



## Research Article

# Plasma free amino acids in Parkinson's disease: An exploratory case–control study

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### Abstract

**Objectives:** Parkinson's disease (PD) is associated with systemic metabolic alterations; however, reproducibility and methodological standardization remain ongoing challenges in metabolomics research. This exploratory case–control study aimed to evaluate whether targeted plasma free amino acid profiling reveals statistically robust differences between PD patients and healthy controls.

**Methods:** Forty-three patients with PD and 43 age- and sex-matched healthy controls were included. Plasma free amino acids were quantified using a targeted triple quadrupole LC–MS/MS platform with Appendix 1 isotope-labeled internal standards. Between-group comparisons were performed with appropriate statistical tests. False discovery rate (FDR) correction and effect size (Cohen's d) calculations were applied. Compound-based KEGG pathway enrichment analysis was conducted using FDR-significant metabolites. ROC analyses were performed for signal strength assessment only.

**Results:** After FDR correction, alanine, arginine, aspartic acid, proline, taurine, threonine, and phenylalanine/tyrosine-related ratios remained significant, with moderate-to-large effect sizes. Compound-based KEGG enrichment demonstrated significant clustering within interconnected amino acid metabolism pathways, including arginine and proline metabolism, taurine and hypotaurine metabolism, glycine, serine and threonine metabolism, and alanine, aspartate and glutamate metabolism (pathway-level FDR <0.05). Exploratory ROC analyses showed moderate signal strength for proline (AUC=0.794), taurine (AUC=0.792), and threonine (AUC=0.780).

**Conclusion:** Targeted plasma amino acid profiling revealed coordinated systemic alterations in amino acid metabolism in PD within a statistically disciplined analytical framework. These findings reflect peripheral metabolic variation and should be interpreted as exploratory and hypothesis-generating. The study primarily contributes an analytically validated and FDR-corrected dataset to the discussion on methodological rigor in PD metabolomics, rather than evidence of diagnostic or mechanistic inference. Validation in longitudinal, clinically well-characterized cohorts is required.

**Keywords:** Alanine, amino acids, arginine, aspartic acid, free amino acids, LC–MS/MS, phenylalanine, plasma, proline, targeted metabolomics, taurine, threonine

**How to cite this article:** Eker Kurt Z, Aydin H, Figul Gokce S. Plasma free amino acids in Parkinson's disease: An exploratory case–control study. Int J Med Biochem 2026;9(2):71–77.

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized primarily by the loss of dopaminergic neurons in the substantia nigra and the presence of intracellular protein aggregates, leading to a wide range of motor and non-motor symptoms [1, 2]. The disease predominantly affects older individuals, with a prevalence of

approximately 1% in those over 60 years of age, increasing to nearly 5% in individuals older than 85 years [3]. Despite extensive research efforts, the pathophysiological mechanisms underlying PD have not been fully elucidated, and no disease-modifying therapy is currently available to halt or slow disease progression [2, 4].

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**Submitted:** November 05, 2025 **Revised:** February 24, 2026 **Accepted:** February 26, 2026 **Available Online:** April 15, 2026

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Although PD has traditionally been regarded as a central nervous system disorder, accumulating evidence indicates that it is a multisystem disease involving widespread metabolic and biochemical alterations beyond the brain [4, 5]. In this context, peripheral biofluids such as plasma have gained increasing attention as accessible matrices for exploring disease-associated metabolic alterations. Metabolomics-based approaches, in particular, have provided valuable insights into systemic metabolic disturbances associated with PD [5, 6].

Amino acids play critical roles in neurotransmitter synthesis, energy metabolism, nitrogen balance, and cellular signaling. Under physiological conditions, plasma amino acid concentrations are tightly regulated through coordinated anabolic and catabolic processes. Disruptions in amino acid homeostasis may therefore reflect altered metabolic pathways associated with neurodegeneration [7]. Several studies have reported significant alterations in amino acid levels in plasma and cerebrospinal fluid (CSF) of patients with PD compared with healthy controls, suggesting a link between amino acid metabolism and disease-related processes [6, 8].

Targeted and untargeted metabolomic investigations have demonstrated changes in multiple amino acids in PD, including alterations in alanine, arginine, glutamate, taurine, tyrosine, and threonine, as well as broader disturbances in nitrogen and energy metabolism [8–10]. These alterations have been reported in the context of several mechanisms proposed in Parkinson's disease, including mitochondrial dysfunction, oxidative stress, altered neurotransmission, and changes in protein metabolism [4, 11, 12]. However, reported findings remain heterogeneous across studies, likely due to differences in analytical platforms, biological matrices, disease stage, medication status, and clinical heterogeneity of patient populations [5, 6].

More recent studies have suggested that metabolomic patterns, including alterations in amino acids, may support future biomarker-oriented research in Parkinson's disease, particularly when combined with standardized clinical phenotyping and complementary biological matrices (e.g., cerebrospinal fluid) [11–13]. Nevertheless, the clinical relevance and reproducibility of plasma amino acid alterations require further investigation in well-characterized cohorts.

In this study, we aimed to investigate plasma amino acid alterations in patients with Parkinson's disease using a targeted LC–MS/MS approach. The objective was to provide a statistically controlled descriptive dataset of circulating amino acids in PD within an exploratory framework.

## Materials and Methods

This study was a clinical case-control study. The research was conducted in a multidisciplinary manner between Sivas Cumhuriyet University Faculty of Medicine Neurology Clinic and Department of Medical Biochemistry. The plasma free amino acid concentrations were measured in Istanbul Ahenk laboratories. A priori power analysis ( $\alpha=0.05$ ,  $1-\beta=0.80$ ) indicated

that 43 participants per group would provide 80.7% statistical power. Patients were diagnosed by experienced neurologists, and atypical parkinsonism was excluded clinically. The sample of the study was composed of patients clinically diagnosed with Parkinson's disease by experienced neurologists at Sivas Cumhuriyet University Faculty of Medicine Neurology Clinic between 01.12.2020 and 01.07.2021. Patients were recruited without any discrimination in terms of age and gender. All patients were evaluated by experienced neurologists at the Neurology Outpatient Clinic and diagnosed with Parkinson's disease based on clinical judgment, including characteristic motor symptoms and neurological examination findings. Patients with secondary parkinsonism, atypical parkinsonian syndromes, or other neurodegenerative diseases were excluded based on clinical assessment and medical history. Standardized diagnostic criteria (e.g., MDS or UK Brain Bank) were not formally documented in the dataset. Detailed clinical characteristics such as disease duration, disease stage, motor severity scores (e.g., UPDRS or Hoehn–Yahr), and dopaminergic treatment status were not systematically recorded and were therefore not included in the analysis.

## Exclusion criteria

Participants with a history of major psychiatric or neurological disorders, head trauma, acute or chronic systemic diseases (including diabetes mellitus, chronic renal failure, malignancy, and hematological disorders), or secondary parkinsonism were excluded. In addition, individuals with neurodegenerative diseases other than Parkinson's disease or those using medications that may affect cognitive function were not included.

## Ethics

Those who underwent anamnesis and physical examination and volunteers who signed the consent form created by the decision of Sivas Cumhuriyet University Faculty of Medicine Ethics Committee dated 04.09.2019 and numbered 2019-09/01 were included in the study. The study was conducted in accordance with the Helsinki Declaration.

## Taking and storing samples

Blood samples were collected under routine clinical conditions. All samples were obtained after an overnight fasting period of at least 12 hours; however, dietary protein intake, circadian variation, and recent physical activity could not be fully standardized. After overnight fasting, venous blood (~10 mL) was collected into EDTA tubes. Samples were kept at room temperature for 5–10 minutes and centrifuged at 4000 rpm for 5 minutes. The plasma supernatant was aliquoted and stored at  $-80^{\circ}\text{C}$  until LC–MS/MS analysis.

## Free amino acid measurement method

Plasma free amino acids were quantified using a targeted LC–MS/MS approach. When the required number of patients for the study was reached, all samples were removed from

–80°C and brought to room temperature. Samples were measured at once on an LC-MS/MS device using Jasem brand kits, using 20 standard amino acids and their metabolites. Analyses of plasma samples were performed on the Agilent HPLC system, consisting of a binary pump (G4220A), column chamber (G1316C), and autosampler (G4226A) coupled with a 6460 triple quadrupole mass spectrometer (6460A, Agilent Technologies, Santa Clara, CA, USA). CE-IVD (In vitro diagnostic) certified and validated Jasem Quantitative Amino Acids LC-MS/MS Analysis Kit was used for the measurement of free amino acid concentrations (Sem Laboratory). Mass spectrometry of the kit analytical method: Drying gas temperature: 150°C, Drying gas flow: 10 L/min, Nebulizer pressure: 40 psi, Sheath gas temperature: 400°C, Sheath gas flow: 10 L/min, Capillary voltage: 2000V. Appendix 1 isotope-labeled internal standards were used for each analyte. Calibration curves were generated using multi-point standard solutions, and quality control samples were analyzed at regular intervals to ensure analytical accuracy and precision.

### Statistical analysis

The obtained data were uploaded to the SPSS 22.0 package program. In the analysis of the data, frequency tables of categorical data and descriptive statistics for quantitative variables were calculated to evaluate the data set. To evaluate quantitative variables with the appropriate test, a normality test was applied using the Shapiro-Wilk test. For difference analysis of quantitative variables suitable for normal distribution, an Independent samples t-test was applied for two-category comparisons. For difference analysis of quantitative variables that do not comply with normal distribution, the Mann-Whitney U test was applied for two-category comparisons, and the Kruskal-Wallis H test was applied for comparisons involving more than two categories. If a statistically significant difference was found as a result of multiple comparisons, the Mann-Whitney U test was used to investigate which two groups caused the difference. Analysis results were interpreted at the 5% significance level. ROC analysis was performed in an exploratory manner to evaluate the discriminatory performance of selected amino acids. Age was compared using an independent samples t-test. To account for multiple comparisons across amino acids, p-values were adjusted using the Benjamini–Hochberg false discovery rate (FDR) procedure. Adjusted p-values (q-values) <0.05 were considered statistically significant. In addition to p-values, effect sizes were calculated to support the interpretation of the magnitude of group differences. Cohen's d was reported for normally distributed variables, while rank-biserial correlation (r) was used for non-normally distributed comparisons. Effect sizes were interpreted as small (0.2), medium (0.5), and large (0.8) for Cohen's d.

### Results

In the study, the age range of a total of 86 volunteers, 43 patients and 43 healthy individuals, was calculated as 32–88 years. There were 18 (42.85%) women and 25 (57.15%) men

in the control group (n=43), and 21 (48.83%) women and 22 (51.17%) men in the patient group. Age was compared using the independent samples t-test, and gender distribution was evaluated using the chi-square test. No statistically significant differences were found between the patient and control groups for age (p=0.295) or gender (p=0.790). Because multiple amino acids were tested simultaneously, p-values were adjusted for multiple comparisons using the Benjamini–Hochberg false discovery rate (FDR) procedure; metabolites that remained significant after adjustment were classified as FDR-significant, whereas those significant only at the nominal level (p<0.05) were reported as nominal findings. Results were interpreted as statistically significant at an FDR-adjusted q-value <0.05. Findings that were significant only at the nominal level (p<0.05) but not after FDR correction were reported as nominally significant. Although several amino acids showed nominal statistical differences between groups, effect size estimates indicated that only a subset of these differences reached moderate-to-large magnitude, supporting cautious interpretation of biological relevance. Effect sizes were calculated to quantify the magnitude of group differences. Cohen's d was used for normally distributed variables, whereas rank-biserial correlation was reported for non-normally distributed variables. KEGG compound-based pathway enrichment analysis identified clustering within amino acid metabolism-related pathways, including arginine and proline metabolism and alanine, aspartate, and glutamate metabolism. KEGG compound-based pathway enrichment analysis was performed using FDR-significant metabolites (alanine, arginine, aspartate, proline, taurine, and threonine). Significant clustering was observed within amino acid metabolism-related pathways. After pathway-level FDR correction, the following pathways remained statistically significant: arginine and proline metabolism (FDR=0.031), taurine and hypotaurine metabolism (FDR=0.031), glycine, serine, and threonine metabolism (FDR=0.031), alanine, aspartate, and glutamate metabolism (FDR=0.031), and arginine biosynthesis (FDR=0.031). These results indicate coordinated alterations within interconnected amino acid metabolic networks.

### Plasma amino acid levels

In our study, a normality test was applied to numerical variables. As a result of the normality test performed with the Shapiro-Wilk test, the suitability of all variables included in the analysis to normal distribution was calculated. Serine, valine, asparagine, methionine, histidine, phenylalanine, tryptophan, glycine, isoleucine, lysine, glutamine, leucine, and  $\beta$ -alanine measurements had a statistically normal distribution (p>0.05). Other variables (alanine, proline, threonine, tyrosine, aspartic acid, glutamic acid, arginine, phenylalanine/tyrosine) did not show a statistically normal distribution (p<0.05) (Table 1). After adjustment for multiple comparisons using the Benjamini–Hochberg FDR method, seven variables remained significantly different between PD patients and

**Table 1. Plasma free amino acid concentrations in Parkinson's disease patients and controls (μmol/L)**

Variable	Control (n=43) Mean±SE (min-max)	Patient (n=43) Mean±SE (min-max)	p	FDR q-value	Cohen's d
Alanine*	499.68±18.07 (246.04–782.10)	578.16±21.04 (254.38–1075.27)	0.006	0.017	0.60
Arginine*	120.39±10.39 (8.64–265.51)	83.03±3.74 (34.91–149.63)	0.001	0.005	-0.72
Asparagine	61.94±2.94 (31.10–122.29)	62.58±1.91 (29.17–87.02)	0.681	0.712	0.05
Aspartic acid*	60.23±5.76 (11.31–202.38)	34.29±2.36 (7.35–82.17)	<0.001	<0.001	-0.90
β-alanine	4.12±3.13 (0.30–4.93)	2.59±0.09 (0.52–2.87)	0.554	0.607	-0.13
Phenylalanine	110.54±5.42 (55.73–223.40)	99.89±2.95 (57.02–148.68)	0.089	0.135	-0.38
Glycine	351.83±16.52 (180.64–653.40)	372.93±17.33 (161.13–669.19)	0.383	0.466	0.19
Glutamic acid	287.54±29.61 (102.38–873.32)	216.67±11.87 (99.98–473.89)	0.030	0.069	-0.48
Glutamine	516.54±27.10 (19.53–791.39)	476.50±28.11 (31.62–781.65)	0.310	0.407	-0.23
Histidine	125.92±3.35 (67.61–166.98)	132.79±2.56 (94.13–206.96)	0.108	0.155	0.34
Isoleucine	135.28±4.70 (75.18–190.68)	145.29±3.67 (98.77–202.31)	0.184	0.256	0.27
Lysine	148.06±6.08 (84.42–211.66)	156.58±4.53 (87.86–248.61)	0.244	0.337	0.22
Leucine	134.41±3.58 (75.18–190.68)	145.28±4.66 (98.77–202.31)	0.508	0.571	0.15
Methionine	34.60±1.21 (17.97–54.69)	36.54±1.01 (19.47–52.64)	0.218	0.320	0.22
Proline*	230.96±10.54 (103.47–404.83)	329.58±14.67 (155.14–560.12)	<0.001	<0.001	1.20
Serine	219.67±8.24 (112.92–339.73)	200.27±7.65 (143.10–388.88)	0.088	0.135	-0.37
Taurine*	120.34±8.89 (47.79–293.65)	189.79±9.61 (47.82–338.74)	<0.001	<0.001	1.13
Tyrosine	82.68±4.13 (47.52–187.71)	93.35±3.52 (54.93–166.72)	0.047	0.093	0.42
Threonine*	164.44±6.31 (91.97–274.10)	209.28±7.13 (73.04–316.90)	<0.001	<0.001	1.00
Tryptophan	69.77±2.42 (39.60–103.27)	74.36±2.69 (27.74–118.55)	0.210	0.320	0.22
Valine	267.46±10.14 (110.39–400.82)	267.90±9.52 (131.20–396.76)	0.970	0.970	0.01
Phenylalanine/tyrosine*	1.345±0.070 (0.420–2.460)	1.110±0.038 (0.620–1.950)	0.002	0.009	-0.66
Tyrosine/phenylalanine*	0.747±0.050 (0.500–2.250)	0.935±0.045 (0.510–1.740)	0.003	0.010	0.64

\*: Non-normally distributed variables analyzed using non-parametric tests. Values are presented as mean±standard error (SE) and minimum–maximum (min–max). p-values are from group comparisons; q-values indicate false discovery rate (FDR)-adjusted significance. Effect size is reported as Cohen's d.

controls: alanine (higher in PD), arginine (lower in PD), aspartic acid (lower in PD), proline (higher in PD), taurine (higher in PD), threonine (higher in PD), as well as the phenylalanine/tyrosine and tyrosine/phenylalanine ratios (both significantly altered). Other amino acids did not show statistically significant differences after FDR correction and were considered nominal. Glutamic acid showed a nominal difference between groups ( $p=0.030$ ); however, this association did not remain statistically significant after false discovery rate (FDR) correction and was therefore interpreted as exploratory.

### ROC analysis of selected amino acids

Exploratory ROC curve analyses were performed for amino acids showing nominal between-group differences to assess their discriminatory performance. Proline (AUC=0.794, 95% CI: 0.703–0.884), taurine (AUC=0.792, 95% CI: 0.697–0.886), and threonine (AUC=0.780, 95% CI: 0.685–0.876) demonstrated moderate discrimination. Alanine (AUC=0.664, 95% CI: 0.552–0.777) and tyrosine (AUC=0.621, 95% CI: 0.506–0.736) showed limited discrimination, while glutamic acid showed poor discrimination (AUC=0.558, 95% CI: 0.436–0.681). Amino acids with AUC<0.5 (aspartic acid and arginine) were considered to show inverse discrimination and were not interpreted as clinically meaningful. These ROC analyses were

hypothesis-generating only and were not intended to establish diagnostic validity, particularly in the absence of external validation (Table 2). Amino acids with AUC<0.5 (aspartic acid and arginine) were considered to show inverse discrimination and are presented in Appendix 1.

### Discussion

This study provides an exploratory cross-sectional comparison of plasma free amino acid concentrations between patients with Parkinson's disease (PD) and healthy controls. The findings should be interpreted as hypothesis-generating, as detailed clinical phenotyping (e.g., disease duration, severity scales, dopaminergic treatment status) and comprehensive pre-analytical standardization were not fully available. Therefore, the observed alterations cannot be interpreted as disease-specific metabolic signatures or as evidence of diagnostic utility.

Parkinson's disease is a progressive neurodegenerative disorder characterized by the degeneration of dopaminergic neurons [2]. Although amino acids are capable of crossing the blood–brain barrier [11], and plasma–CSF concentration ratios have been described, plasma amino acid levels do not directly reflect central neurotransmitter concentrations.

**Table 2. ROC curve analysis of plasma amino acids for exploratory discrimination of Parkinson's disease**

Amino acid	Cut-off ( $\mu\text{mol/L}$ )	Sensitivity	Specificity	p	AUC	95% CI	
						Lower	Upper
Alanine	652.86	0.191	0.045	0.007	0.664	0.552	0.777
Proline	380.09	0.298	0.023	<0.001	0.794	0.703	0.884
Threonine	258.67	0.191	0.023	<0.001	0.780	0.685	0.876
Glutamic acid	157.57	0.744	0.787	0.030	0.558	0.436	0.681
Tyrosine	133.03	0.085	0.023	0.047	0.621	0.506	0.736
Taurine	259.54	0.149	0.023	<0.001	0.792	0.697	0.886

Cut-off values are presented in  $\mu\text{mol/L}$ . ROC analyses were performed for exploratory purposes only and do not indicate diagnostic validity. CI: confidence interval; AUC: Area under the curve.

Cerebrospinal fluid (CSF) amino acid measurements may provide more direct insight into neurochemical alterations; however, CSF sampling was not feasible in the present cohort due to ethical and participant-related constraints. Consequently, mechanistic interpretations at the central nervous system level remain limited.

Several previous metabolomic investigations have reported altered amino acid profiles in PD. Decreased plasma levels of alanine, aspartic acid, arginine, phenylalanine, glutamic acid, histidine, isoleucine, leucine, lysine, methionine, serine, taurine, tyrosine, threonine, tryptophan, and valine, along with increased glutamine, have been described in certain cohorts [8–10, 13]. However, findings across studies are inconsistent. Previous studies have reported heterogeneous alanine-related findings in Parkinson's disease, including stage-related serum differences and inconsistent blood and cerebrospinal fluid (CSF) results [14–19]. For instance, Molina et al. [17] reported decreased CSF alanine levels and reduced CSF/plasma ratios, while the reduction in plasma alanine did not reach statistical significance. In our cohort, plasma alanine levels were significantly increased ( $p=0.006$ ). These discrepancies across studies may reflect differences in analytical platforms, biological matrices, cohort characteristics, medication status, and disease heterogeneity. Disturbances in aromatic amino acid metabolism, including tyrosine- and phenylalanine-related changes, have also been reported in PD [20, 21]. In our study, tyrosine-related alterations were observed, although peripheral measurements cannot be assumed to reflect dopaminergic neurotransmission directly. Similarly, previous studies have described inconsistent findings regarding aspartate and glutamate levels in PD, with both increases and decreases reported across plasma/serum and CSF studies [15, 16, 18, 19]. In the present analysis, plasma aspartic acid and glutamic acid levels were significantly decreased. Given the absence of CSF or functional neurochemical data, these alterations should be interpreted as peripheral metabolic variation rather than central excitotoxic mechanisms.

Arginine metabolism has also been implicated in neurodegenerative processes. Prior studies and meta-analytic evidence suggest that arginine-related alterations may occur in Parkinson's disease, although findings vary across biological matrices and cohorts [14, 19]. In line with these observa-

tions, plasma arginine levels were significantly decreased in our cohort ( $p=0.001$ ). Taurine alterations have been variably reported; Engelborghs et al. [22] reported lower CSF taurine levels in PD, whereas in our cohort taurine levels were significantly increased. These divergent findings further underscore the heterogeneity of amino acid alterations in PD populations. In a previous study, reduced plasma levels of aspartate (Asp), glutamate (Glu), and taurine (Tau) were reported in early Parkinson's disease, and ROC analyses suggested potential discriminatory value for these amino acid neurotransmitters, with AUC values of 0.871, 0.882, and 0.845, respectively [15]. In our study, corresponding AUC values were lower (0.283, 0.558, and 0.792). Several amino acids demonstrated AUC values below 0.5, indicating poor or inverse discriminatory performance. ROC analyses in the present study were conducted solely to assess signal magnitude and should not be interpreted as evidence of diagnostic or biomarker validity, particularly in the absence of external validation cohorts or clinically anchored endpoints. Figura et al. [14] reported stage-related changes in serum threonine levels in PD. In our cohort, threonine levels were significantly elevated ( $p<0.001$ ). While alterations in threonine and related amino acids may reflect systemic changes in protein or intermediary metabolism, direct mechanistic links to neurodegeneration require validation in studies incorporating CSF, imaging, or tissue-based analyses.

In essential tremor, altered plasma aspartate, glutamate, and taurine levels have also been described [23].  $\beta$ -alanine is biochemically related to inhibitory neurotransmission and amino acid metabolism [24]. In our cohort, aspartic acid levels were significantly decreased, while  $\beta$ -alanine showed a non-significant reduction. These observations may indicate coordinated alterations within related metabolic pathways; however, without enzymatic, transcriptomic, or central nervous system-specific data, such interpretations remain speculative. Glutamate-related pathways have frequently been discussed in the context of PD pathophysiology. However, since the majority of glutamic acid is utilized within tissues and peripheral concentrations are relatively low, plasma measurements cannot be assumed to mirror synaptic glutamatergic transmission. Therefore, peripheral amino acid alterations should not be extrapolated directly to central neurotransmission [25–27].

Overall, this study adds to the growing body of targeted LC–MS/MS-based investigations evaluating plasma amino acid alterations in PD. The findings provide descriptive evidence of coordinated peripheral metabolic variation. However, given the cross-sectional design, plasma-only measurements, and limited clinical characterization, the results should be regarded as exploratory and hypothesis-generating rather than definitive.

### Limitations

This study has several limitations. First, the cross-sectional case–control design precludes causal inference and does not allow longitudinal assessment of metabolic changes. Second, detailed clinical phenotyping including disease duration, diagnostic criteria (e.g., MDS or UK Brain Bank), Hoehn–Yahr stage, UPDRS scores, and dopaminergic treatment status was not retained in the analytical dataset, limiting stratified interpretation. Third, although samples were collected under standardized fasting conditions, additional pre-analytical variables such as dietary protein intake, circadian variation, and recent physical activity were not systematically controlled. Fourth, only plasma samples were analyzed; CSF, urine, or imaging biomarkers were not available. Fifth, the targeted LC–MS/MS platform covered a limited metabolite panel, constraining pathway-level inference. Finally, multiple comparisons were performed and ROC analyses were exploratory; therefore, findings should be interpreted cautiously and require confirmation in independent, clinically well-characterized cohorts before any clinical relevance can be inferred.

### Conclusion

In this exploratory cross-sectional case-control study, targeted LC–MS/MS profiling identified statistically significant alterations in selected plasma free amino acids in patients with Parkinson's disease compared with healthy controls. The observed differences were consistent with previously reported metabolic heterogeneity in PD, although the direction and magnitude of specific amino acid changes varied across studies.

Given the absence of detailed clinical phenotyping, standardized diagnostic documentation, and complementary cerebrospinal fluid or functional measurements, the present findings should be interpreted as descriptive and hypothesis-generating. Peripheral plasma amino acid alterations cannot be directly extrapolated to central neurochemical processes, nor do the current results support diagnostic, prognostic, or treatment-guiding application.

The primary contribution of this study is the provision of a targeted, analytically validated plasma amino acid dataset generated under a standardized LC–MS/MS framework. Independent replication in larger, clinically well-characterized, and longitudinal cohorts is required before biological or clinical implications can be established.

### Disclosures

**Ethics Committee Approval:** The study was approved by the Sivas Cumhuriyet University Faculty of Medicine Ethics Committee (no: 2019-09/01, date: 04/09/2019).

**Informed Consent:** Written informed consent was obtained.

**Conflict of Interest Statement:** None declared.

**Funding:** This study was supported by the Scientific Research Projects (BAP) Unit of Sivas Cumhuriyet University within the scope of a doctoral thesis project (Project No: T901).

**Use of AI for Writing Assistance:** None declared.

**Authorship Contributions:** Concept – Z.E.K., H.A., S.F.G.; Design – Z.E.K., H.A., S.F.G.; Supervision – H.A.; Resource – Z.E.K.; Materials – Z.E.K., S.F.G.; Data collection and/or processing – Z.E.K.; Analysis and/or interpretation – Z.E.K., H.A., S.F.G.; Literature review – Z.E.K.; Writing – Z.E.K.; Critical review – H.A.

**Peer-review:** Externally peer-reviewed.

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**Appendix 1. ROC analyses with inverse discrimination (AUC<0.5)**

Amino acid	Cut-off ( $\mu\text{mol/L}$ )	Sensitivity	Specificity	p	AUC	95% CI	
						Lower	Upper
Aspartic acid	19.45	0.872	0.818	<0.001	0.283	0.172	0.395
Arginine	54.13	0.936	0.773	0.019	0.357	0.230	0.484

AUC <0.5 indicates inverse discrimination and was not considered clinically meaningful. CI: confidence interval; AUC: Area under the curve.