



Supplementary Information for

Whole blood metabolomics of dementia patients reveal classes of disease-linked metabolites

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Other supplementary materials for this manuscript include the following:

Dataset S1

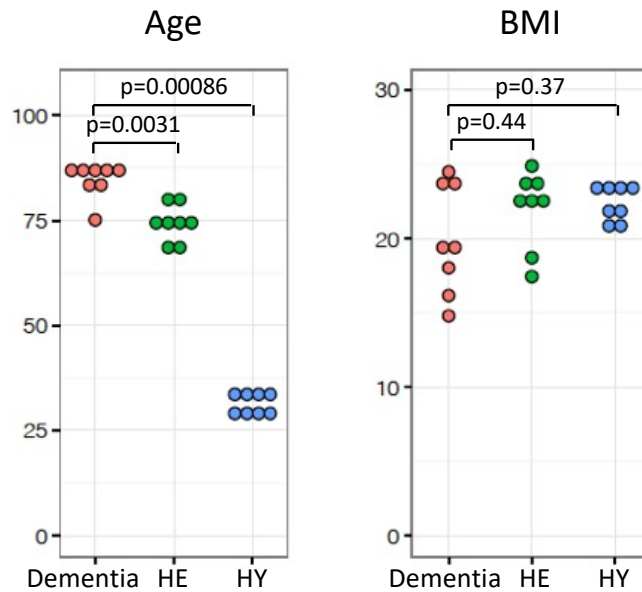


Fig. S1. Patients with dementia, healthy elderly (HE), and healthy young (HY) volunteers. Age and BMI (body mass index) are schematically shown. Ages of dementia patients ranged from 75 to 88 years and their BMIs varied from 14.8 to 24.5. The 8 HE subjects ranged in age from 67-80 with BMIs ranging from 17-25. The 8 HY subjects were between 28 and 34 years with BMIs 21 to 24.

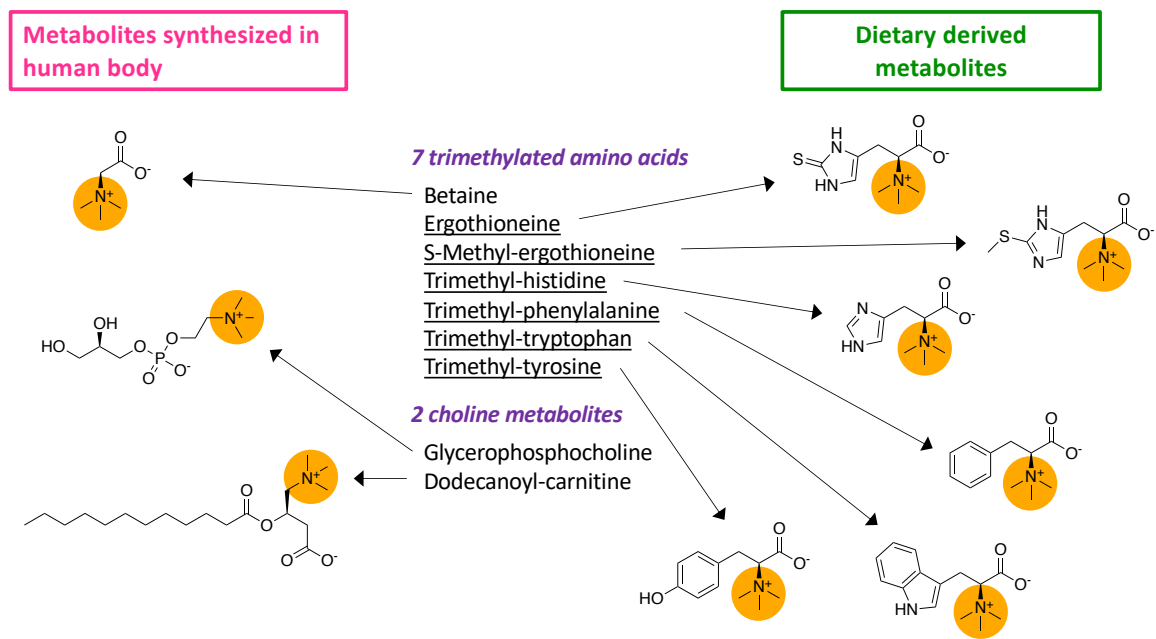


Fig. S2. In dementia patients, the abundance of various trimethylated compounds decreased in blood. Three trimethylated compounds (betaine, glycerophosphocholine, dodecanoyl-carnitine) are synthesized in the human body, whereas six other compounds (ergothioneine, S-methyl-ergothioneine, trimethyl-histidine, trimethyl-phenylalanine, trimethyl-tryptophan, trimethyl-tyrosine) are derived from food. These nine compounds decreased in whole blood of dementia patients.

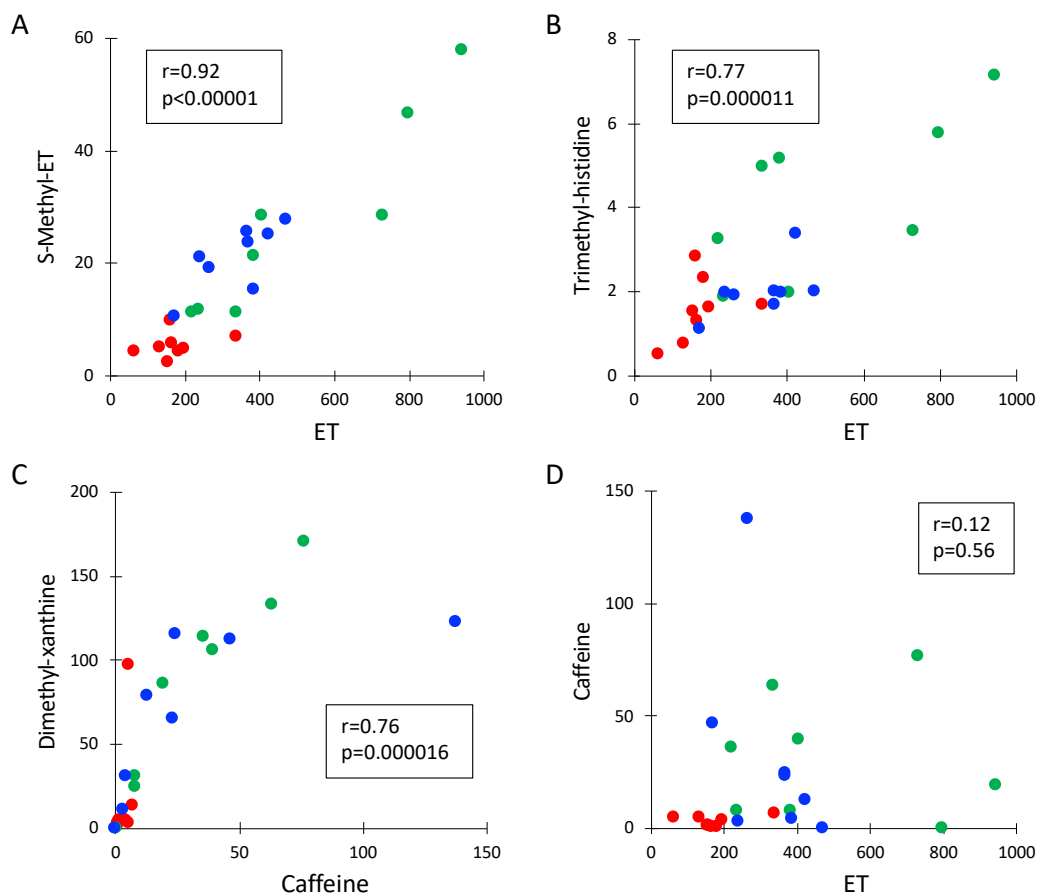


Fig. S3. Scatter plots showing high correlations between related metabolites. Metabolites related to ergothioneine biosynthesis (A and B) and caffeine catabolism (C) are displayed. Note, in contrast, that metabolically unrelated compounds, ET and caffeine, are not correlated at all (D). Metabolite abundance (peak area, $\times 10^6$ AU) of dementia patients (red dots), HE (green), and HY (blue) are represented. Pearson's correlation coefficient r and p -value are shown in boxes.

33 dementia markers			HY								
			#17	#18	#19	#20	#21	#22	#23	#24	
Higher in dementia (7)	A	Quinolinic acid	40.3	40.5	49.8	50.1	50.0	42.1	54.5	45.4	
		Dimethyl-guanosine	41.1	32.9	40.4	52.4	53.8	40.3	44.2	45.3	
		Pseudouridine	45.3	38.0	35.8	45.0	43.3	46.1	43.6	47.2	
		Indoxyl-sulfate	36.6	47.8	43.1	38.8	55.9	45.7	52.6	48.7	
		Kynurenine	44.3	29.5	50.3	52.1	61.1	48.0	53.5	45.1	
		N6-Acetyl-lysine	61.0	53.5	46.1	46.9	48.7	43.7	43.7	43.4	
		Adenosine	48.6	48.0	52.9	59.3	86.2	48.6	48.2	49.5	
Lower in dementia (26)	B	S-Methyl-ergothioneine	55.3	54.1	51.0	57.2	48.2	55.7	44.6	52.3	
		Ergothioneine	53.9	51.4	46.6	56.1	52.2	51.3	42.3	45.4	
		Trimethyl-histidine	54.7	44.5	45.9	46.4	46.3	46.5	41.2	46.3	
		Trimethyl-tryptophan	58.5	42.8	62.5	45.7	50.3	43.0	42.7	43.8	
		Trimethyl-phenylalanine	47.0	47.0	47.1	47.0	46.9	46.9	46.9	47.1	
		Trimethyl-tyrosine	46.4	53.8	50.7	57.0	53.5	48.5	45.5	49.4	
	C	Pantothenate	44.7	41.2	55.2	53.8	52.3	45.4	64.5	41.0	
		Gluconate	47.2	36.8	48.6	59.8	48.4	45.5	48.1	55.6	
		S-Adenosyl-methionine	84.3	66.5	52.1	51.9	44.0	46.1	54.2	59.9	
		NADP+	64.4	50.5	61.3	73.7	59.1	54.2	50.6	55.1	
		Glutathione disulfide	58.2	49.8	48.6	65.7	47.1	43.8	47.5	61.0	
		ATP	49.3	46.8	42.9	67.6	63.2	52.1	35.3	64.6	
	D	Methionine	41.6	39.4	55.9	62.4	63.4	47.1	52.4	57.5	
		Tryptophan	42.4	41.7	59.6	64.8	63.0	45.5	52.4	58.6	
		Glutamine	44.1	47.6	37.4	67.6	55.3	37.9	49.1	56.1	
		Betaine	43.7	38.7	42.2	62.9	72.9	55.5	36.9	53.7	
		Phenylalanine	36.3	37.4	46.6	54.6	69.5	40.4	46.6	55.4	
		Tyrosine	37.7	39.1	69.1	67.4	57.6	39.0	52.1	49.5	
		Histidine	44.7	44.7	42.2	53.4	53.3	49.2	46.1	55.8	
		Uridine	54.0	43.2	35.5	52.9	53.4	56.8	54.1	71.5	
		Keto(iso)leucine	44.3	39.1	37.0	66.4	43.5	53.1	49.9	68.1	
		Glycerophosphocholine	57.0	53.1	41.5	74.1	81.0	43.8	48.1	49.7	
		2-Hydroxybutyrate	55.7	34.7	39.6	42.2	44.2	39.6	49.6	63.6	
		Dodecanoyl-carnitine	67.9	47.7	48.4	42.8	40.6	40.8	68.3	68.2	
		E	Caffeine	47.1	50.8	85.6	43.3	44.6	50.4	57.6	44.2
			Dimethyl-xanthine	54.3	60.9	62.3	39.8	45.4	51.8	60.5	41.8

Fig. S4. A heatmap of standardized metabolite abundances of 33 dementia markers from healthy young (HY) subjects.

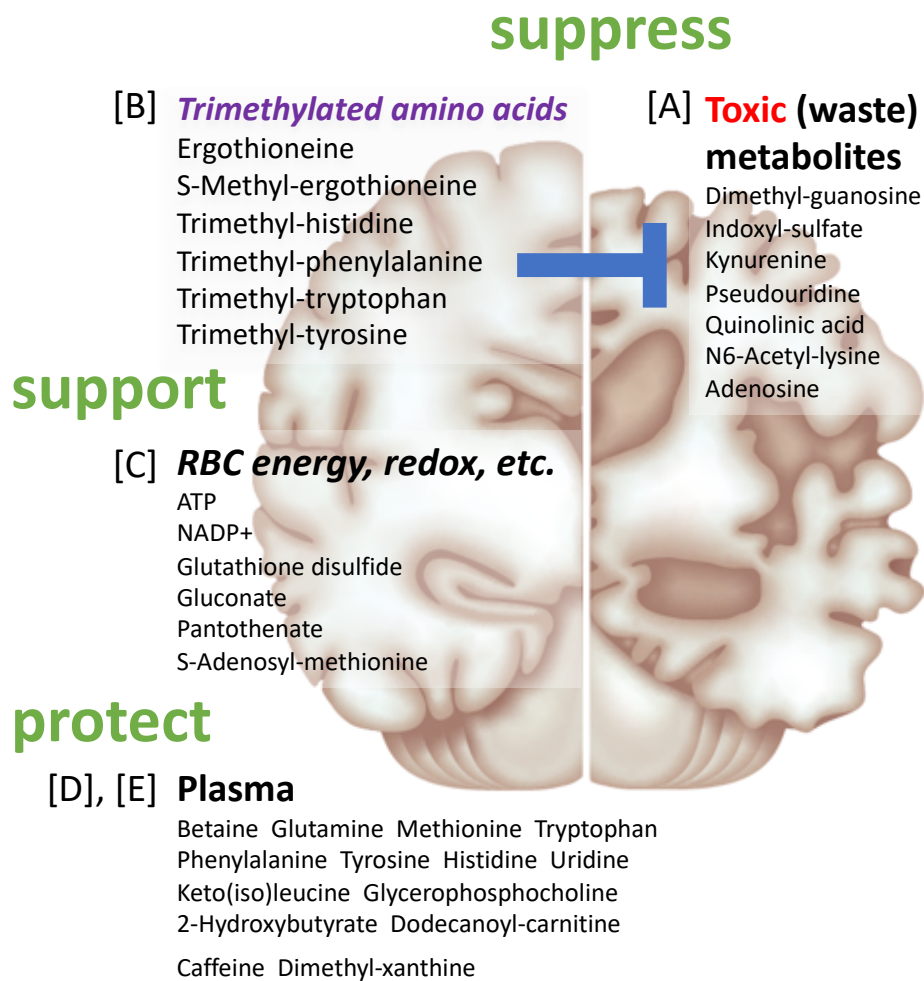


Fig. S5. Subclass A compounds may inhibit normal brain functions, leading to dementia, whereas subclasses B-E oppose dementia, possibly protecting the brain. Some compounds of group D enriched in plasma were previously shown to be dementia markers. Compounds of groups B and C enriched in RBC are mostly novel dementia markers (see text for explanation).

Table S1. Characteristics for dementia patients and healthy young (HY) and elderly (HE) subjects.

Group	Subject	Age	Gender	BMI	Cognitive test			Brain imaging	
					HDS-R score	MMSE score	COGNISTAT total score	MRI finding	VSRAD z-score
Dementia	#1	87	F	16.2	13	11	45	HA, CA	-
	#2	85	F	19.2	10	9	-	HA, WML	4.05
	#3	82	M	19.7	6	12	-	HA, CA	-
	#4	87	M	14.8	12	10	-	HA, WML, OBA	6.27
	#5	87	F	18.0	5	7	-	HA, CA, WML	6.31
	#6	86	F	24.5	18	22	88	HA, CA, OLI	3.55
	#7	88	M	23.4	17	21	63	HA, CA, WHL, OLI	2.03
	#8	75	M	24.0	11	-	-	HA, OBA, OLI	-
HE	#9	67	M	22.3	30	29	-	-	-
	#10	74	M	23.6	-	-	-	-	-
	#11	73	M	23.9	-	-	-	-	-
	#12	70	F	22.1	28	29	-	-	-
	#13	75	F	24.9	30	30	-	-	-
	#14	76	M	23.0	28	29	-	-	-
	#15	80	F	17.4	30	30	-	-	-
HY	#16	80	F	18.7	28	30	-	-	-
	#17	30	M	23.1	-	-	-	-	-
	#18	28	F	21.0	-	-	-	-	-
	#19	33	F	21.9	-	-	-	-	-
	#20	34	M	21.9	-	-	-	-	-
	#21	34	F	20.7	-	-	-	-	-
	#22	33	M	23.0	-	-	-	-	-
#23	30	F	23.3	-	-	-	-	-	
#24	30	M	23.9	-	-	-	-	-	

Dementia was diagnosed by DSM-4 criteria, cognitive tests, and brain imaging (details in the Methods section). BMI, body mass index; HDS-R, Hasegawa's dementia scale-revised; MMSE, mini mental state examination; MRI, magnetic resonance imaging; VSRAD, voxel-based specific regional analysis system for Alzheimer's disease; COGNISTAT, neurobehavioural cognitive status examination; HA, hippocampal atrophy; CA, cerebral atrophy; WML, white matter lesions; OBA, overall brain atrophy; OLI, old lacunar infarction. Short line (-) indicates *not examined*.

Table S2. List of 124 identified blood metabolites

Category/Compound	Peak abundance	CV		Dementia/HE		
		present study	Chaleckis <i>et al.</i> (14)	P-value	Ratio	Q-value
Nucleotides (9)						
* <u>ATP</u>	H	0.20	0.17	0.0047	0.83	0.0171
<u>ADP</u>	H-M	0.30	0.27	0.1949	0.83	
<u>AMP</u>	M	0.35	0.33	0.1605	0.80	
<u>GTP</u>	M	0.21	0.32	0.5737	1.10	
<u>UMP</u>	M	0.20	0.88	0.1304	1.24	
<u>GDP</u>	M-L	0.26	0.38	0.4418	1.21	
<u>CTP</u>	L	0.38	0.33	0.2345	1.22	
<u>UDP</u>	L	0.27	0.31	0.1304	1.28	
<u>UTP</u>	L	0.32	0.44	0.0650	1.38	
Nucleosides, nucleobases, and derivatives (12)						
* Caffeine	H-L	1.49	0.92	0.0104	0.09	0.0344
* Dimethyl-xanthine	H-L	0.98	0.61	0.0281	0.04	0.0422
<u>Urate</u>	M	0.21	0.28	0.0650	0.85	
<u>Adenine</u>	M-L	0.43	0.53	0.2786	0.77	
Cytidine	M-L	0.34	0.33	0.0650	0.67	
Hypoxanthine	M-L	0.46	0.35	0.8785	0.93	
<i>N</i> -Methyl-adenosine	M-L	0.31	0.29	0.7985	0.92	
* Pseudouridine	M-L	0.31	-	0.0148	1.19	0.0304
Xanthine	M-L	1.93	0.56	0.9591	0.91	
* Adenosine	L	0.47	0.49	0.0379	1.51	0.0481
* Dimethyl-guanosine	L	0.40	0.46	0.0499	1.45	0.0549
* Uridine	L	0.30	0.34	0.0030	0.72	0.0122
Vitamins and coenzymes (5)						
<u>Nicotinamide</u>	H-M	0.30	0.56	0.9591	1.04	
<u>NAD+</u>	M	0.35	0.30	0.4418	1.13	
4-Aminobenzoate	M-L	3.47	2.18	0.5054	0.18	
* <u>Pantothenate</u>	M-L	0.46	0.82	0.0148	0.57	0.0287
* <u>NADP+</u>	L	0.33	0.36	0.0104	0.70	0.0312
Nucleotide-sugar derivatives (4)						
<u>UDP-N-acetyl-glucosamine</u>	M	0.55	0.64	0.5737	0.85	
<u>UDP-glucose</u>	M	0.24	0.24	0.5737	0.95	
<u>GDP-glucose</u>	M-L	0.37	0.53	0.4418	0.87	
<u>UDP-glucuronate</u>	L	0.38	0.63	0.9591	0.86	
Sugar phosphates (11)						
<u>Diphosphoglycerate</u>	H	0.18	0.24	0.8785	0.96	
<u>Fructose-1,6-diphosphate</u>	H-M	0.19	-	0.2345	1.15	
<u>Glucose-6-phosphate</u>	M	0.19	0.29	0.4418	1.11	
<u>Glyceraldehyde-3-phosphate</u>	M	0.38	0.99	0.0650	0.59	
<u>Fructose-6-phosphate</u>	M-L	0.21	0.24	0.5054	1.23	
<u>Glycerol-phosphate</u>	M-L	0.28	0.31	0.6454	0.99	
<u>Phosphoglycerate</u>	M-L	0.22	0.29	0.5054	0.93	
<u>6-Phosphogluconate</u>	L	0.20	0.30	0.9591	0.96	
<u>Pentose-phosphate</u>	L	0.22	0.34	0.0650	1.21	
<u>Phosphoenolpyruvate</u>	L	0.18	-	0.2345	0.88	
<u>Sedoheptulose-7-phosphate</u>	L	0.24	0.52	0.0830	1.25	
Sugar derivatives (4)						

1,5-Anhydroglucitol	M	0.30	0.46	0.6454	1.10	
* <u>Gluconate</u>	M	0.17	0.33	0.0379	0.85	0.0464
<u>N-Acetyl-glucosamine</u>	M	0.25	0.26	0.6454	0.88	
myo-Inositol	M-L	0.32	0.24	0.3823	0.86	
<i>Choline and ethanolamine derivatives (7)</i>						
Choline	H-M	0.74	-	0.1949	0.64	
* Glycerophosphocholine	H-M	0.49	0.47	0.0148	0.66	0.0271
Phosphocholine	H-M	0.28	-	0.7209	1.06	
CDP-choline	M-L	0.38	0.41	0.1605	0.80	
CDP-ethanolamine	L	0.42	0.43	0.6454	0.88	
Glycerophosphoethanolamine	L	0.35	-	0.1949	0.83	
Phosphoethanolamine	L	0.27	-	0.4418	1.04	
<i>Organic acids (10)</i>						
* 2-Hydroxybutyrate	M-L	0.34	-	0.0499	0.79	0.0531
Chenodeoxycholate	M-L	1.67	1.33	0.1949	1.99	
Citrate	M-L	0.18	0.31	0.9591	1.03	
Glycochenodeoxycholate	M-L	2.44	1.20	0.5737	1.59	
2-Oxoglutarate	L	0.46	0.54	0.1304	0.73	
3-Hydroxybutyrate	L	0.95	-	0.3282	0.68	
<u>Citramalate</u>	L	0.24	0.36	0.1605	0.87	
Glycerate	L	0.37	0.43	0.5737	0.92	
<u>Malate</u>	L	0.20	0.20	0.2345	0.91	
<u>Succinate</u>	L	0.36	0.60	0.3823	0.81	
<i>Antioxidants (2)</i>						
* <u>Glutathione disulfide</u>	H	0.14	0.18	0.0104	0.82	0.0286
* <u>Ergothioneine</u>	H-M	0.64	0.63	0.0011	0.41	0.0090
<i>Standard amino acids (17)</i>						
Arginine	H	0.14	0.29	0.7985	0.96	
* Glutamine	H	0.14	0.20	0.0207	0.84	0.0341
* Phenylalanine	H	0.18	0.17	0.0281	0.73	0.0404
Proline	H	0.28	0.42	0.1304	0.86	
<u>Glutamate</u>	H-M	0.16	0.28	0.1304	0.84	
Lysine	H-M	0.18	0.39	0.8785	0.96	
* Methionine	H-M	0.16	0.28	0.0379	0.80	0.0447
Threonine	H-M	0.19	0.42	0.5054	0.96	
* Tryptophan	H-M	0.22	0.24	0.0281	0.80	0.0387
* Tyrosine	H-M	0.21	0.27	0.0104	0.75	0.0264
Asparagine	M	0.20	0.23	0.1304	0.90	
* Histidine	M	0.24	0.19	0.0379	0.80	0.0432
Isoleucine	M	0.19	0.32	0.4418	0.86	
Leucine	M	0.18	0.31	0.0830	0.83	
Serine	M	0.20	0.33	0.1304	0.84	
Valine	M	0.20	0.48	0.0650	0.87	
<u>Aspartate</u>	M-L	0.37	0.50	1.0000	0.91	
<i>Methylated amino acids (14)</i>						
* Betaine	H	0.16	0.51	0.0019	0.85	0.0123
<u>Butyro-betaine</u>	H-M	0.23	0.35	0.5054	0.92	
<u>Dimethyl-proline</u>	H-M	0.53	0.79	0.8785	0.95	
<u>Trimethyl-lysine</u>	H-M	0.30	0.38	0.5737	1.04	
* <u>Trimethyl-tryptophan</u>	H-L	1.09	1.67	0.0003	0.10	0.0051

Dimethyl-arginine	M	0.24	0.31	0.7209	1.11	
<i>N</i> 1-Methyl-histidine	M	0.40	0.30	1.0000	0.92	
Dimethyl lysine	M-L	0.64	0.44	0.0830	1.58	
<i>N</i> 3-Methyl-histidine	M-L	0.49	0.30	0.9591	1.01	
<i>N</i> 6-Methyl-lysine	M-L	0.71	0.73	0.7209	0.84	
* <u>S-Methyl-ergothioneine</u>	M-L	0.77	0.63	0.0002	0.20	0.0053
* <u>Trimethyl-phenylalanine</u>	M-L	3.17	1.18	0.0006	0.23	0.0068
* <u>Trimethyl-tyrosine</u>	M-L	1.09	2.50	0.0019	0.08	0.0102
* <u>Trimethyl-histidine</u>	L	0.64	0.57	0.0019	0.37	0.0088
Acetylated amino acids (4)						
<i>N</i> -Acetyl-arginine	L	0.52	0.62	0.7985	1.08	
<i>N</i> -Acetyl-aspartate	L	0.23	0.58	0.2345	1.09	
<i>N</i> 2-Acetyl-lysine	L	0.30	0.50	0.6454	0.86	
* <i>N</i> 6-Acetyl-lysine	L	0.79	0.43	0.0207	1.47	0.0325
Other amino acids (16)						
<u>Creatine</u>	M	0.33	0.29	0.0650	1.39	
Taurine	M	0.18	0.37	1.0000	1.05	
Creatinine	H	0.16	0.35	0.4418	0.93	
* Indoxyl-sulfate	M	0.46	0.59	0.0148	1.93	0.0256
Hippurate	M-L	0.83	1.01	0.1605	0.47	
* Keto(iso)leucine	M-L	0.26	-	0.0104	0.77	0.0245
* Kynurenine	M-L	0.16	0.48	0.0281	1.12	0.0371
Ophthalmic acid	M-L	0.41	0.43	0.1949	0.71	
Ornithine	M-L	0.29	0.48	0.3823	1.17	
4-Guanidinobutanoate	L	0.71	2.05	0.2786	0.60	
Acetyl-carnosine	L	0.65	1.07	0.2786	0.60	
Citrulline	L	0.27	0.30	0.4418	0.95	
<u>Phosphocreatine</u>	L	0.47	0.48	0.7985	1.18	
* Quinolinic acid	L	0.48	1.18	0.0499	1.79	0.0514
<u>S-Adenosyl-homocysteine</u>	L	0.34	0.83	0.1949	1.11	
* <u>S-Adenosyl-methionine</u>	L	1.02	0.88	0.0104	0.35	0.0229
Carnitines (9)						
<u>Acetyl-carnitine</u>	H	0.28	0.41	0.5737	0.96	
Carnitine	H	0.14	0.20	0.2786	1.10	
<u>Propionyl-carnitine</u>	H-M	0.33	0.41	0.5737	0.87	
(iso)Butyryl-carnitine	M-L	0.45	0.37	0.8785	1.12	
Decanoyl-carnitine	M-L	0.75	1.11	0.2345	0.67	
Hexanoyl-carnitine	M-L	0.50	0.76	0.3282	0.73	
Octanoyl-carnitine	M-L	0.78	1.02	0.5737	0.69	
(iso)Valeryl-carnitine	M-L	0.35	1.98	0.1605	0.81	
* Dodecanoyl-carnitine	L	0.91	1.11	0.0499	0.15	0.0499

P-values between patients with dementia and HE were calculated using a Mann-Whitney U-test. Asterisks indicate 33 compounds that showed significant differences ($P < 0.05$) between dementia patients and HE. Coefficients of variation (CV) from the present and a previous study (ref 14) are listed. They are mostly in agreement. Short line (-) indicates not reported. The peak ratio was calculated using the median of peak abundance in dementia patients and HE, respectively. Fifty-one RBC-enriched compounds are underlined (ref 14). To estimate the false discovery rate, *Q*-values were also calculated. *Q*-values were mostly consistent with *P*-values of 33 metabolites.

Table S3. The ratio of the metabolite levels in whole blood between healthy controls, and patients with mild cognitive impairment (MCI), frailty, and dementia.

	MCI Cheah et al. (ref 17)	Frailty Kameda et al. (ref 16)	Dementia present study
Ergothioneine	0.65	0.53	0.41
S-Methyl-ergothioneine	not reported	0.48	0.20
Trimethyl-histidine		0.53	0.37
Tryptophan		0.80	0.80
Methionine		0.81	0.80

The ratio was calculated by dividing the quantitative value of a patient by that of healthy controls.

Table S4. Medication information about dementia patients.

Subject	Medication
#1	Mem, Ari, Esz, Ram, Tul, Bromhexine hydrochloride, Theophylline, Montelukast sodium, Salmeterol xinafoate, Celecoxib
#2	Mem, Ari, Esz, Ram, Val, Nif, Rab, Limaprost alfadex
#3	Mem, Esz, Val, Nif, Sodium ferrous citrate, Magnesium oxide
#4	Mem, Suv, Yokukansan, Potassium gluconate
#5	Galantamine hydrobromide, Esz, Ram
#6	Mem, Esz, Suv
#7	Ram, Tul, Nicorandil, Isosorbide dinitrate, Olmesartan medoxomil, Febuxostat, Tamsulosin hydrochloride, Imidafenacin, Goshajinkigan, Lubiprostone
#8	Rab, Amlodipine besilate

Mem, Memantine hydrochloride; Ari, Aripiprazole; Esz, Eszopiclone; Ram, Ramelteon; Tul, Tulobuterol; Val, Valsartan; Nif, Nifedipine; Rab, Rabeprazole sodium; Suv, Suvorexant.

Dataset S1 (separate file). Compounds were identified using either commercially available standards (STD) or by analysis of MS/MS spectra (MS/MS), if no standard was available.