.93	5.02±5.29	0.72
2-hydroxybutyrate	4.97±7.27	7.12±13.53	4.52±5.14	0.47
2-hydroxyisobutyrate	2.77±0.94	2.82±0.93	2.76±0.94	0.82
2-phenylpropionate	7.48±4.01	8.28±5.20	7.31±3.73	0.39
3-hydroxybutyrate	8.47±5.32	7.35±3.99	8.71±5.56	0.37
3-hydroxykynurenine	3.72±1.87	4.37±1.90	3.58±1.85	0.14
3-methyl-2-oxovalerate	6.12±2.53	5.82±2.36	6.18±2.58	0.61
4-aminohippurate	2.81±1.48	2.82±1.21	2.81±1.54	0.98
Acetate	1.21±0.62	1.25±0.62	1.20 +/-0.63	0.79
Acetoacetate	3.88±1.92	4.32±2.78	3.79±1.70	0.32
Acetoin	3.72±1.83	4.22±2.41	3.61±1.68	0.24
Alanine	8.66±4.48	8.70±4.55	8.65±4.50	0.96
Arabinose	16.31±5.75	17.87±6.64	15.98±5.54	0.25
Arginine	17.21±7.59	16.41±7.02	17.38±7.74	0.65
Betaine	9.88±12.29	7.23+/- 5.73	10.44±13.23	0.36
Carnosine	7.41±5.55	6.16±3.88	7.67±5.83	0.34
Cis-aconite	1.95±1.17	1.81±0.84	1.98±1.23	0.62
Citrate	52.82±31.52	49.56±21.95	53.51±33.27	0.66
Choline	3.84±1.91	3.38±1.48	3.94±1.98	0.31
Creatine	13.04±15.43	21.82±31.97	11.19±8.04	0.22
Creatine phosphate	18.20±16.69	13.78±7.24	19.13±17.96	0.26
Creatinine	154.66±62.58	156.80±39.09	154.21±66.70	0.84
Dimethylamine	3.79±6.31	3.45±4.65	3.86±6.63	0.82
Ethanolamine	10.68±5.28	10.23±4.70	10.78±5.42	0.71
Formate	0.49±0.34	0.42±0.16	0.51±0.36	0.35
Fucose	15.08±6.68	13.51±7.34	15.41±6.54	0.32
Fumarate	0.14±0.08	0.13±0.07	0.14±0.08	0.55
Gentisate	2.44±1.30	2.27±0.83	2.48±1.39	0.58
Glucose	62.80±153.35	21.34±15.35	71.56±167.51	0.07
Glutamate	10.23±4.03	9.83±4.16	10.32±4.07	0.67
Glutamine	19.04±6.35	19.30±5.72	18.99±6.51	0.86
Wisteria	9.86±4.79	9.04±4.02	10.04±4.95	0.46
Hippurate	70.92±60.76	74.23±81.17	70.22±56.24	0.81
Histidine	4.61±3.78	4.65±3.85	4.61±3.79	0.96
Hypoxanthine	0.72±0.59	0.82±0.85	0.71±0.52	0.51
Imidazole	1.70±1.42	1.95±1.29	1.65±1.44	0.45
Indole-3-lactate	10.20±4.95	10.15±4.94	10.21±4.98	0.96
Isobutyrate	1.25±1.80	0.75±0.30	1.36±1.96	0.23
Isoleucine	3.24±1.82	2.90±0.88	3.31±1.95	0.42
Kynurenine	4.95 +/ 2.15	5.05±2.35	4.93±2.12	0.84
Lactate	7.73±6.57	6.33±3.49	8.03±7.03	0.36
Leucine	4.17±1.77	4.22±1.43	4.16±1.84	0.89
Malonate	1.55±2.31	1.24±0.55	1.61±2.53	0.57
Mannose	4.93±3.07	4.18±2.03	5.09±3.24	0.30
Methanol	3.23±2.42	2.72±2.84	3.34±2.33	0.37
Methylamine	1.28±2.04	1.03±1.27	1.34±2.17	0.59
Methylmalonate	7.15±3.61	7.98±4.70	6.97±3.35	0.32
Methylsuccinate	4.50±3.38	4.27±1.76	4.55±3.64	0.76
Myo-inositol	31.11±13.89	30.94±19.67	31.15±12.52	0.95
N-acetylserotonin	5.24±2.45	4.34±1.65	5.43±2.55	0.11
N-methylhydantoin	3.26±1.71	3.41±1.61	3.23±1.74	0.70
N-phenylacetylglycine	32.59±23.26	35.97±29.51	31.88±21.91	0.53
Nicotinamide N-oxide	0.95±0.57	1.05±0.66	0.92±0.55	0.44
O-Acetylcarnitine	6.79±4.97	7.69±7.48	6.60±4.32	0.44
O-Phosphocholine	7.19±6.23	7.41±5.54	7.14±6.41	0.88
Phenylalanine	6.52±5.73	7.25±4.86	6.37±5.92	0.59
Proline	25.96±10.03	22.60±6.36	26.67±10.54	0.15
Pyridoxine	3.82±2.70	3.01±1.56	3.99±2.86	0.07
Pyruvate	1.28±0.64	1.18±0.54	1.30±0.66	0.50
Ribose	25.29±14.15	22.91±12.30	25.80±14.54	0.47
Sarcosine	11.29±5.75	11.75±7.26	11.19±5.44	0.73
Succinate	1.18±1.01	1.04±0.48	1.20±1.09	0.58
Sucrose	12.56±9.26	12.03±6.36	12.67±9.79	0.81
Taurine	22.15±21.03	17.24±9.35	23.18±22.66	0.32
Threonine	4.66±2.81	4.42±1.68	4.70±3.01	0.72
Trigonelline	8.34±6.12	6.21±3.76	8.79±6.45	0.13
Trimethylamine	0.54±0.31	0.52±0.24	0.55±0.32	0.74
Trimethylamine N-oxide	15.19±32.78	27.61±66.19	12.56±19.72	0.39
Tryptophan	6.98±4.45	6.32±2.98	7.12±4.71	0.53
Tyramine	3.51±1.73	3.53±2.08	3.51±1.67	0.96
Tyrosine	4.57±2.32	4.01±1.44	4.69±2.46	0.30
Urea	128.41±112.25	168.72±128.72	119.89±107.54	0.12
Uridina	2.10±1.48	2.25±1.22	2.07±1.53	0.68
Valina	3.41±1.10	3.35±1.02	3.42±1.12	0.83
Xanthosine	3.97±2.55	4.46±3.11	3.86±2.43	0.41

Values are expressed as mean ± standard deviation. Comparison of averages using Mann-Whitney *U* test (P value).

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