

Supplementary information of “Decreased long-chain acylcarnitines from insufficient β -oxidation as potential early diagnostic markers for Parkinson’s disease”

Shinji Saiki,¹ Taku Hatano¹, Motoki Fujimaki¹, Kei-Ichi Ishikawa¹, Akio Mori¹, Yutaka Oji¹, Ayami Okuzumi¹, Takeshi Fukuhara¹, Takahiro Koinuma¹, Yoko Imamichi¹, Miho Nagumo¹, Norihiko Furuya^{1,2} Shuko Nojiri³, Taku Amo⁴, Kazuo Yamashiro¹ and Nobutaka Hattori^{1,2,*}

1. Department of Neurology, Juntendo University School of Medicine, Bunkyo, Tokyo, 113-8421, Japan

2. Department of Research and Therapeutics for Movement Disorders, Juntendo University School of Medicine, Bunkyo, Tokyo, 113-8421, Japan

3. Clinical Research Center, Juntendo University, Bunkyo, Tokyo, 113-8421, Japan

4. Department of Applied Chemistry, National Defense Academy, Yokosuka, Kanagawa, 239-8686, Japan

Supplementary Figure Legends

Supplementary Figure 1 – Additional principal component analysis of plasma metabolites from 32 controls and 32 patients with Parkinson's disease arranged by PC1 and PC3.

A, C, E, G, H PCA from 32 controls and 32 patients with Parkinson's disease randomly selected from the first cohort.

B, D, F, H, J Top 15 factor loading in PC3 listed according to statistical significance.

Abbreviations: AC = acylcarnitine; CTR= control; FA = fatty acid; N-MPEA = N-methylphenylethanolamine; PD = Parkinson's disease

Supplementary Figure 2 – Each long-chain acylcarnitine ratio of Hoehn and Yahr stages II–IV to that in Hoehn and Yahr I in Parkinson's disease with or without motor fluctuations

A, D Statistical analysis was performed using Wilcoxon's test for each long-chain acylcarnitine in each Hoehn and Yahr stage in Parkinson's disease with or without motor fluctuations. No apparent trends commonly observed in both cohorts were detected.

Supplementary Figure 3 – Multiple comparisons of the metabolites associated with skeletal muscle in both cohorts.

A, B, C Statistical analysis was performed using Steel's test for skeletal muscle-associated metabolites and enzymes. It should be mentioned that no changes of carnitine levels were noted in any stage of the disease.

D No significant differences of HbA1c levels among H&Y stages were detected by Steel's test and ANOVA.

Abbreviations: CK = creatine kinase; H&Y = Hoehn and Yahr stage; Hb A1c = haemoglobin A1c; Ctrl = control

Error bars, S.D.; *P<0.05; **P<0.01 (Steel's test), NS = not significant

Supplementary Figure 4 – Levels of 3-methoxytyrosine and homovanillic acid were correlated with levodopa equivalent dose.

A, B Levels of 3-methoxytyrosine in Parkinson's disease in the first and second cohorts correlated with levodopa equivalent dose. Statistical analysis was performed using ANOVA.

C 3-Methoxytyrosine levels were much lower in all cases of normal controls as well as *de novo* Parkinson's disease.

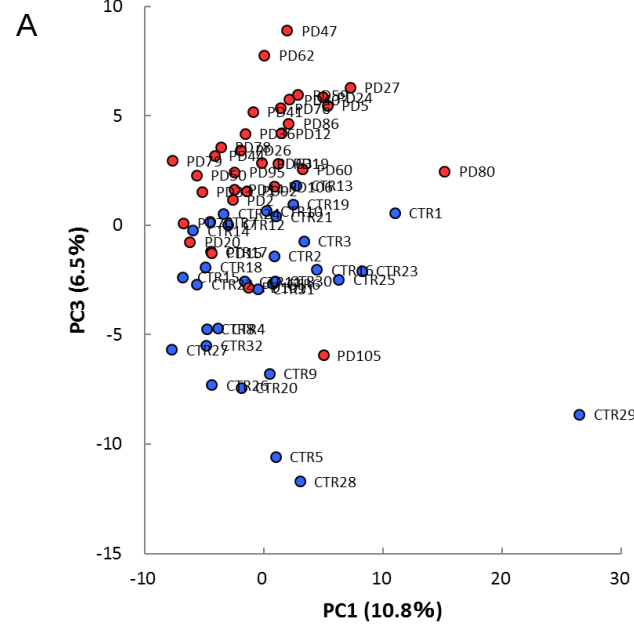
D, E Levels of homovanillic acid in Parkinson's disease in the first and second cohorts correlated with levodopa equivalent dose. Statistical analysis was performed using ANOVA.

F Homovanillic acid levels of *de novo* Parkinson's disease were mildly decreased compared with those in normal controls, suggesting that no homovanillic acid was derived from orally administered levodopa.

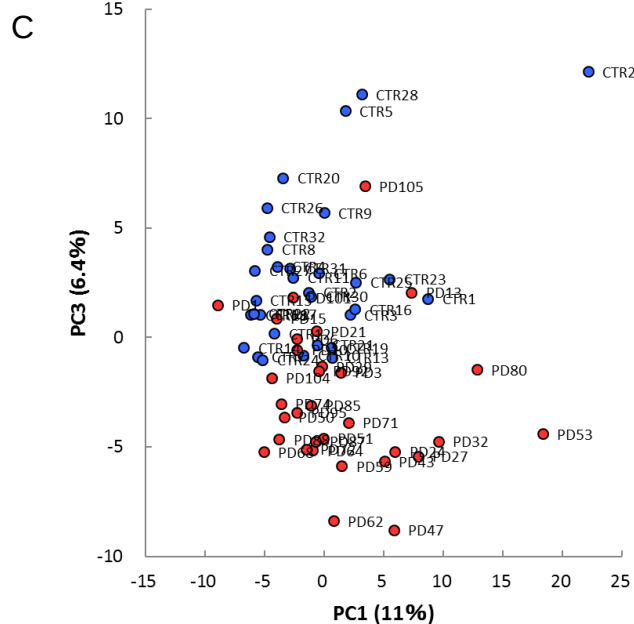
Abbreviations: Ctrl = control; PD = Parkinson's disease

Error bars, S.D.; **P<0.01; ***P<0.001 (Steel-Dwass test)

Supplementary Figure 1

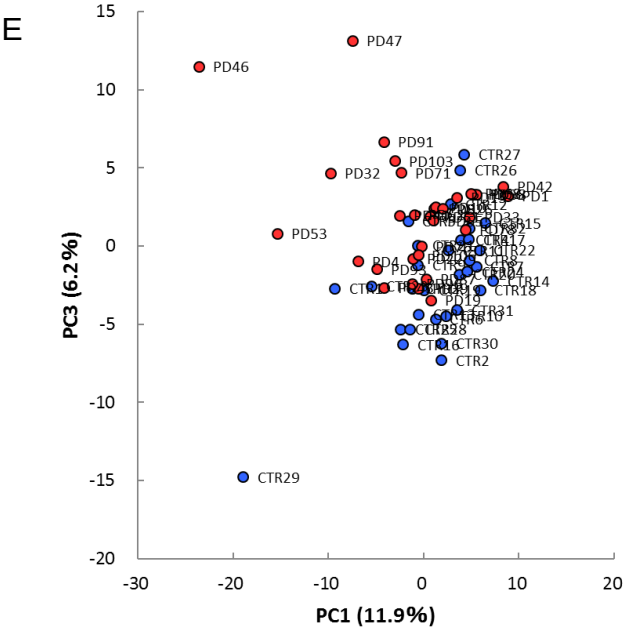


- B Factor loading in PCA (PC3)
1. AC(18:2): -0.164
 2. AC(16:0): -0.160
 3. AC(18:1): -0.158
 4. AC(14:0): -0.147
 5. AC(12:0): -0.144
 6. AC(18:0): -0.134
 7. AC(16:1): -0.134
 8. N-MPEA: -0.128
 9. AC(14:2): -0.127
 10. Pyruvic acid: -0.126
 11. AC(20:1): -0.120
 12. Taurochenodeoxycholic acid: -0.118
 13. Glycochenodeoxycholic acid: -0.115
 14. Glycoursodeoxycholic acid: -0.115
 15. AC(13:1): -0.115



- D Factor loading in PCA (PC3)
1. AC(18:1): 0.169
 2. AC(14:0): 0.168
 3. AC(18:2): 0.166
 4. AC(16:0): 0.163
 5. AC(12:0): 0.162
 6. N-MPEA: 0.156
 7. AC(16:1): 0.155
 8. AC(14:2): 0.142
 9. AC(18:0): 0.137
 10. AC(12:1): 0.135
 11. Glycoursodeoxycholic acid: 0.123
 12. AC(14:1): 0.119
 13. AC(14:3): 0.117
 14. AC(20:1): 0.115
 15. AC(16:2): 0.114

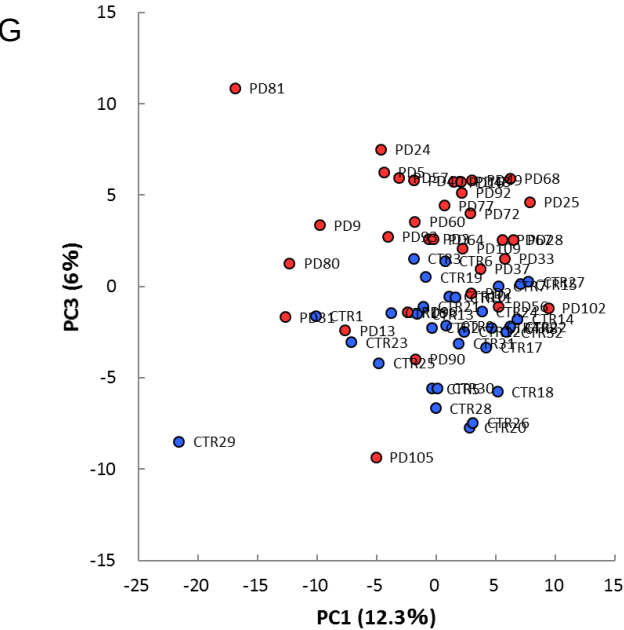
Supplementary Figure 1 (continued)



F

Factor loading in PCA (PC3)

1.	AC(16:2): -0.128
2.	AC(16:1): -0.118
3.	AC(12:0): -0.115
4.	AC(12:1): -0.114
5.	AC(14:1): -0.108
6.	AC(14:0): -0.108
7.	AC(18:1): -0.104
8.	FA(19:2): -0.100
9.	FA(22:2): -0.100
10.	FA(22:3): -0.100
11.	FA(16:3): -0.100
12.	AC(16:0): -0.0993
13.	FA(24:4): -0.0990
14.	AC(14:2): -0.0923
15.	N-MPEA: -0.0916

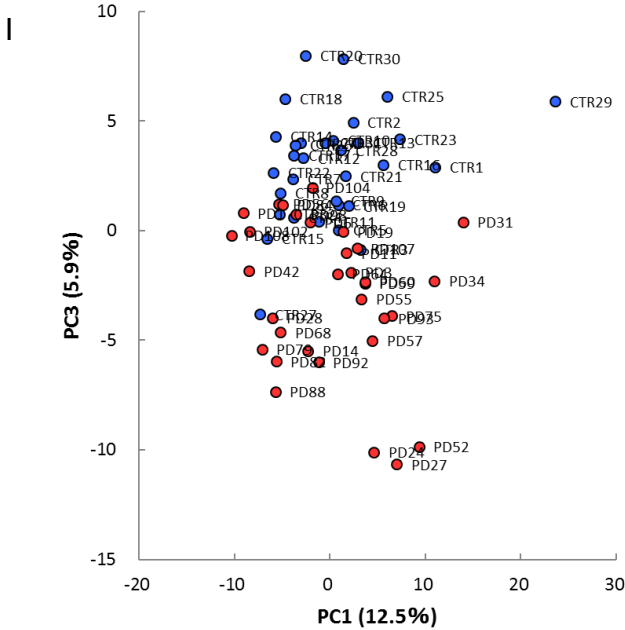


H

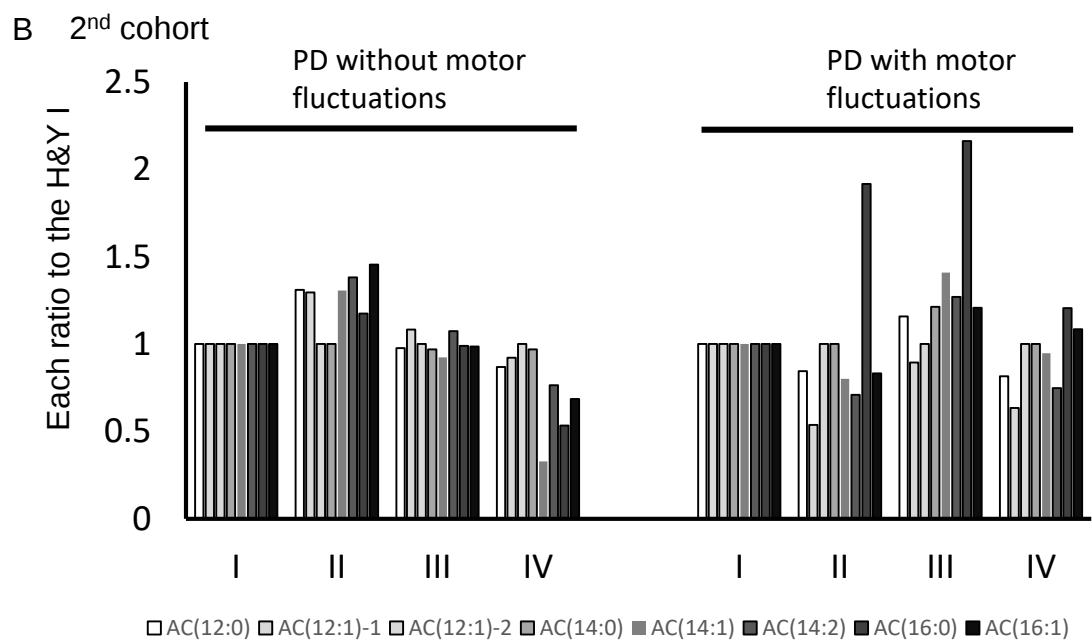
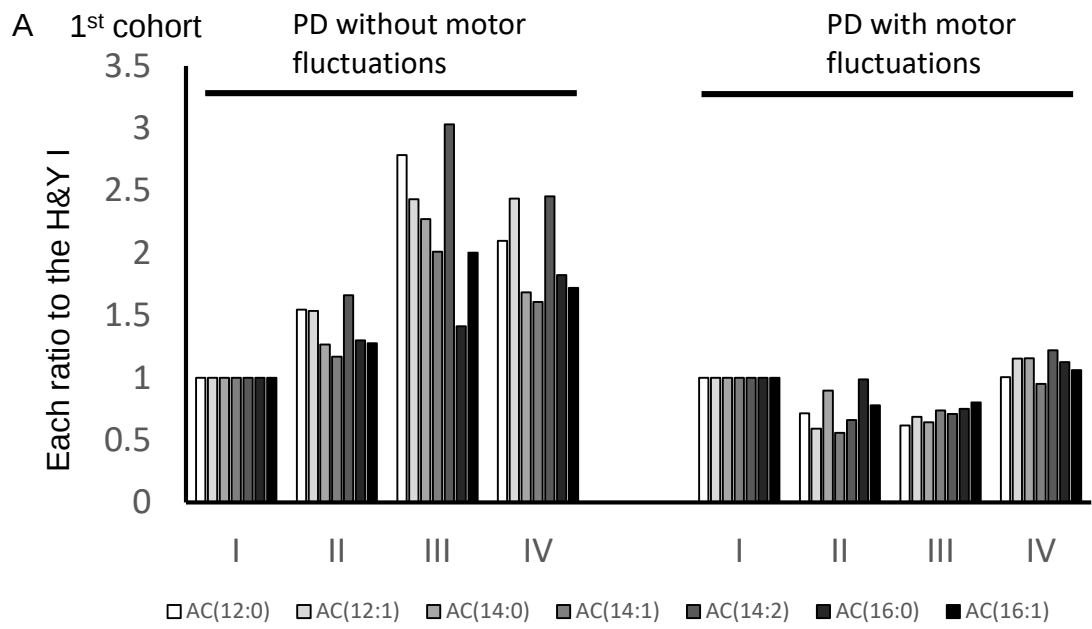
Factor loading in PCA (PC3)

1.	AC(16:0): -0.177
2.	AC(18:1): -0.164
3.	AC(12:0): -0.162
4.	AC(14:0): -0.160
5.	AC(16:1): -0.154
6.	AC(18:2): -0.150
7.	AC(12:1): -0.142
8.	AC(18:0): -0.141
9.	AC(13:1): -0.140
10.	AC(14:2): -0.136
11.	AC(20:1): -0.129
12.	AC(14:3): -0.127
13.	AC(16:2): -0.123
14.	AC(14:1): -0.119
15.	N-MPEA: -0.118

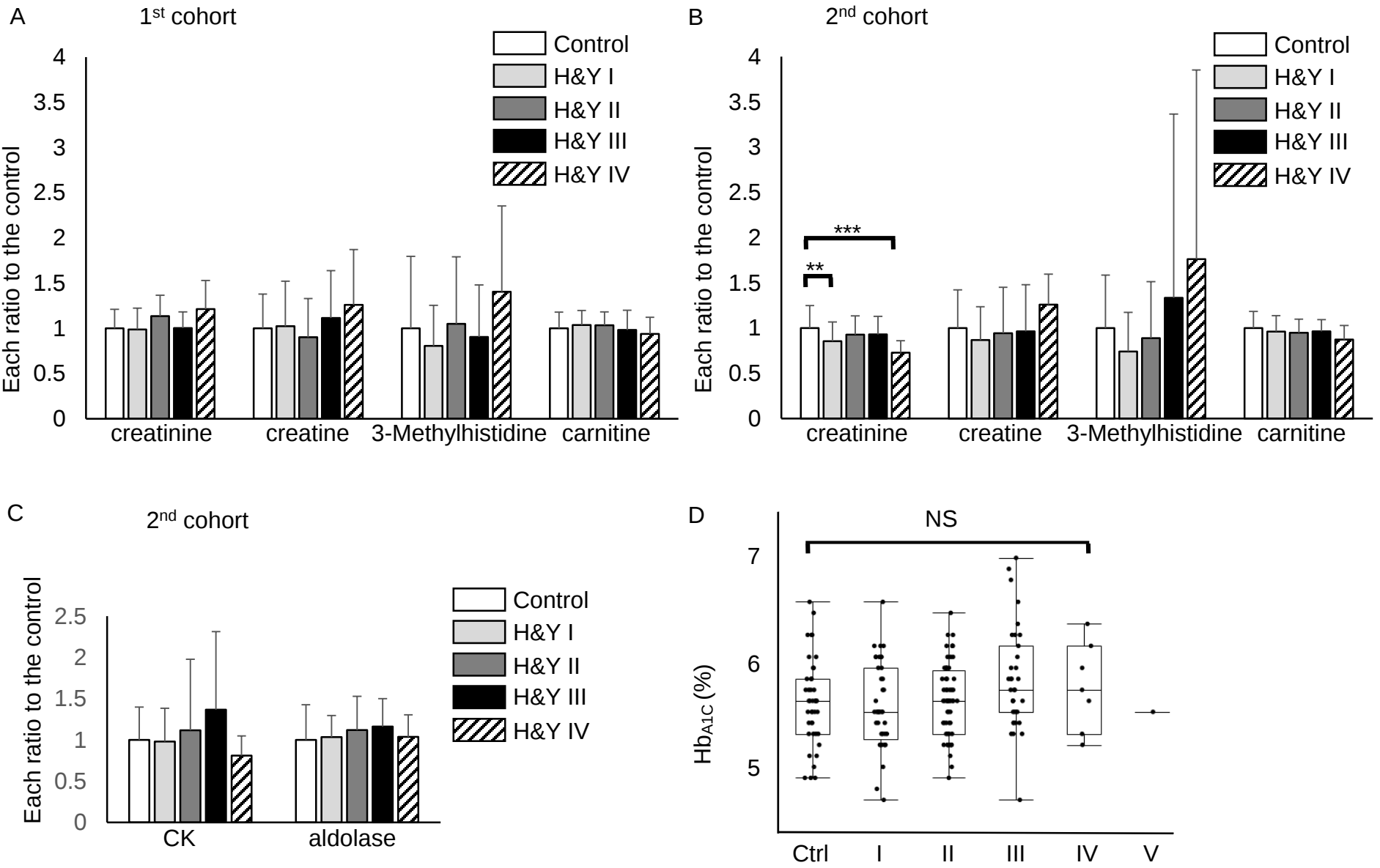
Supplementary Figure 1 (continued)



Supplementary Figure 2

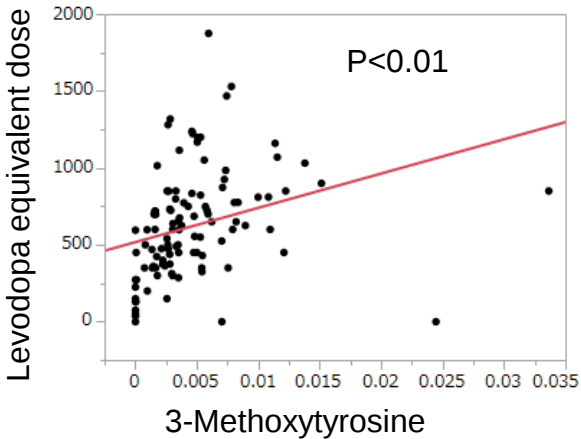


Supplementary Figure 3

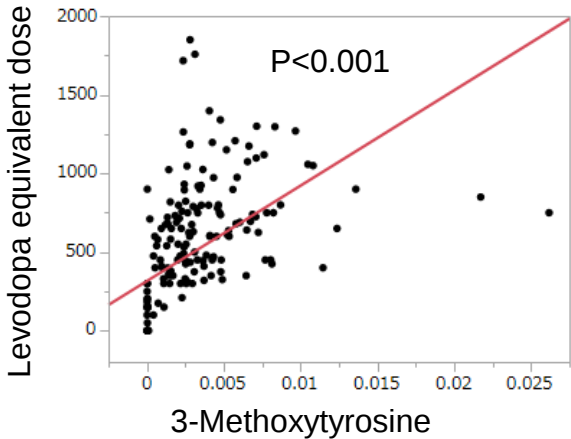


Supplementary Figure 4

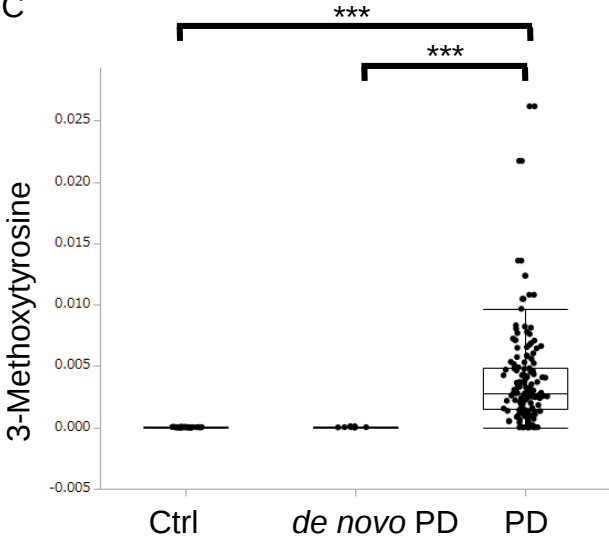
A 1st cohort



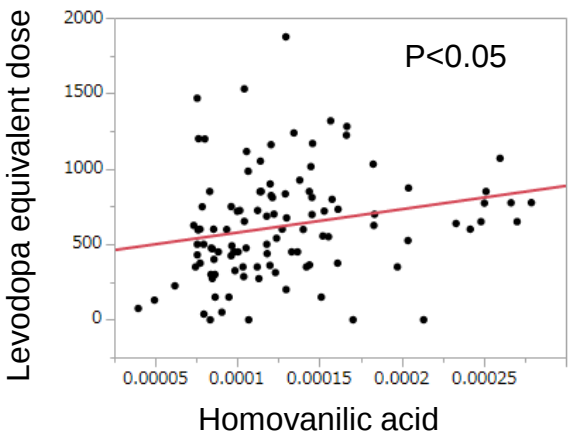
B 2nd cohort



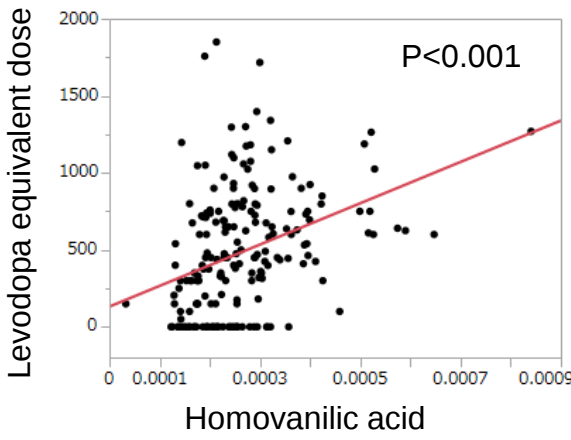
C



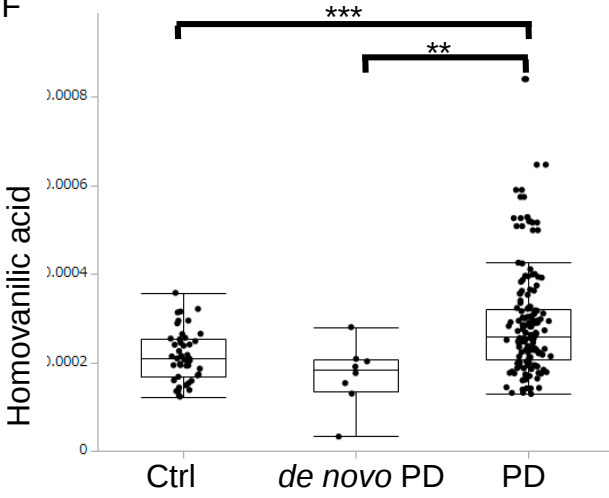
D 1st cohort



E 2nd cohort



F



Supplementary Table S1. Multiple comparisons of clinical parameters according to H&Y stage in both cohorts

	Control		H&Y I		H&Y II		H&Y III		H&Y IV		H&Y V		P-value ^b
	Mean	P-value ^a	Mean	P-value ^a	Mean	P-value ^a	Mean	P-value ^a	Mean	P-value ^a	Mean	P-value ^a	
1st cohort	N=32		N=26		N=52		N=21		N=9		N=1		
Age [years]	62.9	-	63.1	1.000	67.4	0.372	71.0	0.0801	69.0	0.536	67.0	-	0.0492
BMI [kg/m ²]	24.1	-	22.3	0.243	22.3	0.275	21.1	0.0264	20.8	0.143	17.3	-	0.008
disease duration	-	-	3.67	-	5.89	-	7.76	-	14.6	-	10.0	-	<0.0001
UPDRS-III	-	-	5.92	-	13.0	-	17.2	-	27.7	-	34.0	-	<0.0001
LED	-	-	383 (220)	-	590 (319)	-	744 (281)	-	891 (433)	-	1650	-	<0.0001
MMSE	-	-	28.7	-	28.5	-	28.6	-	28.1	-	29.0	-	0.579
2nd cohort	N=45		N=41		N=60		N=35		N=8		N=1		
Age [years]	63.8	-	63.6	0.991	68.0	0.898	70.2	0.378	74.5	0.220	70.0	-	0.0053
BMI [kg/m ²]	23.2	-	22.4	0.441	22.4	0.510	22.7 (4.3)	0.975	21.6	0.208	21.0	-	0.549
disease duration	-	-	4.27	-	6.54	-	9.71	-	11.6	-	21.0	-	<0.0001
UPDRS-III	-	-	7.24	-	13.6	-	22.5	-	27.1	-	31.0	-	<0.0001
LED	-	-	439 (261)	-	582 (319)	-	817 (338)	-	881 (297)	-	1050.0	-	<0.0001
MMSE	28.9	-	28.9	-	28.5 (1.6)	-	28.0	-	28.1	-	28.0	-	0.204

Each parameter was assessed by Steel's test and one-way ANOVA.

Abbreviations: SD = standard deviation; BMI = body mass index; H&Y stage = Hoehn and Yahr stage; UPDRS-III = Unified Parkinson's Disease Rating Scale motor section; LED = levodopa equivalent dose; MMSE = Mini Mental State Examination

^aP-value obtained by Steel's test, comparing with controls. ^bP-value obtained by analysis of covariance among H&Y stages I-IV.

Supplementary Table S2. Multiple comparisons of short- to long-chain acylcarnitines in each cohort, corresponding to Main Figure 3.

compound name	H&Y I/control		H&Y II/control		H&Y III/control		H&Y IV/control		P-value ^b
	Mean	P-value ^a	Mean	P-value ^a	Mean	P-value ^a	Mean	P-value ^a	
1st cohort	N=26		N=52		N=21		N=9		
Carnitine	1.04	0.821	1.03	0.823	0.981	0.979	0.939	0.821	0.157
AC(2:0)	1.05	0.981	1.03	0.941	1.04	0.997	1.00	1.00	0.962
AC(4:0)	1.08	0.945	1.37	0.0355	1.10	0.978	1.33	0.538	0.107
AC(8:0)	1.01	0.994	1.04	0.989	1.23	0.996	1.26	0.607	0.602
AC(12:0)	0.445	<0.0001	0.479	<0.0001	0.588	0.0008	0.662	0.493	<0.0001
AC(12:1)	0.492	0.0004	0.487	<0.0001	0.635	0.0063	0.852	0.993	0.0001
AC(13:1)	0.452	<0.0001	0.640	0.0002	0.581	0.0023	0.909	0.561	<0.0001
AC(14:0)	0.472	<0.0001	0.515	<0.0001	0.584	0.0015	0.678	0.356	<0.0001
AC(14:1)	0.674	0.2	0.563	0.019	0.819	0.328	0.858	1.00	0.0762
AC(14:2)	0.525	0.0001	0.566	0.0004	0.772	0.014	0.930	1.00	0.0002
AC(16:0)	0.485	<0.0001	0.561	<0.0001	0.497	0.0001	0.724	0.347	<0.0001
AC(16:1)	0.530	0.0002	0.544	<0.0001	0.669	0.003	0.745	0.584	<0.0001
AC(18:0)	0.497	<0.0001	0.654	0.0012	0.564	0.0007	0.667	0.276	0.0001
AC(18:1)	0.510	<0.0001	0.575	<0.0001	0.548	0.0008	0.731	0.956	<0.0001
AC(20:1)	0.604	0.0114	0.704	0.0197	0.737	0.154	0.796	0.807	0.0087
2nd cohort	N=41		N=60		N=35		N=8		
Carnitine	0.961	0.560	0.947	0.296	0.963	0.836	0.873	0.222	0.2229
AC(2:0)	0.921	0.698	0.946	0.787	0.949	0.895	0.943	0.941	0.977
AC(4:0)	0.817	0.149	0.983	0.881	1.02	0.994	0.835	0.915	0.0641
AC(8:0)	0.807	0.144	1.04	1.00	1.05	1.00	0.653	0.129	0.0455
AC(12:0)	0.542	0.0092	0.685	0.237	0.816	0.862	0.626	0.718	0.169
AC(12:1)-1	0.671	0.0156	0.743	0.233	0.885	0.808	0.781	0.915	0.232
AC(12:1)-2	0.571	0.0005	0.638	0.0073	0.690	0.135	0.623	0.354	0.491
AC(13:1)	0.791	0.249	0.832	0.649	0.971	1.00	0.772	0.904	0.367
AC(14:0)	0.646	0.0189	0.761	0.293	0.913	0.923	0.771	0.968	0.299
AC(14:1)	0.607	0.0144	0.678	0.199	0.91	0.882	0.710	0.857	0.269
AC(14:2)	0.62	0.0136	0.718	0.269	0.89	0.882	0.714	0.718	0.235
AC(16:0)	0.664	0.0310	0.795	0.286	0.836	0.784	0.769	0.815	0.538
AC(16:1)	0.719	0.0165	0.820	0.431	0.933	0.946	0.881	1.00	0.208
AC(18:0)	0.701	0.0530	0.849	0.654	0.899	0.959	0.867	0.994	0.208
AC(18:1)	0.704	0.0951	0.812	0.700	0.882	0.994	0.876	0.992	0.352
AC(20:1)	0.837	0.344	0.953	1.00	1.06	0.985	0.970	1.00	0.794

Abbreviations: H&Y = Hoehn and Yahr stage; AC = acylcarnitine; NS = not significant

^aP-value obtained using Steel's test, comparing with controls.

^bP-value obtained by Kruskal -Wallis test, comparing H&Y stages I-IV.

Supplementary Table S3. Correlation analysis between long-chain acylcarnitines and age or BMI.

	Age				Body mass index			
	1 st cohort		2 nd cohort		1 st cohort		2 nd cohort	
Compound name	r ^a	<i>P</i> -value ^b	r ^a	<i>P</i> -value ^b	r ^a	<i>P</i> -value ^b	r ^a	<i>P</i> -value ^b
AC(12:0)	0.203	0.0346	0.133	0.110	-0.0512	0.608	0.0276	0.742
AC(12:1)	0.230	0.0160	-	-	-0.112	0.254	-	-
AC(12:1)-1	-	-	0.186	0.0250	-	-	-0.0170	0.839
AC(12:1)-2	-	-	0.00	-	-	-	0.00	-
AC(14:0)	0.219	0.0221	0.129	0.121	-0.0849	0.388	0.0272	0.746
AC(14:1)	0.141	0.144	0.167	0.0452	-0.102	0.297	-0.0394	0.638
AC(14:2)	0.226	0.0182	0.154	0.0648	-0.855	0.385	-0.0452	0.589
AC(16:0)	0.220	0.0215	0.106	0.204	-0.123	0.207	0.0680	0.417
AC(16:1)	0.263	0.00570	0.266	0.00120	-0.132	0.177	-0.0435	0.603

Abbreviations: BMI = body mass index; AC = acylcarnitine; r = correlation coefficient

^{a, b} Correlation coefficient and *P*-value obtained by the analysis of covariance.

Supplementary Table S4. Correlation analysis with MANOVA between long-chain acylcarnitines and age

	Age (normalized by H&Y)				Age (normalized by UPDRS-III)			
	1 st cohort		2 nd cohort		1 st cohort		2 nd cohort	
Compound name	<i>F</i> -value ^a	<i>P</i> -value ^b	<i>F</i> -value ^a	<i>P</i> -value ^b	<i>F</i> -value ^a	<i>P</i> -value ^b	<i>F</i> -value ^a	<i>P</i> -value ^b
AC(12:0)	0.0735	0.394	0.0371	0.401	0.0461	0.0919	0.0184	0.274
AC(12:1)	0.118	0.116		-	0.0560	0.0548	-	-
AC(12:1)-1	-	-	0.0554	0.181		-	0.0359	0.0818
AC(12:1)-2	-	-	0.00	1.00		-	0.00	1.00
AC(14:0)	0.0779	0.354	0.0526	0.206	0.0546	0.0599	0.0299	0.124
AC(14:1)	0.0612	0.523	0.0601	0.146	0.0205	0.341	0.0336	0.0958
AC(14:2)	0.115	0.127	0.0608	0.141	0.0556	0.0570	0.0294	0.127
AC(16:0)	0.107	0.160	0.0288	0.552	0.0530	0.0660	0.0138	0.378
AC(16:1)	0.115	0.126	0.0946	0.0263	0.0747	0.0220	0.0795	0.00440

Abbreviations: MANOVA = multiple analysis of variance; UPDRS-III = Unified Parkinson's Disease Rating Scale motor section; AC = acylcarnitine

^{a, b} *F*-value and *P*-value obtained by the multiple analysis of covariance.

Supplementary Table S5. Correlation analysis between long-chain acylcarnitines and UPDRS-III or levodopa equivalent dose.

	UPDRS-III			
	1 st cohort		2 nd cohort	
Compound name	r ^a	<i>P</i> -value ^b	r ^a	<i>P</i> -value ^b
AC(12:0)	-0.0207	0.831	0.0711	0.395
AC(12:1)	-0.150	0.523	-	-
AC(12:1)-1	-	-	0.0588	0.482
AC(12:1)-2	-	-	0.00950	0.910
AC(14:0)	0.0140	0.885	0.129	0.122
AC(14:1)	0.0347	0.720	0.138	0.0991
AC(14:2)	0.0618	0.720	0.122	0.142
AC(16:0)	-0.0196	0.840	0.0838	0.316
AC(16:1)	0.0109	0.911	0.131	0.117

Abbreviations: UPDRS-III = Unified Parkinson's Disease Rating Scale motor section; AC = acylcarnitine; r = correlation coefficient

^a, ^bCorrelation coefficient and *P*-value obtained by analysis of covariance.

Supplementary Table S6. Correlation analysis between long-chain acylcarnitines and levodopa dose or levodopa equivalent dose.

	Levodopa dose				Levodopa equivalent dose			
	1 st cohort		2 nd cohort		1 st cohort		2 nd cohort	
Compound name	r ^a	P-value ^b	r ^a	P-value ^b	r ^a	P-value ^b	r ^a	P-value ^b
AC(12:0)	-0.112	0.215	0.0814	0.330	0.0621	0.521	0.107	0.201
AC(12:1)	-0.163	0.0910	-	-	0.0864	0.372	-	-
AC(12:1)-1	-	-	0.0910	0.276	-	-	0.0515	0.538
AC(12:1)-2	-	-	0.00	-	-	-	0.0598	0.475
AC(14:0)	-0.162	0.0922	0.228	0.00580	0.106	0.272	0.145	0.0817
AC(14:1)	-0.182	0.0588	0.158	0.0580	0.0347	0.712	0.101	0.229
AC(14:2)	-0.146	0.129	0.111	0.182	0.107	0.268	0.0729	0.383
AC(16:0)	-0.115	0.233	0.167	0.0451	0.131	0.174	0.121	0.149
AC(16:1)	-0.210	0.0284	0.190	0.0218	0.106	0.275	0.110	0.187

Abbreviations: UPDRS-III = Unified Parkinson's Disease Rating Scale motor section; AC = acylcarnitine; r = correlation coefficient

^{a, b}Correlation coefficient and *P*-value obtained by analysis of covariance.

Supplementary Table S7. Characteristics of controls, *de novo* PD, and PD with medication in the 2nd cohort.

	Control	<i>de novo</i> PD	PD with medication	<i>P</i> -value ^a	<i>P</i> -value ^c		
					Control VS <i>de novo</i> PD	Control VS PD with medication	<i>de novo</i> PD VS PD with medication
Number	45	8	137	-	-	-	-
Gender (Male:Female)	23:22	4:4	66:71	0.942 ^b	-	-	-
Age [years], Mean (SD)	63.8 (15.3)	64.6 (11.2)	67.6 (10.1)	-	0.996	0.648	0.780
Duration [years], Mean (SD)	-	1.50 (0.756)	7.37 (5.60)	<0.0001	-	-	-
H&Y stage, Mean (SD)	-	1.63 (0.916)	2.12 (0.892)	0.121	-	-	-
UPDRS III, Mean (SD)	-	12.5 (10.5)	15.0 (9.82)	0.384	-	-	-
MMSE, Mean (SD)	28.9 (2.09)	29.0 (1.69)	28.4 (1.76)	-	0.877	0.0194	0.488
BMI [kg/m ²]	23.2 (3.51)	22.4 (2.54)	22.4 (3.34)	-	0.980	0.745	0.223

Abbreviation: PD = Parkinson's disease; H&Y stage = Hoehn and Yahr stage; UPDRS-III = Unified Parkinson's Disease Rating Scale motor section; MMSE = Mini Mental State Examination; BMI = Body Mass Index

^a*P*-value obtained by analysis of covariance comparing *de novo* PD and PD with medication.

^b*P*-value obtained by chi-squared test.

^c*P*-value obtained by the Steel-Dwass test.

Supplementary Table S8. Lists of acylcarnitines and fatty acids in *de novo* PD and PD with medication.

Compound name	<i>de novo</i> PD/Control		PD with medication/Control	
	Mean	<i>P</i> -value ^a	Mean	<i>P</i> -value ^a
Carnitine	0.995	0.997	0.946	0.072
AC(2:0), O-Acetylcarnitine	1.03	0.616	0.935	0.255
AC(4:0), Butyrylcarnitine	0.898	0.841	1.16	0.149
AC(4:0), Isobutyrylcarnitine	0.795	0.532	0.943	0.444
AC(8:0), Octanoylcarnitine	0.84	0.616	0.963	0.554
AC(12:0)	0.411	0.0366	0.690	0.0463
AC(12:1)-1	0.421	0.313	0.782	0.573
AC(12:1)-2	0.445	0.011	0.643	0.0007
AC(13:1)	0.694	0.452	0.863	0.304
AC(14:0)	0.497	0.0366	0.782	0.0783
AC(14:1)	0.503	0.416	0.733	0.0537
AC(14:2)	0.534	0.0966	0.747	0.0519
AC(15:0)-1	0.228	0.582	0.296	<0.0001
AC(15:0)-2	0.0926	0.0913	0.244	<0.0001
AC(16:0), Palmitoylcarnitine	0.567	0.0966	0.779	0.0568
AC(16:1)	0.588	0.0768	0.838	0.117
AC(18:0)	0.664	0.209	0.829	0.174
AC(18:1)	0.595	0.190	0.815	0.179
AC(18:2)-1	0.703	0.516	0.842	0.272
AC(18:2)-2	0.698	0.972	1.61	0.916
AC(18:2)-3	0.897	0.532	2.436	<0.0001
AC(20:1)	0.929	0.916	0.953	0.717
FA(12:0)	0.882	1.00	1.39	0.007
FA(14:0), Myristic acid	0.904	0.992	1.24	0.537
FA(14:1)-1	0.748	0.749	1.18	0.506
FA(14:1)-2	0.774	0.980	0.976	0.826
FA(14:3)	0.0174	0.0069	1.04	0.0121

FA(15:0), Pentadecanoic acid	1.02	0.421	1.08	0.0625
FA(16:0), Palmitic acid	1.10	0.437	1.12	0.190
FA(16:1), Palmitoleic acid	0.879	0.947	1.25	0.249
FA(17:0), Heptadecanoic acid	1.04	0.765	1.04	0.708
FA(17:0)	1.01	0.868	1.01	0.984
FA(17:1)	0.837	0.684	1.02	0.979
FA(18:0), Stearic acid	1.10	0.285	1.05	0.464
FA(18:1), Oleic acid	1.08	0.532	1.15	0.324
FA(18:1), Ricinoleic acid	0.588	0.005	0.955	0.0714
FA(18:2), Linoleic acid	1.28	0.199	1.13	0.289
FA(18:3), Linolenic acid	1.20	0.273	1.23	0.245
FA(18:4), Stearidonic acid	0.925	0.650	0.924	0.837
FA(19:1)	0.997	0.955	1.00	0.957
FA(20:1), cis-11-Eicosenoic acid,	1.41	0.199	1.20	0.217
FA(20:2), cis-11,14-Eicosadienoic acid	1.06	0.684	1.05	0.958
FA(20:3), cis-8,11,14-Eicosatrienoic acid	0.913	0.955	0.956	0.611
FA(20:3)	0.790	0.781	1.066	0.959
FA(20:4), Arachidonic acid	1.02	0.781	0.956	0.492
FA(20:5), cis-5,8,11,14,17-Eicosapentaenoic acid	1.49	0.392	0.971	0.999
FA(22:1), Erucic acid	0.334	1.00	0.400	0.335
FA(22:4)	0.865	0.977	1.08	1.00
FA(22:5)-1	0.970	0.927	0.974	0.567
FA(22:5)-2	1.18	0.363	1.05	0.982
FA(22:6), cis-4,7,10,13,16,19-Docosahexaenoic acid	1.13	0.437	1.06	0.926
FA(24:1), Nervonic acid	1.09	0.273	1.05	0.172
FA(24:5)	0.919	0.947	0.959	0.804
NEFA	1.08	0.933	1.07	0.669

Abbreviations: PD = Parkinson's disease; AC = acylcarnitine; FA = fatty acid; NEFA = non-esterified fatty acid

^aP-value obtained by Steel's test.

Supplementary Table S9. Comparison of 7 long-chain acylcarnitines in PD with/without motor fluctuations.

Comparative Analysis				
Compound	PD with motor fluctuations/PD without motor fluctuations			
	1 st cohort		2 nd cohort	
	Ratio	<i>P</i> -value ^a	Ratio	<i>P</i> -value ^a
AC(12:0)	0.952	0.825	1.11	0.613
AC(12:1)	0.867	0.248		
AC(12:1)-1	-	-	1.22	0.211
AC(12:1)-2	-	-	1.00	-
AC(14:0)	0.854	0.557	1.05	0.552
AC(14:1)	0.926	0.439	1.39	0.0800
AC(14:2)	0.892	0.658	1.17	0.448
AC(16:0)	0.943	0.978	1.18	0.149
AC(16:1)	0.839	0.279	1.51	0.00650

Abbreviations: PD = Parkinson's disease; AC = acylcarnitine

^a*P*-value obtained by Wilcoxon's test between PD with and without motor fluctuations.

Supplementary Table S10. Comparison of long-chain acylcarnitines in each H&Y stage of PD with or without motor fluctuations

	PD without motor fluctuations (N)	PD with motor fluctuations (N)	Comparative Analysis																		
			PD with motor fluctuations/PD without motor fluctuations																		
			AC(12:0)		AC(12:1)		AC(12:1)-1		AC(12:1)-2		AC(14:0)		AC(14:1)		AC(14:2)		AC(16:0)		AC(16:1)		
			P-		P-		P-		P-		P-		P-		P-		P-		P-		
			Ratio	value ^a	Ratio	value ^a	Ratio	value ^a	Ratio	value ^a	Ratio	value ^a	Ratio	value ^a	Ratio	value ^a	Ratio	value ^a	Ratio	value ^a	
1 st cohort	I	16	10	1.99	0.848	1.87	0.219	-	-	-	-	1.34	0.886	1.65	0.789	1.99	0.763	1.27	0.895	1.33	0.859
	II	26	26	0.923	0.817	0.719	0.237	-	-	-	-	0.950	0.902	0.786	0.261	0.794	0.484	0.967	0.862	0.812	0.483
	III	8	13	0.443	0.913	0.530	0.364	-	-	-	-	0.379	0.446	0.605	0.561	0.467	0.385	0.676	0.971	0.531	0.538
	IV	5	4	0.955	1.00	0.885	0.793	-	-	-	-	0.919	0.901	0.973	0.893	0.991	0.903	0.785	1.00	0.820	0.903
2 nd cohort	I	31	10	1.29	0.323	-	-	1.89	0.0748	1.00	-	0.969	0.61	1.44	0.937	1.43	0.229	1.01	0.963	1.66	0.191
	II	33	27	0.834	0.216	-	-	0.780	0.179	1.00	-	0.968	0.388	0.885	0.683	0.731	0.0891	1.05	0.858	0.948	0.876
	III	11	24	1.53	0.232	-	-	1.56	0.184	1.00	-	1.21	0.242	2.20	0.0441	1.69	0.173	1.41	0.213	2.03	0.0405
	IV	1	7	1.21	-	-	-	1.30	-	1.00	-	1.00	-	4.17	-	1.39	-	1.02	-	2.63	-

Abbreviations: PD = Parkinson’s disease; AC = acylcarnitine

^aP-value obtained by Wilcoxon’s test between PD with and without motor fluctuations.

Supplementary Table S11. Area under the curve of each long-chain acylcarnitine in both cohorts.

Compound name	Area under the curve value	
	1 st cohort	2 nd cohort
AC(12:0)	0.802	0.612
AC(12:1)	0.764	0.617
AC(12:1)-1	-	0.685
AC(12:1)-2	-	0.685
AC(13:1)	0.779	0.571
AC(14:0)	0.783	0.601
AC(14:1)	-	0.618
AC(14:2)	0.745	0.605
AC(14:3)	0.575	-
AC(15:0)	0.509	-
AC(15:0)-1	-	0.721
AC(15:0)-2	-	0.765
AC(16:0)	0.805	0.607
AC(16:1)	0.757	0.590
AC(16:2)	0.641	-
AC(18:0)	0.756	0.587
AC(18:1)	0.769	0.584
AC(18:2)	0.791	-
AC(18:2)-1	-	0.574
AC(18:2)-2	-	0.507
AC(18:2)-3	-	0.713
AC(20:1)	0.670	0.522

Abbreviation: AC = acylcarnitine

Supplementary Table S12. List of amino acids and fatty acids detected in each cohort.

Comparative Analysis				
Amino Acid	PD/Control			
	1 st cohort		2 nd cohort	
	Ratio	<i>P</i> -value ^a	Ratio	<i>P</i> -value ^a
Leu	0.998	0.918	0.973	0.254
Ile	0.984	0.949	0.962	0.180
Val	0.980	0.532	0.948	0.0759
Gly	1.10	0.0311	1.04	0.299
Arg	1.08	0.059	0.953	0.137
Gln	1.06	0.0074	1.03	0.232
Phe	1.03	0.516	0.990	0.166
His	1.02	0.832	0.946	0.0027
Pro	0.99	0.491	0.994	0.582
Tyr	0.986	0.168	0.982	0.319
Lys	0.986	0.626	0.911	0.0005
Ser	0.982	0.708	0.951	0.120
Asn	0.970	0.164	0.959	0.0403
Trp	0.948	0.318	0.875	<0.0001
Asp	0.903	0.129	0.982	0.823
Thr	0.938	0.268	0.952	0.127
Met	0.936	0.249	0.935	0.0867
Ala	0.920	0.0392	0.921	0.0169
Glu	0.876	0.148	0.970	0.806
FA(12:0)	1.21	0.39	1.36	0.0055
FA(14:0), Myristic acid	1.14	0.193	1.22	0.0351
FA(14:1)-1	0.989	0.898	1.15	0.375
FA(14:1)-2	1.0896539	0.958	0.965	0.812
FA(14:3)	2.60	<0.0001	0.983	0.003
FA(15:0), Pentadecanoic acid	-	-	1.08	0.0294

FA(16:0), Palmitic acid	1.08	0.389	1.12	0.0908
FA(16:1), Palmitoleic acid	1.13	0.291	1.23	0.17
FA(17:0)	1.09	0.321	1.02	0.838
FA(17:0), Heptadecanoic acid	1.15	0.051	1.04	0.443
FA(17:1)	1.11	0.348	1.01	0.792
FA(18:0), Stearic acid	1.10	0.138	1.06	0.226
FA(18:1), Oleic acid	1.12	0.259	1.14	0.165
FA(18:1), Ricinoleic acid	-	-	0.934	0.0184
FA(18:2), Linoleic acid	1.20	0.146	1.14	0.126
FA(18:3), Linolenic acid	1.20	0.223	1.23	0.11
FA(18:4), Stearidonic acid	-	-	0.924	0.553
FA(19:1)	1.09	0.249	1.00	0.835
FA(20:1), cis-11-Eicosenoic acid,	1.29	0.0418	1.21	0.0926
FA(20:2), cis-11,14-Eicosadienoic acid	0.946	0.293	1.05	0.738
FA(20:3)	0.842	0.883	1.05	0.754
FA(20:3), cis-8,11,14-Eicosatrienoic acid	0.946	0.99	0.954	0.392
FA(20:4), Arachidonic acid	0.947	0.939	0.959	0.36
FA(20:5), cis-5,8,11,14,17-Eicosapentaenoic acid	1.40	0.242	0.999	0.916
FA(22:4)	0.849	0.885	1.07	1.00
FA(22:5)	1.13	0.703	-	-
FA(22:6), cis-4,7,10,13,16,19-Docosahexaenoic acid	1.14	0.511	1.07	0.642

Abbreviations: PD = Parkinson's Disease; FA = fatty acid

^aP-value obtained by Wilcoxon's test, comparing between PD and controls.

Supplementary Table S13. Multiple comparisons of the long-chain fatty acids in each H&Y stage of both cohorts.

Comparative Analysis																		
Compound name	1 st cohort									2 nd cohort								
	H&Y I/Control		H&Y II/Control		H&Y III/Control		H&Y IV/Control		P-value ^b	H&Y I/Control		H&Y II/Control		H&Y III/Control		H&Y IV/Control		P-value ^b
	N=41		N=60		N=35		N=8			N=41		N=60		N=35		N=8		
	Ratio	P-value ^a	Ratio	P-value ^a	Ratio	P-value ^a	Ratio	P-value ^a		Ratio	P-value ^a	Ratio	P-value ^a	Ratio	P-value ^a	Ratio	P-value ^a	
	FA(12:0)	1.31	0.859	1.19	0.978	0.983	1.00	1.35	0.913	0.199	0.958	0.926	1.59	0.0275	1.38	0.0332	1.67	0.102
FA(14:0), Myristic acid	1.19	0.517	1.12	0.734	1.02	1.00	1.25	0.938	0.202	1.11	0.913	1.31	0.137	1.14	0.51	1.45	0.115	0.1198
FA(14:1)-1	-	-	-	-	-	-	-	-	-	1.01	1.00	1.23	0.635	1.00	1.00	1.50	0.180	0.227
FA(14:1)-2	-	-	-	-	-	-	-	-	-	0.984	0.980	0.973	1.00	0.926	0.996	0.837	1.00	0.716
FA(14:3)	2.73	<0.0001	2.42	<0.0001	2.97	<0.0001	2.52	0.0005	<0.0001	1.13	0.0318	1.04	0.166	0.754	0.0318	0.941	0.701	0.0539
FA(15:0), Pentadecanoic acid	-	-	-	-	-	-	-	-	-	1.03	0.780	1.10	0.101	1.08	0.429	1.13	0.593	0.266
FA(16:0), Palmitic acid	1.09	0.802	1.08	0.801	0.997	1.00	1.14	1.00	0.455	1.13	0.751	1.15	0.445	1.03	0.877	1.19	0.311	0.377
FA(16:1), Palmitoleic acid	1.18	0.665	1.09	0.986	1.08	0.992	1.32	0.538	0.223	1.18	0.973	1.30	0.467	1.06	1.00	1.58	0.136	0.166
FA(17:0), Heptadecanoic acid	1.15	0.409	1.16	0.191	1.11	0.703	1.06	1.00	0.286	1.01	1.00	1.06	0.837	0.988	1.00	1.13	0.718	0.720
FA(17:0)	1.14	0.830	1.10	0.822	1.05	0.983	0.936	1.00	0.832	1.02	1.00	1.01	0.991	0.978	1.00	1.16	0.752	0.921
FA(17:1)	1.12	0.839	1.13	0.838	1.02	1.00	1.11	0.999	0.542	0.992	0.817	1.07	0.990	0.890	0.857	1.14	0.784	0.479
FA(18:0), Stearic acid	1.12	0.539	1.12	0.312	1.05	0.998	0.983	0.994	0.335	1.05	0.865	1.06	0.890	1.02	0.992	1.21	0.190	0.336
FA(18:1), Oleic acid	1.15	0.772	1.11	0.838	1.07	0.995	1.20	0.982	0.341	1.12	0.987	1.19	0.458	1.03	0.982	1.36	0.190	0.281
FA(18:1), Ricinoleic acid	-	-	-	-	-	-	-	-	-	0.922	0.0240	0.959	0.631	0.814	0.0563	1.40	0.830	0.0268

FA(18:2), Linoleic acid	1.19	0.653	1.19	0.777	1.13	0.888	1.36	0.720	0.380	1.10	0.938	1.21	0.522	1.05	0.836	1.17	0.370	0.397
FA(18:3), Linolenic acid	1.15	0.821	1.19	0.982	1.10	0.919	1.51	0.367	0.352	1.13	0.956	1.33	0.658	1.13	0.574	1.32	0.0955	0.258
FA(18:4), Stearidonic acid	-	-	-	-	-	-	-	-	-	0.856	1.00	0.900	0.997	0.900	0.967	1.33	0.385	0.314
FA(19:1)	1.13	0.573	1.06	0.833	1.05	0.995	1.12	1.00	0.579	0.993	0.932	1.02	0.999	0.941	0.882	1.18	0.575	0.533
FA(20:1), cis-11-Eicosenoic acid	1.25	0.495	1.23	0.367	1.29	0.383	1.44	0.783	0.173	1.16	0.801	1.16	0.709	1.21	0.759	1.65	0.0405	0.081
FA(20:2), cis-11,14-Eicosadienoic acid	1.06	0.741	1.06	0.771	0.987	0.992	0.971	0.989	0.351	1.09	1.00	1.08	0.981	0.939	0.994	1.10	0.784	0.702
FA(20:3), cis-8,11,14-Eicosatrienoic acid	0.963	0.987	0.964	1.00	0.880	0.927	0.880	0.888	0.470	0.988	0.999	0.977	1.00	0.867	1.693	0.961	0.370	0.864
FA(20:3)	0.754	0.998	0.883	0.984	0.808	0.997	0.857	1.00	0.363	1.14	0.953	1.12	0.971	0.785	0.771	1.28	1.00	0.221
FA(20:4), Arachidonic acid	0.930	1.00	0.971	0.999	0.851	0.933	0.990	1.00	0.673	1.02	0.956	0.972	0.855	0.830	0.752	1.09	0.941	0.579
FA(20:5), cis-5,8,11,14,17-Eicosapentaenoic acid	1.24	0.986	1.53	0.65	0.992	0.993	1.59	0.902	0.717	1.10	0.999	0.958	1.00	0.889	1.00	1.16	0.857	0.731
FA(22:4)	0.863	0.927	0.850	0.999	0.797	0.968	0.828	0.902	0.323	1.23	1.00	1.05	1.00	0.847	0.962	1.28	0.469	0.274
FA(22:5)	1.04	0.990	1.18	0.989	0.951	1.00	1.17	0.957	0.920	1.19	0.909	1.01	0.932	0.953	0.952	1.09	0.990	0.494
FA(22:6), cis-4,7,10,13,16,19-Docosahexaenoic acid	1.06	1.00	1.20	0.949	1.01	0.968	1.05	0.999	0.927	1.10	1.00	1.02	1.00	1.06	0.985	1.14	0.956	0.799
FA(24:1), Nervonic acid	1.29	0.471	1.29	0.111	1.66	0.0222	1.34	0.399	0.0326	0.994	0.978	1.03	0.578	1.07	0.482	1.31	0.0122	0.0182
NEFA	-	-	-	-	-	-	-	-	-	1.06	0.999	1.07	0.962	1.05	0.988	1.16	0.675	0.795

Abbreviations: H&Y = Hoehn and Yahr stage; FA = fatty acid; NEFA = non-esterified fatty acid

^aP-value obtained by Steel's test, comparing with controls.

^bP-value obtained by Kruskal-Wallis test, comparing between H&Y stages I-IV.