Tng *et al.*
Translational Medicine Communications (2024) 9:12
https://doi.org/10.1186/s41231-024-00169-9

Translational Medicine Communications

REVIEW

Open Access

Systematic analysis of multi-omics data reveals component-specific blood-based biomarkers for Parkinson's disease

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Abstract

Parkinson's disease (PD) is a prevalent neurodegenerative disorder affecting millions of elderly individuals worldwide. Clinically, PD is diagnosed based on the presentation of motoric symptoms. Other methods such as F-DOPA PET scan or α-Synuclein detection from the cerebral spinal fluid are either too expensive or invasive for routine use. Omics platforms such as transcriptomics, proteomics, and metabolomics may identify PD biomarkers from blood, which can reduce cost and increase efficiency. However, there are many biological moieties being measured and issues with false positives/negatives. It is also unknown which omics platform offers most useful information. Therefore, it is important to assess the reliability of these omics studies. Here, we shortlisted and analysed nearly 80 published reports across transcriptomics, proteomics and metabolomics in search of overlapping blood-based biomarkers for PD. The top biomarkers were reported across 29%, 42% and 12.5% of shortlisted papers in transcriptomics, proteomics and metabolomics respectively. These percentages increased to 42%, 60% and 50% accordingly when studies were grouped by specific blood subtypes for analysis, demonstrating the need for test kits to be blood-subtype specific. Following systematic analyses, we propose six novel PD biomarkers: two mRNAs (Whole blood, WB) – Arg1 and SNCA, two proteins (Plasma EV) – SNCA and APOA1, and two metabolites (WB) – 8-OHdG and uric acid for further validation. While these proposed biomarkers are useful, they are also snapshots, representing subsets of larger pathways of origin where the different omics levels corroborate. Indeed, identifying the interconnections across different biological layers can strengthen contextual reasoning, which in turn, would give rise to better quality biomarkers. Knowledge integration across the omics spectrum revealed consistent aberrations on the same neuroinflammation pathway, showcasing the value of integrative (i)-omics agreements for increasing confidence of biomarker selection. We believe that our findings could pave the way for identifying reproducible PD biomarkers, with potential for clinical deployment.

Keywords Multi-omics, Parkinson's, Biomarker, Blood-subtype

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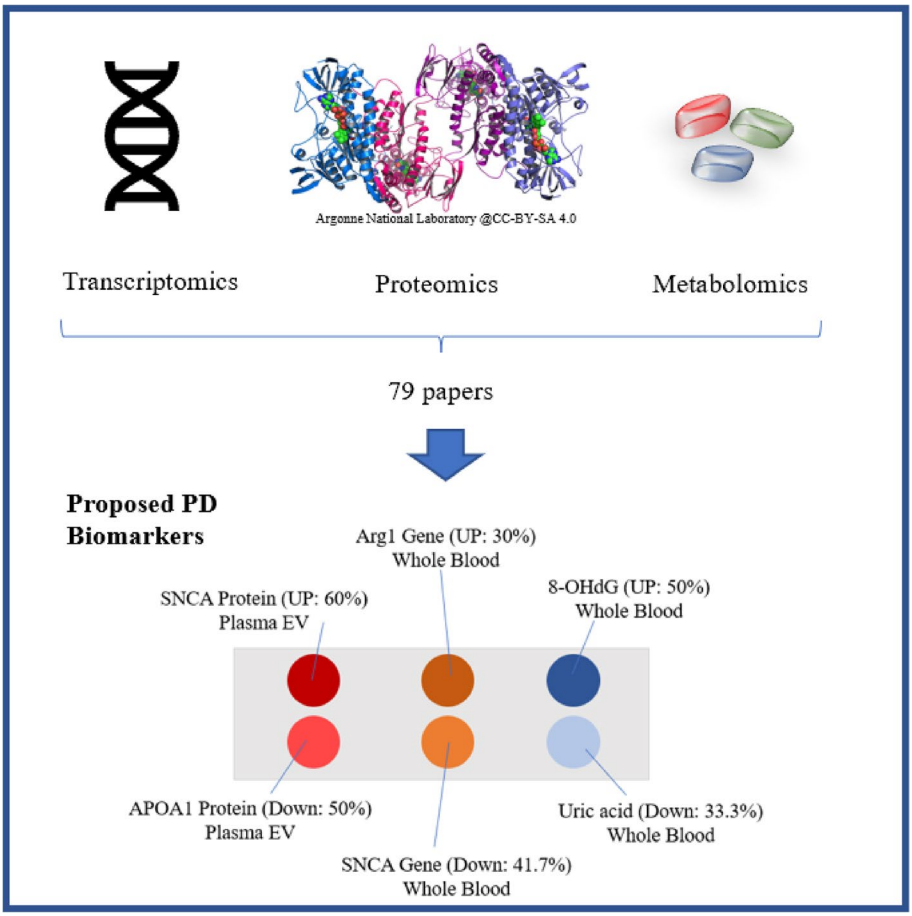
Graphical Abstract

Six Proposed blood-based biomarkers. Seventy-nine publications across transcriptomics, proteomics and metabolomics were shortlisted and analysed for reported biomarkers. The proposed biomarkers are SNCA, APOA1, Arg1, 8-OHdG and Uric acid.



Background: Parkinson's Disease and Current Diagnostic Tools

Parkinson's disease (PD) is a prevalent neurological disorder [1] characterized by the loss of dopaminergic neurons in the substantia nigra pars compacta of the midbrain [2]. Since its discovery in 1817, the incidence rates of PD have increased by 10 times over the past nine decades [3]. PD prevalence is strongly associated with the elderly, a phenomenon that aligns with the notion that age is a major risk factor for the development of PD [4]. As the global population continues to age rapidly, PD poses a significant threat in deteriorating the quality of life for many afflicted individuals as well as their caregivers.

PD is a progressive neurodegenerative disease typically diagnosed via neurological examination for classical motor symptoms such as bradykinesia and resting tremors [5]. Clinically, PD diagnosis is usually conducted using the Unified Parkinson's Disease Rating Scale (UPDRS), which assesses an individuals' PD-related motor and non-motor deficits such as rigidity and olfactory dysfunction [6]. Additionally, the UPDRS score is used to track disease progression, with increasing score indicating worsening of PD disability [7]. However, even with clinical markers, the misdiagnosis rate for PD remains high at 42% [8, 9]. Clearly, less subjective phenotypic biomarkers would be helpful to improve PD diagnosis. To reduce subjectivity in PD diagnosis, brain imaging tools such as Magnetic resonance imaging (MRI) and Positron Emission Tomography (PET) have been used as a supplement and have shown potential to achieve a reliable diagnosis for parkinsonism. In particular, the

123I-ioflupane DaTSCAN was approved by the US FDA in 2011 for doctors to confirm a PD diagnosis [10]. However, these imaging tools are expensive to conduct, and a negative result does not absolutely rule out PD [11, 12]. Therefore, a cheaper but feasible alternative is needed for PD diagnosis.

Recently, mounting evidence suggested that biofluids can reflect the pathophysiology of PD [13–15]. This, in part, is fuelled by the Braak's hypothesis that sporadic PD begins via the olfactory or gastrointestinal system before affecting the central nervous system, suggesting that PD is not confined to the brain [16]. Biofluids such as urine, blood, cerebrospinal fluid (CSF) and tear fluid have been studied for the metabolites of dopamine since PD is driven by the loss of dopaminergic neurons. CSF analysis could be a reliable prognostic tool as it better mirrors pathological changes of the brain [13, 17]. However, collecting CSFs is invasive and is not practically suitable in clinical settings for suspected cases of PD [13]. Hence, this had led to the investigations of using non-invasive blood-based biomarkers such as serum, whole blood, and exosomes to facilitate PD diagnosis. With the rise of sequencing technologies, many studies have used omics to analyse diverse biological modalities of the genome, transcriptome, proteome, and metabolome in the blood of PD patients, with the view that they may provide valuable insights into the etiology of PD. However, current studies tend to be within the respective omics field and lacks cross-platform corroborations. This was demonstrated by Redenšek et al. where only 4% (5 out of 107 papers examined) of PD-related omics study from 2005 to 2017 were integrative [18]. The lack of corroboration represents an important research gap. The overall contributory value of each individual study can be greatly enhanced by careful data mining and knowledge integration, to demonstrate how functionally coherent (or discordant) the targets reported across the omics spectrum are. Integrating multi-layered studies and identifying corroborations could reduce false-positive and false-negative results. It could also strengthen our contextual reasoning and understanding of the interconnections across different biological layers, thus deepening our insights into PD prognosis. To achieve this, we examined a total of 79 papers spanning transcriptomics, proteomics, and metabolomics, which revealed six blood-based biomarkers suitable for PD diagnosis.

Individual omics platform demonstrate diagnostic potential with improved agreement on biomarkers across studies for specific blood components

Advances in high-throughput "omics" technologies provides new ways of studying diseases. Omics refers to a field of biological study that has the suffix -omics, which include genomics, transcriptomics, proteomics, metabolomics and more recently microbiomics [19, 20]. Omics technologies provide insight not only at single biological moieties, but also at higher order functional structures such as biological mechanisms or pathways critical for initiating various diseases. For example, genome-wide association studies (GWAS) have identified common PD risk loci consisting of *PARK16*, *ITPKB*, *MCCC1*, *SNCA*, *FAM47E-SCARB2*, *DLG2*, *LRRK2*, *RIT2* and *FYN* [21]. Common risk loci like *SNCA* provides a handle for researchers to investigate the pathology of PD, for instance alternative splicing of SNCA risk loci can result in a SNCA112 transcript that results in SNCA proteins that are structurally more prone to aggregation [22]. The aggregation of α-synuclein protein into Lewy bodies is the histopathological hallmark of PD [23]. Through omics studies, we also now know that LRRK2 interacts with many other important proteins and play a central role in pathways underlying PD [24].

To exploit omics for potential blood-based biomarkers, we gathered papers about PD spanning transcriptomics, proteomics and metabolomics studies via PubMed or Google Scholar between 2015 to 2021. The screening criteria is summarised by the PRISMA flow diagram (Fig. 1). Briefly, in our predefined timeframe, the search engine pairings of "Parkinson" and "Metabolomics" in PubMed yielded 260 results, while "Parkinson" and "Proteomics" yielded 568 results, whereas "Parkinson" with "Transcriptomics" yielded 104 results. The search results suggest that proteins are most popularly studied in the field of PD, which reflects the widely accepted role of misfolded protein aggregates in PD pathogenesis. As our focus is on blood-based biomarkers, we finetuned our search terms accordingly. The following final combinations of search terms were used: "PD", "Parkinson's Disease", "Blood" / "Blood-based biomarkers", "Plasma EV", "Serum EV" with "Transcriptomics" or "mRNA", "Proteomics" or "Proteins", "Metabolomics" or "Metabolites" for the respective omics. Only original research articles were selected. Review papers were examined for the original research articles that were cited so as to avoid double counting, thus resulting in some of the older original research articles being included in this analysis. This resulted in 18 transcriptomics- [25–42], 34 proteomics- [14, 43–74] and 27 metabolomics-related papers [46, 59, 72, 75–99], with a total of 6 different blood subtypes covered across the 79 papers. These included whole blood (24.0%), peripheral blood mononuclear cell (6.3%), serum (16.5%), plasma (26.6%), plasma extracellular vesicle (EV) (15.2%) and serum EV (11.1%). Interestingly, transcriptomic studies tend to focus on whole blood samples (66.7%), while

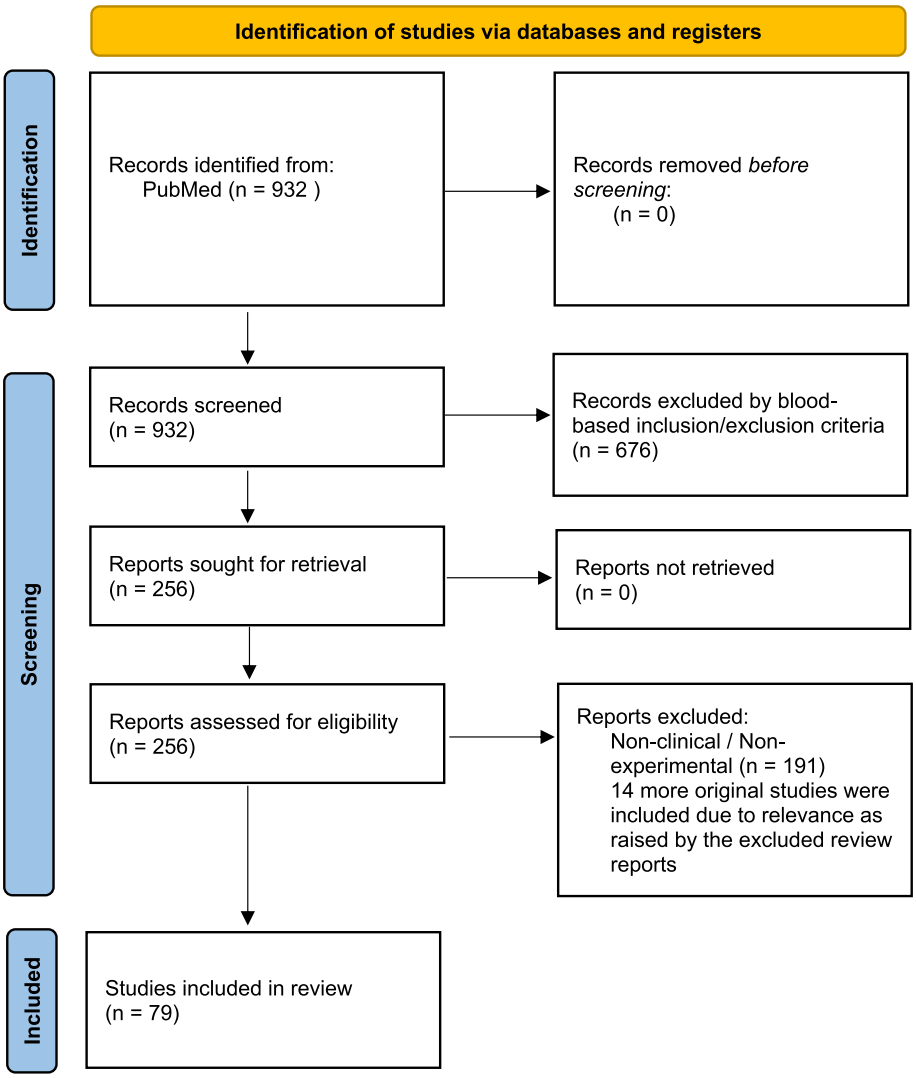


Fig. 1 PRISMA 2020 flow diagram for new systematic reviews. The screening process of 932 papers on PubMed related to Parkinson, Transcriptomics, Proteomics and Metabolomics to a final 79 papers for review that are blood-based specific

proteomic studies focused on EVs (55.9%) and metabolomic studies on plasma (44.4%).

Individually, omics technology reveals some potential for identifying risk factors, diagnostic biomarkers and therapeutic targets for PD as summarized in Supplementary Table 1, 2, 3 and 4 across the 79 papers we examined. For each of the 18 transcriptomics-, 34 proteomics-, 27 metabolomics-related papers, we noted the list of reported targets and examined the agreement rate of reported targets across papers within each omics field. For each transcript, protein or metabolite, agreement rate is defined as the number of papers that reported it as biomarker divided by the total number of papers examined in the corresponding omics field. The highest agreement rate was 29% amongst papers publishing transcriptome signatures between 2007–2021, 42% amongst papers publishing proteome signatures between 2009–2021 and 12.5% amongst papers publishing metabolite signatures between 2009–2021 (Table 1). For each omic field, we further subdivided the transcript, protein or metabolite into the blood

Table 1 Comparison of highest agreement rates across all papers within transcriptomics, proteomics and metabolomics. Percentage agreement increased with specificity of blood component

	Transcriptomics	Proteomics	Metabolomics
ALL Blood types	29%	42%	12.5%
Blood Type-Specific	42%	60%	50%

subtypes (ie. Serum, plasma, EV etc.) that they were reported in and calculated the agreement rate within the blood subtypes. Importantly, the highest agreement rates increased to 42%, 60% and 50% for transcriptomics, proteomics and metabolomics respectively, when we grouped the papers by specific blood subtypes. This suggests that different blood subtypes reflect varying differential changes to PD that can be used as biomarkers and future studies should be specific about the component of blood being examined.

Multi-omics analysis suggests 6 blood component specific parkinson's disease biomarkers

Genetic analysis of biomarkers suggests the use of Arg1 and SNCA gene in whole blood as potential blood-based mRNA biomarkers of PD

We included 15 studies that looked at gene upregulation and 17 studies that looked at gene downregulation across distinct blood subgroups (Table 2). Across all studies, commonly reported upregulated genes include Arginase 1 (ARG1) (26.67%) and Thrombomodulin (THBD) (26.7%). These genes were also reported in papers specific for whole blood. Interestingly, Arg1, an anti-inflammatory marker for anti-inflammatory microglia polarisation, is reported to be supressed in MPP + PD model [100] and affected by micro-RNA miR-155 in AAV2-SYN PD model [101]. Given the role of neuroinflammation and microglia polarisation [102] in PD, we selected Arg1 as a candidate biomarker. On the other hand, the most downregulated gene across all papers is SNCA (29.4%). SNCA is also the most reported downregulated gene in papers on whole blood (42%).

Protein analysis of the various blood subtypes suggest use of SNCA and APOA1 in plasma EV as potential protein biomarkers of PD

We identified 31 papers studying protein upregulation across various blood subtypes and 18 papers studying protein downregulation suitable for our intersection analysis (Table 3). The more commonly reported upregulated proteins across all blood subtypes in these papers are SNCA (42%), MAPT (10%), TTR (10%) and VWF (10%) (Table 3). When examining the specific blood subtypes, SNCA is still the most reported upregulated protein in plasma EV (60%), serum EV (57%) and plasma (25%). The more commonly reported downregulated proteins across all blood subtypes in the 18 papers are APOA1 (16.7%), FGG (16.7%), IGKV3-20 (16.7%) and SNCA (16.7%). Surprisingly, SNCA protein that is most reported as upregulated is also reported to be downregulated by other studies [53, 55, 73]. Although it seems puzzling to observe downregulation in αSYN gene expression and upregulation in SNCA protein, various explanations have been proposed. For example, a downregulation in αSYN gene could be induced by upregulation in DNA methylation in the CpG sites that lead to the exhibition of PD phenotypes [103]. Contrastingly, an upregulation of SNCA protein could be observed after autophagy-lysosomal pathway failure, where low-aggregated SNCA will predominantly be released via exosomes, in line with our observations of increased SNCA reported in plasma EVs

Table 2 Analysis of top transcriptomic hits categorised by blood subtypes

ALL UP (15)			WB UP (10)			PBMC UP (3)			Serum UP (2)		
Name	**Freq**	**percentage**	**Name**	**Freq**	**percentage**	**Name**	**Freq**	**percentage**	**Name**	**Freq**	**percentage**
ARG1	4	26.66667	THBD	4	40	ALAS2	1	33.33333	ADAP2	2	100
THBD	4	26.66667	ADARB2	3	30	AP1S2	1	33.33333	ATAT1	2	100
ADARB2	3	20	ALOX5A	3	30	ARG1	1	33.33333	CCL19	2	100
ALOX5A	3	20	ARG1	3	30	ARSB	1	33.33333	IL20	2	100
ARHGAP	3	20	ARHGAP	3	30	ASAH1	1	33.33333	MAPKAP	2	100
ATP6V0D	3	20	BASP1	3	30	Atg7	1	33.33333	MOB3C	2	100
BASP1	3	20	CLIC3	3	30	ATP6V0D	1	33.33333	MRPL19	2	100
CLIC3	3	20	GPX3	3	30	CD68	1	33.33333	RPS18	2	100
GPX3	3	20	GSTM3	3	30	CLTCL1	1	33.33333	BRSK1	1	50
GSTM3	3	20	IDS	3	30	CTSA	1	33.33333	BUD31	1	50

ALL Down (17)			WB Down (12)			PBMC Down (5)		
Name	**Freq**	**percentage**	**Name**	**Freq**	**percentage**	**Name**	**Freq**	**percentage**
SNCA	5	29.41176	SNCA	5	41.66667	NURR1	2	40
LRRN3	4	23.52941	LRRN3	4	33.33333	ALAS2	1	20
ALAS2	3	17.64706	CNTNAP	3	25	AMBRA1	1	20
CNTNAP	3	17.64706	CTSE	3	25	APAF1	1	20
CTSE	3	17.64706	FCER2	3	25	ARHGAP	1	20
FCER2	3	17.64706	FCRL2	3	25	ARPC5L	1	20
FCRL2	3	17.64706	HBG1	3	25	ATG12	1	20
HBD	3	17.64706	IGFBP2	3	25	ATG16L1	1	20
HBG1	3	17.64706	ITLN1	3	25	ATG2	1	20
HBM	3	17.64706	KCNH8	3	25	ATG4B	1	20

Table 3 Analysis of top proteomic hits categorised by blood subtypes

ALL UP (31)

Name	Freq	percentage
SNCA	13	41.93548
MAPT	3	9.677419
TTR	3	9.677419
VWF	3	9.677419
AGT	2	6.451613
APCS	2	6.451613
Aß1–42	2	6.451613
CALM3	2	6.451613
CLU	2	6.451613
CRP	2	6.451613
F2	2	6.451613

Plasma EV UP (10)

Name	Freq	percentage
SNCA	6	60
CALM3	2	20
HLA-B	1	10
HLA-C	1	10
ABCA7	1	10
ABCB6	1	10
ACTN1	1	10
ACTN2	1	10
ACTN3	1	10
ACTN4	1	10
ADD1	1	10

Serum EV UP (7)

Name	Freq	percentage
SNCA	4	57.14286
AGT	2	28.57143
CRP	2	28.57143
FGB	2	28.57143
IGKV3-2(	2	28.57143
VWF	2	28.57143
COL1A2	1	14.28571
ACTB	1	14.28571
AHSG	1	14.28571
APCS	1	14.28571
APOA1	1	14.28571

Plasma UP (8)

Name	Freq	percentage
SNCA	2	25
APCS	1	12.5
APOE	1	12.5
Aß1–42	1	12.5
C3	1	12.5
CHI3L	1	12.5
CHL1	1	12.5
DEFA1	1	12.5
F2	1	12.5
FN1	1	12.5
FN1-10	1	12.5

Serum UP (5)

Name	Freq	percentage
TTR	2	40
APOA4	1	20
APOH	1	20
AZGP1	1	20
C4B	1	20
CF1	1	20
CFH	1	20
CLU	1	20
FGG	1	20
FIGNL1	1	20
FIGNL2	1	20

WB UP (1)

Name	Freq
Aβ1–42	1
MAPT	1

ALL DOWN (18)

Name	Freq	percentage
APOA1	3	16.66667
FGG	3	16.66667
IGKV3-2(	3	16.66667
SNCA	3	16.66667
C1R	2	11.11111
C4B	2	11.11111
CRLF3	2	11.11111
EPB41	2	11.11111
F2	2	11.11111
IGHG1	2	11.11111

Plasma EV DOWN (2)

Name	Freq	percentage
CRLF3	2	100
EPB41	2	100
FGG	2	100
PDIA3	2	100
RAD23A	2	100
ABCC1	1	50
ABCC4	1	50
ABCG2	1	50
ABHD14I	1	50
ACAT2	1	50

Serum EV DOWN (5)

Name	Freq	percentage
IGKV3-2(	3	60
IGHG1	2	40
A2M	1	20
ADIPOQ	1	20
ANPEP	1	20
ATP5A	1	20
C1QB	1	20
C1QC	1	20
C1R	1	20
C1S	1	20

Plasma DOWN (6)

Name	Freq	percentage
ACY1	1	16.66667
ANTXR2	1	16.66667
APOA1	1	16.66667
B2M	1	16.66667
BAG2	1	16.66667
CFH	1	16.66667
CR2	1	16.66667
CTSD	1	16.66667
F8	1	16.66667
FGG	1	16.66667

Serum DOWN (4)

Name	Freq	percentage
ALB	1	25
AMBP	1	25
APOA1	1	25
APOA4	1	25
APOE	1	25
DYNC1H	1	25
F2	1	25
HP	1	25
HPR	1	25
HSA	1	25

WB DOWN (1)

Name	Freq
SNCA	1

ALL Pathway UP (31)

Name	Freq	percentage
Parkinson	15	48.3871
Pathways	15	48.3871
Alzheimer	14	45.16129
Compleme	5	16.12903
ECM-rece	5	16.12903
Coronavir	4	12.90323
Nil	4	12.90323
Staphyloc	4	12.90323
MAPK sig	3	9.677419
Neutrophi	3	9.677419

ALL Pathway Down (18)

Name	Freq	percentage
Compleme	6	33.33333
African try	4	22.22222
Parkinson	4	22.22222
Pertussis	4	22.22222
Staphyloc	4	22.22222
Cholesterc	3	16.66667
Coronavir	3	16.66667
Fat digesti	3	16.66667
Nil	3	16.66667
Pathways	3	16.66667

[104]. Nonetheless, we selected SNCA as our biomarker of choice since its expression is reported by most as being affected in PD. We have also chosen APOA1 alongside SNCA as a downregulated biomarker for ease of testing using a common plasma EV blood subtype and also due to its relation to PD such as risk of having mild cognitive impairment as reported by other literatures [105–107].

Metabolites analysis of various blood subtypes suggests 8-OhDG markers for PD patients

We studied 26 and 24 papers on metabolites upregulation and downregulation in PD patients, respectively. The more commonly reported downregulated metabolites in PD patients are Uric acid (12.5%), Catechol sulfate (8.33%) and Cis-aconitic acid (8.33%) (Table 4). When examining the specific blood subtypes, the percentage for uric acid increased from 12.5% to 33% in whole blood. On the other hand, the more frequently reported upregulated metabolites across all studies are Proline (11.5%), 8-OhDG (7.69%) and Alanine (7.69%) (Table 4). 8-OhDG was chosen as our choice of upregulated biomarker for ease of testing using a common whole blood subtype and also due to its relation to PD via oxidative stress as reported by others [108, 109].

Table 4 Analysis of top metabolomic hits categorised by blood subtypes

Up Whole blood - 4 papers

Name	Freq	percentage
8-OHdG	2	50
13-hydro	1	25
Arachido	1	25
Cholester	1	25
Ferritin	1	25
Glutathio	1	25
MDA	1	25
Nitrite	1	25
Stearic ac	1	25

Serum - 7 papers

Name	Freq	percentage
Ornithin	2	28.6
Phosphoe	2	28.6
Proline	2	28.6
Tyrosine	2	28.6
1-methyll	1	14.3
3-hydroxy	1	14.3
3-hydroxy	1	14.3
3-hydroxy	1	14.3
Aliphatic	1	14.3
Biliverdir	1	14.3

Blood plasma - 11 papers

Name	Freq	percentage
Bile acid	2	18.2
Carnitine	2	18.2
LysoPC(1	2	18.2
Succinate	2	18.2
Alanine	2	18.2
1,3-Dime	1	9.09
2-Octenoi	1	9.09
2-oxoisoc	1	9.09
3-carboxy	1	9.09
3-ketosph	1	9.09

Plasma EV -1 paper

Name	Freq	percentage
ex-miR-3	1	100
miR-125a	1	100
miR-137	1	100
miR-181c	1	100
miR-193a	1	100
miR-331-	1	100
miR-454	1	100
miR196-p	1	100

Serum EV -1 paper

Name	Freq	percentage
ex-let-7d	1	100
ex-miR-2	1	100
ex-miR-2	1	100
miR-29a	1	100
ex-miR-2	1	100
ex-miR-1	1	100
ex-miR-2	1	100
ex-miR-1	1	100

ALL - Total 26 papers

Name	Freq	percentage
Proline	3	11.5
8-OHdG	2	7.69
Alanine	2	7.69
Bile acid	2	7.69
Carnitine	2	7.69
LysoPC(1	2	7.69
Ornithin	2	7.69
Phosphoe	2	7.69
Succinate	2	7.69
Tyrosine	2	7.69

Down Whole blood - 6 papers

Name	Freq	percentage
Uric acid	2	33.3
12-hydro	1	16.7
17,18-dih	1	16.7
Acetate	1	16.7
Amino m	1	16.7
Ascorbat	1	16.7
Br-GSH	1	16.7
Butanoic	1	16.7
Catalase	1	16.7
Cholester	1	16.7

Serum - 6 papers

Name	Freq	percentage
Catechol	2	33.3
Citrulline	2	33.3
1-myristo	1	16.7
1,3-dimet	1	16.7
2-myristo	1	16.7
3-methyl-	1	16.7
Arg/3-AA	1	16.7
Bilirubin	1	16.7
Caffeine	1	16.7
Ergothior	1	16.7

Blood plasma- 10 papers

Name	Freq	percentage
Cis-aconi	2	20
FFA 11:1	2	20
Formic ac	2	20
Kynureni	2	20
Linoleic a	2	20
Oleic acid	2	20
Palmitole	2	20
Trigonelli	2	20
1,5-anhyd	1	10
186.1189	1	10

Plasma EV- 1 paper

Name	Freq	percentage
ex-miR-5	1	100
miR-626	1	100
miR-505	1	100

Serum EV- 1 paper

Name	Freq	percentage
ex-miR-1	1	100

All - total 24 papers

Name	Freq	percentage
Uric acid	3	12.5
Catechol	2	8.33
Cis-aconi	2	8.33
Citrulline	2	8.33
Ethanola	2	8.33
FFA 11:1	2	8.33
Formic ac	2	8.33
Hypoxant	2	8.33
Kynureni	2	8.33
Linoleic a	2	8.33

Overlap of biomarkers from multi-omics integration likely to be true signal amongst false positives or false negatives artefact from omics methodologies

Given that there are usually more expressed genes relative to acquired samples, transcriptomic analyses typically suffer from a lack of statistical power (curse-of-dimensionality) or produce many false positives/negatives due to erroneous assumptions on data distribution [110]. To counter such issues, more appropriate bioinformatics algorithms were developed such as DESeq2 [111] or limma-voom [112]. In addition, fluid-based proteomics also face many challenges and complexities. High abundance proteins such as albumin can mask low abundance proteins and must be removed to facilitate observation of lower abundance proteins. However, the removal of albumin might result in unintended removal of non-targeted low abundance proteins [113, 114]. Many peptides in serum also give highly intense signals that makes identification of endogenous peptides difficult. In addition, there is a wide array of technical variables that can influence the proteomic results [115]. Variables such as blood withdrawal site or simply letting serum samples sit beyond 60 min can lead to detection of false targets arising from hemolysis caused by residual disinfecting alcohol [116] or unwanted cell lysis [117] respectively.

The current way transcriptomics and proteomics are conducted and analyzed, may produce many false positives and false negatives. Multi-omics integration

can value add by reducing the extent of false positives and false negatives presented. With the large amount of transcriptomics, proteomics, and metabolomics data available, targets that are repeatedly reported as common across the different omics layers are more likely to be true signals rather than technical artefacts. We define a cross-omics agreement rate as the intersection of transcriptomics and proteomics set of targets divided by the minimum number of targets of the 2 sets [ie. $A \cap B/\min(A, B)$]. Analysis between the lists of compiled upregulated mRNAs and proteins identified 53 overlaps (13.7%) amongst the 2515 mRNAs and 386 proteins reported by the various studies. Similarly, between the lists of compiled downregulated mRNAs and proteins, there were 66 overlaps (12.9%) amongst the 1504 mRNAs and 508 proteins reported by the various studies. However, not all mRNA expression is positively correlated with protein expression [118]. Hence, we examined cross-omics agreement regardless of direction of expression as well. There was an increase in percentage overlap with a total of 211 overlaps (23%) between the 4019 mRNAs and 894 proteins reported by the various studies. The Hypergeometric test is an important statistical instrument used to estimate the probability of chance occurrences of overlapping genes between 2 genes sets [119]. Using the phyper() function in R and the assumption of 19,950 protein coding genes in the GRCh38 human genome [120], cross-omics biomarker overlap between all mRNAs and proteins reported is significant ($p\text{-}val = 0.005339753$). This suggests that overlapping biomarkers via multi-omics integration are likely true signals and should be further validated, including our proposed biomarkers SNCA and Arg1.

Both transcriptomics and proteomics point to common theme of neuroinflammation and metabolic processes despite seemingly different list of biomarkers

Omics technologies can easily produce a large number of differentially expressed targets which is often overwhelming for researchers to look at individually. Hence, a common strategy is to identify the higher-order functional perspective by summarizing observed differential genes in light of their parent pathway (for instance, via DAVID) [121]. Pathway analysis can improve experiment credibility by locating the most important pathways. For instance, when the protein list from each study was ran through EnrichR to obtain pathways affected for each study (Table 5), 33% reported downregulation of Complement and coagulation cascades for proteomics (vs 17% overlap in protein targets). Pathway analysis may also reduce the study's scope to a few hundred pathways rather than thousands of DEGs. Hence, in addition to intersections analysis, we exploited popular public repositories like Gene Ontology (GO) to study the pathways associated with PD.

We observed unifying themes for GO pathways derived from the compiled list of transcripts and proteins (Fig. 2). We pooled together upregulated differentially expressed genes from all the literature analysed and ran a pathway analysis using enrichGO function from clusterProfiler package in R (v4.0.5). The same was done for downregulated DEGs, upregulated proteins and downregulated proteins. Significant GO pathways from upregulated DEGs point towards IL6 and immune system changes, which were also observed in significant GO pathways from upregulated proteins. A study by Fielding et al. reported that IL6 is the key signal for neutrophil trafficking during inflammation, chemokine production and leukocyte apoptosis [122]. Similarly, we observed significant pathways of IL6 production, regulation of IL6 production and positive regulation of IL6 production from upregulated DEGs, which were supported by significant pathways of neutrophil degranulation, neutrophil activation involved in immune response, cell killing and other inflammatory pathways involving MHC I from upregulated proteins. Despite seemingly modest overlap in the list of upregulated DEGs and proteins, PD is associated with neuroinflammation when evaluated collectively across omics layers as illustrated in the upset plot (Fig. 3A). The upset plot shows that different pathways were upregulated predominantly across the different omics, with neuroinflammation dominating transcriptomics; amino acid metabolism dominating metabolomics and blood related changes dominating proteomics (Fig. 3A).

Many significant pathways from downregulated DEGs and proteins are inflammation-related (eg. T-cell activation, lymphocyte proliferation and antigen processing / presentation). Other than neuroinflammation, the significant pathways from both downregulated DEGs and proteins converged on downregulated metabolic pathways such as cellular response to toxic substance, hydrogen peroxide catabolic process, ubiquitin-dependent protein catabolic process and regulation of cellular amino acid metabolic process. This reaffirms the proposition that PD is a metabolic disease [123] and also demonstrated in the upset plot, where the top hits other than neuroinflammation are protein and lipid metabolism (Fig. 3B).

Inter i-omics agreement on pathways can deepen confidence in selected biomarkers

The importance of integrating multi-layered studies is demonstrated when we observe how pathways across transcriptomics, proteomics and metabolomics corroborate. Pathways for the list of compiled metabolites

Table 5 Related pathways across transcriptomics, proteomics and metabolomics

S No.	Blood Component	Top 10 pathways affected	Level in PD (compared to healthy controls)
Transcriptomics			
1	Whole blood	Nil	Downregulated
		Dilated cardiomyopathy, Hypertrophic cardiomyopathy, Cardiac muscle contraction, ECM-receptor interation, Platelet activation, Arrhythmogenic righ ventricular cardiomyopathy, Antigen processing and presentation, Adrenergic signaling in cardiomyocytes, Focal adhesion,	Upregulated
2	Whole blood	Nil	Downregulated
		Staphylococcus aureus infection, Complement and coagulation cascades	Upregulated
3	Whole blood	Nil	Downregulated
		Osteoclast differentiation, Leishmaniasis, Tuberculosis,	Upregulated
4	Whole blood	Parkinson disease, Alzheimer disease, Pathways of neurodegeneration	Downregulated
5	Whole blood	Nil	Downregulated
		Osteoclast differenciation, Natural killer cell mediated cytotoxicity, Antigent processing and presentation, B cell receptor signalling pathway, Graft-versus-host disease, Lysosome, Phagosome, Chemokine signaling pathway, Fc gamma R-mediated phagocytosis, Tuberculosis	Upregulated
6	Whole blood	Parkinson Disease, Perussis, Prion Disease, Huntington disease,	Downregulated
		Nil	Upregulated
7	peripheral blood mononuclear cells (PBMCs)	Th1 and Th2 cell differentiation, Th17 cell differentiation, Epstein-Barr virus infection, Toxoplasmosis, Leukocyte transendothelial migration, Human immunodeficiency virus 1 infection, Tuberculosis, Leishmaniasis, Pathways in cancer, Human cytomegalovirus infection	Downregulated
		Porphyrin and chlorophyll metabolism, Amoebiasis	Upregulated
8	peripheral blood mononuclear cells (PBMCs)	Sulfur metabolism, Collecting duct acid secretion, Glycine, serine and threonine metabolism, Porphyrin and chlorophyll metabolism, Malaria	Downregulated
9	Whole blood	Nil	Downregulated
		Endocrine and other factor-regulated calcium reabsorption, Nitrogen metabolism,	Upregulated
10	Whole blood / leukocytes	Citrate cycle (TCA cycle), Pyruvate metabolism, Cocaine addiction, Aminoacyl-tRNA biosynthesis, Amphetamine addiction, Salmonella infection, Parkinson disease, Prion disease, Huntington Disease, Amyotrophic lateral sclerosis	Downregulated
		Nil	Upregulated
11	peripheral blood mononuclear cell (PBMC)	Autophagy, Mitophagy, RIG-I-like receptor signaling pathway, NOD-like recptor signaling pathway, Shigellosis	Downregulated
		Lysosome, Glycosaminoglycan degradation, Other glycan degradation, Glycosphingolipid biosynthesis, Phagosome, Sphinagolipid metabolism, Collecting duct acid secretion, Apoptosis, Tuberculosis, Synaptic vesicle cycle	Upregulated
12	Whole blood	Nil	Downregulated
		Nil	Upregulated

Table 5 (continued)

S No.	Blood Component	Top 10 pathways affected	Level in PD (compared to healthy controls)
13	peripheral blood mononuclear cells (PBMCs)	Nil	Downregulated
		Inflammatory bowel disease, Cytokine-cytokin receptor interaction, African trypanosomiasis, Malaria, Perussis, Leishmaniasis, Asthma, IL-17 signaling pathway, Hematopoietic cell lineage, Amoebiasis	Upregulated
14	Whole blood	Nil	Downregulated
15	Whole blood	Ubiquitin mediated proteolysis, Protein processing in endoplasmis reticulum, Parkinson disease	Downregulated
		Fructose and mannose metabolism, Inflammatory bwel disease	Upregulated
16	Whole blood	Nil	Downregulated
		Arachidonic acid metabolism	Upregulated
17	Peripheral blood	Nil	Downregulated
18	Blood Serum	Nil	Upregulated
	Blood Serum	Viral protein interaction with cytokine and cytokine receptor,	Upregulated
Proteomics			
1	Plasma EV	Parkinson disease, MAPK signaling pathway, Alzheimer disease, Pathways of neurodegeneration	Upregulated
2	Plasma EV	Parkinson disease, Alzheimer disease, Pathways of neurodegeneration	Upregulated
3	Plasma EV	Parkinson disease, Alzheimer disease, Pathways of neurodegeneration	Upregulated
4	Plasma EV	Parkinson disease, Alzheimer disease, Pathways of neurodegeneration	Upregulated
5	Plasma EV	Parkinson disease, Alzheimer disease, Pathways of neurodegeneration	Upregulated
6	Plasma EV	Parkinson disease, Alzheimer disease, Pathways of neurodegeneration	Upregulated
7	Plasma EV	Parkinson disease, Alzheimer disease, Pathways of neurodegeneration,	Upregulated
8	Plasma EV	Ferroptosis, Prion disease, Pathways of neurodegeneration,	Upregulated
9	Plasma EV	Nil	Upregulated
10	Plasma EV	Complement and coagulation cascades, Staphylococcus aureus infection, Coronavirus disease, Vitamin digestion and absorption, African trypanosomiasis, Fat digestion and absorption, Cholesterol metabolism, PPAR signaling pathway, Pertussis, Platelet activation,	Downregulated
11	Plasma EV	Proteasome, Gastric acid secretion, Endocytosis, Parkinson disease, Phagosome, Aldosterone-regulated sodium reabsorption, Salivary secretion, Adrenergic signaling in cardiomyocytes, Aldosterone synthesis and secretion, Glycolysis / Gluconeogenesis, Endocrine and other factor-regulated calcium reabsorption,	Upregulated
		Proteasome, Pentose phosphate pathway, Spinocerebellar ataxia, Prion disease, Parkinson disease, Amyotrophic lateral sclerosis, Huntington disease, Pathways of neurodegeneration, Glycolysis / Gluconeogenesis, Cysteine and methionine metabolism,	Downregulated
12	Serum EV	Parkinson disease, Alzheimer disease, Pathways of neurodegeneration	Upregulated
		Nil	Downregulated

Table 5 (continued)

S No.	Blood Component	Top 10 pathways affected	Level in PD (compared to healthy controls)
13	Serum EV	Parkinson disease, Alzheimer disease, Pathways of neurodegeneration	Downregulated
14	Serum EV	Asthma, African trypanosomiasis, Allograft rejection, Graft-versus-host disease, Type I diabetes mellitus, Type II diabetes mellitus, Malaria, Legionellosis, Inflammatory bowel disease, Fc epsilon RI signaling pathway,	Upregulated
		Thermogenesis, Citrate cycle (TCA cycle), Asthma, Oxidative phosphorylation, Non-alcoholic fatty liver disease, Diabetic cardiomyopathy, Parkinson disease, Melanoma, Prion disease, Huntington disease,	Downregulated
15	Serum EV	Parkinson disease, Alzheimer disease, Pathways of neurodegeneration	Upregulated
16	Serum EV	Parkinson disease, Alzheimer disease, Pathways of neurodegeneration	Upregulated
17	Serum EV	Parkinson disease, Alzheimer disease, Pathways of neurodegeneration	Upregulated
18	Serum EV	Complement and coagulation cascades, Platelet activation, Pertussis, Systemic lupus erythematosus, Neutrophil extracellular trap formation, ECM-receptor interaction, Hypertrophic cardiomyopathy, Coronavirus disease, Staphylococcus aureus infection, Dilated cardiomyopathy,	Upregulated
		Complement and coagulation cascades, Pertussis, Staphylococcus aureus infection, Systemic lupus erythematosus, Coronavirus disease, Renin-angiotensin system, Ferroptosis, Porphyrin and chlorophyll metabolism, Type II diabetes mellitus, Glutathione metabolism,	Downregulated
19	Serum EV	Complement and coagulation cascades, Platelet activation, Coronavirus disease, ECM-receptor interaction, Staphylococcus aureus infection, Neutrophil extracellular trap formation, Focal adhesion, , , ,	Upregulated
		Allograft rejection, Staphylococcus aureus infection, Autoimmune thyroid disease, Viral myocarditis, Systemic lupus erythematosus, Pertussis, Complement and coagulation cascades, Dilated cardiomyopathy, Chagas disease, Coronavirus disease,	Downregulated
20	Blood Plasma	Complement and coagulation cascades, Coronavirus disease, Neuroactive ligand-receptor interaction	Upregulated
		Complement and coagulation cascades, Staphylococcus aureus infection, Vitamin digestion and absorption, African trypanosomiasis, Fat digestion and absorption, Cholesterol metabolism, PPAR signaling pathway, Platelet activation, Neutrophil extracellular trap formation, Lipid and atherosclerosis,	Downregulated
21	Blood Plasma	Nil	Upregulated
		Nil	Downregulated
22	Blood Plasma	PI3K-Akt signaling pathway, ECM-receptor interaction, Growth hormone synthesis, secretion and action, JAK-STAT signaling pathway, Focal adhesion, Cytokine-cytokine receptor interaction, Human papillomavirus infection, Neuroactive ligand-receptor interaction,	Upregulated
		Arginine biosynthesis	Downregulated

Table 5 (continued)

S No.	Blood Component	Top 10 pathways affected	Level in PD (compared to healthy controls)
23	Blood Plasma	ECM-receptor interaction, Staphylococcus aureus infection	Upregulated
		Pantothenate and CoA biosynthesis, Complement and coagulation cascades,	Downregulated
24	Blood Plasma	African trypanosomiasis, Malaria, AGE-RAGE signaling pathway in diabetic complications, NF-kappa B signaling pathway, TNF signaling pathway, Leukocyte transendothelial migration, Fluid shear stress and atherosclerosis, Cell adhesion molecules, Lipid and atherosclerosis,	Upregulated
		African trypanosomiasis, Graft-versus-host disease, Malaria, Legionellosis, Inflammatory bowel disease, Pertussis, Antigen processing and presentation, Epstein-Barr virus infection, Human T-cell leukemia virus 1 infection, Human cytomegalovirus infection,	Downregulated
25	Blood Plasma	Sphingolipid signaling pathway, Lysosome, Estrogen signaling pathway, Autophagy, Apoptosis, Protein processing in endoplasmic reticulum, Tuberculosis, Diabetic cardiomyopathy	Downregulated
26	Blood Plasma	ECM-receptor interaction	Upregulated
27	Blood Plasma	Parkinson disease, Alzheimer disease, Pathways of neurodegeneration, MAPK signaling pathway	Upregulated
28	Blood Plasma	Parkinson disease, Alzheimer disease, Pathways of neurodegeneration	Upregulated
29	Blood Serum	Nil	Upregulated
		Nil	Downregulated
30	Blood Serum	Cholesterol metabolism, Thyroid hormone synthesis, Complement and coagulation cascades	Upregulated
		Vasopressin-regulated water reabsorption	Downregulated
31	Blood Serum	Complement and coagulation cascades, Staphylococcus aureus infection, Platelet activation, Neutrophil extracellular trap formation, Coronavirus disease	Upregulated
		Vitamin digestion and absorption, Fat digestion and absorption, Cholesterol metabolism, African trypanosomiasis, Lipid and atherosclerosis, PPAR signaling pathway, Complement and coagulation cascades, Platelet activation, Phospholipase D signaling pathway,	Downregulated
32	Blood Serum	Nil	Upregulated
		Asthma, JAK-STAT signaling pathway, Cytokine-cytokine receptor interaction	Downregulated
33	Blood Serum	Parkinson disease, Alzheimer disease, Pathways of neurodegeneration	Upregulated
34	Whole Blood	Parkinson disease, MAPK signaling pathway, Alzheimer disease, Pathways of neurodegeneration	Upregulated
		Parkinson disease, Alzheimer disease, Pathways of neurodegeneration	Downregulated
Metabolomics			
1	Blood plasma	Sphingolipid metabolism	Upregulated
		names not identified	Downregulated
2	Blood plasma	names not identified	Upregulated
3	Blood plasma	Citrate cycle (TCA cycle), Arginine biosynthesis	Upregulated
		NIL	Downregulated

Table 5 (continued)

S No.	Blood Component	Top 10 pathways affected	Level in PD (compared to healthy controls)
4	Blood plasma	Nicotinate and nicotinamide metabolism, Tyrosine metabolism	Upregulated
		Tryptophan metabolism	Downregulated
5	Blood Plasma	Steroid hormone biosynthesis	Upregulated
		NIL	Downregulated
6	Blood Serum	Arginine biosynthesis, Aminoacyl-tRNA biosynthesis, Pantothenate and CoA biosynthesis, beta-Alanine metabolism, Glutathione metabolism, Alanine, aspartate and glutamate metabolism ,Pyrimidine metabolism Phenylalanine, tyrosine and tryptophan biosynthesis, Tyrosine metabolism, D-Glutamine and D-glutamate metabolism, Nitrogen metabolism	Upregulated
		Thiamine metabolism ,Taurine and hypotaurine metabolism, Pantothenate and Co biosynthesis, Glutathione metabolism, Glycine, serine and threonine metabolism, Cysteine and methionine metabolism	Downregulated
7	Blood Plasma	Arginine biosynthesis, Butanoate metabolism	Upregulated
		Taurine and hypotaurine metabolism, Biotin metabolism, Lysine degradation	Downregulated
8	Blood Plasma	Linoleic acid metabolism, alpha-Linolenic acid metabolism	Upregulated
		Glycerophospholipid metabolism, Glycosylphosphatidylinositol (GPI)-anchor biosynthesis	Downregulated
9	Blood Plasma	Valine, leucine and isoleucine biosynthesis, Aminoacyl-tRNA biosynthesis	Upregulated
		Biosynthesis of unsaturated fatty acids, Linoleic acid metabolism	Downregulated
10	Blood Plasma	Phenylalanine, tyrosine and tryptophan biosynthesis, Phenylalanine metabolism	Upregulated
		NIL	Downregulated
11	Plasma EV	names not identified	Upregulated
		names not identified	Downregulated
12	Serum EV	names not identified	Upregulated
		names not identified	Downregulated
13	Whole blood	Glutathione metabolism	Upregulated
		Purine metabolism	Downregulated
14	Whole blood	names not identified	Downregulated
15	Whole blood	NIL	Upregulated
		NIL	Downregulated
16	Blood Plasma	Glycerophospholipid metabolism	Upregulated
		Biosynthesis of unsaturated fatty acids, Linoleic acid metabolism, Arachidonic acid metabolism, Synthesis and degradation of ketone bodies, Tryptophan metabolism	Downregulated
17	Whole blood	Biosynthesis of unsaturated fatty acids, Arachidonic acid metabolism	Upregulated
		Biosynthesis of unsaturated fatty acids	Downregulated
18	Blood Plasma	Purine metabolism	Downregulated
19	Blood Plasma	NIL	Upregulated
20	Blood Serum	D-Glutamine and D-glutamate metabolism, Nitrogen metabolism, Arginine biosynthesis	Upregulated
		Arginine and proline metabolism	Downregulated

Table 5 (continued)

S No.	Blood Component	Top 10 pathways affected	Level in PD (compared to healthy controls)
21	Blood Serum	Arginine and proline metabolism, Arginine biosynthesis	Upregulated
		Arginine biosynthesis	Downregulated
22	Blood Serum	Histidine metabolism	Upregulated
		NIL	Downregulated
23	Blood Serum	Sphingolipid metabolism, Glycerophospholipid metabolism, Arginine and proline metabolism	Upregulated
		Arginine biosynthesis	Downregulated
24	Whole blood	Biosynthesis of unsaturated fatty acids	Upregulated
		Butanoate metabolism, Alanine, aspartate and glutamate metabolism, Arginine and proline metabolism, Nitrogen metabolism, D-Glutamine and D-glutamate metabolism	Downregulated
25	Blood Serum	Tyrosine metabolism, Phenylalanine, tyrosine and tryptophan biosynthesis, Ubiquinone and other terpenoid-quinone biosynthesis, Phenylalanine metabolism, Arginine biosynthesis	Upregulated
		Tryptophan metabolism, caffeine metabolism	Downregulated
26	Whole blood	Citrate cycle (TCA cycle), Glyoxylate and dicarboxylate metabolism, Pyruvate metabolism, Galactose metabolism,Alanine, aspartate and glutamate metabolism	Downregulated
27	Blood Serum	NIL	Upregulated

(Table 5) were obtained using MetaboAnalyst 5.0 [124]. Arginine biosynthesis was the most frequent pathway, followed by biosynthesis of unsaturated fatty acids and ROS related pathways like aspartate and glutamate metabolism and glutathione metabolism. The metabolomic pathways exhibit great relevance to the transcriptomic and proteomic pathways identified. Arginine has been shown to inhibit acute microglia-mediated inflammation [125] while fatty acids can serve as inflammatory response signalling molecules [126]. Additionally, glutathione metabolism is related to hydrogen peroxide catabolic process [127] that was picked up by proteomics. The interconnectedness across different biological layers comes to light with metabolomic pathways supporting the major neuroinflammation and metabolic themes highlighted by both transcriptomics and proteomics. Integration of cross omics findings can also result in greater confidence in differentiating targets with clinical value amongst a pool of false positives. For instance, knowledge integration of our proposed biomarkers SNCA, ARG1 and 8-OhDG reveals complex biological relationships. SNCA (α-syn) was shown to increase ARG1 in bone marrow derived macrophages [128], suggesting that SNCA may be responsible for triggering inflammation and immune response. In turn, there is observable increase in 8-OhDG, as interestingly, ARG1 also showed positive correlation with 8-OhDG levels [129]. Hence, via i-omics agreement, there is increased confidence in our proposed biomarkers.

Pathway changes with age, motor severity and medication status agrees with disease progression

Information on the age range, UPDRS III assessment and medication status were used to subset the cohort into younger or older (>67yrs.old for transcriptomics and proteomics, >65 for metabolomics), less or more severe (>UPDRS mean) and not medicated or medicated for further pathway analysis.

When segregated by age, transcriptomics of younger patient cohorts exhibited downregulation in detox and oxidative stress response pathways and upregulation of movement related pathways. Older patient cohorts subsequently exhibited upregulation of *IL6* and inflammation pathways. In terms of proteomics, both young and old cohorts experienced dysregulation of various immune response. Younger cohorts also experienced downregulation of TCA cycle related metabolites.

We divided the cohorts obtained from studies that reported UPDRS III values based on their UPDRS scores (into low: <22 for transcriptomics and proteomics, <18 for metabolomics and high). At low UPDRS III scores, transcriptomic changes mainly involved downregulation of autophagy and upregulation of lipid metabolism related processes which was supported by metabolomic

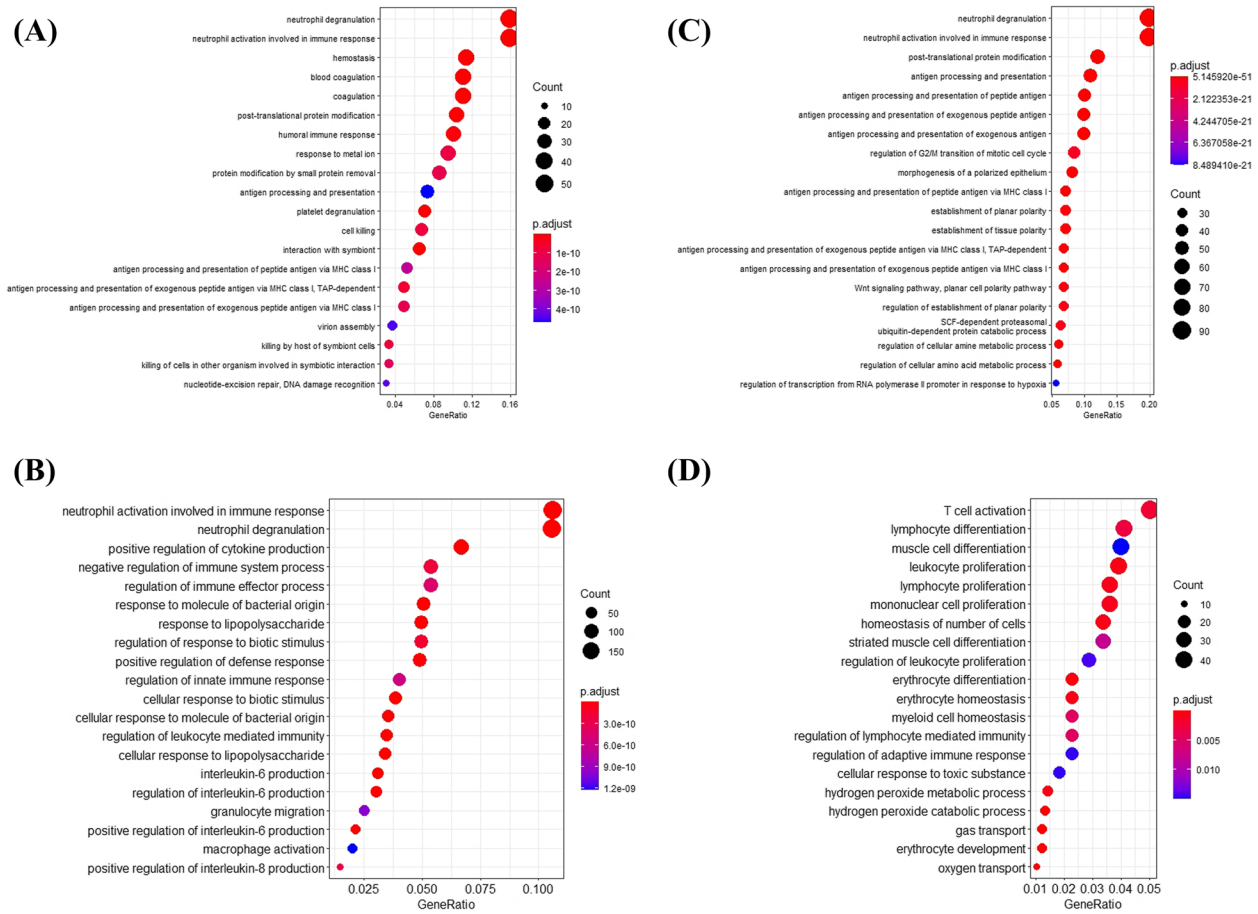


Fig. 2 Gene Ontology (GO) Biological Processes (BP) Pathway Analysis indicates immunological and metabolic implications. **A** GO—BP pathways for compiled upregulated protein list. **B** GO – BP pathways for compiled upregulated transcript list. **C** GO—BP pathways for compiled downregulated protein list. **D** GO – BP pathways for compiled downregulated transcript list

changes involving steroid hormone and fatty acid metabolism. Interestingly, proteomic changes at low UPDRS highlighted downregulation of many pathways involving dopamine uptake and biosynthesis as well as protein stability suggestive that changes start at low UPDRS status which leads to disease progression.

Lastly, the transcriptomics of drug-naïve patient cohorts generally exhibited downregulated ROS processing and upregulated *IL-6* production and inflammation. Downregulated phagocytosis but upregulated defence response to bacterium, killing of symbiont cells and immune responses were observed in proteomics. Metabolically, drug-naïve patient cohorts had downregulated energy production related to citrate cycle and pyruvate, but upregulated arginine and tryptophan metabolism related to inflammation as previously discussed. Interestingly, the drug treated patient cohorts showed some counteracting pathways. When treated, the transcriptomics of medicated patient cohorts had downregulated T cell activation and upregulated regulation of immune responses. Downregulated post translational protein modification and upregulated antigen processing, response to stress and protein removal were observed in proteomics. Metabolically, medicated patients had downregulated biosynthesis of unsaturated fatty acid and upregulated arginine biosynthesis. Taken together, medication had a positive effect on reducing inflammation and regulating protein stress. These pathway changes should however be taken with caution as they were not derived from paired studies of before and after treatment of the same patient but instead via comparison of different cohorts.

Taken together, by comparing age, motor severity and medication status, we observed that older, more severe and unmedicated patient cohorts exhibit dysregulation in energy [130], inflammation [131], lipid [132] and dopamine related pathways [133] which are in agreement with disease progression. A further examination of our 6 proposed biomarkers revealed preferences for different age, motor severity and medication status (Table 6),

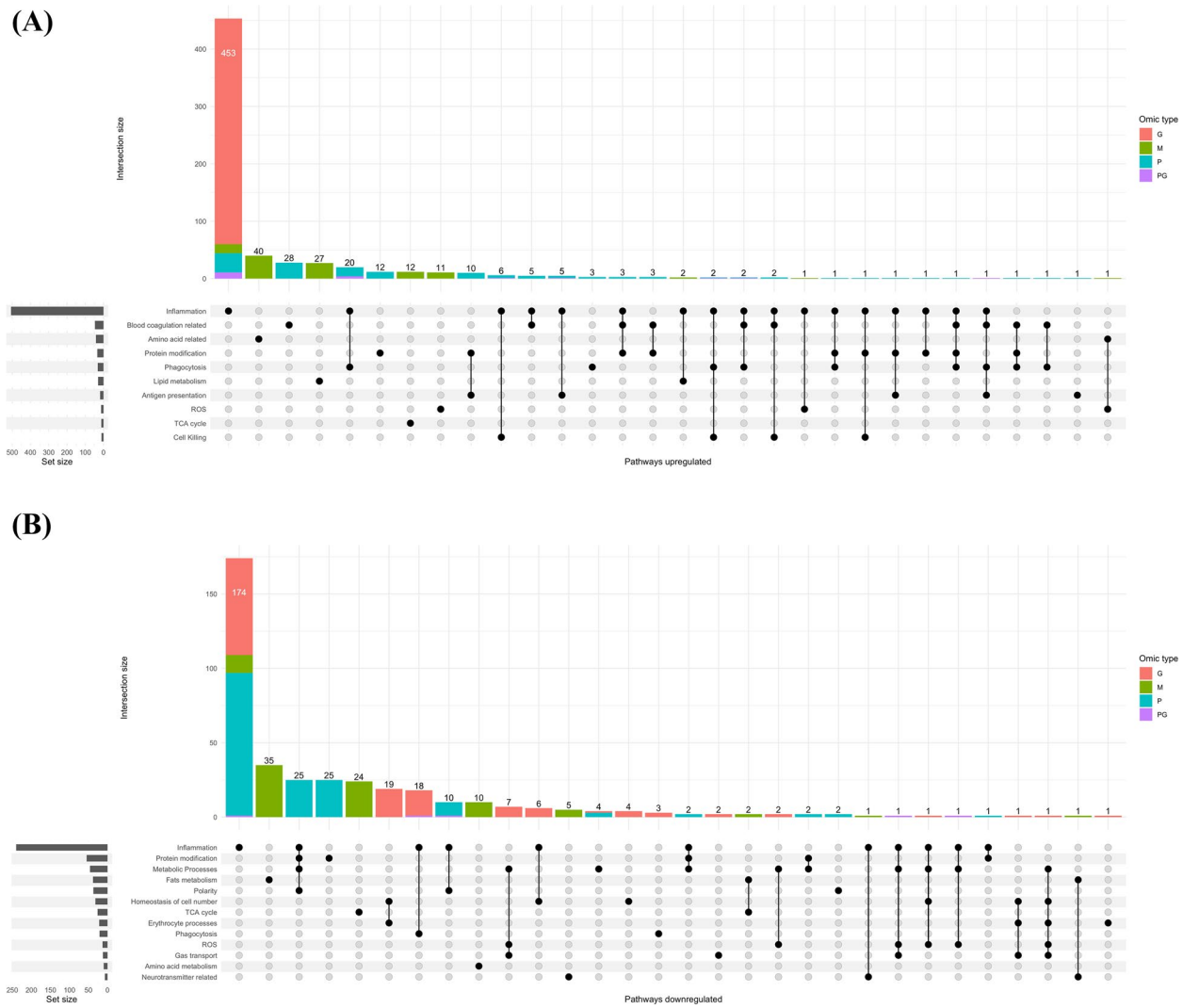


Fig. 3 Upset Plots Reveal Different Pathway Changes Dominated Different Source of Omics Analysis. **A** Upset plot for all pathways upregulated. Inflammation, amino acid metabolism and blood related changes dominated transcriptomics, metabolomics and proteomics respectively. **B** Upset plot for all pathways downregulated. Inflammation, Lipid metabolism and combination of Inflammation, Protein modification, Metabolic processes and polarity dominated transcriptomics, metabolomics and proteomics respectively

with the majority of the biomarkers favouring detection of younger PD patients. In agreement with our findings, previous studies have also identified reduced levels of APOA1 [134, 135] and uric acid [136] to be associated with greater motor severity.

Standardisation of analysis and UPDRS patient staging can improve reproducibility and accuracy of biomarker identification

We observe modest overlap in agreement of targets across studies. However, this is unsurprising since the statistical thresholds, and multiple test corrections used in each independent study differed. We demonstrate this point by comparing the results produced via publicly available database and analytical tools on Gene Expression Omnibus (GEO)

Table 6 Preference for age, UPDRS scoring and medication status of our proposed biomarkers. (-) indicates no information or no preference

UP	***Arg1***	**SNCA**	**8-OHdG**
Age	Younger	Younger	-
UPDRS	-	High	-
Medication	-	No medication	Medication
Down	***SNCA***	**APOA1**	**Uric Acid**
Age	Younger	Older	Younger
UPDRS	-	High [134, 135]	High [136]
Medication	-	Medication	Medication

repository and those reported by the original literature. We analyzed five microarray datasets to detect DEGs in PD patients, including GSE62283, GSE165083, GSE22491, GSE100054, GSE99039 datasets. Using the inbuilt GEO2R function on GEO repository and an intersection analysis (adjpvalue < 0.05, logFC > 1|logFC < -1) for the 5 datasets coded using R, we found that the most upregulated DEGs are CLTCL1, COMMD6, GNS, HGSNAT, LAMP2, LSM 3.00, LSMEM1, MANBA, SCARB2, SDPR, TCIRG1, and TPP1 as appeared twice in those microarray datasets (Table 7), while a lack of overlapping upregulated DEG is detected from the corresponding literature papers (Table 7). The full table of analysis from GEO2R can be found in Supplementary Table 5. Furthermore, the corresponding literature papers suggested BCL2 (using GSE6613 and GSE22491 datasets) [31, 33] and TRAF6 (using GSE99039 and GSE22491 datasets) [30, 31] are the most downregulated DEGs in PD patients, while a lack of overlapping downregulated DEG is identified using GEO2R analysis (Table 7). Interestingly, we found that XIST is more upregulated, and EIF1AY and KDM5D are more downregulated in females than in male PD patients using GSE7475 and GSE100054 datasets (Table 7), but this phenomenon has not been documented thus far. The lack of consistent overlapping DEGs between GEO2R analysis and the corresponding literature papers may be due to non-obvious confounding or batch factors that were known and corrected by the authors but not available in the inbuilt GEO2R function on GEO repository or due to our strict foldchange cut-off. In addition, the lack of overlapping genes seen in RNA sequencing data can also be attributed to the different analysis packages used (ie DESeq2 and edgeR). Dealing with these issues requires good quality meta-data. We therefore propose for more transparency in batch effects and standardisation of analysis pipelines by various studies to enhance reproducibility of results.

Another observation was that studies do not properly synchronize and stage patient severity (or not published) via the universal UPDRS scale (~ 30% among our reviewed papers did not use UPDRS), which makes it difficult to properly find early diagnostic markers. In addition, some papers classified early PD as Hoehn and Yahr stage ≤ 2 [46], while other papers used Hoehn and Yahr stage 1 to 3 [14]. This means that they are not comparing patients at the same time point and given how PD is a progressive disease, it is thus expected that different targets will be reported. Clearly, there is a need for better alignment between researchers and their clinician partners on how patient samples should be categorized and stored. A community-wide standardized framework for characterizing patient's PD status and disease severity using the UPDRS scale with a defined range of scores that distinguishes early PD from advanced PD patients would be useful. We also recommend that researchers use samples from the same PD staging and treatment to minimise confounding factors. This will aid in better comparisons and understanding of omics changes in PD progression.

Future directions: human-microbe i-omics

The gut microbiome is an exciting area to explore for PD given the emerging acceptance of a gut-brain axis in the pathogenesis of sporadic PD [16, 137]. Supporting the hypothesis, gut microbiome alterations have been reported by Toh et al., namely increased *Akkermansia* and reduced *Roseburia* in PD patients [138]. In addition, Sampson et al. also demonstrated that gut microbiome from PD patients can induce enhanced motor deficits when introduced into germ-free mice [139]. With the average 70 kg adult male hosting a total of 39 trillion bacteria in the body, humans are thus considered as supra-organisms and subjected to mutualistic microbiota-host interactions [140]. Microbiomics hence hold great potential to identify new ways to screen risk factors, diagnostic factors and therapeutics to various human diseases [141] such as PD. Future research can focus on studying human-microbe interactions by integrating genomic, proteomic and metabolic analysis, which can lead to some novel and interesting insights.

Concluding remarks

We analysed 79 papers related to transcriptomics, proteomics and metabolomics profile of PD. Individual omics platforms revealed potential in identifying PD blood-based biomarkers with increased reporting rates when grouped by blood subtypes, suggesting that different blood subtypes reflect different expression changes. Our study integrating the different omics datasets have validated SNCA as a potential useful biomarker and suggest

Table 7 GEO2Ranalysis of top transcriptomic hits categorised by blood subtypes

GEO2R Up adjpvalue<0.05 - 5 datasets			GEO2R Up Sex Female VS Male - 2 datasets			GEO2R DOWN adjpvalue<0.05 - 6 datasets			GEO2R DOWN Sex Female VS		
Name	Freq	percentage	Name	Freq	percentage	Name	Freq	percentage	Name	Freq	percentage
CLTCL1	2	40	XIST	2	100	AARD	1	16.7	EIF1AY	2	100
COMMD	2	40	RPL36A-	1	50	ABHD17	1	16.7	KDM5D	2	100
GNS	2	40				ACADL	1	16.7	RPS4Y1	2	100
HGSNAT	2	40				ACBD4	1	16.7	DDX3Y	1	50
LAMP2	2	40				ACTL6B	1	16.7	MALAT1	1	50
LSM 3.00	2	40				ACVRL1	1	16.7	PRKY	1	50
LSMEM	2	40				ADAM2	1	16.7	TXLNGY	1	50
MANBA	2	40				AGAP3	1	16.7	USP9Y	1	50
SCARB2	2	40				AGBL3	1	16.7			
SDPR	2	40				AGTPBP	1	16.7			

Arg1 expression, APOA1 protein level and two metabolites (WB) – 8-OHdG and uric acid could add further value as additional biomarkers. It is also possible that some of these potential biomarkers are more sensitive or specific for certain PD subtypes (tremor dominant, gait disorder etc.) and these should be further validated in future studies. Corroboration of targets from different omics platforms can help overcome the false positives / negatives artefacts from current omics methodologies. Interestingly, when we pool all the genes and proteins together, there is indication of immunological and metabolic implications for pathways associated with PD. Neuroinflammation and metabolic disruption are common themes around the pathogenesis of PD, suggesting that despite the seemingly different biomarkers, i-omics agree in terms of overarching pathways. We also demonstrated how knowledge integration of cross omics findings show that our proposed targets SNCA, ARG1 and 8-OhDG work in the same pathway and increase our confidence in the proposed biomarkers. While current methodologies can yield biomarkers with potential for PD diagnosis, there can be greater transparency by authors in terms of the data cleaning and processing for greater reproducibility. Different data corrections used and thresholds selected for differential expression will naturally lead to different targets reported. Publicly available datasets from some of the 79 papers analysed using tools on GEO database resulted in different sets of targets reported by the literature. Lastly, we suggest that future studies standardise the usage of UPDRS scale and analyse samples from the same PD staging to minimise confounding factors for more accurate biomarkers. We also propose future research to make use of i-omics to gain deeper understanding of PD progression via human-microbe interactions related to the proposed the gut-brain axis in PD, the clarification of which holds promise to reveal prodromal markers for the disease.

Abbreviations

8-OHdG	8-Hydroxy-2′-deoxyguanosine
AAV2-SYN	Adeno-associated virus synuclein
APOA1	Apolipoprotein A1
Arg1	Arginase 1
BCL2	B-cell lymphoma 2
CLTCL1	Clathrin Heavy Chain Like 1
COMMD6	COMM Domain Containing 6
CpG	CG Islands
CSF	Cerebral Spinal Fluid
DEGs	Differentially Expressed Genes
DLG2	Discs Large MAGUK Scaffold Protein 2
DNA	Deoxyribonucleic acid
EIF1AY	Eukaryotic translation initiation factor 1A
EV	Extracellular Vesicle
FAM47E-SCARB2	Family With Sequence Similarity 47 Member E-Scavenger receptor class B member 2
F-DOPA PET	Dopamine Positron Emission Tomography
FGG	Fibrinogen
FYN	Proto-oncogene tyrosine-protein kinase Fyn
GEO	Gene Expression Omnibus
GNS	N-acetylglucosamine-6-sulfatase
GO	Gene Ontology
HGSNAT	Heparan-Alpha-Glucosaminide N-Acetyltransferase
IGKV3-20	Immunoglobulin Kappa Variable 3–20
IL6	Interleukin 6
ITPKB	Inositol-trisphosphate 3-kinase B
KDM5D	Lysine-specific demethylase 5D
LAMP2	Lysosome-associated membrane protein 2
LRRK2	Leucine-rich repeat kinase 2
LSM 3.00	LSM3 Homolog, U6 Small Nuclear RNA And mRNA Degradation Associated
LSMEM1	Leucine Rich Single-Pass Membrane Protein 1
MANBA	Mannosidase Beta
MAPT	Tau
MCCC1	3-Methylcrotonoyl-CoA carboxylase
MHC I	Major Histocompatibility Complex I
MPP + PD	1-Methyl-4-phenylpyridinium induced Parkinson's Disease
mRNAs	Messenger Ribonucleic Acid
PARK16	Parkin
PD	Parkinson's Disease
RIT2	GTP-binding protein Rit2
ROS	Reactive Oxygen Species
SCARB2	Lysosomal integral membrane protein 2
SDPR	Serum deprivation-response protein
SNCA	Alpha-Synuclein
TCIRG1	T cell immune regulator 1
THBD	Thrombomodulin
TPP1	Tripeptidyl-peptidase 1
TRAF6	Tumour necrosis factor receptor associated factor (TRAF) protein
TTR	Transthyretin
UPDRS	Unified Parkinson's Disease Rating Scale
US FDA	United States Food and Drug Administration
VWF	Von Willebrand factor
WB	Whole Blood
XIST	X-inactivation process gene

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s41231-024-00169-9.

Additional file 1: Table 1. Overall Summary. **Table 2**. Transcriptomics. **Table 3**. Proteomics. **Table 4**. Metabolomics. **Table 5**. Geo2R.

Acknowledgements
This work was supported by grant from the Singapore Ministry of Health Open Fund-Large Collaborative Grant (MOH-OFLCG18May-0002) awarded to K-L.L.

Authors' contributions
Conceptualization: T.J.W.T. and B.W.Y.W.; writing-original draft preparation: T.J.W.T., B.W.Y.W. and E.H.Y.S.; writing-review and editing: T.J.W.T., B.W.Y.W., E.K.T., W.W.B.G. and K.L.L.; visualization, T.J.W.T. and B.W.Y.W.; supervision, E.K.T., W.W.B.G. and K.L.L. All authors have read and agreed to the published version of the manuscript.

Funding
This work was supported by grant from the Singapore Ministry of Health Open Fund-Large Collaborative Grant (MOH-OFLCG18May-0002) awarded to K-L.L.

Availability of data and materials
All data generated or analysed during this study are included in this published article.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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Received: 28 December 2023 Accepted: 17 March 2024
Published online: 23 April 2024

References

1. Jankovic J, Tan EK. Parkinson's disease: etiopathogenesis and treatment. J Neurol Neurosurg Psychiatry. 2020;91(8):795–808.
2. Bernheimer H, et al. Brain dopamine and the syndromes of Parkinson and Huntington Clinical, morphological and neurochemical correlations. J Neurol Sci. 1973;20(4):415–55.
3. Marras, C., et al., Prevalence of Parkinson's disease across North America. npj Parkinson's Disease, 2018. 4(1): p. 21.
4. Rees, R.N., et al., An early diagnosis is not the same as a timely diagnosis of Parkinson's disease. F1000Research, 2018. 7: p. F1000 Faculty Rev-1106.
5. Hustad E, Aasly JO. Clinical and Imaging Markers of Prodromal Parkinson's Disease. Front Neurol. 2020;11:395.
6. Regnault A, et al. Does the MDS-UPDRS provide the precision to assess progression in early Parkinson's disease? Learnings from the Parkinson's progression marker initiative cohort. J Neurol. 2019;266(8):1927–36.
7. Holden SK, et al. Progression of MDS-UPDRS Scores Over Five Years in De Novo Parkinson Disease from the Parkinson's Progression Markers Initiative Cohort. Movement Disorders Clinical Practice. 2018;5(1):47–53.
8. Newman EJ, et al. Accuracy of Parkinson's disease diagnosis in 610 general practice patients in the West of Scotland. Mov Disord. 2009;24(16):2379–85.
9. Beach TG, Adler CH. Importance of low diagnostic Accuracy for early Parkinson's disease. Mov Disord. 2018;33(10):1551–4.
10. Pagano G, Niccolini F, Politis M. Imaging in Parkinson's disease. Clin Med (Lond). 2016;16(4):371–5.
11. Loane C, Politis M. Positron emission tomography neuroimaging in Parkinson's disease. American journal of translational research. 2011;3(4):323–41.
12. Heim B, et al. Magnetic resonance imaging for the diagnosis of Parkinson's disease. Journal of neural transmission(Vienna Austria 1996). 2017;124(8):915–64.
13. Parnetti L, et al. CSF and blood biomarkers for Parkinson's disease. The Lancet Neurology. 2019;18(6):573–86.
14. Chang CW, et al. Plasma and Serum Alpha-Synuclein as a Biomarker of Diagnosis in Patients With Parkinson's Disease. Front Neurol. 2020;10:1388.
15. Seol W, Kim H, Son I. Urinary Biomarkers for Neurodegenerative Diseases. Experimental neurobiology. 2020;29(5):325–33.
16. Rietdijk CD, et al. Exploring Braak's Hypothesis of Parkinson's Disease. Front Neurol. 2017;8:37.
17. Parnetti L, et al. Parkinson's and Lewy body dementia CSF biomarkers. Clin Chim Acta. 2019;495:318–25.
18. Redenšek S, Dolžan V, Kunej T. From Genomics to Omics Landscapes of Parkinson's Disease: Revealing the Molecular Mechanisms. OMICS. 2018;22(1):1–16.
19. Vailati-Riboni M, Palombo V, Loor JJ. What are omics sciences? In: Periparturient diseases of dairy cows. Springer; 2017. p. 1–7.
20. Hasin Y, Seldin M, Lusis A. Multi-omics approaches to disease. Genome Biol. 2017;18(1):83.
21. Foo JN, et al. Identification of Risk Loci for Parkinson Disease in Asians and Comparison of Risk Between Asians and Europeans: A Genome-Wide Association Study. JAMA Neurol. 2020;77(6):746–54.
22. Magistrelli L, Contaldi E, Comi C. The Impact of SNCA Variations and Its Product Alpha-Synuclein on Non-Motor Features of Parkinson's Disease. Life (Basel). 2021;11(8):804.
23. Wakabayashi K, et al. The Lewy body in Parkinson's disease and related neurodegenerative disorders. Mol Neurobiol. 2013;47(2):495–508.
24. Cookson MR. LRRK2 Pathways Leading to Neurodegeneration. Curr Neurol Neurosci Rep. 2015;15(7):42–42.
25. Infante J, et al. Comparative blood transcriptome analysis in idiopathic and LRRK2 G2019S–associated Parkinson's disease. Neurobiol Aging. 2016;38:214.e1–214.e5.
26. Infante J, et al. Identification of candidate genes for Parkinson's disease through blood transcriptome analysis in LRRK2-G2019S carriers, idiopathic cases, and controls. Neurobiol Aging. 2015;36(2):1105–9.
27. Craig DW, et al. RNA sequencing of whole blood reveals early alterations in immune cells and gene expression in Parkinson's disease. Nature Aging. 2021;1(8):734–47.
28. Locascio JJ, et al. Association between α-synuclein blood transcripts and early, neuroimaging-supported Parkinson's disease. Brain. 2015;138(Pt 9):2659–71.
29. Wang C, et al. Identification of potential blood biomarkers for Parkinson's disease by gene expression and DNA methylation data integration analysis. Clin Epigenetics. 2019;11(1):24.
30. Shamir R, et al. Analysis of blood-based gene expression in idiopathic Parkinson disease. Neurology. 2017;89(16):1676–83.
31. Mutez E, et al. Transcriptional profile of Parkinson blood mononuclear cells with LRRK2 mutation. Neurobiol Aging. 2011;32(10):1839–48.
32. Mutez E, et al. Involvement of the immune system, endocytosis and EIF2 signaling in both genetically determined and sporadic forms of Parkinson's disease. Neurobiol Dis. 2014;63:165–70.
33. Scherzer CR, et al. Molecular Markers of Early Parkinson's Disease Based on Gene Expression in Blood. Proc Natl Acad Sci USA. 2007;104(3):955–60.
34. Henderson AR, et al. DNA Methylation and Expression Profiles of Whole Blood in Parkinson's Disease. Front Genet. 2021;12:640266.
35. Miki Y, et al. Alteration of autophagy-related proteins in peripheral blood mononuclear cells of patients with Parkinson's disease. Neurobiol Aging. 2018;63:33–43.
36. Calligaris R, et al. Blood transcriptomics of drug-naïve sporadic Parkinson's disease patients. BMC Genomics. 2015;16:876.
37. Li T, et al. Alterations of NURR1 and Cytokines in the Peripheral Blood Mononuclear Cells: Combined Biomarkers for Parkinson's Disease. Front Aging Neurosci. 2018;10:392.
38. Montarolo F, et al. Altered NR4A Subfamily Gene Expression Level in Peripheral Blood of Parkinson's and Alzheimer's Disease Patients. Neurotox Res. 2016;30(3):338–44.
39. Kurvits L, et al. Transcriptomic profiles in Parkinson's disease. Exp Biol Med (Maywood). 2021;246(5):584–95.
40. Jiang F, et al. Identification of potential diagnostic biomarkers for Parkinson's disease. FEBS Open Bio. 2019;9(8):1460–8.
41. Yang Z, et al. Altered Expression Levels of MicroRNA-132 and Nurr1 in Peripheral Blood of Parkinson's Disease: Potential Disease Biomarkers. ACS Chem Neurosci. 2019;10(5):2243–9.
42. DeMarshall CA, et al. Potential utility of autoantibodies as blood-based biomarkers for early detection and diagnosis of Parkinson's disease. Immunol Lett. 2015;168(1):80–8.
43. Shi M, et al. CNS tau efflux via exosomes is likely increased in Parkinson's disease but not in Alzheimer's disease. Alzheimer's Dementia. 2016;12(11):1125–31.
44. Shi M, et al. Plasma exosomal α-synuclein is likely CNS-derived and increased in Parkinson's disease. Acta Neuropathol. 2014;128(5):639–50.
45. Cerri S, et al. The Exosomal/Total α-Synuclein Ratio in Plasma Is Associated With Glucocerebrosidase Activity and Correlates With Measures of Disease Severity in PD Patients. Front Cell Neurosci. 2018;12:125–125.
46. Niu M, et al. A longitudinal study on α-synuclein in plasma neuronal exosomes as a biomarker for Parkinson's disease development and progression. Eur J Neurol. 2020;27(6):967–74.

47. Zou J, et al. Long Noncoding RNA POU3F3 and α-Synuclein in Plasma L1CAM Exosomes Combined with β-Glucocerebrosidase Activity: Potential Predictors of Parkinson's Disease. Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics. 2020;17(3):1104–19.
48. Stuendl A, et al. α-Synuclein in Plasma-Derived Extracellular Vesicles Is a Potential Biomarker of Parkinson's Disease. Mov Disord. 2021;36(11):2508–18.
49. Zhao Z-H, et al. Increased DJ-1 and α-Synuclein in Plasma Neural-Derived Exosomes as Potential Markers for Parkinson's Disease. Frontiers in aging neuroscience. 2019;10:438–438.
50. Leng B, et al. Plasma exosomal prion protein levels are correlated with cognitive decline in PD patients. Neurosci Lett. 2020;723: 134866.
51. Anastasi F, et al. Proteomics Profiling of Neuron-Derived Small Extracellular Vesicles from Human Plasma: Enabling Single-Subject Analysis. Int J Mol Sci. 2021;22(6):2951.
52. Kitamura Y, et al. Proteomic Profiling of Exosomal Proteins for Blood-based Biomarkers in Parkinson's Disease. Neuroscience. 2018;392:121–8.
53. Lamontagne-Proulx J, et al. Portrait of blood-derived extracellular vesicles in patients with Parkinson's disease. Neurobiol Dis. 2019;124:163–75.
54. Agliardi C, et al. Oligomeric α-Syn and SNARE complex proteins in peripheral extracellular vesicles of neural origin are biomarkers for Parkinson's disease. Neurobiol Dis. 2021;148: 105185.
55. Si X, et al. Central Nervous System-Derived Exosomal Alpha-Synuclein in Serum May Be a Biomarker in Parkinson's Disease. Neuroscience. 2019;413:308–16.
56. Picca A, et al. Mitochondrial Signatures in Circulating Extracellular Vesicles of Older Adults with Parkinson's Disease: Results from the EXosomes in PArkiNson's Disease (EXPAND) Study. J Clin Med. 2020;9(2):504.
57. Fu Y, et al. Facile Impedimetric Analysis of Neuronal Exosome Markers in Parkinson's Disease Diagnostics. Anal Chem. 2020;92(20):13647–51.
58. Dutta S, et al. α-Synuclein in blood exosomes immunoprecipitated using neuronal and oligodendroglial markers distinguishes Parkinson's disease from multiple system atrophy. Acta Neuropathol. 2021;142(3):495–511.
59. Jiang C, et al. Serum neuronal exosomes predict and differentiate Parkinson's disease from atypical parkinsonism. J Neurol Neurosurg Psychiatry. 2020;91(7):720–9.
60. Tomlinson PR, et al. Identification of distinct circulating exosomes in Parkinson's disease. Annals of clinical and translational neurology. 2015;2(4):353–61.
61. Jiang R, et al. Differential proteomic analysis of serum exosomes reveals alterations in progression of Parkinson disease. Medicine. 2019;98(41):e17478–e17478.
62. Alberio T, et al. Parkinson's disease plasma biomarkers: An automated literature analysis followed by experimental validation. J Proteomics. 2013;90:107–14.
63. Chen H-M, Lin C-Y, Wang V. Amyloid P component as a plasma marker for Parkinson's disease identified by a proteomic approach. Clin Biochem. 2011;44(5):377–85.
64. Posavi M, et al. Characterization of Parkinson's disease using blood-based biomarkers: A multicohort proteomic analysis. PLoS Med. 2019;16(10): e1002931.
65. Zhao, Y., et al., Plasma proteome profiling using tandem mass tag labeling technology reveals potential biomarkers for Parkinson's disease: a preliminary study. PROTEOMICS – Clinical Applications. n/a(n/a): p. 2100010.
66. O'Bryant SE, et al. Potential two-step proteomic signature for Parkinson's disease: Pilot analysis in the Harvard Biomarkers Study. Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring. 2019;11:374–82.
67. Kang J, et al. Identification of BAG2 and Cathepsin D as Plasma Biomarkers for Parkinson's Disease. Clin Transl Sci. 2021;14(2):606–16.
68. Pan C, et al. Targeted Discovery and Validation of Plasma Biomarkers of Parkinson's Disease. J Proteome Res. 2014;13(11):4535–45.
69. Lin W-C, et al. Linking Stage-Specific Plasma Biomarkers to Gray Matter Atrophy in Parkinson Disease. Am J Neuroradiol. 2021;42(8):1444–51.
70. Goldknopf IL, et al. Abnormal serum concentrations of proteins in Parkinson's disease. Biochem Biophys Res Commun. 2009;389(2):321–7.
71. Lu W, et al. Specific Changes of Serum Proteins in Parkinson's Disease Patients. PLoS ONE. 2014;9(4): e95684.
72. Calvani R, et al. A novel multi-marker discovery approach identifies new serum biomarkers for Parkinson's disease in older people: an EXosomes in PArkiNson Disease (EXPAND) ancillary study. GeroScience. 2020;42(5):1323–34.
73. Daniele S, et al. α-Synuclein Heterocomplexes with β-Amyloid Are Increased in Red Blood Cells of Parkinson's Disease Patients and Correlate with Disease Severity. Front Mol Neurosci. 2018;11:53.
74. Zhao X, et al. Proteome analysis of the sera from Chinese Parkinson's disease patients. Neurosci Lett. 2010;479(2):175–9.
75. Xicoy H, et al. Shared genetic etiology between Parkinson's disease and blood levels of specific lipids. npj Parkinson's Disease. 2021;7(1):23.
76. Bolner A, et al. Plasma and urinary HPLC-ED determination of the ratio of 8-OHdG/2-dG in Parkinson's disease. Clin Lab. 2011;57(11–12):859–66.
77. Saiki S, et al. Decreased long-chain acylcarnitines from insufficient β-oxidation as potential early diagnostic markers for Parkinson's disease. Sci Rep. 2017;7(1):7328.
78. Chang K-H, et al. Alternations of Metabolic Profile and Kynurenine Metabolism in the Plasma of Parkinson's Disease. Mol Neurobiol. 2018;55(8):6319–28.
79. Shao Y, et al. Comprehensive metabolic profiling of Parkinson's disease by liquid chromatography-mass spectrometry. Mol Neurodegener. 2021;16(1):4.
80. Klatt S, et al. A six-metabolite panel as potential blood-based biomarkers for Parkinson's disease. npj Parkinson's Disease. 2021;7(1):94.
81. Pathan M, et al. Plasma Metabolite Markers of Parkinson's Disease and Atypical Parkinsonism. Metabolites. 2021;11(12):860.
82. Stoessel D, et al. Promising Metabolite Profiles in the Plasma and CSF of Early Clinical Parkinson's Disease. Front Aging Neurosci. 2018;10:51.
83. Trupp M, et al. Metabolite and peptide levels in plasma and CSF differentiating healthy controls from patients with newly diagnosed Parkinson's disease. J Parkinsons Dis. 2014;4(3):549–60.
84. Zhao H, et al. Potential biomarkers of Parkinson's disease revealed by plasma metabolic profiling. J Chromatogr B. 2018;1081–1082:101–8.
85. Y.-F. Yao, M.-W.Q., G.-C. Li, F.-B. Zhang, H.-C. Rui, Circulating exosomal miRNAs as diagnostic biomarkers in Parkinson's disease. Eur Rev Med Pharmacol Sci, 2018. 22(16): p. 5278–5283.
86. Bogdanov M, et al. Metabolomic profiling to develop blood biomarkers for Parkinson's disease. Brain. 2008;131(Pt 2):389–96.
87. Campolo J, et al. Antioxidant and inflammatory biomarkers for the identification of prodromal Parkinson's disease. J Neurol Sci. 2016;370:167–72.
88. Wei Z, et al. Oxidative Stress in Parkinson's Disease: A Systematic Review and Meta-Analysis. Front Mol Neurosci. 2018;11:236.
89. Gonzalez-Riano C, et al. rognostic biomarkers of Parkinson's disease in the Spanish EPIC cohort: a multiplatform metabolomics approach. npj Parkinson's Disease. 2021;7(1):73.
90. Johansen KK, et al. Metabolomic profiling in LRRK2-related Parkinson's disease. PLoS ONE. 2009;4(10): e7551.
91. Plewa S, et al. The Metabolomic Approach Reveals the Alteration in Human Serum and Cerebrospinal Fluid Composition in Parkinson's Disease Patients. Pharmaceuticals (Basel). 2021;14(9):935.
92. Picca A, et al. Circulating amino acid signature in older people with Parkinson's disease: A metabolic complement to the EXosomes in PArkiNson Disease (EXPAND) study. Exp Gerontol. 2019;128: 110766.
93. Burté F, et al. metabolic profiling of Parkinson's disease and mild cognitive impairment. Mov Disord. 2017;32(6):927–32.
94. Troisi J, et al. A metabolomic signature of treated and drug-naïve patients with Parkinson's disease: a pilot study. Metabolomics. 2019;15(6):90.
95. Taku H, et al. Identification of novel biomarkers for Parkinson's disease by metabolomic technologies. J Neurol Neurosurg Psychiatry. 2016;87(3):295.
96. Ahmed SSSJ, et al. Metabolic profiling of Parkinson's disease: evidence of biomarker from gene expression analysis and rapid neural network detection. J Biomed Sci. 2009;16(1):63.
97. Roede, J.R., et al., Serum Metabolomics of Slow vs. Rapid Motor Progression Parkinson's Disease: a Pilot Study. PLOS ONE, 2013. 8(10): p. e77629.
98. Zhang J, et al. Targeted fatty acid metabolomics to discover Parkinson's disease associated metabolic alteration. J Mass Spectrom. 2021;56(10): e4781.
99. Barbagallo C, et al. Specific Signatures of Serum miRNAs as Potential Biomarkers to Discriminate Clinically Similar Neurodegenerative and Vascular-Related Diseases. Cell Mol Neurobiol. 2020;40(4):531–46.

100. Chen T, et al. Donepezil Regulates 1-Methyl-4-phenylpyridinium-Induced Microglial Polarization in Parkinson's Disease. ACS Chem Neurosci. 2015;6(10):1708–14.
101. Thome AD, et al. microRNA-155 Regulates Alpha-Synuclein-Induced Inflammatory Responses in Models of Parkinson Disease. J Neurosci. 2016;36(8):2383–90.
102. Guo S, Wang H, Yin Y. Microglia Polarization From M1 to M2 in Neurodegenerative Diseases. Front Aging Neurosci. 2022;14:815347.
103. Kantor B, et al. Downregulation of SNCA Expression by Targeted Editing of DNA Methylation: A Potential Strategy for Precision Therapy in PD. Mol Ther. 2018;26(11):2638–49.
104. Poehler AM, et al. Autophagy modulates SNCA/α-synuclein release, thereby generating a hostile microenvironment. Autophagy. 2014;10(12):2171–92.
105. Qiang JK, et al. Plasma apolipoprotein A1 as a biomarker for Parkinson disease. Ann Neurol. 2013;74(1):119–27.
106. Swanson CR, et al. Lower plasma apolipoprotein A1 levels are found in Parkinson's disease and associate with apolipoprotein A1 genotype. Movement disorders : official journal of the Movement Disorder Society. 2015;30(6):805–12.
107. Deng X, et al. Identifying clinical features and blood biomarkers associated with mild cognitive impairment in Parkinson disease using machine learning. Eur J Neurol. 2023;30(6):1658–66.
108. Sato S, Mizuno Y, Hattori N. Urinary 8-hydroxydeoxyguanosine levels as a biomarker for progression of Parkinson disease. Neurology. 2005;64(6):1081–3.
109. Kikuchi Y, et al. Urinary 8-OHdG elevations in a partial lesion rat model of Parkinson's disease correlate with behavioral symptoms and nigrostriatal dopaminergic depletion. J Cell Physiol. 2011;226(5):1390–8.
110. Goh WWB, Wong L. Dealing with Confounders in Omics Analysis. Trends Biotechnol. 2018;36(5):488–98.
111. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 2014;15(12):550.
112. Law, C.W., et al., RNA-seq analysis is easy as 1–2–3 with limma, Glimma and edgeR. F1000Research, 2016. 5: p. ISCB Comm J-1408.
113. Chandramouli K, Qian P-Y. Proteomics: challenges, techniques and possibilities to overcome biological sample complexity. Human genomics and proteomics : HGP. 2009;2009: 239204.
114. Bellei E, et al. High-abundance proteins depletion for serum proteomic analysis: concomitant removal of non-targeted proteins. Amino Acids. 2011;40(1):145–56.
115. Lista S, Faltraco F, Hampel H. Biological and methodical challenges of blood-based proteomics in the field of neurological research. Prog Neurobiol. 2013;101–102:18–34.
116. Tammen H. Specimen collection and handling. In: Clinical Proteomics. Springer; 2008. p. 35–42.
117. Timms JF, et al. Preanalytic influence of sample handling on SELDI-TOF serum protein profiles. Clin Chem. 2007;53(4):645–56.
118. Fournier ML, et al. Delayed correlation of mRNA and protein expression in rapamycin-treated cells and a role for Ggc1 in cellular sensitivity to rapamycin. Molecular & cellular proteomics : MCP. 2010;9(2):271–84.
119. Fury, W., et al. Overlapping Probabilities of Top Ranking Gene Lists, Hypergeometric Distribution, and Stringency of Gene Selection Criterion. in 2006 International Conference of the IEEE Engineering in Medicine and Biology Society. 2006.
120. Breschi A, Gingeras TR, Guigó R. Comparative transcriptomics in human and mouse. Nat Rev Genet. 2017;18(7):425–40.
121. Huang da W, Sherman BT, Lempicki RA. ystematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc. 2009;4(1):44–57.
122. Fielding CA, et al. IL-6 regulates neutrophil trafficking during acute inflammation via STAT3. J Immunol. 2008;181(3):2189–95.
123. Peng, Z., et al., Association Between Metabolic Syndrome and Mild Parkinsonian Signs Progression in the Elderly. Frontiers in Aging Neuroscience, 2021. 13.
124. Pang, Z., et al., MetaboAnalyst 5.0: narrowing the gap between raw spectra and functional insights. Nucleic Acids Research, 2021. 49(W1): p. W388-W396.
125. Chen S-F, et al. Arginine is neuroprotective through suppressing HIF-1α/LDHA-mediated inflammatory response after cerebral ischemia/reperfusion injury. Mol Brain. 2020;13(1):63.
126. Melo HM, Santos LE, Ferreira ST. Diet-Derived Fatty Acids, Brain Inflammation, and Mental Health. Front Neurosci. 2019;13:265.
127. Smeyne M, Smeyne RJ. Glutathione metabolism and Parkinson's disease. Free Radical Biol Med. 2013;62:13–25.
128. Alam MM, et al. Alpha synuclein, the culprit in Parkinson disease, is required for normal immune function. Cell Rep. 2022;38(2): 110090.
129. Ogino K, et al. Evaluation of serum arginase I as an oxidative stress biomarker in a healthy Japanese population using a newly established ELISA. Clin Biochem. 2013;46(16–17):1717–22.
130. Halliwell B. Oxidative stress and neurodegeneration: where are we now? J Neurochem. 2006;97(6):1634–58.
131. Stone DK, et al. Innate and adaptive immunity for the pathobiology of Parkinson's disease. Antioxid Redox Signal. 2009;11(9):2151–66.
132. Dixon SJ, et al. Ferroptosis: an iron-dependent form of nonapoptotic cell death. cell. 2012;149(5):1060–72.
133. Dong-Chen X, et al. Signaling pathways in Parkinson's disease: molecular mechanisms and therapeutic interventions. Signal Transduct Target Ther. 2023;8(1):73.
134. Swanson CR, et al. Plasma apolipoprotein A1 associates with age at onset and motor severity in early Parkinson's disease patients. Mov Disord. 2015;30(12):1648–56.
135. Deng X, et al. Biomarker characterization of clinical subtypes of Parkinson Disease. NPJ Parkinsons Dis. 2022;8(1):109.
136. Dănău A, et al. Serum Uric Acid Levels in Parkinson's Disease: A Cross-Sectional Electronic Medical Record Database Study from a Tertiary Referral Centre in Romania. Medicina (Kaunas). 2022;58(2):245.
137. Del Tredici K, Braak H. Sporadic Parkinson's disease: development and distribution of α-synuclein pathology. Neuropathol Appl Neurobiol. 2016;42(1):33–50.
138. Toh TS, et al. Gut microbiome in Parkinson's disease: New insights from meta-analysis. Parkinsonism Relat Disord. 2022;94:1–9.
139. Sampson TR, et al. Gut Microbiota Regulate Motor Deficits and Neuroinflammation in a Model of Parkinson's Disease. Cell. 2016;167(6):1469–1480.e12.
140. Sender R, Fuchs S, Milo R. Revised Estimates for the Number of Human and Bacteria Cells in the Body. PLoS Biol. 2016;14(8):e1002533–e1002533.
141. Turnbaugh PJ, et al. The Human Microbiome Project. Nature. 2007;449(7164):804–10.

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