Supplementary figures and legends to

High glucosylceramides and low anandamide contribute to sensory loss and pain in Parkinson's Disease

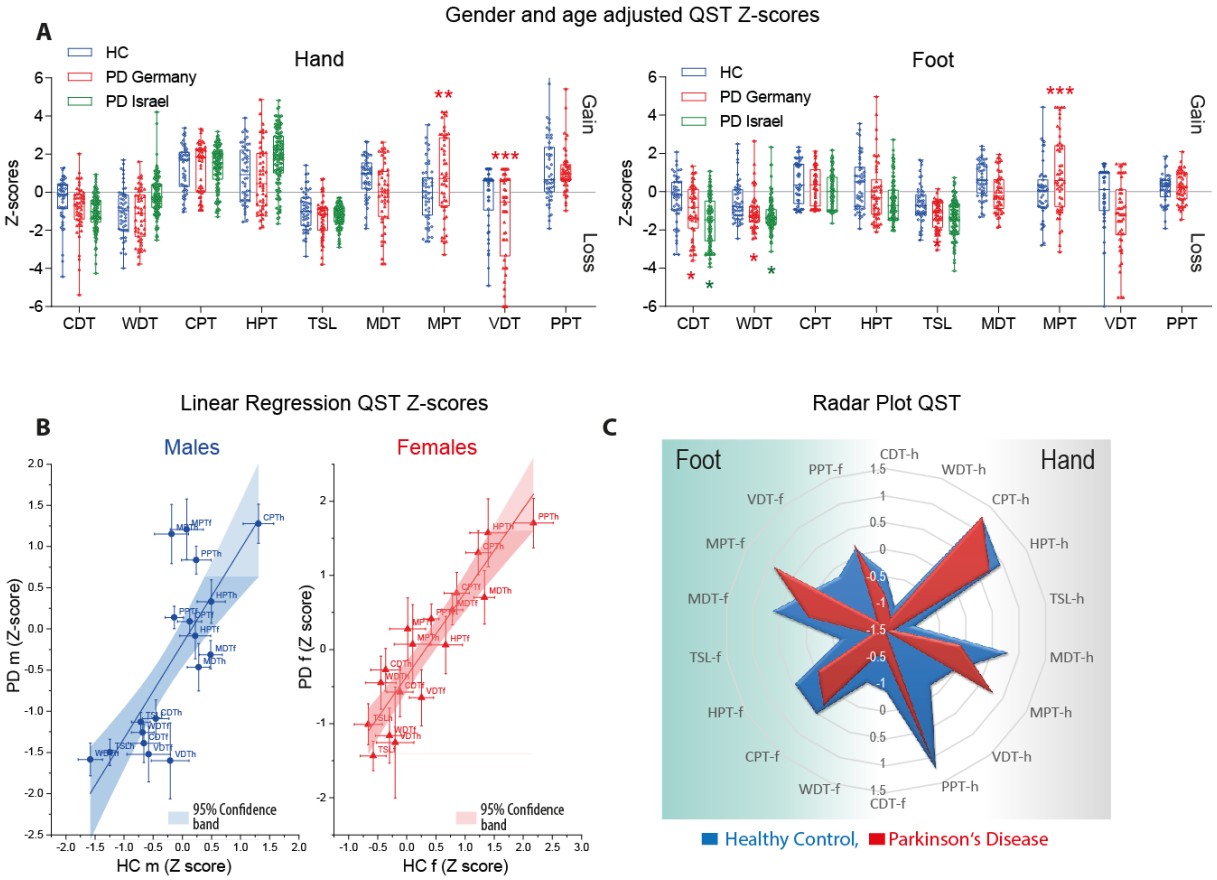
Katharina Klatt-Schreiner[1], Lucie Valek, PhD[1], Jun-Suk Kang, MD[2], Alexander Khlebtovsky, MD[3,7], Sandra Trautmann, PhD[1], Lisa Hahnefeld[1], Yannick Schreiber, BSc[5], Robert Gurke, PhD[1], Dominique Thomas, PhD[1], Annett Wilken-Schmitz[1], Sabine Wicker, MD[4], Georg Auburger, MD[2], Gerd Geisslinger, MD, PhD,[1,5,6], Jörn Lötsch, MD, PhD[1,5,6], Waltraud Pfeilschifter, MD[2], Ruth Djaldetti, MD[3,7], Irmgard Tegeder, MD[1]

[1]Institute of Clinical Pharmacology, Goethe-University, Medical Faculty, Frankfurt, Germany
[2]Department of Neurology, Goethe University Hospital Frankfurt, Germany
[3]Department of Neurology, Rabin Medical Center, Petach Tiqva, Israel
[4]Occupational Health Service, Goethe-University Hospital Frankfurt, Germany
[5]Fraunhofer Institute for Molecular Biology and Applied Ecology, Branch Translational Medicine, Frankfurt, Germany
[6]Fraunhofer Cluster of Excellence for immune mediated diseases (CIMD)
[7]Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv Israel.

Abstract

BACKGROUND Parkinson's disease (PD) causes chronic pain in two-third of patients, in part originating from sensory neuropathies. METHODS The present observational study assessed associations of quantitative sensory tests (QST), pain ratings and questionnaires with plasma levels of multiple bioactive lipid species using untargeted and targeted lipidomic analyses. The study comprised two sets of patients and healthy controls (HC): the first 128 Israeli PD and 224 German young HC for exploration, the second 50/50 German PD and matched HC for deeper analyses. RESULTS The data show a 70% prevalence of PD pain and sensory neuropathies with a predominant phenotype of thermal sensory loss plus mechanical hypersensitivity. Multivariate analyses of lipids revealed major differences between PD and HC, mainly originating from glucosylceramides and endocannabinoids. GlcCer's were increased, whereas anandamide and lysophosphatidic acid LPA20:4 were reduced, stronger in patients with ongoing pain and with a linear relationship with pain intensity and sensory losses, particularly for GlcCer24:1 and GlcCer18:1. CONCLUSIONS GlcCer metabolism is mediated by glucocerebrosidase, GBA1, whose mutations are associated with PD and aggravate the toxicity of mutant synuclein. Our data suggest that PD-associated sensory neuropathies and PD-pain are in part caused by accumulations of glucosylceramides, raising the intriguing possibility to reduce PD pain and sensory loss by GBA1 refolding treatments.

Suppl. Fig. 1



CDT, WDT: Cold and warm detection threshold
CPT, HPT: Cold and heat pain threshold
MDT, MP: Mechanical detection and pain threshold
VDT:Vibration detection threshold
PPT: Pressure pain threshold
TSL: Thermal sensory limen

Suppl. Figure 1

Quantitative sensory tests (QST) in PD patients and healthy controls – additional analyses

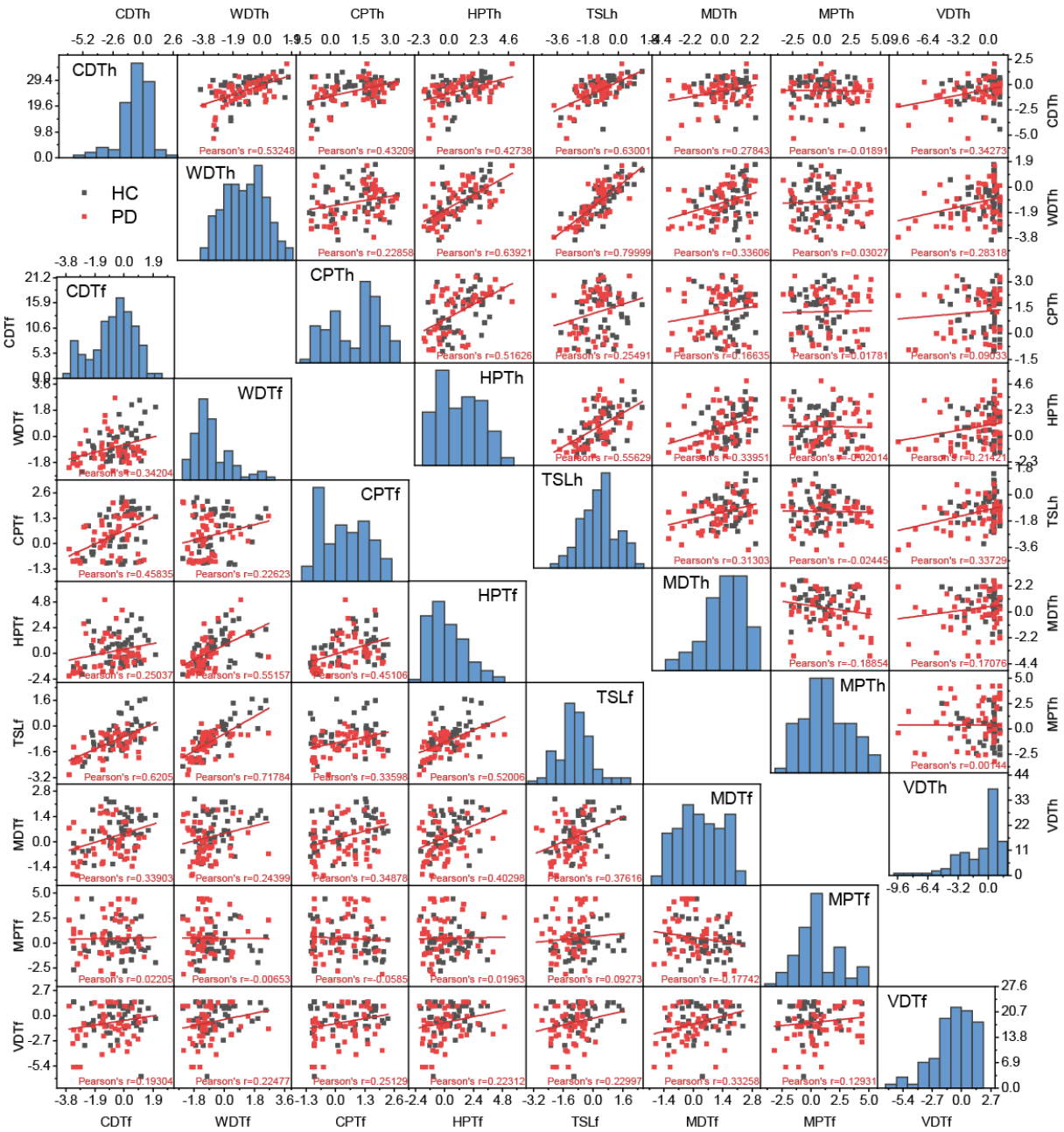
A: Box/scatter plots of the age and gender adjusted QST z-scores of 50 healthy controls (HC), 50 PD patients from Germany and 128 PD patients from Israel. QST data were normalized for age class and gender according to QST reference data. The box shows the interquartile range, the line is the median, the whisker show the 10-90% percentile and the dots are individual subjects. Z-scores were submitted to 2-way ANOVA for "QST-parameter" X "group" for hand and foot separately. Groups were subsequently compared with t-tests for each QST parameter using an adjustment of alpha according to Šidák. Asterisks indicate significant differences between groups (adjusted P * < 0.05, *** < 0.001).

B: Linear regression analysis of QST z-scores of 50 healthy controls (HC) and 50 PD patients from Germany. The dots show the group mean, the whisker show the SD, the line is the regression line, the color shaded area shows the 95% CI. Dots outside of the 95% CI band reveal significant differences between PD and healthy controls.

C: Radar plot showing the group means of QST z-scores in 50 healthy controls (HC) and 50 PD patients from Germany. The only parameter, which shows a gain-of-function (more pain) is the mechanical pain threshold for hand and feet (MPTh, MPTf), but mostly the shrinkage of the resulting asterisk reveals the sensory loss in PD.

Suppl. Fig. 2

Multiscatter with linear regression of QST parameters



Suppl. Figure 2

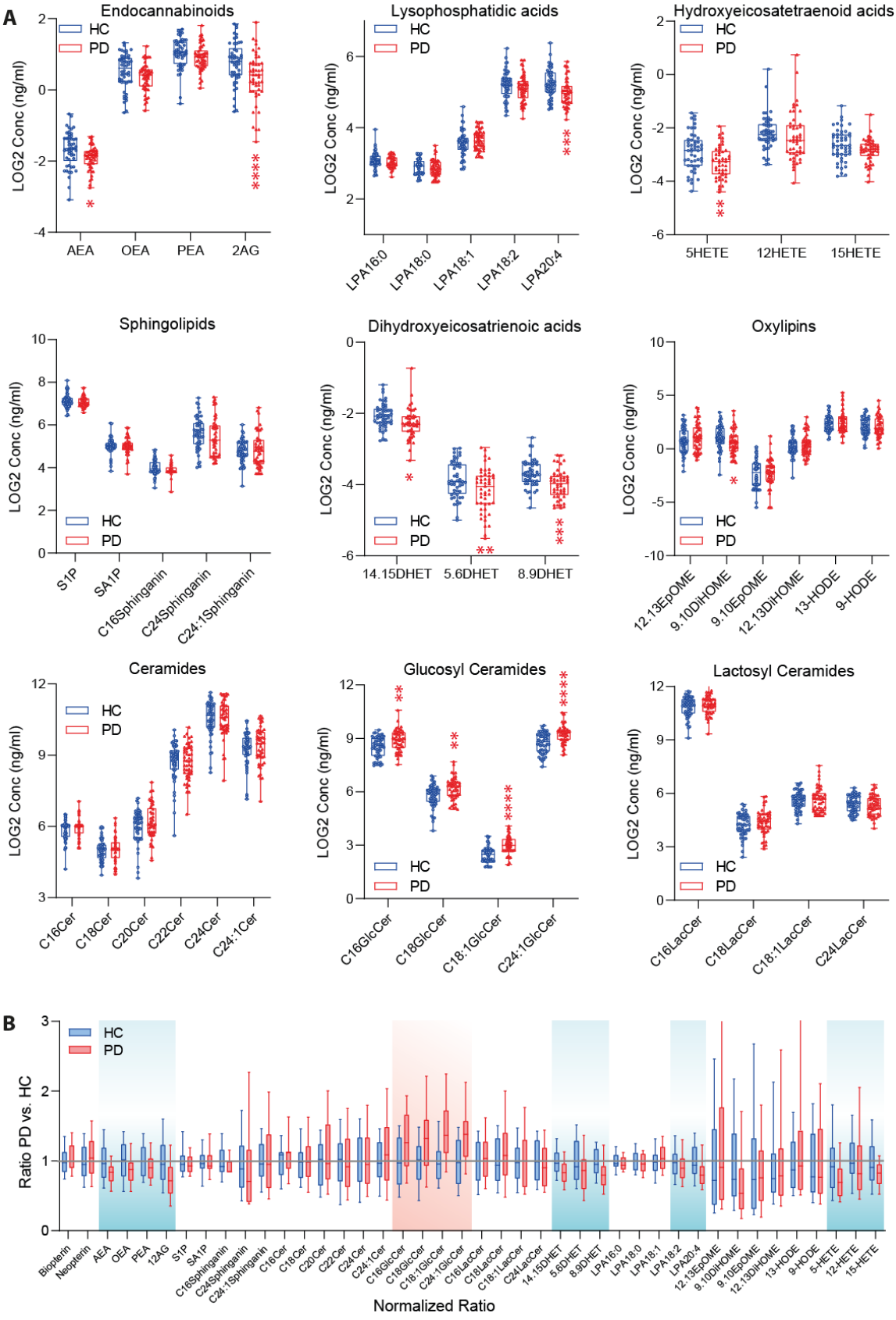
QST z-scores scatter matrix with linear regression analysis

The scatter matrix shows the linear associations of QST parameters for hand and feet. The dots show individual subjects (50 healthy controls, HC in grey and 50 PD patients in red). The middle panels show the frequency distribution, which mostly fit to a Gauss distribution or sum of two Gauss curves (CPT).

Thermal QST parameters were linearly associated with each other in both healthy controls and PD patients. In line with previous studies {Lotsch, 2015 #25906}, CPT frequency distributions were biphasic in both groups.

Suppl. Fig. 3

Targeted Lipids Frankfurt PD patients versus age-matched Healthy Controls



Suppl. Figure 3

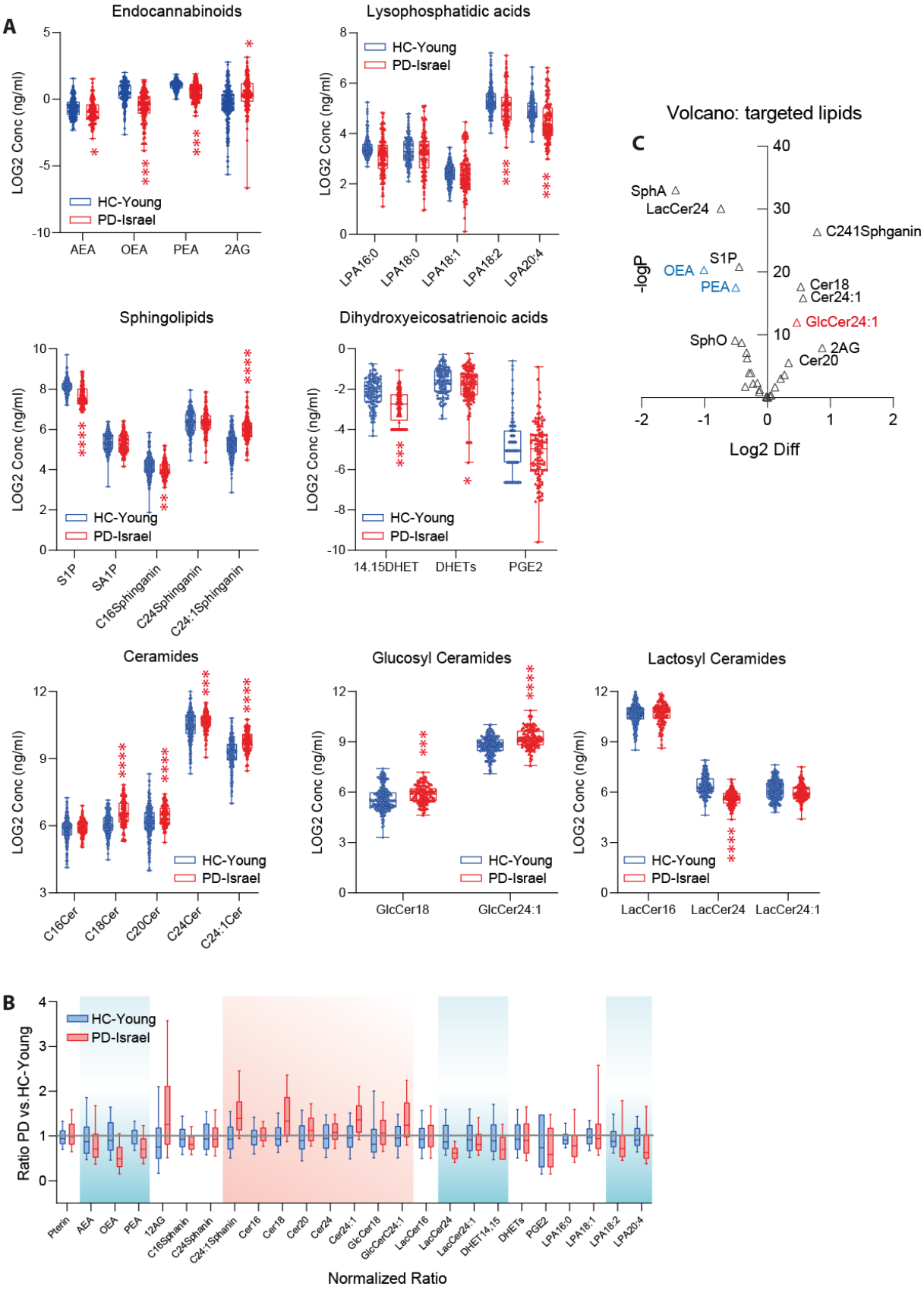
Plasma concentrations of lipid species in German PD patients and age matched healthy controls

A: Box/scatter plots of plasma lipids of different classes, analyzed with targeted LC-MS/MS. The box shows the interquartile range, the line is the median, the whiskers show the 95% CI and the dots are individual results of 50 healthy controls (HC) and 50 PD patients. Concentrations were submitted to 2-way ANOVA for "lipid" X "group" for each class of lipids. Groups were subsequently compared per t-tests using an adjustment of alpha according to Šidák. Asterisks indicate significant differences between groups. Adjusted P value: * < 0.05, ** < 0.01, *** < 0.0001, **** P < 0.0001.

B: Ratios of lipid concentrations versus the mean of healthy controls set to 1. To allow for multivariate comparisons of lipids of different classes, concentrations (ng/ml) were normalized to the mean of healthy controls. The blue and red shaded areas show lipids, which were reduced or increased in PD, respectively.

Suppl. Fig. 4

Targeted Lipids Israeli PD patients versus Young Healthy Controls



Suppl. Figure 4

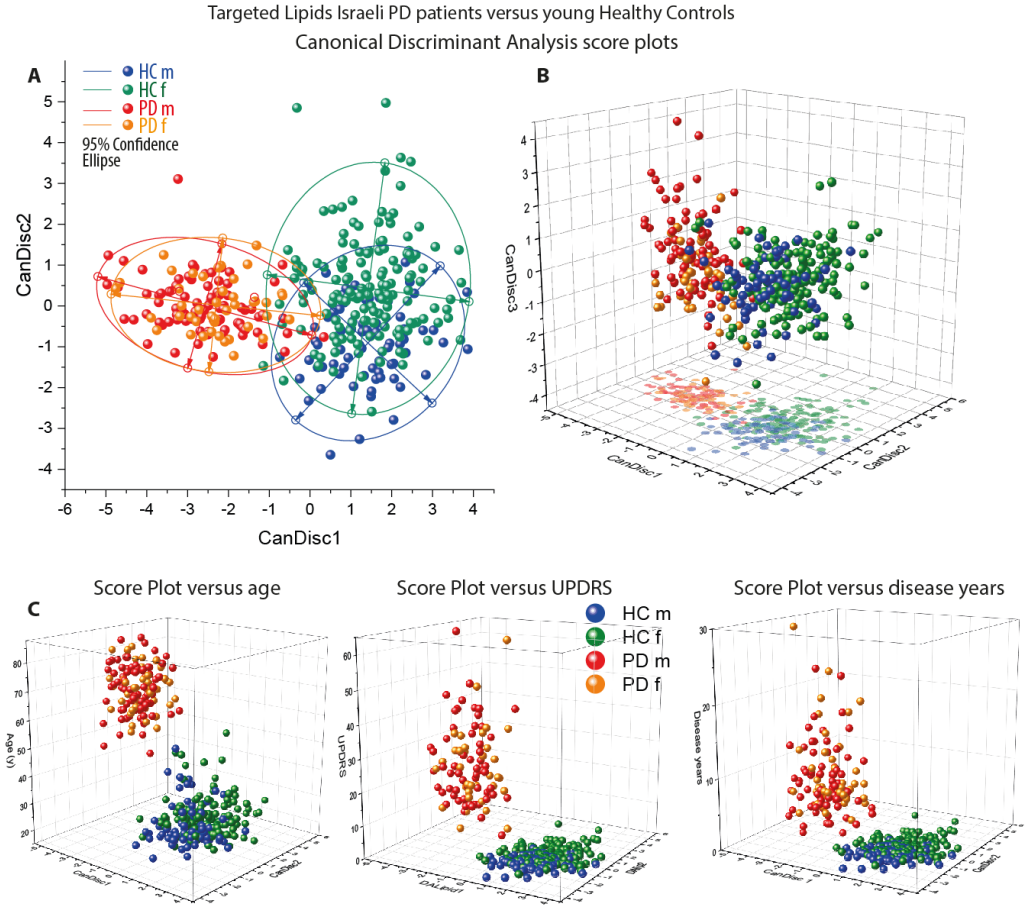
Plasma concentrations of lipid species in Israeli PD patients versus young controls

A: Box/scatter plots of plasma lipids of different classes, analyzed with targeted LC-MS/MS. The box shows the interquartile range, the line is the median, the whiskers show the 95% CI and the dots are individual results of 224 young healthy controls and 128 PD patients. Concentrations were submitted to 2-way ANOVA for "lipid" X "group" for each class of lipids. Groups were subsequently compared with t-tests for using an adjustment of alpha according to Šidák. Asterisks indicate significant differences between groups. Adjusted P value: * < 0.05, ** < 0.01, *** < 0.0001, **** P < 0.0001.

B: Ratios of lipid concentrations versus the mean of healthy controls set to 1. To allow for multivariate comparisons of lipids of different classes, concentrations (ng/ml) were normalized to the mean of healthy controls. The blue and red shaded areas show lipids, which were reduced or increased in PD, respectively.

C: Volcano plots showing the log2 difference of lipid concentrations (x-axis) versus the negative log10 of the P-value (y-axis) comparing 128 PD patients versus 224 young healthy controls. Lipids reduced or increased in PD appear on the left or right side of the y-axis, respectively. A -logP >2 was considered significant.

Suppl. Fig. 5



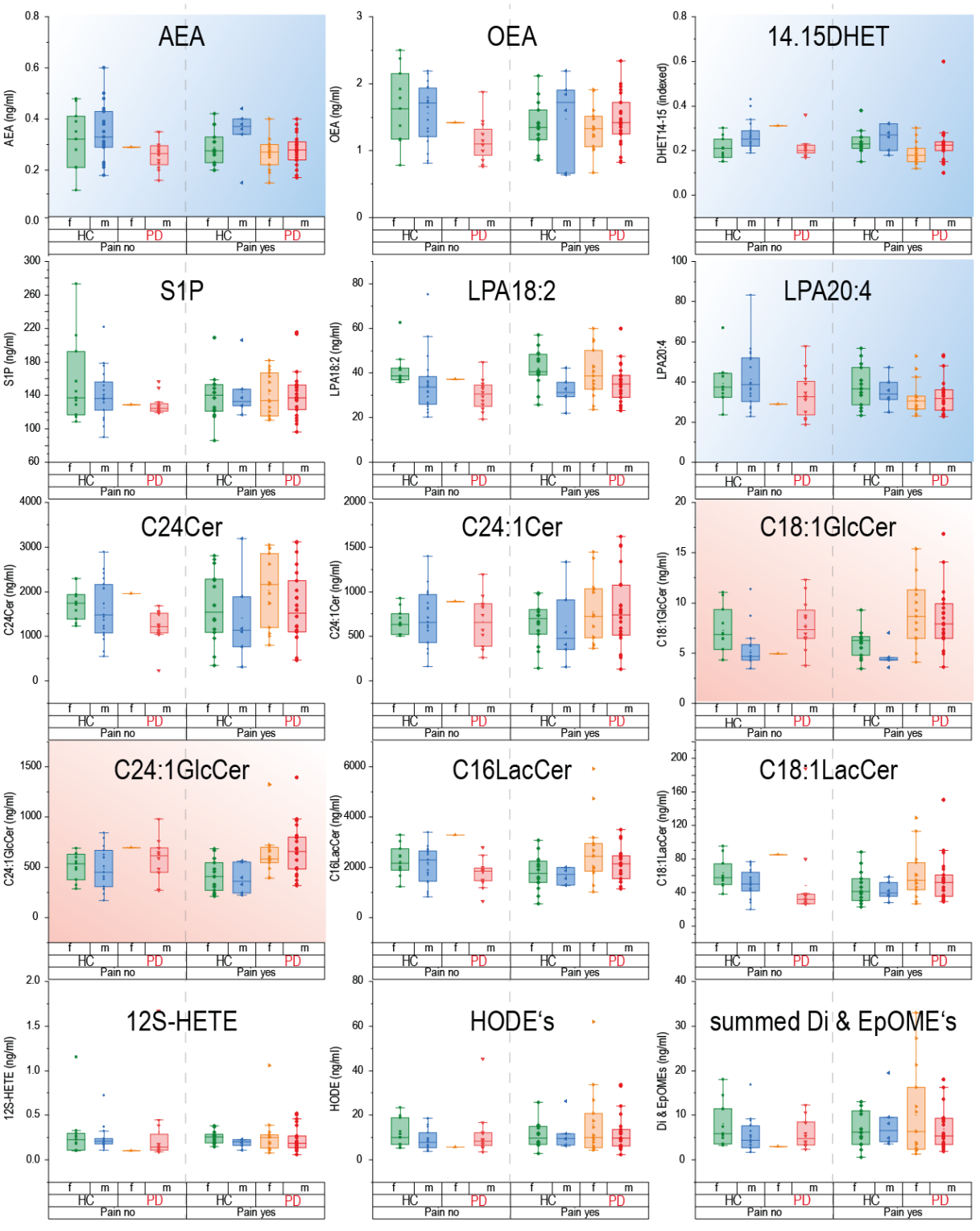
Suppl. Figure 5

Canonical Discriminant analysis of lipids in Israeli PD patients versus young healthy controls

A, B: Canonical discriminant analysis 2D and 3D score plots with 95% CI ellipsoid extracted from 29 targeted lipids. The dots represent 224 healthy controls (72 male, 152 female) and 128 Israeli PD patients (85 male, 42 female). Normalized lipid ratios shown in Suppl. Fig. 3B were used as input.

C: 3D scatter plots of the discriminant factors CanDisc1 and CanDisc2 (x-axis and z-axis) versus age, UPDRS and number of disease years, each on the y-axis.

Suppl. Fig. 6



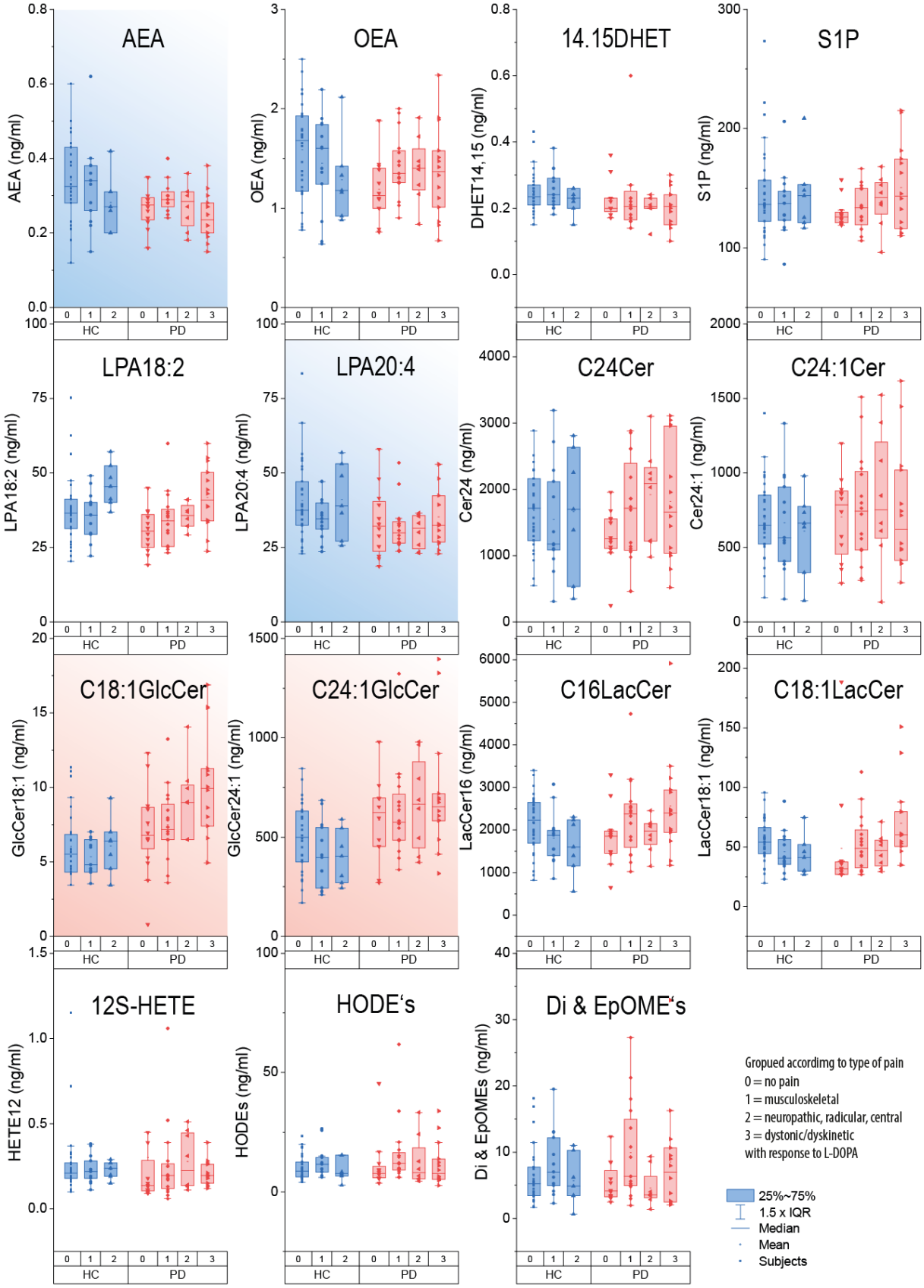
Pain yes/no binomial discrimination based on BPI and VAS rating

Suppl. Figure 6

Grouped box/scatter plots of the most prominent lipids according to gender and pain

Subjects were categorized as having or not having pain based on Brief Pain Inventory and VAS ratings. The boxes show the interquartile range, the line is the median, the whisker show 1.5 x IQR, the dots are individual results of 50 healthy controls (25m, 25f) and 50 PD patients (34m, 16f). Lipid concentrations were submitted to 2-way ANOVA. Graphs showing lipids, which were significantly reduced in PD are highlighted in blue, increased lipids in red.

Suppl. Figure 7



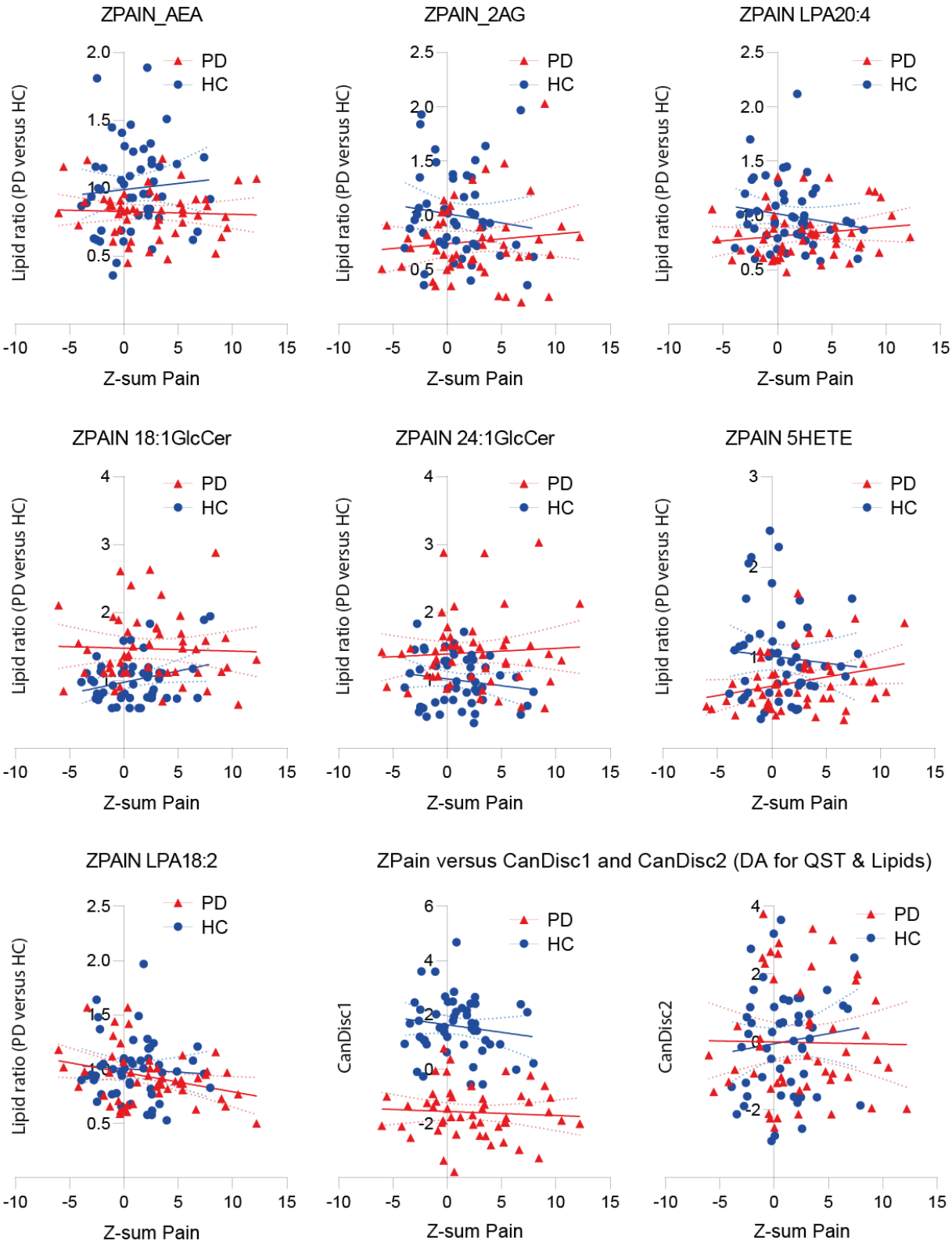
Grouped according to type of pain

Suppl. Figure 7

Grouped box/scatter plots of plasma lipids according to yes/no pain and pain type

Subjects were categorized as having no pain, musculoskeletal pain, neuropathic pain or dyskinetic pain the latter defined as pain responding to L-Dopa on the basis of the Brief Pain Inventory and VAS ratings. The boxes show the interquartile range, the line is the median, the whisker show 1.5 x IQR, the dots are individual results of 50 healthy controls and 50 PD patients. Graphs showing lipids, which were significantly reduced in PD are highlighted in blue, increased lipids in red.

Suppl. Fig. 8



Z-sum PAIN = HPTh + MPTh + MPTf

Suppl. Figure 8

Scatter plots and linear regression analyses of QST-pain versus plasma lipids in 50 PD patients and 50 healthy controls

The QST z-score representing pain was calculated as the sum of the QST scores for HPT-hand, MPT-hand and MPT-foot, and was plotted versus the lipid ratios (ratio versus the mean of HC) for candidate lipids, which showed the strongest regulation in PD patients. The bottom last two graphs shows the summed z-score versus the discriminant scores of multivariate analysis. The line shows the linear regression line, the dotted lines show the 95% CI. There was no significant linear association of QST-pain versus any of the lipids.