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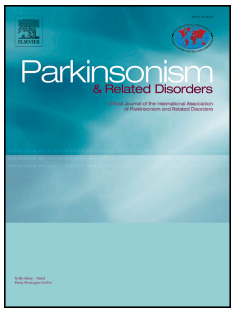

Plasma acyl-carnitines, bilirubin, tyramine and tetrahydro-21-deoxycortisol in Parkinson's disease and essential tremor. A case control biomarker study

Silvia M. Albillos, PhD, Olimpio Montero, PhD, Sara Calvo, BS, Berta Solano-Vila, MD, José M. Trejo, MD PhD, Esther Cubo, MD PhD

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Graphical TOC

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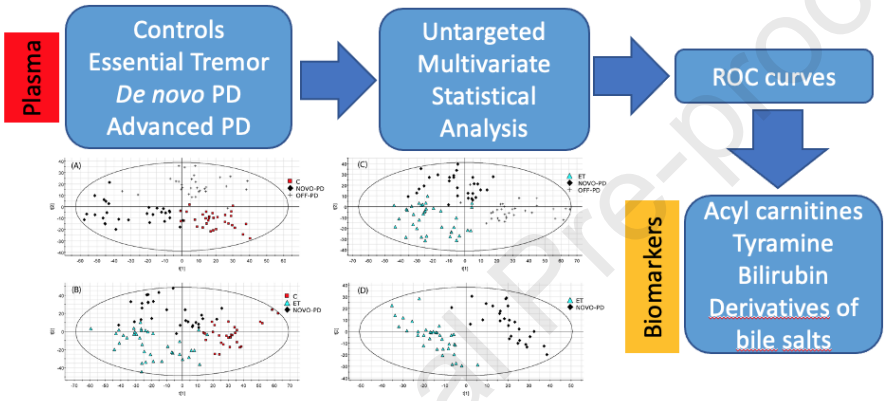
Silvia M Albillos, PhD*; Olimpio Montero, PhD; Sara Calvo, BS; Berta Solano-Vila MD; José M Trejo, MD, PhD; Esther Cubo, MD, PhD.

Summary

We performed a metabolomics study of plasma samples of patients suffering from PD and ET, as well as controls. Bilirubin, several acyl-carnitines, tyramine and some adrenal gland derived metabolites showed up as potential biomarkers to differentiate between groups. The ROC curves obtained for combined metabolites had predictive accuracy with AUC >0.8 for differentiating NOVO-PD and advanced PD from controls and ET. In multivariate regression analysis, metabolite levels could not be associated with motor and non-motor severity in PD.



Plasma acyl-carnitines, bilirubin, tyramine and tetrahydro-21-deoxycortisol in Parkinson's disease and essential tremor. A case control biomarker study.

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Availability of data
The data supporting the findings of this study are available from the corresponding author upon reasonable request.

ABSTRACT

Background and purpose: Given the overlapping clinical manifestations and pathology, the differentiation between Essential tremor (ET) and Parkinson´s disease (PD) is difficult. Our aims were to examine the plasma metabolomics profiling and their association with motor and non-motor symptoms (NMS) in patients with PD, and to determine differences between *de novo* PD compared to moderate-advanced PD *vs.* controls and patients with ET.

Methods: Plasma samples were collected from 137 subjects including 35 age matched controls, 29 NOVO-PD, 35 PD and 38 ET patients. PD severity, motor and NMS including cognitive function were assessed using the UPDRS, NMS and PD cognitive rating scales, respectively. Metabolomics analysis was performed by UPLC-ESI-QToF-MS followed by unsupervised multivariate statistics. The area under the curve of the biomarkers according to distribution of their concentrations and the diagnosis of PD (NOVO-PD, advanced PD) *vs* ET and healthy controls was used as a measurement of diagnostic ability.

Results: Several acyl-carnitines, bilirubin, tyramine and tetrahydro-21-deoxycortisol (THS) presented good predictive accuracy (AUC higher than 0.8) for differentiating *de novo* PD and advanced PD from controls and ET, suggesting an alteration in the lipid oxidation pathway. In multivariate regression analysis, metabolite levels were not significantly associated with motor and NMS severity in PD.

Conclusions: Diverse acyl-carnitines, bilirubin, tyramine and some adrenal gland derived metabolites are suggested as potential biomarkers able to distinguish between PD from controls and ET.

INTRODUCTION

The field of biomarker discovery in Parkinson´s disease (PD) has attracted significant attention recently [1]. PD diagnosis is based on clinical criteria, with poor overall validity and accuracy rates using pathologic examination. Frequently false positives (26.5%) had a final diagnosis of essential tremor (ET) [2].

Despite a growing interest in non-motor symptoms (NMS), occurring early in PD, biological markers are not easily accessible [3]. The search for biomarkers in biofluids related to diminished dopamine neurotransmission, proteinopathy, or altered colon bacterial population, able to differentiate PD from controls and to monitor disease progression has been unsuccessful [4-6]. So far, metabolomics is the most promising method for this search [7,8], but transition of these biomarkers to clinical practice is lacking. Potential biomarkers from biofluids still need validation in large cohorts and longitudinal studies [6,9,10].

Due to the low diagnostic accuracy particularly relevant in early stages of PD, and the differentiation between *de novo* PD and ET, the aim of this prospective study was 3-fold: 1) to examine the plasma metabolomics profiling in PD, controls, and patients with ET; 2) to determine differences between plasma metabolomics profiling in *de novo* PD (NOVO-PD) *vs* ET and moderate-advanced PD, and 3) to investigate the associations of plasma metabolomics profiling with motor and NMS in PD.

METHODS

Participants

This study was approved by the Burgos and Soria Health Area Institutional Review Board. Written informed consent was obtained from all subjects. Non demented out-patients followed in a movement disorder clinic at University Hospital of Burgos, Spain, diagnosed with idiopathic PD according to the UK PD Society brain bank criteria [11], including NOVO-PD, (drug naive PD), and PD patients with Hoehn Yahr stages from 1 to 4 [12], with a Mini Mental State Examination [MMSE] $\geq$ 26) [13], patients diagnosed with ET based on

established international criteria [14], and healthy controls were included. NOVO-PD diagnosis was confirmed by a presynaptic dopaminergic depletion in the [123] I-FP-CIT SPECT. Besides, patients with features of atypical or secondary parkinsonism, and patients and controls with arterial hypertension or diagnosed with other medical conditions (i.e. diabetes) and significant comorbidity according to the investigator criteria were excluded.

Sociodemographic, bio specimen and clinical data collection occurred on one visit. For PD patients, the biological sample was collected on the clinically defined OFF state (12 hours after the last dose of antiparkinsonian drugs).

Assessments

The severity of PD was assessed by using the Unified Parkinson´s disease Rating Scale (UPDRS) [15]. The UPDRS part 3 (motor UPDRS) was assessed in the OFF state. The UPDRS parts 1, 2 and 4 were assessed in the ON state (one hour after intake of antiparkinsonian medication). Severe motor fluctuations were defined as having UPDRS part 4 score >4.

The cognitive status was evaluated by using the Parkinson´s Disease Cognitive Rating Scale (PDCRS) [16], and severity of NMS using the Non-Motor symptoms questionnaire (NMSQ) [17], higher scores indicating worse status.

Collection procedures

Blood samples were collected and immediately centrifuged. Plasma supernatant transferred to Eppendorf containers and kept at -80ºC until analysis.

For metabolomics analysis, to 100 μL of plasma, 400 μL of cold methanol were added for protein precipitation and kept for 1 h at 4ºC. After centrifugation, 350 μL of the supernatant were recovered and evaporated in a Speed-Vac, following, 200 μL of acetonitrile:water (9:1, v/v) were added and the pellet resuspended. This solution was kept at 4ºC and analysed by UPLC-MS as soon as possible. Water submitted to the same treatment was used as blank.

UPLC-MS analysis

Liquid-chromatography analysis (LC) was performed in an Acquity Ultraperformance LC (UPLC) from WATERS (Barcelona, Spain). An Acquity UPLC HSS T3 1.8 µm, 2.1 × 100 mm column was used. The flow was 0.35 mL/min, and 7.5 µL of each sample were injected. Samples were randomly distributed to disperse error propagation. A binary gradient elution was used where solvent A was methanol:water (2:8, v/v) + 0.1% formic acid, and solvent B was 100% acetonitrile + 0.1% formic acid. The eluent was directly connected to a mass spectrometer SYNAPT HDMS G2 (WATERS, Barcelona, Spain) fitted with an electrospray ionization source (ESI, Z-spray®) and time of flight analyser (ESI-QToF-MS).

UPLC-MS data analysis

A three-dimensional Pareto-scaled data array comprising the variables plasma sample (including blanks), retention time_m/z values (molecular features), and normalized (scaled to Pareto variance) signal intensity of the m/z value was generated after UPLC-MS data were processed by using MarkerLynx® software (WATERS, Manchester, UK). Following, m/z values were manually checked and those present in the blank samples considered as noise or contaminants excluded. The resulting data arrays were used afterwards for untargeted multivariate statistical analysis.

The Extended Statistics (XS) application included in the MarkerLynx® software was used for the multivariate statistical analysis. The XS application includes principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) tools of the SIMCA-P+ software package (Umetrics EZ info 2.0; Umea, Sweden). Values of m/z with a defined chromatographic peak were only accepted to potentially arise from any true compound. Selected differential metabolites were tentatively identified by comparison of their m/z and elemental composition with available databases HMDB, METLIN, KEGG, ChEBI and LipidMaps. Additionally, comparison of retention time, adduct formation and fragments in the MS^E spectrum with commercial pure compounds when available (i.e.

bilirubin, biliverdin, tyramine and acyl-carnitines) was conducted. The chromatographic peak area of the selected metabolites was afterwards measured by integration using the QuantLynx® application (Waters, Manchester, UK).

Statistical Analysis

IBM SPSS Statistics 19 was used for data analysis. Normal distribution was confirmed using the Kolmogorov-Smirnov test. Demographic data, disease characteristics, and biomarker concentrations were compared between PD, ET and controls using the ANOVA test (several groups) or the Student t test (two groups), or by using their corresponding non-parametric tests for the medians when data did not follow a normal distribution. Gender and dichotomous variables were compared between groups applying chi-square tests. Pearson and Spearman rho correlations were used to examine the relationships among age, and biomarker concentrations. Regression models controlling for age and gender were used to examine biomarker concentrations predictors of PD diagnosis.

A multivariate linear regression analysis was conducted in order to determine a panel of biomarkers that might predict motor impairment (UPDRS motor scores), cognitive impairment and NMS (PDCRS and NMS scores, respectively). The ROC curve of combined biomarkers according to distribution of their concentrations and the diagnosis of PD (NOVO-PD, OFF-PD) *vs* ET and healthy controls, was used as a measurement of diagnostic ability. For each group type, sample size was verified fixing initial power $(1-\beta) = 80\%$, confidence level $(1-\alpha) = 95\%$ and minimum AUC=0.75, according to maximum requirement as per Obuchowski *et al.*, [18]. Final results obtained were admissible with power $(1-\beta) = 90\%$. Backward multivariate logistic regression was calculated, in which metabolites were excluded one by one according to Wald statistic when satisfying simultaneously two conditions: highest p-value and p-value > 0.05. The process was finished when all metabolites had p-value < 0.05. ROC curves were obtained according to the final logistic

regression using Real Statistics Resource Pack software (Release 6.8). The optimum cutoff values were calculated using Youden index and Accuracy maximums.

RESULTS

Participants Characteristics

For this exploratory study, a total of 137 subjects including 35 age matched controls, 29 NOVO-PD, 35 PD and 38 ET patients were included. Clinical characteristics of participants are presented in Table 1.

Metabolomics

OFF-PD and controls could be separated in the PLS-DA scoreplot, and clear separation was shown for NOVO-PD from both OFF-PD and controls (Figure 1A). Components 1 and 2 explained 22% and 40% (R^2Y[cum]) of variance respectively, with predictability (Q^2[cum]) being 21% and 32% for each respective component. Controls, NOVO-PD and ET were separated as well in the PLS-DA scoreplot (Figure 1B), with 31% and 59% of experimental variance being explained by components 1 and 2 (28% and 53% predictability), respectively. The same can be said for ET, NOVO-PD and OFF-PD groups (Figure 1C), with 35% and 65% of experimental variance being explained by components 1 and 2 (34% and 57% predictability), respectively. Paired comparisons (OPLS-DA) were also conducted between OFF-PD and control groups, between NOVO-PD and control groups, between OFF-PD and NOVO-PD groups, and between NOVO-PD and ET groups (the scoreplot for this latter comparison in Figure 1D). From the respective scoreplots, several differential metabolites were selected and receiver operating characteristic curves (ROC) were obtained with combination of metabolites for significant differences between two groups at a time (Table 2). Finally, a set of 12 metabolites was chosen as potential biomarkers, namely: bilirubin, seven acyl-carnitines, tetrahydro-21-deoxycortisol (THS), and tyramine.

Higher contents of bilirubin and biliverdin in patients seem to be a characteristic feature of these related pathologies (PD and ET), with increasing values as the pathology progresses (Figure 2). Metabolite comparisons and diagnostic accuracy are presented in Supplementary Table. Overall, bilirubin and biliverdin contribute to distinguish PD and ET *vs* controls as well as between OFF-PD and NOVO-PD, whereas specific carnitines distinguished PD *vs* controls and ET. Interestingly, C16:1-carnitine along with tyramine seem to be specific biomarkers for ET *vs* NOVO-PD, whereas THS together with almost the whole set of carnitines are prompted to be biomarkers for the differentiation between ET and OFF-PD. Short-chain carnitines were shown to be a characteristic feature of the differentiation of OFF-PD (Figure 2, Table 2).

Apart from C16:1-carnitine, mid- and long-chain acyl carnitines, in particular C18:1-carnitine, were shown to have significantly higher values in NOVO-PD and ET than in controls and OFF-PD, but the levels of these carnitines in OFF-PD are comparable to controls, except for C18:2-carnitine (Figure 2). Tetrahydro-21-desoxycortisol had higher values in NOVO-PD than in controls and ET. Lower values of tyramine in ET than controls and PD groups were found (Figure 2).

ROC analysis results are presented in Table 2. The full set of acyl-carnitines apart from C16:1-carnitine along with THS, bilirubin and biliverdin, were able to differentiate OFF-PD from ET (AUC = 0.929; PPV: 0.840 to 0.998; NPV: 0.737 to 0.962). Short chain carnitines and bilirubin were shown to distinguish OFF-PD from NOVO-PD (AUC = 0.931; PPV: 0.732 to 0.986; NPV: 0.704 to 0.945) and from controls (AUC = 0.932; PPV: 0.804 to 0.998; NPV: 0.652 to 0.902). C16:1-carnitine, considered a long-chain carnitine, seems to be specific for the differentiation of ET from NOVO-PD, along with tyramine (AUC = 0.787; PPV: 0.560 to 0.896; NPV: 0.629 to 0.894), and C18:1-carnitine, another long-chain carnitine, together with bilirubin, differentiates ET from controls (AUC = 0.815; PPV: 0.629 to 0.915; NPV: 0.557 to 0.837).

Relationship of metabolomics profiling with motor and nonmotor symptoms and their diagnostic accuracy

Correlations between NMS, PDCRS and UPDRS scores in the group of PD are presented in Supplementary Table. In NOVO-PD, indolacetic acid and tyrosine were significantly correlated with NMS scores. Instead, C16:1 and C:18-carnitines were inversely correlated with UPDRS-III and total UPDRS scores, and C18:1 and Biliverdin IX with UPDRS-II scores. In the group of patients with more advanced PD, several metabolites were inversely correlated with NMS scores (C8:0, C10:1 and THS), and with UPDRS-II scores (C18:1-carnitine and THS).

In multivariate regression analysis, after adjusting for age and disease duration, metabolite levels were not significantly associated with UPDR-III, IV, PDCRS and NMS scores and were similar when PD patients with/without severe motor fluctuations were compared (data not shown).

DISCUSSION

In this cross-sectional, observational study, the antioxidant-bound bilirubin and acyl-carnitines were affected in PD *vs* controls and ET. These differences being independent of dopaminergic therapy were observed in NOVO-PD and in PD at advanced disease stages, thus providing robustness.

For the first time, metabolic differences between ET and PD are provided. These specific biomarker differences found in NOVO-PD are not biased by the intake of dopaminergic drugs, providing a metabolomics scenario outside dopaminergic pathways. However, although our analysis discerned a biochemical profile linked to early and moderate/advanced PD, these findings do not clarify whether the observed metabolites represent primary biochemical manifestations of the disease or epiphenomena of PD as a neurodegenerative disorder.

Plasma or serum bilirubin and/or biliverdin contents in PD higher than in controls have been reported with positive correlations of serum bilirubin levels with UPDRS III, along with better motor outcomes [9, 19]. Overall, this increase in bilirubin and/or biliverdin contents might be due to heme oxygenase (HO) overexpression as a compensatory response to oxidative stress occurring from early stages of PD [19]. This result is in agreement with up regulation of the HO enzyme in the *substantia nigra* [20]. The neuronal origin of the augmented contents of bilirubin was indicated by diverse studies on HO-1 mRNA level in peripheral tissues and dynamic equilibrium with extravascular tissues [19]. Even though Hatano *et al.* [9] reported higher plasma levels of biliverdin but lower plasma levels of bilirubin in PD compared to controls, there is no contradiction with the HO up regulation because biliverdin is the direct product of HO along with Fe and CO, with further reduction of bilirubin by the biliverdin reductase taking place. Hence, the activity of biliverdin reductase might have been down regulated in concurrence with HO over-activity [9]. In the present study biliverdin was found to exhibit the same trend as bilirubin but with much lower contents (Figure 2). Up regulation of HO may be a counteracting response to increased oxidative stress [19], but an opposite view is also possible in which excessive release of iron ions from the heme group could have a damaging effect for mitochondria in PD neurons with consequent dysregulation of the energetic pathway of fatty acid oxidation, altering acyl-carnitine levels as observed in our study.

Levels of diverse acyl-carnitines, (C8 to C18), were higher in NOVO-PD than controls. Some of them were also augmented in OFF-PD, and ET. Elevated levels of acyl-carnitines in plasma are currently ascribed to dysfunctional β-oxidation of fatty acids in the mitochondria, though long chain acyl-carnitines might also indicate impaired functioning of the peroxisomes. In our study, we observed indolacetic acid, a serotonin catabolite, tyrosine and acyl-carnitines were inversely correlated with NMS in the group of patients with more advanced PD and with motor impairment in NOVO-PD, suggesting disturbances of

dopaminergic and serotonin neurons in PD [21]. In this regard, high contents of acyl-carnitines and acyl-glutamines in serum of PD patients as compared with controls were recently reported and correlated with mild cognitive impairment [10]. Other authors propose the impairment of mitochondrial respiratory chain, likely at the complex I [22], as one of the features, if not the main, taking place at the onset of PD. The rather wide survey of acyl-carnitines, from C8 to C18, found in the present study might arise from dopaminergic and non-dopaminergic neurons at diverse stages of degeneration with progressive proteins of the mitochondrial fatty acid oxidation pathway being dysfunctional. The over-activity of the monoamine-oxigenases (MAOs) in the outer membrane of the mitochondria has been shown to play a role in mitochondria impairment [23, 24], a feature that would agree with the finding of tyramine as a putative biomarker for NOVO-PD versus ET.

A peak with m/z 368.27 ($C_{21}H_{38}NO_4$) was ascribed to tetrahydro-21-deoxycortisol (THS, $[M+NH_4]^+$ adduct), the corresponding ion with m/z 395.24 ($C_{22}H_{35}O_6$ $[M+HCOOH-H]^-$ adduct for THS) being detected under negative ionization. According to Human Metabolome Database (HMDB) THS (ID: HMBD0005972) is a mineralocorticoid derived from 11-deoxycortisol metabolism and its excretion is significantly associated with tetrahydroaldosterone excretion. The fact that THS level was increased in NOVO-PD and OFF-PD as compared to the controls might be indicative of malfunctioning of the adrenal gland during PD progression. In spite of gluco- and mineralo-corticoids being synthesized in different areas of the adrenal gland, deregulation of the interplay between (gluco)corticoids and their receptors in dopaminergic neurons has indeed been proposed as a secondary unregulated inflammatory response taking place in PD progression [25], possibly concurrent with depositions of α-syn in the adrenal gland [26].

Tyramine (HMDB0000306) is a tyrosine-derived monoamine which may act as a neurotransmitter. It belongs to the so called “trace amines”, and its function in brain is bound to the trace amine-associated receptors 1 and 4 (TAAR1 and TAAR4), they being G-protein

coupled receptors [27]. Tyramine, as other trace amines, may prolong the action of adrenergic transmitters and prompt their release, this activity being implicated in aminergic dysregulation as a consequence of imbalance, and thereby this monoamine has been associated to PD [27,28]. It is shown in this study as a potential biomarker to distinguish NOVO-PD from ET, with significantly higher values in NOVO-PD than ET. D'Andrea *et al.* [29] have recently reported the circulating concentrations of different monoamines, this study pointing out tyramine as a putative biomarker for both early stages and progression of PD, with significantly higher values in PD than in healthy controls. In our study, with a bigger cohort, NOVO-PD and PD also showed higher values than controls but without statistically significant differences. Indolacetic acid (HDMB0000197) and tyrosine (HDMB0000158) did not give significant differences between groups, but they showed positive correlation with NMS (Supplementary Table). Tyrosine is a precursor of dopamine, and it is closely related to tyramine, which may be synthesized from dopamine. Indolacetic acid may arise from the tryptophan and gut microbial metabolism. Plasma tyrosine levels have been reported to be lower in treated PD patients than in controls [30], which agrees with our results (data not shown). No significant effects of tyrosine supplementation, mainly regarding blood pressure, were reported in treated PD patients despite of significantly risen plasma levels [30].

The strength of our conclusions is tempered by some limitations, including a relatively small sample size and the cross-sectional design. In addition, we do not have the overall hepatic profile in our participants, and we cannot exclude that higher bilirubin values could be produced by hepatic dysfunction in the group of patients with PD. On the other hand, our results were obtained in a single center and the biosamples were standardly collected and analysed in all participants, thus decreasing collection and measurement bias.

In conclusion, bilirubin, diverse acyl-carnitines, tyramine and some adrenal gland derived metabolites are suggested as potential biomarkers to differentiate early and more

advanced PD from ET and controls. Enzymatic changes related to oxidative and peroxisomes dysfunction are suggested to play a key role in PD etiology. However, further research studies using larger longitudinal cohorts are necessary to confirm our results.

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FIGURES

Figure 1. Scoreplots showing separated grouping of (A) control, OFF and NOVO-PD groups; (B) control, NOVO-PD and ET groups; (C) OFF-PD, NOVO-PD and ET groups; and (D) NOVO-PD and ET groups. Legend: C, control group; OFF-PD, diagnosed PD patients whose blood sample was collected 12 hours after the last dose of antiparkinsonian drugs; NOVO-PD, *de novo* diagnosed PD group; and ET, essential tremor group.

Figure 2. Representation of mean contents (nanomol/mL plasma, left panel, or micromol/mL plasma, right panel) and standard error values for each metabolite with statistically significant difference between groups. Legend: C, control group; OFF-PD, diagnosed PD patients whose blood sample was collected 12 hours after the last dose of antiparkinsonian drugs; NOVO-PD, *de novo* diagnosed PD group; and ET, essential tremor group. Tyramine concentration is plotted as divided by 10 to fit within the Y-axes scale.

TABLES

Table 1. Participant´s characteristics.

Table 2. Receiver operating characteristic curves (ROC) of combination of metabolites obtained by the backward multivariate logistic regression method showing the diagnostic accuracy discriminating between paired comparisons.

Supplementary Table. Significant bivariate correlations for metabolites with clinical parameters.

Table 1. Participant´s characteristics.

	NOVO-PD N=29	**PD** N=35	**ET** N=38	**Controls** N=35
Males (%) [a]	16 (64)	24 (68.6)	18 (58.1)	11 (31.3)
Females (%)	9 (36)	11 (31.4)	13 (41.9)	24 (68.6)
Age, mean (SD) [b]	66.9 (7.9)	63.8 (8.8)	67.7 (11.5)	61.4 (7.3)
Disease duration (years), mean (range)	1.4 (0.6; 3)	9.9 (2; 25)	11.4 (1; 30)	
UPDRS-I, median (range)	3 (0; 7)	3 (0; 9)		
UPDRS-II, median (range)	5 (0; 19)	12.5 (3; 27)		
UPDRS-III [d], median (range)	18 (5; 41)	24 (5; 49)		
UPDRS-IV [e], median (range)	0 (0; 1)	3.5 (0; 23)		
NMS total, median (range)	48 (4; 89)	69 (12; 119)		
PDCRS total, median (range)	74 (58; 112)	80 (36; 109)		

PD= Parkinson´s disease; ET=Essential Tremor; a= There was a higher proportion of females in the control group compared to other groups (p=0.01); b= Patients with ET were older compared to controls (p=0.002); c= Disease duration was higher in the PD group compared to the NOVO-PD group (p<0.0001); d, e= The UPDRS scores were higher in the PD-OFF state compared to the NOVO-PD (p=0.006, <0.001, respectively). UPDRS= Unified Parkinson´s disease Rating Scale; NMS= Non motor symptoms severity score: PDCRS= Parkinson´s disease cognitive rating scale; ET=Essential Tremor.

Table 2. Receiver operating characteristic curves (ROC) of combination of metabolites obtained by the backward multivariate logistic regression method showing the diagnostic accuracy discriminating between paired comparisons.

Paired Comparisons	Metabolites	(p-value [A])	AUC (95% CI) [B]	PPV (95% CI)	NPV (95% CI)	Accuracy	Youden Index	p-Value [C]
ET *vs* NOVO-PD	C16:1-carnitine Tyramine Glycoperine? (feature 2.76_409.1621)	(0.00069) (0.00139) (0.04401)	0.825 (0.726-0.925)	0.719 (0.529-0.845)	0.848 (0.673-0.938)	0.785	0.578	$1.5\ E^{-10}$
C *vs* NOVO-PD	C18:2-carnitine Bilirrubin 5-AMMU (5-acetylamino-6-amino-3-methyluracyl)	(0.00056) (0.00847) (0.02816)	0.940 (0.880-0.999)	0.893 (0.710-0.970)	0.914 (0.761-0.976)	0.905	0.807	$< 1.0\ E^{-20}$
C *vs* OFF-PD	C8:0-carnitine Bilirrubin	(0.01762) (0.00065)	0.878 (0.795-0.962)	1.000 (0.838-1.000)	0.729 (0.577-0.832)	0.878	0.618	$< 1.0\ E^{-20}$
C *vs* ET	C18:1-carnitine Bilirrubin 5-AMMU (5-acetylamino-6-amino-3-methyluracyl)	(0.01066) (0.00750) (0.02379)	0.857 (0.768-0.946)	0.780 (0.617-0.880)	0.839 (0.655-0.934)	0.806	0.608	$4.0\ E^{-15}$
OFF-PD *vs* NOVO-PD	C8:0-carnitine C10:0-carnitine C12:0-carnitine	(0.00364) (0.00540) (0.00907)	0.826 (0.723-0.928)	0.706 (0.522-0.831)	0.857 (0.665-0.950)	0.774	0.563	$4.9\ E^{-10}$
OFF-PD *vs* ET	Bilirrubin Biliverdin IX Glycodeoxycholic acid	(0.00110) (0.00441) (0.01565)	0.817 (0.716-0.918)	0.857 (0.665-0.950)	0.698 (0.535-0.811)	0.761	0.531	$7.10\ E^{-10}$

In this table, metabolites directly related to levodopa were not included. [A] p-Value of each metabolite according to the Wald statistics in the backward logistic regression. [B] AUC= Area under the curve with 95% CI (confidence interval). [C] p-Value of the ROC curve based on the AUC Z-score compared to AUC=0.5. Final results obtained were admisible with power (1-β)= 90%. NOVO-PD= *de novo* Parkinson´s disease, C=Healthy control, ET= essential tremor, OFF-PD= OFF-State Parkinson´s disease, PPV= Positive predictive value, NPV= Negative predictive value.

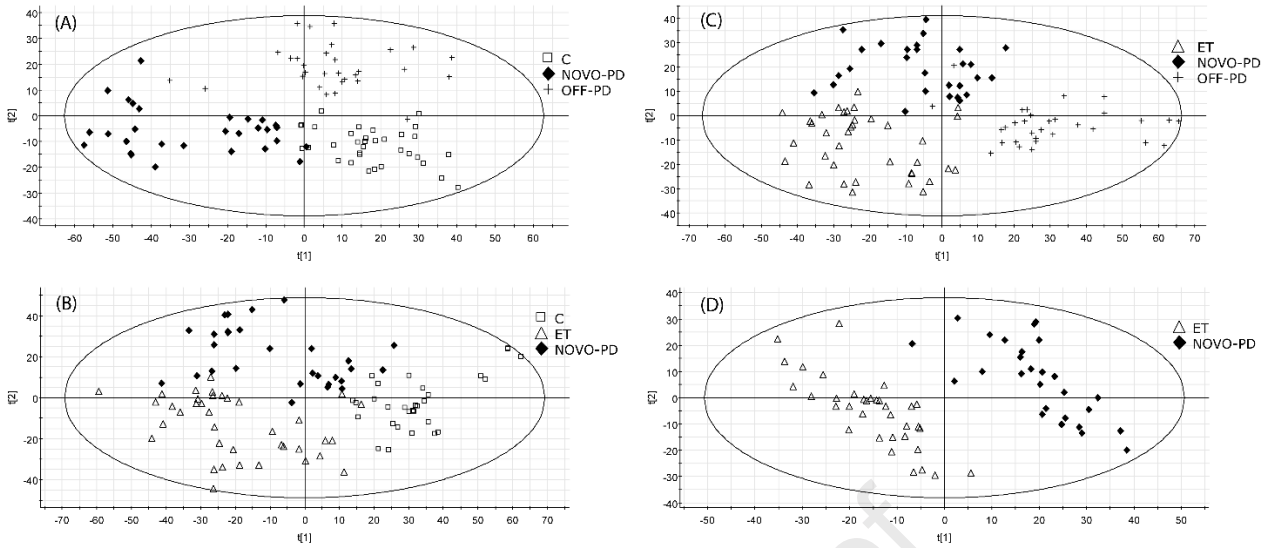

Figure 1. Scoreplots showing separated grouping of (A) control, OFF and NOVO-PD groups; (B) control, NOVO-PD and ET groups; (C) OFF-PD, NOVO-PD and ET groups; and (D) NOVO-PD and ET groups. Legend: C, control group; OFF-PD, diagnosed PD patients whose blood sample was collected 12 hours after the last dose of antiparkinsonian drugs; NOVO-PD, *de novo* diagnosed PD group; and ET, essential tremor group.

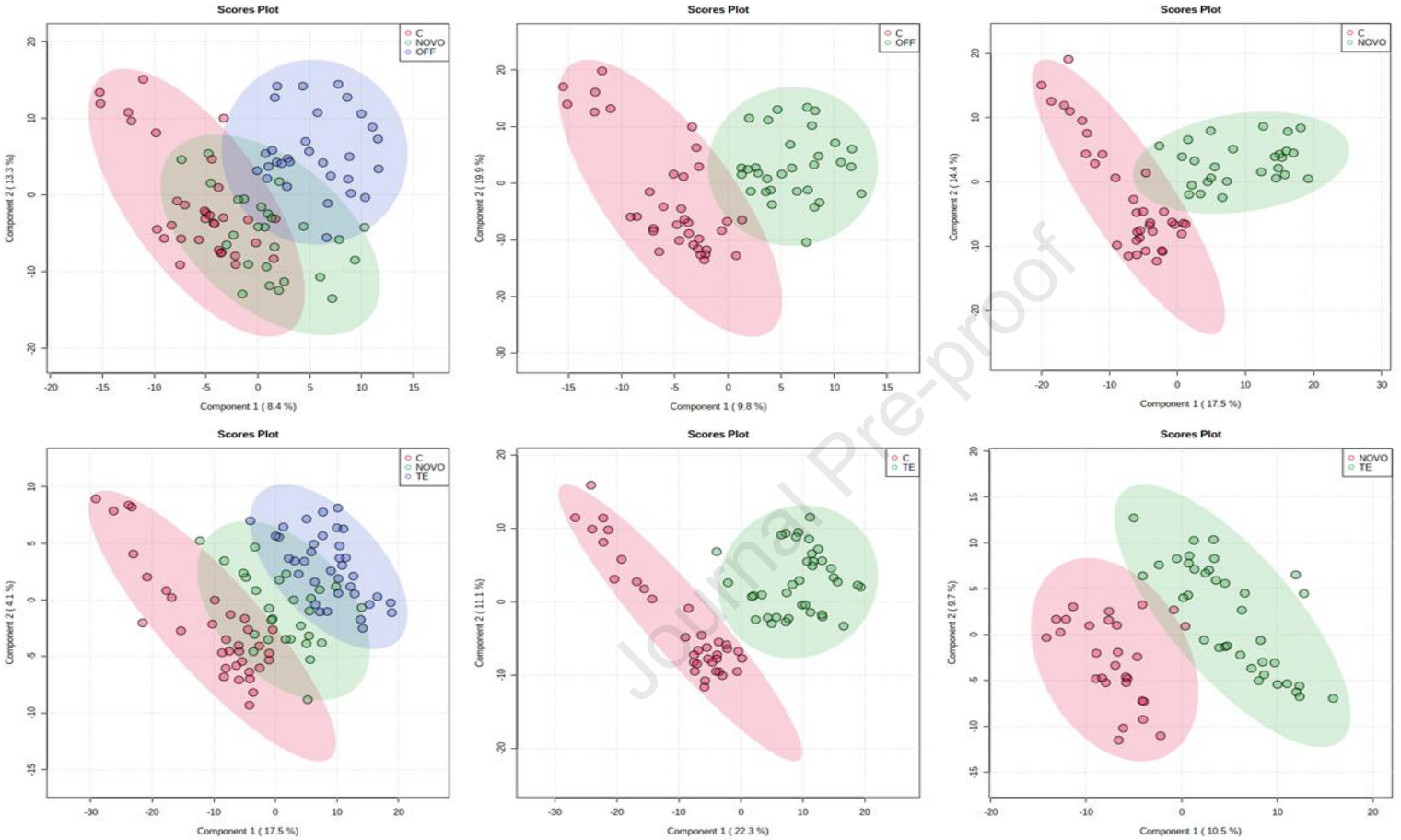

Figure 2. Scoreplots from the MetaboAnalyst (MA) software showing separated grouping of control, OFF and NOVO-PD groups (upper left corner); control, NOVO-PD and ET groups (lower left corner); control and OFF-PD groups (mid upper); control and ET groups (mid lower); control and NOVO-PD (upper right corner); and NOVO-PD and ET groups (lower right corner). Legend: C, control group; OFF, diagnosed PD patients whose blood sample was collected 12 hours after the last dose of antiparkinsonian drugs; NOVO, *de novo* diagnosed PD group; and ET, essential tremor group.

There are overlapping clinical manifestations and pathology in Parkinson´s disease, and Essential tremor.

Plasma metabolomics profiling differences between Parkinson´s disease and essential tremor were analyzed.

Several acyl-carnitines, bilirubin, tyramine, and some adrenal gland differentiated Parkinson´s disease from Essential Tremor.

Metabolomics can be used as a research tool in movement disorders