Identification of mitophagy-associated proteins (MAPs) profile as potential plasma biomarkers of idiopathic Parkinson’s disease

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Article

Keywords: Mitophagy-associated proteins, MAPs, Biomarkers, Diagnosis, Parkinson's disease

Posted Date: December 1st, 2022

DOI: https://doi.org/10.21203/rs.3.rs-2301788/v1

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Abstract

It is noteworthy that despite many efforts to screen biochemical plasma markers for PD diagnosis, there is still not an accepted and validated surrogate biomarker. To decipher the role of the mitophagy-associated proteins (MAPs) in idiopathic PD subjects and investigate whether the diagnosis is related to MAP levels and whether the levels predict motor and cognitive progression. This prospective study totally enrolled 150 PD patients. 71 age-matched controls (CN) alongside 41 PDs in two cohorts: modeling cohort (cohort 1), including 121 PD, 52 CN, and 29 PDs; validated cohort (cohort 2), including 29 PD, 19 CN, and 12 PDs. The MAPs (PINK1, Parkin, PGAMS, BNIP3, and p-TBK1) and a-synuclein-related proteins (ASPs: total a-synuclein, phosphorylated a-synuclein, and a-synuclein oligomer) levels were measured using an electrochemiluminescence immunoassay. MAPs are elevated in the plasma of PD patients. The PINK1, Parkin, and PGAMS displayed the top three measurable increase trends in amplitude compared to BNIP3 and p-TBK1. Moreover, the AUCs of PINK1, PGAMS, and Parkin were ranked the top three MAP candidates in diagnosis accuracy for PD from CN, but the MAPs hard to differentiate the PD from PDs. In addition, Plasma PINK1 positively correlated with total UPDRS, UPDRS part III, and H-Y stage, with no significant correlations with HAMA, HAMD, and RBD scores. As expected, higher plasma PINK1-Parkin levels and prominent diagnostic accuracy in A-synuclein (+) subjects than in A-synuclein (-) subjects. These results uncover that plasma MAPs (PINK1, Parkin, and PGAMS) may be potentially useful target biomarkers for PD diagnosis. Studies on larger cohorts would be required to test whether elevated plasma MAP levels are related to PD risk or prediction.

Introduction

Parkinson’s disease (PD) is the most prevalent devastating neurodegenerative movement disorder, suffering roughly 1% of the population aged 65 or older worldwide for which there is no causative treatment (Goedert et al., 2013). The clinical motor symptoms of PD as a result of the dopaminergic neurons insufficient in the Substantia Nigra pars compacta (SNpc) in the midbrain, simultaneously occur in their axon terminals, which project to the dorsal striatum (Hornykiewicz, 1962). The well-known pathologic hallmark of PD is the Lewy body, a neuronal inclusion largely made up of a-synuclein misfolding and aggregations (Bengoa-Vergnory et al., 2017). At present, diagnosis of PD is mainly based on individuals’ history, physical examination, and response to dopaminergic medication. History can include diverse prodromal features, characteristic movement disturbances (such as tremors, bradykinesia, rigidity), and psychological as well as cognitive problems et al (Armstrong and Okun, 2020). Moreover, the clinical diagnosis can be benefited through imaging tools that are advantageous to detect neurodegenerative damage. Notably, even when the aforementioned criteria are correctly used, the risk of misdiagnosis is still moderate to high and inevitable because of numerous clinical overlaps among parkinsonian disorders (Tolosa et al., 2006). Although PET and SPECT techniques are very sensitive, they are expensive and not specific for PD, and as well involve radiation exposure (Walker et al., 2018). Thus, exploring new mechanisms and corresponding feasible biomarkers for the early identification of patients with PD are urgently needed. Compared to CSF, plasma-based biomarkers are under promising investigation because they would provide a non-invasive method.

Of interest, damaged mitochondrial and its associated oxidative stress, ATP depletion, ROS production, and neuroinflammation are common characteristics in PD subject brain samples, and PD cross-species models (Bose and Beal, 2019; Lin and Beal, 2006). Biochemical and genetic researches uncover two genes mutated in autosomal recessive PD, namely PINK1 (PTEN-induced kinase 1) and Parkin, which typically collaborate in the same pathway to modulate the mitophagy pathway and mitochondria quality control (Pickrell and Youle, 2015). To our knowledge, it has been widely tested that mitophagy is dedicated to eliminating damaged mitochondria to maintain cellular integrity and homeostasis and is beneficial for neuroprotection (Lou et al., 2019). PINK1 localizes on the outer membrane of damaged mitochondria, and its kinase activity is required for Parkin translocation to the mitochondria surface. Then, Parkin ubiquitimates outer membrane proteins (such as FUNDCl, BNIP3, FKBP8, etc) to trigger selective autophagy (Palikaras et al., 2018). Historically, Nicholas et al reported that hereditary early-onset PD was caused by mutations in PINK1, as the core factor to initiate the mitophagy process. They identified two homozygous mutations influencing the PINK1 kinase domain from three consanguineous PARK6 carrier PD families (Valente et al., 2004). Moreover, mitochondrial dysfunction in PINK1<sup>−/−</sup> human dopaminergic cells along with lowered autophagic flux, can be rescued by parkin expression, indicating PINK1-parkin-dependent mitophagy involves in PD pathogenesis (Gegg and Schapira, 2011).

It is noteworthy that despite many efforts to screen biochemical plasma markers for PD diagnosis, there is still not an accepted and validated surrogate biomarker. Mitophagy-associated proteins (MAPs), including PINK1, Parkin, PGAMS, BNIP3, and phosphorylated-TBK1 (p-TBK1), to our best knowledge, are not well studied as a panel of biomarkers of neurodegeneration in PD. The primary aim of this study was to decipher the role of the mitophagy pathway proteins in idiopathic PD subjects and investigate whether the diagnosis is related to MAP levels and whether the levels predict motor and cognitive progression. Moreover, considering the basic mitophagy molecular mechanism identified in cross-species PD models, we hypothesized that MAPs represent dyshomeostasis brought on by compromised mitochondria.

Results

Baseline phenotypic characteristics of the cohorts

Table 1 shows demographic data for each group investigated here. For the modeling cohort, A total of 202 participants were selected, comprising Fifty-two individuals with healthy control (CN), one hundred and twenty-one clinically diagnosed with PD, and twenty-nine individuals were subsequently characterized as having PDs. The basic information includes average age, sex ratio, height, weight, BMI, education, disease duration, UPDRS, Hoehn and Yahr stage, HAMA, HAMD, RBD, ADL, and the levels of a-synuclein as well as MAPs provided. Patients with PDs were older at the time of recruitment and sampling of biomaterials. There was a high male/female ratio with PD and PDs (55.37% of PD and 68.97% of PDs) compared to CN (44.23%) and mild to low education levels in the PD group but no significance. To explore a possible link between impaired mitophagy-associated proteins (MAPs) and PD in this cohort, we quantified our cohort’s abundance of MAPs in plasma. Moreover, PDs were characterized by more serious motor symptoms and...
As a first step, we tested the distribution of plasma-derived MAP levels (e.g., PINK1, Parkin, PGAM5, BNIP3, and p-TBK1) in the modeling cohort. We hypothesized that patients with PD exhibit higher MAP levels compared to healthy control subjects due to impaired mitochondria, in which inverse feedback induces the mitophagy process. Following this hypothesis, plasma PINK1, Parkin and PGAM5 concentrations were significantly higher in PD (92.7 ± 20.1 ng/ml, 21.4 ± 4.47 ng/ml, 902 ± 146 pg/ml, respectively) and PDs (96.9 ± 24.7 ng/ml, 22.9 ± 6.15 ng/ml, 910 ± 143 pg/ml, respectively) as compared to the CN group (77.4 ± 17.5 ng/ml, 19.3 ± 4.67 ng/ml, 833 ± 171 pg/ml, respectively), suggesting higher levels of PINK1, Parkin, and PGAM5 were associated with an increased likelihood of PD or PDs relative to the CN group (Table 2 and Fig. 2a-c). Oppositely, the levels of p-TBK1 (CN: 9.95 ± 1.96 ng/ml, PD: 10.4 ± 2.15 ng/ml, PDs: 9.56 ± 2.55 pg/ml, respectively) and BNIP3 (CN: 14.2 ± 3.49 ng/ml, PD: 15.4 ± 3.80 ng/ml, PDs: 14.3 ± 3.70 pg/ml, respectively) were associated with an increased likelihood of PD or PDs relative to the CN group (77.4 ± 17.5 ng/ml, 19.3 ± 4.67 ng/ml, 833 ± 171 pg/ml, respectively), suggesting higher levels of PINK1, Parkin, and PGAM5

### Table 1
Baseline characteristics for the modeling and validated cohorts

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Modeling cohort</th>
<th></th>
<th>Validated cohort</th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CN (N = 52)</td>
<td>PD (N = 121)</td>
<td>PDs (N = 29)</td>
<td>PD vs CN</td>
</tr>
<tr>
<td>Age (years)</td>
<td>64.1 (7.60)</td>
<td>65.9 (8.31)</td>
<td>67.4 (8.15)</td>
<td>0.596</td>
</tr>
<tr>
<td>Female (%)</td>
<td>29 (55.77%)</td>
<td>54 (44.63%)</td>
<td>9 (31.03%)</td>
<td>0.189b</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>161 (6.63)</td>
<td>161 (8.28)</td>
<td>161 (9.06)</td>
<td>1.000</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>62.00 (10.12)</td>
<td>60.00 (13.50)</td>
<td>62.00 (12.50)</td>
<td>ns</td>
</tr>
<tr>
<td>Education (years)</td>
<td>4.00 [7.50]</td>
<td>4.00 [6.00]</td>
<td>5.00 [7.00]</td>
<td>ns</td>
</tr>
<tr>
<td>Disease History (years)</td>
<td>-</td>
<td>3.00 [5.00]</td>
<td>-</td>
<td>0.052</td>
</tr>
<tr>
<td>UPDRS</td>
<td>42.9 (22.1)</td>
<td>49.8 (22.6)</td>
<td>4.00 [2.00]</td>
<td>-</td>
</tr>
<tr>
<td>I</td>
<td>2.00 [3.00]</td>
<td>3.00 [2.50]</td>
<td>2.00 [2.50]</td>
<td>-</td>
</tr>
<tr>
<td>II</td>
<td>11.00 [9.00]</td>
<td>13.00 [9.50]</td>
<td>11.00 [8.90]</td>
<td>-</td>
</tr>
<tr>
<td>III</td>
<td>25.1 (13.2)</td>
<td>28.3 (16.10)</td>
<td>2.00 [0.91]</td>
<td>-</td>
</tr>
<tr>
<td>IV</td>
<td>2.00 [3.00]</td>
<td>1.00 [2.50]</td>
<td>-</td>
<td>2.06 [1.06]</td>
</tr>
<tr>
<td>H-Y stage</td>
<td>2.40 (1.06)</td>
<td>4.09 (6.27)</td>
<td>0.16</td>
<td>-</td>
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<tr>
<td>MMSE</td>
<td>22.8 (4.58)</td>
<td>21.5 (6.30)</td>
<td>16.8 (6.99)</td>
<td>0.634</td>
</tr>
<tr>
<td>HAMD</td>
<td>3.27 (3.34)</td>
<td>6.62 (5.01)</td>
<td>6.93 (4.45)</td>
<td>0.000</td>
</tr>
<tr>
<td>HAMA</td>
<td>5.33 (5.00)</td>
<td>9.25 (5.86)</td>
<td>9.34 (5.69)</td>
<td>0.000</td>
</tr>
<tr>
<td>RBDQ-HK</td>
<td>7.04 (9.23)</td>
<td>20.5 (20.2)</td>
<td>20.7 (21.4)</td>
<td>0.000</td>
</tr>
<tr>
<td>ADL</td>
<td>20.00 [0.00]</td>
<td>27.00 [13.00]</td>
<td>34.00 [29.50]</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Data are expressed as mean (SD), median [IQR] or n (%); a: p-values obtained from One-Way ANOVA or Kruskal-Wallis test followed by Bonferroni corrected post hoc comparisons, and UPDRS and H-Y stage were only compared between PD and PDs by Student’s t-test; b: p-values obtained from chi-squared test corrected by Bonferroni, ns: not significant; CN: Control; PD: Parkinson disease; PDs: Parkinsonian syndrome; UPDRS: unified Parkinson’s disease rating scale; MMSE: Mini-Mental State Examination; HAMD: Hamilton Depression Scale; HAMA: Hamilton Anxiety Scale; RBDQ-HK: REM sleep behavior disorder questionnaire-Hong Kong; ADL: Activity of Daily Living Scale; BMI: Body Mass Index.

**MAPs are elevated in the plasma of PD patients**

As a first step, we tested the distribution of plasma-derived MAP levels (e.g., PINK1, Parkin, PGAM5, BNIP3, and p-TBK1) in the modeling cohort. We hypothesized that patients with PD exhibit higher MAP levels compared to healthy control subjects due to impaired mitochondria, in which inverse feedback induces the mitophagy process. Following this hypothesis, plasma PINK1, Parkin and PGAM5 concentrations were significantly higher in PD (92.7 ± 20.1 ng/ml, 21.4 ± 4.47 ng/ml, 902 ± 146 pg/ml, respectively) and PDs (96.9 ± 24.7 ng/ml, 22.9 ± 6.15 ng/ml, 910 ± 143 pg/ml, respectively) as compared to the CN group (77.4 ± 17.5 ng/ml, 19.3 ± 4.67 ng/ml, 833 ± 171 pg/ml, respectively), suggesting higher levels of PINK1, Parkin, and PGAM5 were associated with an increased likelihood of PD or PDs relative to the CN group (Table 2 and Fig. 2a-c). Oppositely, the levels of p-TBK1 (CN: 9.95 ± 1.96 ng/ml, PD: 10.4 ± 2.15 ng/ml, PDs: 9.56 ± 2.55 pg/ml, respectively) and BNIP3 (CN: 14.2 ± 3.49 ng/ml, PD: 15.4 ± 3.80 ng/ml, PDs: 14.3 ± 3.70 pg/ml,
respectively) have no obvious differences among these three groups (Table 2 and Supple Fig. 1a, b). Further comparison, regarding PD and PDs, the MAPs showed a similar pattern using post hoc comparison (p > 0.05). According to the aforesaid results, the PINK1, Parkin, and PGAM5 displayed the top three measurable increase trends in amplitude compared to BNIP3 and p-TBK1. PINK1 localizes on the damaged mitochondria outer membrane and induces the Parkin and a series of membrane proteins to trigger selective autophagy. Therefore, we observed a parallel tendency in these MAPs. Moreover, we supposed that PD or PDs subjects exhibit higher total α-synuclein (34.9 ± 6.85 ng/mL, 34.1 ± 6.43 ng/mL, respectively), phosphorylated α-synuclein (17.9 ± 3.21 ng/mL, 18.2 ± 2.73 ng/mL) and α-synuclein oligomer (3238 ± 634 pg/mL, 3163 ± 458 pg/mL) levels than CN group. Indeed, we confirmed the expected order consistent with the MAPs pattern (Table 2 and Supple Fig. 1c-e), but the differences in values between PD and PDs did not reach significance, indicating the MAPs and α-synuclein-related proteins (ASP) were unsuitable as potential biomarkers to differentiate the PD from PDs. The distribution dots in Fig. 1g and h demonstrated the high value of MAPs and ASPs located in PD and PDs regions.

### Table 2

Plasma-derived multiple biomarker levels of these two cohorts

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>Modeling cohorta</th>
<th></th>
<th></th>
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<tr>
<td></td>
<td>CN (N = 52)</td>
<td>PD (N = 121)</td>
<td>PDs (N = 29)</td>
<td>PD vs CN</td>
<td>PD vs PDs</td>
<td>PD vs CN</td>
<td>CN (N = 19)</td>
<td>PD (N = 27)</td>
<td>PDs (N = 12)</td>
<td>PD vs CN</td>
<td>PD vs PDs</td>
<td>PD vs CN</td>
</tr>
<tr>
<td>PINK1 (ng/mL)</td>
<td>77.4 (17.5)</td>
<td>92.7 (20.1)</td>
<td>96.9 (24.7)</td>
<td>0.000</td>
<td>0.289</td>
<td>0.017</td>
<td>17.1 (1.75)</td>
<td>18.6 (2.66)</td>
<td>17.9 (1.88)</td>
<td>0.000</td>
<td>1.000</td>
<td>0.018</td>
</tr>
<tr>
<td>Parkin (ng/mL)</td>
<td>19.3 (4.67)</td>
<td>21.4 (4.47)</td>
<td>22.9 (6.15)</td>
<td>0.001</td>
<td>1.000</td>
<td>0.027</td>
<td>9.24 (0.79)</td>
<td>9.59 (0.83)</td>
<td>9.46 (1.17)</td>
<td>0.084</td>
<td>0.944</td>
<td>1.000</td>
</tr>
<tr>
<td>PGAM5 (pg/mL)</td>
<td>833 (171)</td>
<td>902 (146)</td>
<td>910 (143)</td>
<td>0.331</td>
<td>0.362</td>
<td>1.000</td>
<td>13.7 (0.74)</td>
<td>13.4 (1.06)</td>
<td>13.6 (1.09)</td>
<td>0.567</td>
<td>1.000</td>
<td>1.000</td>
</tr>
<tr>
<td>BNIP3 (ng/mL)</td>
<td>14.2 (3.49)</td>
<td>15.4 (3.80)</td>
<td>14.3 (3.70)</td>
<td>0.062</td>
<td>0.549</td>
<td>1.000</td>
<td>15.3 (1.26)</td>
<td>16.5 (1.50)</td>
<td>16.2 (1.33)</td>
<td>0.822</td>
<td>1.000</td>
<td>1.000</td>
</tr>
<tr>
<td>P-TBK1 (ng/mL)</td>
<td>9.95 (1.96)</td>
<td>10.4 (2.15)</td>
<td>9.56 (2.55)</td>
<td>0.000</td>
<td>0.285</td>
<td>0.059</td>
<td>778 (63.7)</td>
<td>828 (62.5)</td>
<td>795 (87.8)</td>
<td>0.052</td>
<td>0.526</td>
<td>1.000</td>
</tr>
<tr>
<td>T-asyn (ng/mL)</td>
<td>29.8 (6.17)</td>
<td>34.9 (6.85)</td>
<td>34.1 (6.43)</td>
<td>0.000</td>
<td>1.000</td>
<td>0.003</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P-asyn (ng/mL)</td>
<td>16.5 (2.87)</td>
<td>17.9 (3.21)</td>
<td>18.2 (2.73)</td>
<td>0.001</td>
<td>1.000</td>
<td>0.033</td>
<td>2406 (209)</td>
<td>2499 (244)</td>
<td>2549 (199)</td>
<td>0.022</td>
<td>1.000</td>
<td>0.334</td>
</tr>
<tr>
<td>Asy-no (pg/mL)</td>
<td>2805 (802)</td>
<td>3238 (634)</td>
<td>3163 (458)</td>
<td>0.000</td>
<td>1.000</td>
<td>0.002</td>
<td>66.6 (4.62)</td>
<td>74.2 (6.55)</td>
<td>73.0 (6.94)</td>
<td>0.514</td>
<td>1.000</td>
<td>0.265</td>
</tr>
</tbody>
</table>

Data are expressed as mean (SD); CN: Control; PD: Parkinson disease; PDs: Parkinsonian syndrome; p-values obtained from One-Way ANOVA adjusted by Bonferroni; T-asyn: total α-syn; P-asyn: phosphorylated α-syn in ser129; Asy-no: oligomeric α-syn; PINK1: PTEN induced putative kinase 1; P-TBK1: Phosphorylated TANK binding kinase 1; PGAM5: phosphoglycerate mutase 5; BNIP3: BCL2 interacting protein 3.

a Less participants took the examination of three plasma BNIP3 and P-TBK1 in the modeling cohort (CN = 31, PD = 65, PDs = 14).

b Less participants took the examination of three plasma α-synuclein levels in the modeling cohort (CN = 31, PD = 65, PDs = 14).

c Participants in validated cohort did not take the examination of plasma T-asyn.

### Diagnostic accuracy of plasma MAPs biomarkers for PD

Assessing the utility of MAP levels to discriminate between clinically defined idiopathic PD and CN, we found an area under the ROC curve (AUC) of 0.764 (95% CI: 0.691 to 0.836) for PINK1, 0.665 (95% CI: 0.578 to 0.752) for Parkin, and 0.721 for PGAM5 (95% CI: 0.632 to 0.810; Fig. 1). Similarly, ROC tests compared PDs patients against the CN group (Fig. 1j), the AUC for PINK1 was 0.714 (95% CI: 0.597 to 0.830), which was mild higher than for Parkin (AUC 0.676; 95% CI: 0.554 to 0.797) and PGAM5 (AUC 0.636; 95% CI: 0.515 to 0.757). Notably, in terms of secondary outcomes analysis that compared participants of PD vs PDs (Fig. 1k), the AUCs for PINK1 (0.598; 95% CI: 0.493 to 0.703), Parkin (0.499; 95% CI: 0.384 to 0.614), and PGAM5 (0.601; 95% CI: 0.480 to 0.722) were comparable and all the AUC levels less than 0.61 in this analysis domain, indicating the MAPs hard to differentiate the PD from PDs. Regarding the ASPs as a kind of classical diagnosis marker, the AUC values are comparable to the MAPs (Fig. 1l-n). Next, the discriminative accuracy values of plasma p-TBK1, and BNIP3 in primary and secondary outcomes were displayed in Supple Fig. 1 and corresponding detailed AUCs quantitative values in these analyses can be found in Supple Table 1. Notably, machine learning such as random forest can improve the performance of risk predictions based on the decision tree to identify vital features and rank the characteristics. In this issue, we found Asy-no, PGAM5, and PINK1 are the top 3 important candidates to discriminate PD from CN using the random forest algorithm (Fig. 3a). For RBD, the AUC is 0.720, which was mild to moderately higher than other neuropsychological domains of PD vs CN (e.g., MMSE, HAMD, and HAMA; Fig. 3b). Next, we assessed the accuracy of combined MAPs to identify PD from CN participants (Fig. 3c and Supple Table 2). The AUC was 0.842 (95% CI: 0.771 to 0.912) when combined with analysis of MAPs (PINK1, PGAM5, and Parkin), which was alike to the AUCs for Bio-Top3 (Asy-no, PINK1 plus Parkin) and MAPs plus Asy-no of 0.856 (95% CI: 0.785 to 0.926), indicating MAPs is the promising candidate biomarkers to differentiate the PD from CN in the modeling cohort. Interestingly, we tested the same analysis in the validated cohort and found similar effects (Fig. 3d).
Association of plasma MAPs biomarkers with motor and Nonmotor Features

The linear correlation at cross-sectional between MAP levels (PINK1, Parkin, and PGAM5) and neuropsychological evaluation scales was displayed in Fig. 4a and Supple Table 3, including total UPDRS, UPDRS part III, H-Y stage, MMSE, HAMD, HAMA, RBD, and ADL. Based on the coefficients r values through this analysis after adjusting age, education, and sex, we found plasma PINK1 positively correlated with total UPDRS (r = 0.247, p = 0.001), UPDRS part III (r = 0.243, p = 0.001), and H-Y stage (r = 0.234, p = 0.001). Moreover, we then analyzed the correlation of Parkin and motor and nonmotor features in all three groups, Parkin was positively associated with UPDRS (r = 0.191, p = 0.006), RBD (r = 0.168, p = 0.017) and ADL (r = 0.197, p = 0.005) scores, as well as negatively related to MMSE (r = -0.482, p = 0.008) in the PDs group. Additionally, it should be noted that PGAM5 also correlated with the UPDRS (r = 0.217, p = 0.002), UPDRS part III (r = 0.214, p = 0.002), and RBD score (r = 0.204, p = 0.004). Next, when examining ASPs as the target indexes, the association of plasma ASPs with neuropsychological scales was displayed in Fig. 4b. In addition, we used non-linear smoothing spline regressions RCS model between MAPs as a continuous value and neuropsychological evaluation scales after adjusting age, and sex. Interestingly, we observed no significant positive association between MAPs (PINK1, Parkin, and PGAM5) and UPDRS, MMSE, HAMD, HAMA, and RBD scores in PD subjects (Fig. 5a-c and Supple Table 4). Of note, even though no significance, PGAM5 displayed a positive correlation to the low total UPDRS scores (< 50) but an inverse interaction with high UPDRS scores (> 50), suggesting an inverted U-shaped curve between PGAM5 and PD symptoms severity (Fig. 5c). Using MMSE scores as the outcome, RCS analysis revealed an inverse association of Parkin with high MMSE scores and a forward correlation of Parkin with low MMSE scores (Fig. 5b). Furthermore, detailed information concern on the association of p-TBK1, BNI3P, and ASPs with UPDRS, MMSE, HAMD, HAMA, and RBD scores can be found in Supple Fig. 2a, b and Supple Fig. 3.

Higher plasma MAP levels and prominent diagnostic accuracy in a-synuclein-positive subjects

Notably, as a key hallmark of PD pathogenesis, numerous studies revealed a-synuclein as a potential meritorious biomarker for PD diagnosis (Foulds et al., 2011; Stuendl et al., 2021; Wang et al., 2020). Linear models were applied to evaluate the association between plasma MAPs and ASPs. As shown in Fig. 6a, higher plasma PINK1 level was correlated with higher total a-syn (r = 0.292), phosphorylated a-syn (r = 0.358), and a-syn oligomer (r = 0.363). Similarly, a high level of Parkin was closely related to total a-syn (r = 0.358), phosphorylated a-syn (r = 0.405), and a-syn oligomer (r = 0.326). In addition, Plasma PGAM5 was associated with measures of total a-syn (r = 0.311), and phosphorylated a-syn (r = 0.233), except for a-syn oligomer which was no association. Hence, to test the MAP levels and diagnostic accuracy in a-syn positive subjects. A-syn distinguishes a-Syn (+) from a-Syn (-) by the cut-off value obtained from PD and CN groups through the prediction value of the logistic regression model using total a-syn, phosphorylated a-syn, and a-syn oligomer (Fig. 6b). We found abnormally higher PINK1 (CN: 84.7 ± 16.2 vs 73.6 ± 14.5 ng/ml; PD: 94.1 ± 16.7 vs 82.8 ± 18.8 ng/ml, respectively), and Parkin (CN: 23.0 ± 3.20 vs 17.9 ± 3.91 ng/ml; PD: 22.1 ± 3.56 vs 20.4 ± 4.65 ng/ml, respectively) levels in the a-Syn (+) subjects compared to a-Syn (-) populations (Fig. 6c-e and Supple Table 5). Of interest, the levels of p-TBK1 were also higher in the a-Syn (+) than a-Syn (-) subjects, but not BNP3 (Supple Fig. 2c, d). Moreover, comparing PD vs CN, plasma PINK1 level discriminated abnormal vs normal a-synuclein status with an AUC of 0.775 in A-syn (+) vs 0.595 in A-syn (-) subjects (Fig. 6f, g), which was mild higher than the AUCs for plasma levels of Parkin (0.694 vs 0.567), and PGAM5 (0.751 vs 0.773), indicating PINK1 showed higher diagnostic accuracy than established plasma Parkin and PGAM5 biomarkers in A-syn (+) subjects. See Supple Fig. 2e, f for the AUCs of p-TBK1 and BNI3P in the A-syn (+) or (-) subjects.

Correlation and concordance among PINK1, Parkin, and PGAM5

To investigate the relationship and concordance between plasma PINK1, Parkin, and PGAM5. A linear correlation analysis model displayed that plasma PINK1 was sensitive to the Parkin level (r = 0.409, p < 0.001, Supple Fig. 4a). Notably, the relationship between plasma PINK1 and PGAM5 was mild and low r value (r = 0.127, p = 0.073, Supple Fig. 4b). Of interest, for the biomarker of Parkin and PGAM5, we found that plasma Parkin was concordant with the PGAM5 distribution (r = 0.158, p = 0.025, Supple Fig. 4c). These results indicate that plasma PINK1, Parkin, and PGAM5 have similar and related expression trajectories.

Discussion

To the best of our knowledge, this is the first study of such a topic in the PD biomarker fields and we detail baseline plasma MAP (e.g., PINK1, Parkin, and PGAM5) levels of PD and PDs compared with healthy controls. We have provided several neoteric observations and produced such hypotheses that need additional longitudinal follow-up studies. Notably, the most important observations in the study were as follows: (1) Subjects with PD or PDs exhibit higher MAP levels compared to the CN group, but the differences in values between PD and PDs did not reach significance. (2) The AUCs of PINK1, PGAM5, and Parkin were ranked the top three MAP candidates in diagnosis accuracy for PD from CN, but the MAPs hard to differentiate the PD from PDs. It is possible that we were underpowered to detect differences of smaller effect size due to the sample size. (3) Plasma PINK1 positively correlated with total UPDRS, UPDRS part III, and H-Y stage. Additionally, Parkin and PGAM5 related to the UPDRS, and RBD scores, and no significant correlations between MAPs and HAMA and HAMD. (4) Higher plasma PINK1-Parkin levels and prominent diagnostic accuracy in A-syn (+) subjects than in A-syn (-) subjects. Thus, we pinpointed that plasma PINK1, PGAM5, and Parkin as promising candidate MAP biomarkers of the target mitophagy process in PD.

Few data are available about the change in plasma MAP levels in human physiological and pathological circumstances. The most crucial discovery in this project is higher levels and better diagnosis values of MAPs in PD and PDs than in healthy controls. Although numerous studies have described the potential role of mitophagy in PD pathogenesis according to basic research (Malpartida et al., 2021; Ryan et al., 2015), few reports have assessed MAPs activity in biofluids from PD cases. Our data are in line with the theory that patients with PD exhibit higher MAP levels due to impaired mitochondria, in

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which inverse feedback induces the mitophagy process. However, here, we found at the late disease stage (such as UPDRS scores > 50) the PGAM5 levels lean to decline, emerging an inverted U-shaped curve between PGAM5 and PD symptoms severity. Furthermore, the utility of the PINK1, PGAM5, and Parkin as potential biomarkers was strengthened by our finding that these markers discriminated PD from control serum in two independent cohorts, even though the sample size is relatively small in the second cohort. Notably, although the p-TBK1 and BNIP3 are involved in the mitophagy process (Heo et al., 2018; Lin et al., 2021), we found that the levels of these two markers were not obvious differences among these three groups, indicating the complexity of the mitophagy pathway and the whole veil still not yet unmasked. These results present some of the first insights regarding the different types of plasma MAP levels and how they might change in PD subjects.

Previous works identified mitochondrial dysfunction and impaired mitophagy as associated with PD pathogenesis (Pickrell and Youle, 2015). This theory is supported by several animal experiments, where similar Parkinsonism signs were detected in MAPs knockout animals, including PINK1−/−, Parkin−/−, and PGAM5−/− (Lu et al., 2014; Sliter et al., 2018; Valente et al., 2004). Recently, emerging evidence focused on the relationship between mitophagy roles and PD genes such as the LRRK2 mutations (Wauters et al., 2020) and observations that α-synuclein interact with outer mitochondrial membrane substrates, disrupting the mitophagy pathway (Shaltouki et al., 2018). According to biochemical studies in transfected cells and transgenic mice suggest that PINK1 and Parkin act in concert with a mitochondrial quality control system in neuroprotection (Lou et al., 2019; Whitworth and Pallanck, 2017). Correspondingly, mendelian genetics attributes loss-of-function mutations in PINK1 and Parkin, two key mitophagy regulators, to early-onset PD (Valente et al., 2004). Based on such critical findings, we set out to explore the roles of MAPs in the PD diagnosis domain. As mentioned earlier, we have previously speculated that MAPs could be increased in the PD background due to the impaired mitochondria leading to inverse feedback. More than that, the link between impaired mitophagy and PD has opened a window of opportunity for multiple therapeutics targeting mitochondria quality control. The regimen of interventions ranges from boosting mitophagy activity via specific activators or mitophagy pathway-related gene therapy.

The observed close association of the PINK1 and PGAM5 in this cohort with clinical scales commonly utilized to assess the severity of motor symptoms may be explained for several potential reasons. It is possible that the PINK1 and PGAM5 levels are probably reactive or coincidental in more severely progressing individuals. Alternatively, the PINK1 and PGAM5 values measured in plasma may reflect part of the PD process driving worse disease in some individuals. Oppositely, there was no association between the PINK/PGAM5 and the MMSE score, displaying that cognitive dysfunction in PD patients is not related to the reduction of the PINK1 and PGAM5 levels. However, Parkin, p-TBK1, and BNIP3 did not show a tight relationship with the clinical parameters which might reflect aspects of different MAPs function heterogeneity in the mitophagy process that is not well understood. Because Parkin was numerically higher in PD or PDs compared with controls, we posit that Parkin may play a disparate role in the mitophagy pathway compared to PINK1 and disease features progression (Villa et al., 2018). In addition, such clinical parameters also have some limitations, including sensitivity and assessment accuracy et al. Moreover, further investigations in larger cohorts with biochemical, and advanced measurement tools may be revealing in this regard. More importantly, studies examining MAP levels in the CSF in addition to plasma may firmly test its relevance as a disease biomarker for PD.

Interestingly, we also found increased MAP levels in A-syn (+) subjects compared to in A-syn (-) subjects, and we provide here the first evidence for a significant augment of diagnosis capacity in A-syn-positive patients supporting the hypothesis that MAP levels or mitophagy process close interacted with α-synuclein aggregation in this disease. The increase of plasma MAP levels detected in the A-syn (+) background may be due to more serious mitochondria dysfunction in this issue. The loss of mitochondrial dysfunction and the appearance of Lewy bodies (A-syn aggregation) are two prevailing pathological hallmark features of PD. Under mild to moderate mitochondrial stress conditions, PINK1, Parkin, and α-synuclein form a regulatory circuit to manage the mitochondrial stress response (Norris et al., 2015). Moreover, Xiaoxi et al reported that human α-syn A53T overexpression in transgenic mice induces pervasive mitophagy impairment preceding dopaminergic neuron degeneration. They found that mitochondria hubs are the main targets of α-syn and their defective mitophagy plays a vital role during disease pathogenesis (Chen et al., 2015), indicating the MAPs interaction with α-syn tightly and offer the reason behind the higher MAP levels in A-syn (+) subjects.

Despite some interesting observations on differences in MAP signatures in PD and PDs, our project has still some limitations. This study employed the relatively low-throughput way of centrifugation of blood biospecimens and laborious ELISA to analyze the target proteins. This assay is often performed manually and therefore difficult to standardize. The precision issue might be limited, and hence higher throughput assays with more precision may facilitate additional insights. For instance, single-molecule-based assays (Simoa) or real-time quaking-induced conversion (RT-QuIC) have been successfully developed for ultralow concentrations of interested proteins in biofluids (Kuhle et al., 2016; Rossi et al., 2020), they have been shown to be more sensitive than traditional immunoassays. Furthermore, given the effect of storage time on the MAPs expression in our serum samples, it needs attention that future studies should carefully control for this variation and take it into consideration when analyzing the data.

**Conclusions**

In summary, beyond in vitro studies, these results uncover that plasma MAPs (PINK1, PGAM5, and Parkin) may be potentially useful target biomarkers for PD diagnosis, and PINK1 reflects disease symptoms progression closely. Studies on larger cohorts would be required to test whether elevated plasma MAP levels are related to PD risk or prediction.

**Methods**

**Standard protocol approvals, registrations, and patients consent**

All patients in this study were consecutively recruited from the First Affiliated Hospital of Wenzhou Medical University. The study was approved by the institutional Ethics Board Committee of the Wenzhou Medical University First Affiliated Hospital. All participants provided their written informed consent...
prior to joining this study.

**Study population**

See Fig. 1 for a flowchart of this study's selection process. All participants were recruited from the First Affiliated Hospital of Wenzhou Medical University between October 2018 and February 2022 and were divided into three groups (CN, PD, and PDs). Patients with PD were classified as having clinically established or probable PD based on the Movement Disorder Society Clinical Diagnostic Criteria (Postuma et al., 2015). Participants in PDs groups were defined as patients with parkinsonian syndrome including MSA (multiple system atrophy), PSP (progressive supranuclear palsy), DLB (dementia with Lewy bodies), or Vascular PD. Controls (CN) comprised outpatients or inpatients who were neurologically unaffected participants without a history of Parkinsonism, psychiatric disorders, brain trauma or stroke, and cancer et al. The participants recruited after March 2020 were labeled as cohort 1 while the rest were labeled as cohort 2.

**Diagnostic criteria for PD, PDs and CN**

All the individuals included in the study were clinically featured using standard scales that evaluated neuropsychiatric, cognitive, and movement disorder components. PD patients were diagnosed according to the MDS clinical diagnostic criteria (Postuma et al., 2015). PDs comprised MSA, PSP, DLB, or VPD. In all subjects, those who had other neurodegenerative diseases, such as Alzheimer's disease (AD), Wilson's disease (WD), acute infectious diseases, tumors, etc., were excluded. CN participants were spouses or friends of patients with PD or PDs and were in neurologically normal condition. Clinical PD and PDs diagnoses were all performed by experienced movement disorders specialty-trained neurologists.

**Neuropsychological evaluation**

Participants were analyzed using the Unified Parkinson's Disease Rating Scale (UPDRS) (Elton, 1987) and Hoehn-Yahr staging (Hoehn and Yahr, 1967) to evaluate the motor symptoms and progression stage of PD. The Chinese version of the Mini-mental State Examination (MMSE) was used for cognitive examination, with adjustment of the cutoff score for cognitive impairment according to the years of education as follows: illiterate, ≤ 17 points; primary school education, ≤ 20 points; and postsecondary education or above, ≤ 24 points (Cui et al., 2011; Katzman et al., 1988). Examination of emotional aspects was performed using the Hamilton Depression Rating Scale (HAM-D) and Hamilton Anxiety Rating Scale (HAM-A). Specifically, depression and anxiety were defined as HAMD score ≥ 17 points and HAMA score ≥ 14 points, respectively. The REM sleep behavior disorder questionnaire-Hong Kong (RBDQ-HK) was used to detect REM sleep behavior disorder (RBD), with a cutoff value of ≥ 18 points (Li et al., 2010). The Activity of Daily Living Scale (ADL) was chosen to assess the ability of participants to deal with daily affairs with tools or without tools. All the examinations were done in the "on" state of the disease.

**Measurement of plasma biomarkers**

Plasma samples were obtained through blood centrifugation (3,000×g for 10 min) and stored at − 80°C until subsequent analysis. Plasma PNK1, Parkin, PGAM5, BNIP3, p-TBK1, total a-syn, phosphorylated a-syn (p-asyn), and a-syn oligomer levels were determined using enzyme-linked immunosorbent assay (Jianglai Biotechnology Company, Shanghai, China). The detailed information of these kits as follows: No: JL11175 (PINK1), JL11195 (Parkin), JL11208 (PGAM5), JL34153 (BNIP3), JL11283 (p-TBK1), JL12231 (total a-syn), JL12598 (p-asyn) and JL41188 (a-syn oligomer), respectively. The samples were processed by a laboratory technician blinded to all clinical data following the manufacturer's instructions. Briefly, 80 µl of standard solution and 20 µl of samples (5×diluted) were pipetted into the wells of 96-well plates. Next, 100 µl of antibody-horse radish peroxidase conjugate (MyBioSource, United States) was added to standard and sample wells, which were covered using an adhesive strip and incubated for 60 min at 37°C. After washing four times, the plates were incubated in tetramethylbenzidine substrate for 15 min at 37°C. Additionally, the reactions were stopped using 2 mol/l H2SO4. The plates were read at the 450-nm wavelength. All samples were run in triplicate.

**Statistical analyses**

Continuous variables were assessed for normality using the Kolmogorov-Smirnov test, histogram, and Q-Q plot. Variables were expressed as the mean (SD) or median [IQR] and were assessed by analysis of covariance (ANCOVA) or Kruskal-Wallis test followed by Bonferroni corrected post hoc comparisons. Categorical variables were presented as numbers (percentages) and were compared using the Chi-square test followed by Bonferroni correction. The associations between plasma biomarkers and neuropsychological evaluation scales were tested using Pearson correlations and restricted cubic spline (RCS) (Desquilbet and Mariotti, 2010). RCS was fitted between biomarkers and neuropsychological scores in the PD group adjusted for age, sex, and education, with 3 knots fixed at the 10th, 50th, and 90th percentiles, and p values for nonlinearity were calculated using the Wald chi-square test. Random forest classifier (Cha et al., 2021) was performed with all the plasma biomarkers, neuropsychological scores and demographic factors ranked according to the proportion of importance (the number of decision trees was 100). Partial participants (CN = 49, PD = 90) were analyzed for the model of Random Forest and followed combined models in cohort 1 due to the lack of plasma a-syn examination, while the lack of data of plasma BNIP3 and p-TBK1 was added by multiple imputations. Then, optimal model indices were further screened using logistic regression analysis and Akaike information criterion (AIC) (Vrieze, 2012). Finally, the area under the curve (AUC) in receiver-operating characteristic (ROC) curves was used to evaluate the predictive utility of plasma biomarkers alone or combined models, and the difference in the AUC was determined using DeLong statistics. All the basic analyses were practiced in cohort 1 and the combined models were also validated in cohort 2 to see the stability of the constructed models. Statistical analyses were performed using R version 4.1 and python version 3.9. Statistical significance was set at a two-tailed p < 0.05.

**Declarations**

**CONFLICT OF INTERESTS**
No commercial or financial relationships could be construed as a potential conflict of interest.

**FUNDING**

Supported by the Projects of the National Science Foundation of China (No. 81600977 and 82271469).

**DISCLOSURES**

The authors report no disclosures relevant to the manuscript.

**References**


Figure 1

Flow chart of the study design and selection process.
Figure 2

Plasma-derived MAPs and ASPs concentrations and diagnostic accuracy across clinically defined diagnostic profiles. (a-c) Distribution of MAPs (PINK1, Parkin, and PGAM5) concentrations across clinically defined diagnostic groups; (d-f) Levels of ASPs (total a-synuclein, phosphorylated a-synuclein, and a-synuclein oligomer) in different groups. The distribution dots of MAPs (g) and ASPs (h) in three-dimensional space across three groups. The diagnosis accuracy of MAPs (i-k) and ASPs (l-n) in the context of PD vs CN, PDs vs CN, and PD vs PDs. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to CN group.
Figure 3

The receiver operating characteristic (ROC) curve analyses the diagnosis accuracy of combined MAPs to identify PD from CN participants in the modeling cohort and validated cohort. (a) The random forest can improve the performance of risk predictions based on the decision tree to identify vital features. Rank the important candidates to discriminate PD from CN using the random forest algorithm. (b) Receiver operating characteristic (ROC) curve analyses of neuropsychological domains (MMSE, HAMA, HAMD, and RBD) to differentiate PD from CN. Diagnosis accuracy of combined MAPs to identify PD from CN participants in the modeling cohort (c) and validated cohort (d).
Figure 4

Association of plasma MAPs biomarkers with motor and Nonmotor Features. (a) The linear correlation at cross-sectional between MAPs (PINK1, Parkin, and PGAM5) and neuropsychological evaluation scales, including total UPDRS, UPDRS part III, H-Y stage, MMSE, HAMD, HAMA, RBD, and ADL. (b) The linear correlation at cross-sectional between ASPs and neuropsychological evaluation scales.
Correlations between MAPs and neuropsychological domains in PD patients using the Restricted cubic spline curves (RCS) fitting model. Using non-linear smoothing spline regressions RCS model between PINK1 (a), Parkin (b), and PGAM5 (c) as a continuous value and neuropsychological evaluation scales after adjusting age, and sex.
Figure 6

Higher plasma MAP levels and prominent diagnostic accuracy in a-synuclein-positive subjects. (a) The linear correlation at cross-sectional between MAPs and ASPs. (b) Receiver operating characteristic (ROC) curve analyses of ASPs to get cut-off value. Levels of PINK1 (c), Parkin (d), and PGAM5 (e) in a-Syn (+) and a-Syn (-) subjects. Diagnosis accuracy of plasma PINK1, Parkin, and PGAM5 to differentiate PD from CN in a-Syn positivity (f) and a-Syn negativity (g) subjects, respectively, as the reference standard.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarydocuments.pdf