RESEARCH ARTICLE

High Glucosylceramides and Low Anandamide Contribute to Sensory Loss and Pain in Parkinson's Disease

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ABSTRACT: Background: Parkinson's disease (PD) causes chronic pain in two-thirds of patients, in part originating from sensory neuropathies. The aim of the present study was to describe the phenotype of PD-associated sensory neuropathy and to evaluate its associations with lipid allostasis, the latter motivated by recent genetic studies associating mutations of glucocerebrosidase with PD onset and severity. Glucocerebrosidase catalyzes the metabolism of glucosylceramides.

Methods: We used quantitative sensory tests, pain ratings, and questionnaires and analyzed plasma levels of multiple bioactive lipid species using targeted lipidomic analyses. The study comprised 2 sets of patients and healthy controls: the first 128 Israeli PD patients and 224 young German healthy controls for exploration, the second 50/50 German PD patients and matched healthy controls for deeper analyses.

Results: The data showed 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 patients and healthy controls, mainly originating from glucosylceramides and endocannabinoids. Glucosylceramides were increased, whereas anandamide and lysophosphatidic acid 20:4 were reduced, stronger in patients with ongoing pain and with a linear relationship with pain intensity and sensory losses, particularly for glucosylceramide 18:1 and glucosylceramide 24:1.

Conclusions: Our data suggest that PD-associated sensory neuropathies and PD pain are in part caused by accumulations of glucosylceramides, raising the intriguing possibility of reducing PD pain and sensory loss by glucocerebrosidase substituting or refolding approaches.

Key Words: endocannabinoids; lipidomic analysis; pain; quantitative sensory testing; sensory neuropathy

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Parkinson's disease (PD) is a chronic progressive neurodegenerative disorder with a predominant loss of dopaminergic neurons in the midbrain, resulting essentially in motor dysfunctions. However, PD also causes a number nonmotor and premotor phenomena including autonomic, sensory, mental, and psychological symptoms.[1-3] Pain is the most common and primary nonmotor manifestation.[4] It manifests as musculoskeletal, dyskinetic, dystonic, radicular, or central neuropathic and visceral pain,[5,6] and about two-thirds of PD patients experience pain related to the disease,[4,7,8] some responding to antiparkinsonian medication, but others suffering from persistent pain.

Some relief is often provided by cannabis,[9-11] pointing to putative dysfunctions of the endogenous cannabinoid system,[10] supported by associations of PD pain with polymorphisms of fatty acid amide hydrolase (FAAH), a key enzyme of anandamide metabolism.[12] Importantly, cannabinergic and dopaminergic circuits regulate each other in reward-associated brain centers[13] and likely contribute to the feeling of pain relief.[14,15] Functional magnetic resonance brain imaging studies have shown alterations of inhibitory pain control in PD[16,17] and increased bold signals in parietal cortex, insula, and striatum contributing to heightened pain processing in PD.[18-22]

Above these central mechanisms, PD pain likely originates from damage of peripheral somatosensory and autonomic neurons, leading to sensory deficits but also heightened pain sensitivity[23-25] with abnormal somatosensory evoked potentials,[19,26,27] pathological quantitative sensory tests (QSTs),[8,16,28] and loss of epidermal nerve fiber density. Sensory fiber damage concurs with depositions of α-synuclein,[23-25,29] and it has been suggested that α-synuclein spreading may originate from the sensory nervous system,[25,30,31] giving rise to the intriguing idea that early diagnosis of premotor sensory loss may allow for motor-preserving, progression-slowing treatments in the future.

Above endocannabinoids, recent research has strengthened the idea of pathogenic lipid allostasis owing to the discovery of heterozygous mutations of the lipid-degrading enzyme glucocerebrosidase (GBA1) in association with early-onset and rapidly progressive sporadic PD.[32-35] Importantly, in vitro models revealed that mutant GBA1 increases the toxicity of α-synuclein.[36-38]

Given the putative therapeutic implications and the importance of the involvement of the sensory system in PD, we combined quantitative sensory testing with complex analyses of signaling lipids using targeted lipidomic analyses, including endocannabinoids, sphingolipids, ceramides, glycerophospholipids, and oxylipids to evaluate the contribution of lipid allostasis in the etiopathology of PD-associated pain and sensory loss.

Methods

Study Objectives and Design

The first and second parts of the study were done successively and included 2 separate populations of PD patients, one from Israel, where GBA1 mutations are highly prevalent[39] and one from Frankfurt, Germany, and 2 sets of healthy controls botrh recruited in Frankfurt.

The first exploratory pilot part compared plasma levels of bioactive lipids of Israeli PD patients (85 men, 43 women; mean age ± SD, 69 ± 25 years; range, 45–87 years) with 224 young healthy controls (72 men, 152 women; 28 ± 8 years; range, 18–56 years). Samples of PD patients and controls were analyzed in successive analytical sessions. QSTs for thermal parameters were performed in PD patients only. The normalization of QSTs to age and sex[40] pointed to sensory losses.

The second study used the experiences of the exploratory pilot study and addressed its shortcomings in terms of age differences, QST settings, and preanalytical procedures. The second part compared sensory functions according to standardized QSTs in an accredited QST laboratory by a certified investigator, and lipids were studied more extensively with up-to-date technology in a quality management-regulated laboratory. This part comprised 50 PD patients (34 men: 68 ± 8 years; range, 50–79 years; 16 women: 64 ± 9 years; range, 51–79 years) and 50 age-matched healthy controls (25 men: 65 ± 9 years; range, 51–79 years; 25 women: 61 ± 7 years; range, 50–79 years), who were recruited consecutively within 1 year. The sex distribution reflects a higher prevalence of PD in males. The sample size was estimated on endocannabinoid results of cohort 1 using a power of 80% and α error of 0.05. The samples were collected, preprocessed, and analyzed according to standard operating procedures. The primary statistical analyses are based on the optimized second part of the study.

The studies were approved by the respective institutional ethics committees, and all patients and controls signed an informed consent form to participate in the study. Data acquisition and blood sample collection adhered to the Declaration of Helsinki. Detailed information about patient recruitment and assessment of pain and sensory functions are provided as the supplementary file Supplementary Methods_Pain and QST. Demographic data are provided in Supplementary Tables 1A–D (Excel file).

Pain Assessment and Quantitative Sensory Testing

Patients were interviewed using a structured questionnaire. Items included demographic and clinical data including age, sex, ethnicity, disease duration and

course, comorbidities, and medication including analgesics. Patients were asked if they experienced pain and if yes, to describe the location, distribution, intensity (on a scale of 0–10, where zero is no pain and 10 is extreme pain) during rest and during movement, quality, duration (hours per day), and time of onset in relation to the motor symptoms, and first diagnosis of PD. The patients also filled the short McGill questionnaire (Israel) or the Brief Pain Inventory (Germany) for evaluation of pain intensity and the Pain Detect questionnaire (Germany) for detection of neuropathic pain.[41] Based on interview and questionnaires, pain was categorized as musculoskeletal, central, or radicular/neuropathic pain and dystonic/dyskinetic pain. Participants with 1 or more of these types were considered positive for PD-related pain.[8,12,42]

Quantitative sensory tests (QSTs)[43,44] were performed to assess somatosensory manifestations of PD including small-fiber neuropathies.[45] Subjects were instructed and tested according to standardized protocols in a comfortable position in a quiet room. During QST sessions, the skin of the dorsum of the hand or foot of the right or left side was tested. Because the first study did not reveal significant side differences, subsequent QST studies in the Frankfurt population were done on the hand and foot dorsum of the most affected side. If there was no side difference, the side was randomly chosen as in the control group. For patients with response fluctuations, QST was assessed during the ON period.

The standardized QST battery according to the German Research Network on Neuropathic Pain protocol[40] begins with the determination of thermal thresholds for perception and then for pain, followed by mechanical thresholds and vibration detection. The QST procedures can be chronologically listed as follows. Cold and warm detection thresholds (CDT, WDT), number of paradoxical heat sensations (PHSs) during testing of thermal sensory limen (TSL), cold and heat pain thresholds (CPT, HPT), mechanical detection, and pain thresholds, vibration detection threshold (VDT), and pressure pain threshold.[40,46] Owing to the age and condition of our patients, testing of dynamic mechanical allodynia and windup ratio were left out. Hence, the set consisted in each nine parameters for hand and foot. Detailed descriptions of the stimuli are provided in Supplementary Methods — Pain and QST.

Analysis of Lipid-Signaling Molecules

Blood samples were collected in K_3-ethylenediaminetetraacetic acid tubes (Microvette Sarstedt), kept on ice, and centrifuged in a tabletop centrifuge (Eppendorf) at 2000*g* and 4°C for 10 minutes within 15–20 minutes. Plasma aliquots were immediately frozen after centrifugation and kept at −80°C until analysis.

Bioactive lipids including sphingolipids and ceramides, lysophosphatidic acids, endocannabinoids, eicosanoids (oxylipids), and pterins were analyzed in plasma by liquid chromatography–electrospray ionization–tandem mass spectrometry as described in detail in reference 47. The analytical methods were optimized on the basis of methods reported in our previous studies.[48-52] Detailed analytical protocols are provided in Supplementary Analytical Protocols, which have been deposited at BioSciences with the accession S-BSST389 and are an updated version of the protocols in reference 47.

Data Analysis and Statistics

Lipid concentrations are presented as scatterplots with mean ± standard deviation (SD) or box-scatter plots, in which the box is the interquartile range, the whiskers show minimum to maximum, and the line is the median. Data were analyzed with SPSS 25, Origin Pro 2020, and GraphPad Prism 8.0. The frequency distributions of lipids were fitted according to Gauss curves, log-Gauss curves, or the sum of 2 Gauss curves, based on the best-fit values. Normality of distribution was assessed with the Shapiro-Wilk test and Anderson-Darling test and quantile-quantile plots, and variances with the Levene's test. For Volcano plot analyses, lipid concentrations were log2-transformed.

QST data were normalized to the respective age, site, and sex-dependent reference values using a reference database.[53] Thresholds are log-transformed, except for CPT and heat pain threshold (HPT), and then transformed to *z* scores. Raw QST thresholds are provided as Supplementary Table 2 (supplementary Excel file). Thresholds of patients and controls >70 years for whom reference data are not available were normalized to the reference values of the 60- to 70-year class. Volcano plots compared the *z* score differences between patient and control groups versus the $-\log P$ value of the *t* test.

For multivariate analyses, lipid data (linear concentrations) were normalized to the mean of the healthy control group because lipids of different classes differ by several orders of magnitude. The ratios were used for further analyses. Canonical discriminant analysis (CanDisc) and partial least-squares analysis were used to reduce the dimensionality and identify the factors, which discriminated the best between groups. Group (HCs versus PD patients), sex, age, body mass index (BMI), pain intensity, type of pain, and medication were considered independent factors. CanDisc was used to assess the predictability of group membership. It was performed without and with bootstrapping, the latter

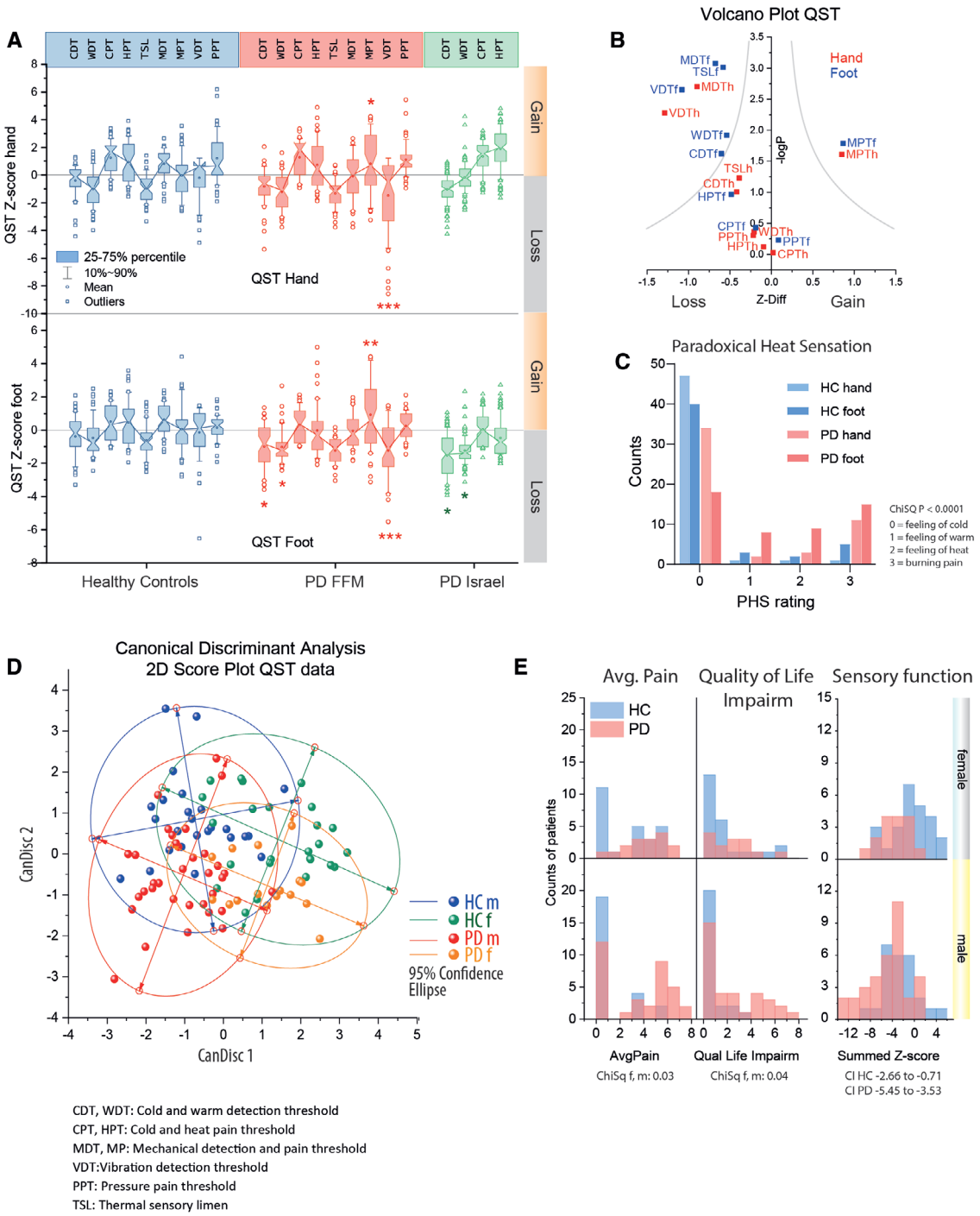

FIG. 1. Quantitative sensory tests of PD patients and healthy controls. (**A**) Notched box-plot *z* scores of QST results of 50 healthy controls, 50 PD patients from Germany, and 128 PD patients from Israel. The QST results were normalized with QST reference data for age class and sex. Non-normalized thresholds are presented in Supplementary Table 2. The notched box shows the interquartile range, the line is the median, the whiskers show the 10%–90% percentile, and the dots are outliers. *Z* scores were submitted to 2-way ANOVA for QST-parameter × group for hand and foot separately. Groups were subsequently compared per *t* tests for each QST parameter using an adjustment of alpha according to Šidák. Asterisks indicate significant differences between groups (adjusted $^{*}P < 0.05$, $^{***}P < 0.001$). (**B**) Volcano plot showing the *z*-score difference of QST data (*x* axis) versus the negative logarithm of the *P* value (*y* axis) comparing 50 PD patients with 50 healthy controls. (**C**) Frequency distribution of paradoxical heat in 50 PD patients versus 50 healthy controls showing a strong increase of paradoxical heat pain (score 3) in PD patients on cold stimulation of the foot. Frequencies were compared with chi-square statistics, $P < 0.0001$. (**D**) Canonical discriminant analysis of QST *z* scores. The 2-D scatter plot with 95% CI ellipses shows the scores (CanDisc) for the first 2 discriminant factors. The dots represent 50 healthy controls (25 male, 25 female) and 50 PD patients (34 male, 16 female) form Germany. (**E**) Frequency distribution of ratings for average pain intensity and quality of life (QLife) impairment (ordinal VAS from 0 to 10: 0 = no pain/no QLife impairment; 10 = extreme pain/extreme QLife impairment) and for sensory functions, obtained as a summed QST *z* value in 50 PD patients versus 50 healthy controls. Chi-square statistics for VAS pain and quality of life (QLife), and confidence intervals (CI) of a Gausssian fit for sensory loss using combined female and male data. [Color figure can be viewed at wileyonlinelibrary.com]

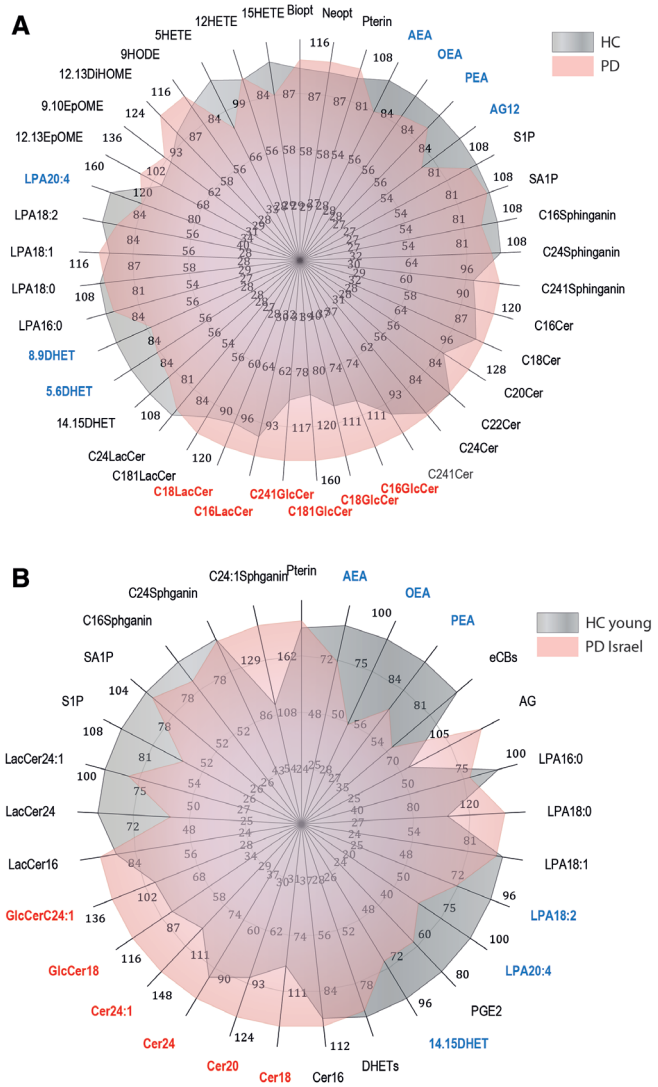

FIG. 2. Polar plots of plasma lipids. (**A**) Polar plots show the means of normalized lipids (percentages of the mean of the respective healthy control group) of targeted lipid analyses in 50 German PD patients versus 50 age matched German healthy controls (HCs). (**B**) Polar plots show the means of normalized lipids (percentages of the mean of the respective healthy control group) of 128 Israeli PD patients versus 224 young German healthy controls. Increased lipids are highlighted in red, reduced lipids in blue. [Color figure can be viewed at wileyonlinelibrary.com]

using a stratified random-sampling approach considering sex and age and 100 iterations.

Plasma concentrations were compared between groups using univariate or 2-way analyses of variance (ANOVA) or *t* tests according to the data subgroup structure and distribution. In case of violations of normality and log-normality, nonparametric tests were used (Mann-Whitney *U* or Kruskal-Wallis). Age, sex, BMI, and visual analogue scale (VAS) ratings for pain intensities (from 0 = no pain to 100 = extreme pain) were introduced as covariates, and if significant, further analyses of group differences were performed. For example, 2-way ANOVAs compared group × sex, group × pain, and group × sensory loss. In case of significant results of ANOVAs, groups were mutually compared using *t* tests, The *P* values were adjusted according to the procedures of Dunnett (versus 1 control group) or Šidák. Further analyses consisted of χ^2 statistics for nominal data and linear regression analyses. The α level was set at 0.05 for all comparisons, and asterisks in the figures refer to multiplicity-adjusted *P* values.

Data Availability Statement

All data generated or analyzed during this study are included in the article and its supplementary information files or have been deposited to BioStudies, which are available via the accession number S-BSST389.

Results

Similar Frequency of Chronic Pain in Israeli and German PD Patients

Comparisons of demographic data including age, sex, disease duration and severity, medication, analgesics, and pain ratings (Supplementary Tables 1A–D) show that key parameters are very similar in both PD populations. The average age in the German PD group was 66.8 years, and the Hoehn and Yahr scale was 2.5. Israeli patients were 69 years old and had a mean UPDRS of 25. Two-thirds of PD patients were male (34 men, 16 women German PD; 85 men, 43 women Israeli PD). Chronic PD-associated musculoskeletal, neuropathic, or dyskinetic/dystonic pain was prevalent in 74% of German and 66% of Israeli PD patients. Age-matched healthy controls with a mean age of 63 years reported chronic pain in 40%. Half the PD patients (51%) with chronic pain received analgesic medications, whereas 80% of the controls with pain got analgesics. Pain medication included opioid and nonopioid analgesics, benzodiazepines for muscle pain, pregabalin and similar antiepileptic agents, antidepressants, and medical cannabis, the latter only in Israeli patients (medications summarized in Supplementary Table 1B; Association of Medication with Lipids in Supplementary Table 4).

Sensory Loss in PD Patients

The predominant QST phenotype of PD patients was a loss of thermal cold and warm sensation and vibration detection, particularly of the feet accompanied by mechanical pain hypersensitivity, which was stronger on hands than feet (Fig. 1A,B; descriptive QST results in Supplementary Table 2). The sensory phenotype — loss of thermal sensation plus mechanical hypersensitivity — agrees with QST phenotypes of small- or mixed-fiber sensory neuropathies according

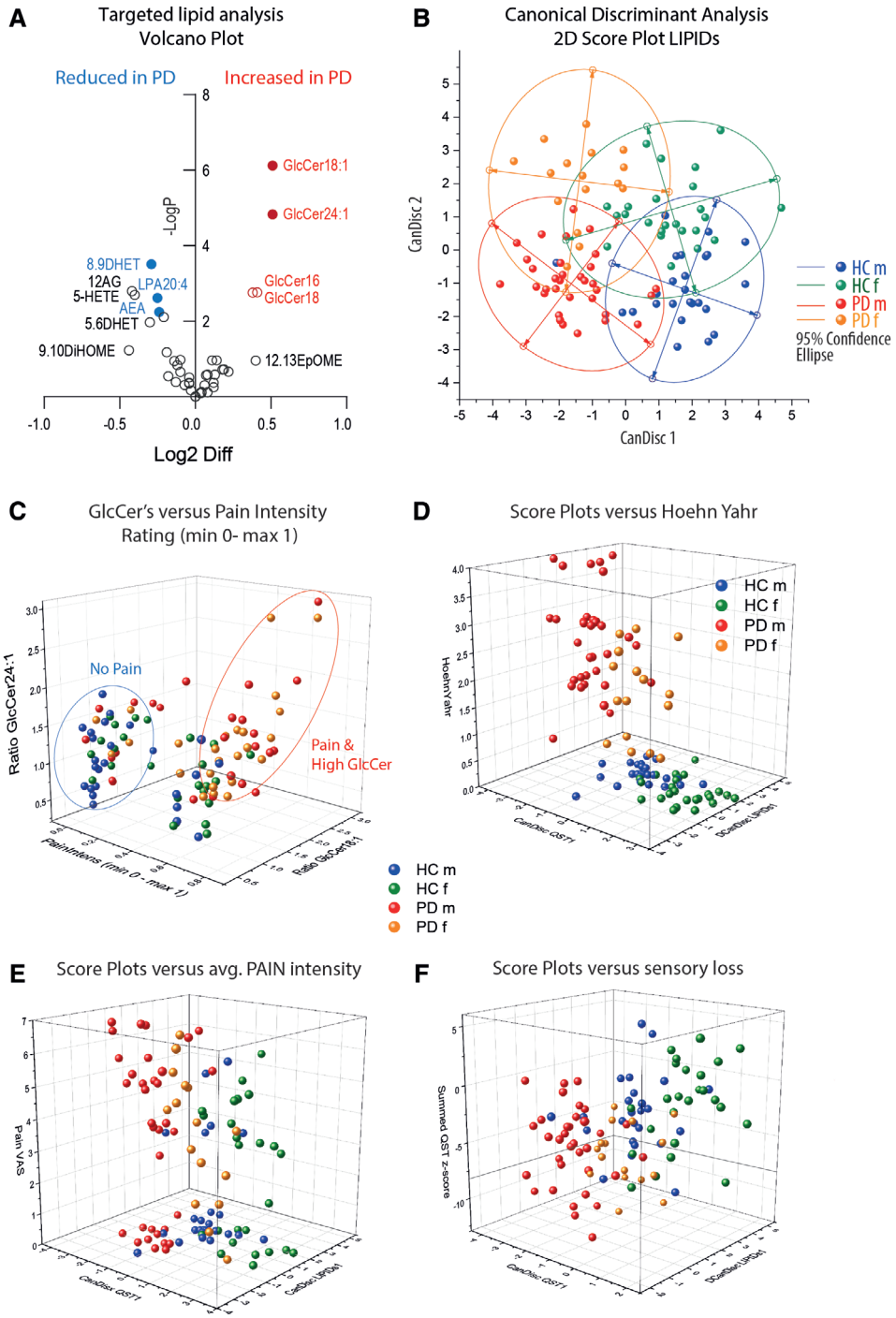

FIG. 3. Volcano plots of plasma lipids and multivariate analyses of lipids versus pains and PD scores. (**A**) Volcano plots showing the log2 difference of lipid concentrations (*x* axis) versus the negative log10 of the *P* value (*y* axis) comparing 50 PD patients versus 50 healthy controls. Lipids reduced or increased in PD appear on the left or right side of the *y* axis, respectively. A difference of $-\log P > 2$ was considered significant. (**B**) Score plot of canonical discriminant analysis using plasma lipids as input. The 2-dimensional scatterplot with 95% CI ellipse shows the scores (CanDisc) for 2 discriminant factors. The dots represent 50 healthy controls (25 male, 25 female) and 50 PD patients (34 male, 16 female) form Germany. Normalized lipid ratios were used as input. (**C**) Three-dimensional (3-D) scatterplot of pain intensity ratings (from no pain = 0 to extreme pain = 1) versus the most prominent glucosylceramides, Glc18:1 and Glc24:1, transformed to ratios versus the mean of controls. (**D–F**) 3-D scatterplots of the first discriminant factors for QST (*x* axis) and for plasma lipids (*z* axis) versus the Hoehn-Yahr score, VAS pain intensity, and sensory loss *z* score, each on the *y* axis. [Color figure can be viewed at wileyonlinelibrary.com]

to Vollert et al.[54] The loss of thermal sensation was accompanied by PHS and paradoxical heat pain on cold stimulation of the feet (Fig. 1C). Based on the QST data, canonical discriminant analyses separated PD patients from controls and men from women, however with overlapping confidence intervals

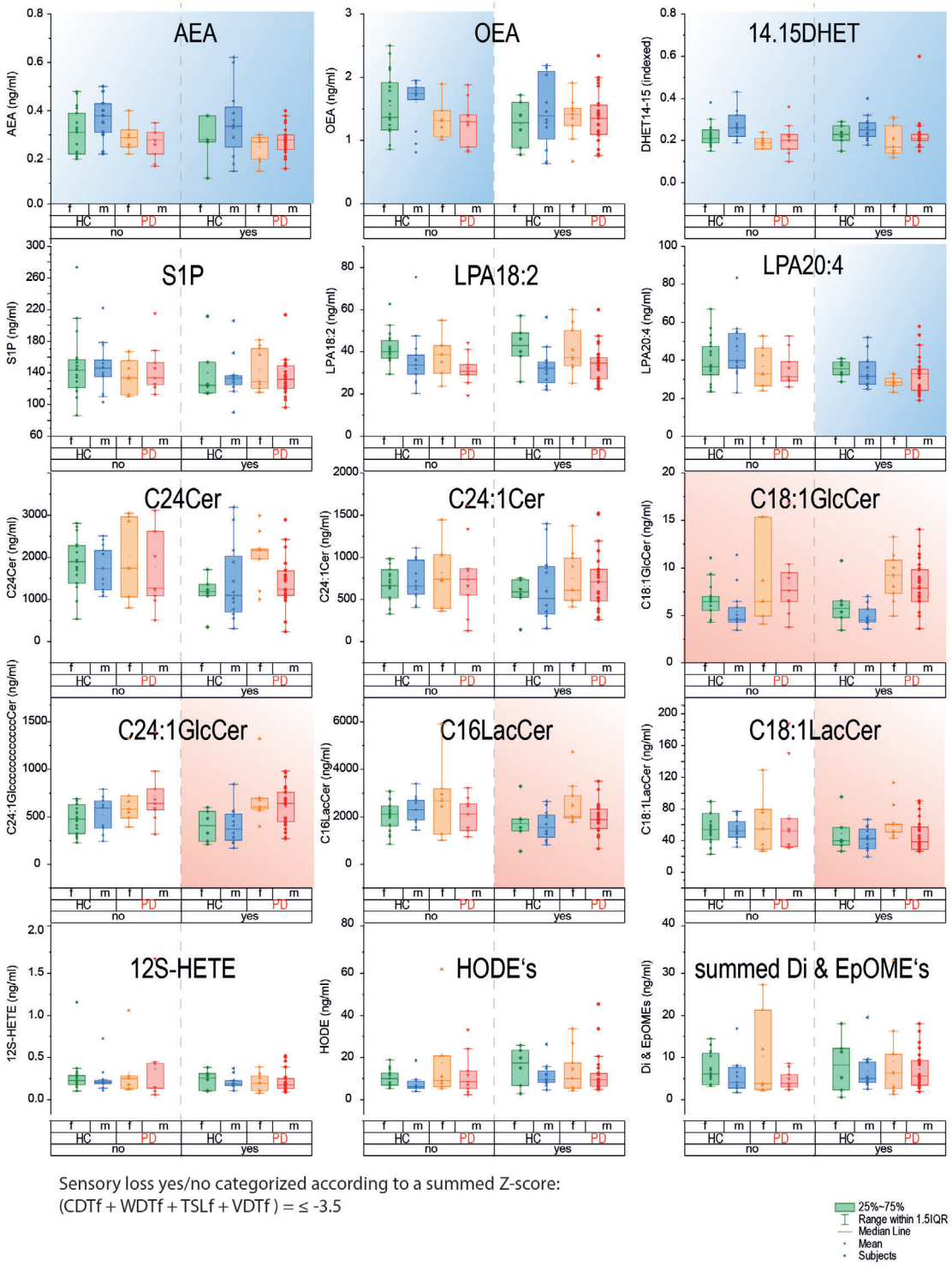

FIG. 4. Grouped box/scatterplots of plasma lipids according to sensory loss. Subjects were categorized as having or not having sensory loss based on a sum of QST *z* scores. Sensory loss was defined as CDTf + WDTf + TSLf + VDTf ≤ −3). The boxes show the interquartile range, the line is the median, the whiskers show 1.5 × IQR, the dots are individual results of 50 healthy controls (HCs) and 50 PD patients. Graphs showing lipids, which were significantly reduced in PD are highlighted in blue, increased lipids in red (2-way ANOVA group × sex; subsequent *t* test for corresponding groups, $P < 0.05$). [Color figure can be viewed at wileyonlinelibrary.com]

(Fig. 1D). A left shift of the frequency distribution of summed QST *z* scores revealed the sensory loss, and a right shift of the frequency distributions of VAS pain ratings revealed more pain, both stronger in male PD patients than in female PD patients (Fig. 1E). Further analyses of the QST data and distributions of QST *z* scores are provided in Supplementary Figures 1 and 2.

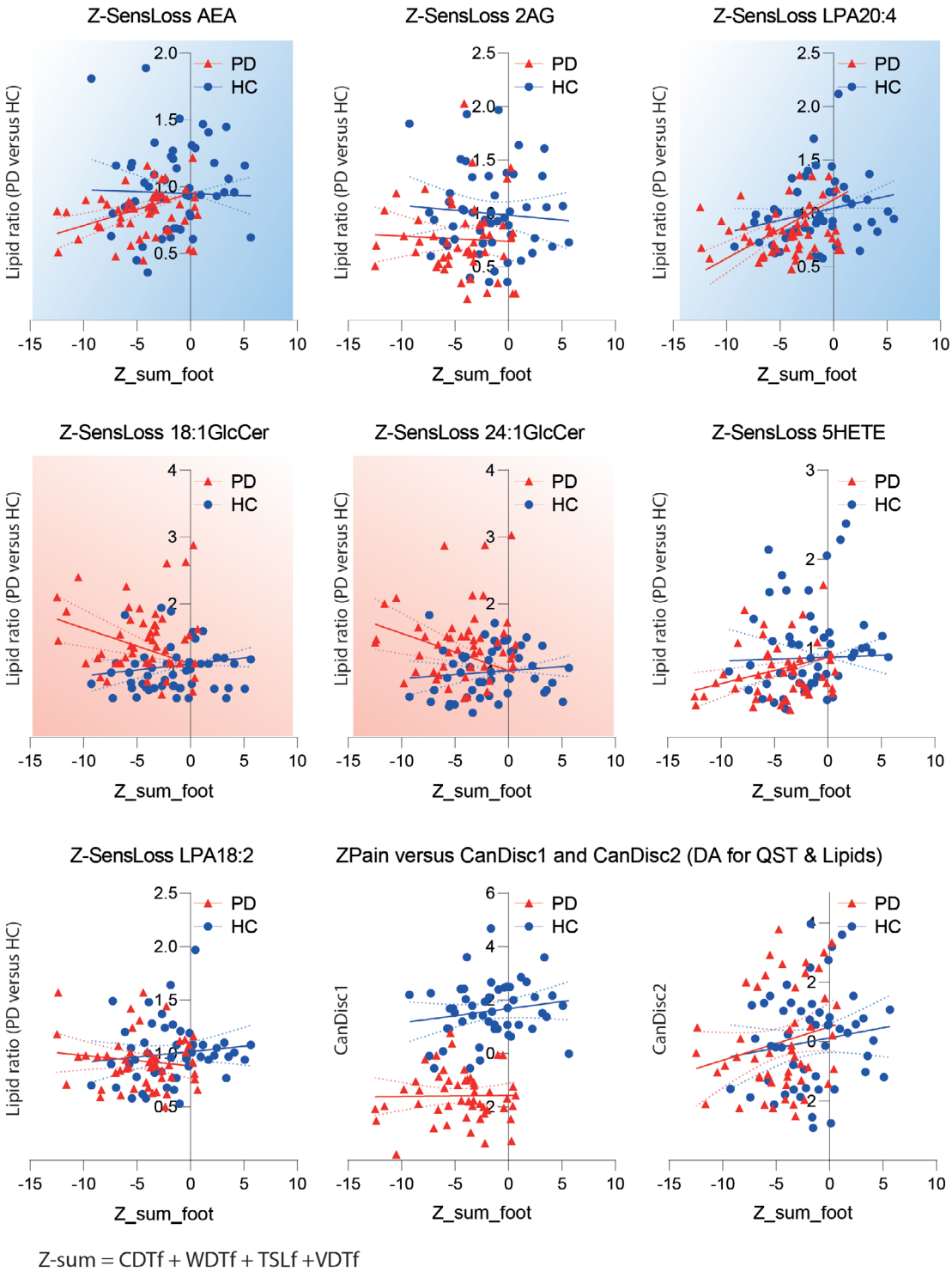

FIG. 5. Scatterplots and linear regression analyses of sensory loss versus plasma lipids. The QST *z* score representing sensory loss of the feet was calculated as the sum of the QST scores for CDT, WDT, TSL, and VDT and was plotted versus the lipid ratios (versus mean of HCs) for candidate lipids that showed the strongest regulation in PD patients. The bottom last 2 graphs show the *z* score versus the scores of canonical discriminant analysis. The line shows the linear regression line, the dotted lines show the 95% CI. A significant association of sensory loss with plasma lipid ratios was detected for the color-coded lipids, with blue indicating that low lipids were associated with strong sensory loss (the lower, the stronger the loss), red indicating the inverse (the higher the lipid, the stronger the sensory loss). Significance required that 95% CIs of the slopes of the regression lines did not overlap and that the slope in PD was significantly nonzero (F test). [Color figure can be viewed at wileyonlinelibrary.com]

Anandamide Deficiency and Glucosylceramide Accumulation in PD

Motivated by the association of PD pain with FAAH polymorphisms and by the associations of PD incidence, disease onset, and course with GBA1 mutations, we analyzed plasma lipidomic profiles, which are presented as polar plots (Fig. 2A,B, German and Israeli patients, respectively). Detailed raw and transformed data are presented as Supplementary Figures 3 and 4, and descriptive lipid statistics are shown in Supplemenetary Table 3 (Excel file). PD patients had reduced plasma levels of anandamide compared with age- and sex-matched healthy controls. All endocannabinoids tended to be reduced. In contrast, glucosylceramides (GlcCers) were strongly increased in PD patients. In addition, PD patients had low levels of polyunsaturated lysophosphatidic acids (LPA18:2, LPA20:4) and of omega oxylipids (dihydroxyeicosatrienoic acid [DHET] species). The changes of the lipid profile were consistent in both populations of PD patients (Fig. 2A,B and Supplementary Figs. 3 and 4). An additional increase in non-glycosylated ceramides was only observed in Israeli PD patients compared with young healthy controls, likely owing to raising plasma ceramide levels on aging[47] or owing to a higher prevalence of GBA1 mutations in Israel. The most robust and reproducible alterations were a loss of anandamide, loss of lysophosphatidic acid, LPA20:4 and increase of all glucosylceramides, and these candidate lipids were also revealed in Volcano plots (Fig. 3A). Canonical discriminant analysis using lipids as input clearly separated PD patients from healthy controls (Fig. 3B), and in particular, high levels of GlcCers were associated with higher pain intensity ratings (Fig. 3C). Three-dimensional (3-D) plots of CanDisc scores versus Hoehn & Yahr (Fig. 3D) or with summed QST parameters (Fig. 3E,F) suggested an association of Hoehn & Yahr with sensory loss, and of lipid allostasis with pain and with sensory loss (Fig. 3E,F). Corresponding 3-D plots of the Israeli PD population are shown in Supplementary Figure 5. Analysis of medication subgroups receiving or not receiving antiparkinsonian medication did not reveal significant effects for specific drugs or the number of different drugs (Supplementary Table 4, Excel file). Nine patients receiving 4 or 5 different drugs had particularly high GlcCer18:1 levels (significant at $P < 0.05$), associated with the highest Hoehn & Yahr scores, hence pointing to serious disease courses.

Associations of Lipid Profile Changes With Pain and Sensory Loss

To assess further the relevance of specific lipids for pain, pain type, and sensory loss, we used a binominal categorization of patients with/without pain or with/without sensory loss, the latter based on summed QST *z* scores. The observed lipid changes (GlcCer increase; anandamide (AEA), DHET, and LPA20:4 reductions) appear to be stronger in patients with sensory loss (Figs. 4 and 5), but occur in patients with and without pain, with some differences between male and female patients (Supplemental Fig. 6), and the lipid alterations tend to depend on the type of pain (Supplemental Fig. 7). Patients with neuropathic pain and dyskinetic pain had higher GlcCer and lower AEA than patients without pain or patients with musculoskeletal pain. The subgroup analysis points to a pathophysiologic role, particularly of high GlcCer and low AEA and low LPA20:4, for the development of PD-associated sensory neuropathies. This conclusion is further supported by linear regression analyses of lipid concentrations versus summed QST scores, CDTf + WDTf + TSLf + VDTf for sensory loss (Fig. 5; "f" for foot) and HPTh + MPTh + MPTf for pain (Supplementary Fig. 8; "h" for hand). Indeed, there was a linear relationship with sensory loss: the lower the AEA or LPA20:4 and the higher the GlcCer, the stronger was the sensory loss (low QST *z* score; Fig. 5). There was no such association with the summed QST score for pain (Supplementary Fig. 8).

Discussion

The encoding of noxious stimulus intensity and unpleasantness consists in sensory-discriminative, affective, and cognitive dimensions of pain, and numerous studies have investigated possible causes for pain in PD, mostly searching for alterations of central circuits. Chudler et al suggested that the basal ganglia constitute a gate for regulation of nociceptive information processing.[55] Indeed, abnormal pain processing in PD was confirmed by several electrophysiology studies measuring somatosensory-evoked potentials.[19,26-28,56] or blood-oxygen-level-dependent (BOLD) signals in functional imaging studies.[18-22] We show here that PD also causes somatosensory dysfunction, mainly loss of thermal sensation coupled with mechanical hypersensitivity, pointing to sensory PD-associated polyneuropathy, in agreement with previous reports.[27,57] The predominant QST phenotype was loss of thermal sensation with paradoxical heat pain. Somatosensory or autonomic neuropathies often cause burning and visceral pain, irrespective of sensory losses, and they contribute to muscle pain, likely including PD-associated back pain. Indeed, central muscle pain syndromes like fibromyalgia were proposed to arise from small-fiber neuropathies in subsets of patients,[58,59] supporting the view that musculoskeletal pain not only originates from rigidity but is contributed by alterations of somatosensory input from the periphery. It is presently unknown why sensory neurons including olfactory,

somatosensory, and autonomic sensory neurons are particularly vulnerable to the pathophysiology of PD, which is believed to arise from pathological protein aggregates, inefficient protein waste removal, and dysfunctional mitochondria. It is important to note that the peripheral nervous and the olfactory systems have been suggested as starting points for the spreading of α-synuclein.[25,60-62]

Our lipidomic studies reveal that QST readouts of sensory loss and pain intensity ratings are associated with low levels of endocannabinoids and elevated levels of glucosylceramides, to name the most prominent lipid derangements. These associations do not establish causality, but in combination with the known clinical benefit of cannabis[9-11,63] and the high prevalence of GBA1 mutations in PD,[34,64,65] and the reproducibility in 2 very different cohorts argue for a pathogenic role of these lipids for PD-associated sensory neuropathies and pain. Indeed, cannabinoids are neuroprotective in models of MPP + -evoked Parkinson's disease in rodents,[66] and loss of anandamide in dorsal root ganglia on sciatic nerve injury is associated with nociceptive hypersensitivity,[67,68] which also occurs in mice deficient of the cannabinoid-1 receptor specifically in somatosensory neurons.[69] The data point to a prominent function of anandamide in sensory neurons.

From a mechanistic viewpoint, it is presently unknown how high levels of glucosylceramides affect sensory neurons, but there is strong evidence that low activity of the GlcCer-metabolizing enzyme GBA1 increases the toxicity of α-synuclein.[34,38,70-74] Importantly, low GBA1 activity has been observed in PD patients carrying or not carrying GBA1 mutations,[75] suggesting that a loss of GBA1 activity is highly prevalent in PD and possibly contributes to the development or progression to PD-associated sensory neuropathy.[74] Indeed, we found similarly elevated levels of glucosylceramides in both our patient populations, although known GBA1 mutations are frequent in Jews[76,77] but rare in German PD patients. Accumulation of glucosylceramides in lysosomes may result in defects of lysosomal trafficking[78,79] membrane leaks and possibly expelling of GlcCer-laden lysosomes, which would explain high extracellular levels in plasma. We previously showed in PD mice that ceramides and glucosylceramides are strongly increased in the olfactory bulb,[80] that is, a site from which α-synuclein spreading may originate.[30,31,81,82] Pathologically high levels already occurred at the age of about 6 months when these mice were still asymptomatic in terms of PD-associated motor dysfunctions.[80] Based on the functional GBA1 evidence provided in a number of in vitro studies, it may be speculated that glucosylceramides are not only markers of but functionally relevant factors in the development and progression of PD-associated sensory neuropathies and PD-associated pain.

Our first exploratory study had some limitations in terms of ages and differences in preanalytical sample handling and the availability of deuterated analytical standards. Absolute lipid levels were therefore not all directly comparable between the Israeli and the Frankfurt PD populations. However, the major findings for the key lipid candidates and QST alterations were consistent and reproducible irrespective of the site where and the time when the patients were recruited. Hence, we concluded that anandamide loss and glucosylceramide accumulations are robust lipid manifestations of the disease. The similarity of the PD-associated QST phenomena with sensory neuropathies in metabolic diseases suggests that glucosylceramides may have a broader pathogenic role in the metabolic damage of sensory neurons, supported by studies showing a glycosphingolipid-dependent increase in insulin resistance in diabetes[83] and of obesity-evoked inflammation.[84] Importantly, both anandamide loss and glucosylceramide accumulation are theoretically amenable to available medication, anandamide via cannabis and glucosylceramides via GBA1 refolding, enzyme replacement, or substrate depletion. Hence, it is conceivable that patients with PD-associated sensory losses and pain would benefit from Gaucher-directed treatments.

In summary, we have shown that both PD patients suffering and not suffering from chronic pain have QST alterations pointing to sensory neuropathies with loss of thermal sensation and mechanical hypersensitivity, which are associated with lipid derangements, most prominently with loss of anandamide and accumulation of glucosylceramides, supporting the recent concept of a GBA1-directed treatments. ■

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Supporting Data

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