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Reproducibility and replicability in Parkinson's disease and age-related movement disorders

Edited by
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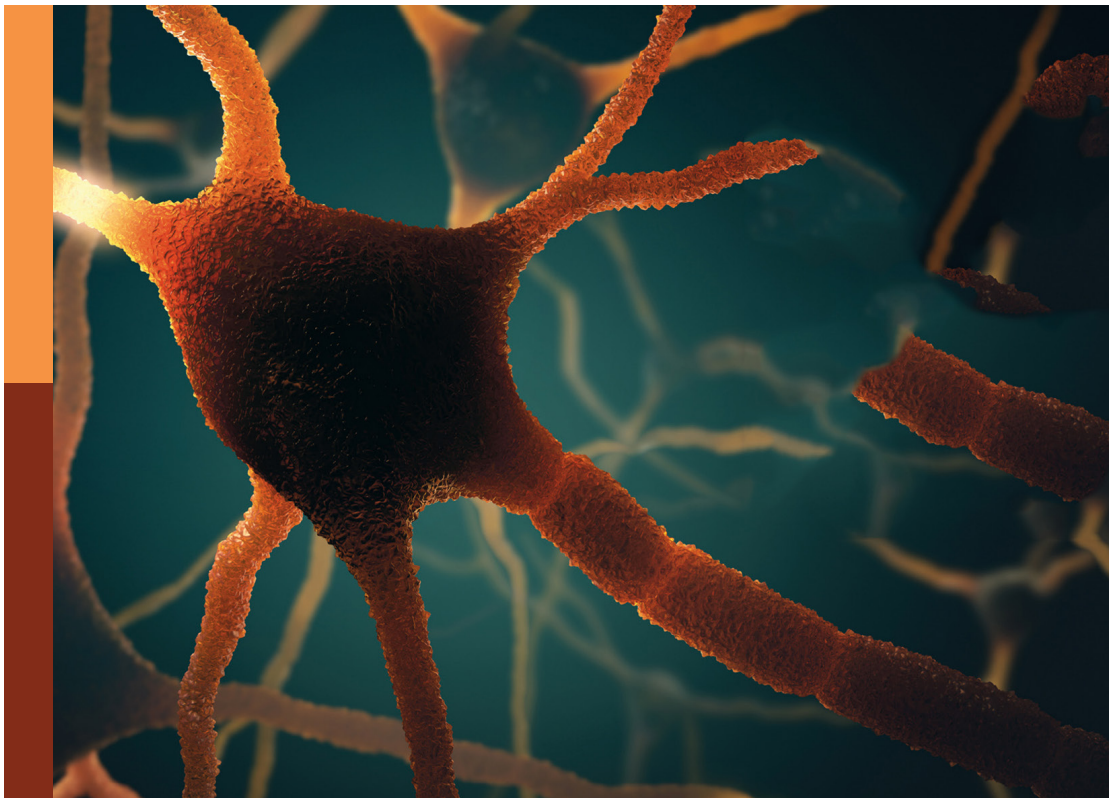

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Reproducibility and replicability in Parkinson's disease and age-related movement disorders

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Table of contents

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SYSTEMATIC REVIEW
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Efficacy and Safety of Probiotics for the Treatment of Alzheimer's Disease, Mild Cognitive Impairment, and Parkinson's Disease: A Systematic Review and Meta-Analysis

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Background: Alzheimer's disease (AD) and Parkinson's disease (PD) are two of the most common neurodegenerative diseases, and mild cognitive impairment (MCI) is considered a prodromal stage of clinical AD. Animal studies have shown that probiotics can improve cognitive function and mitigate inflammatory response, however, results from randomized controlled trials in humans are still unclear.

Objectives: A systematic review and meta-analysis was conducted to evaluate the efficacy and safety of probiotic therapy on cognitive function, oxidative stress, and gastrointestinal function in patients with AD, MCI, and PD.

Methods: We searched the electronic databases such as PubMed, EMBASE, Cochrane Library until October 2020 for the eligible randomized controlled trials, as well as the unpublished and ongoing trials. Our primary endpoints were cognitive function, inflammatory and oxidative stress biomarkers, gastrointestinal function, and adverse events.

Results: After screening 2,459 titles and abstracts about AD or MCI, we selected 6 eligible studies ($n = 499$ patients). After screening 1,923 titles and abstracts about PD, we selected 5 eligible studies ($n = 342$ patients). Compared with the control group, treatment with probiotics improved the cognitive function of patients with AD in the intervention group ($P = 0.023$). Cognitive function also improved in MCI patients ($P = 0.000$). Inflammation-related indicators: Malondialdehyde (MDA) was significantly reduced ($P = 0.000$); and hs-CRP decreased ($P = 0.003$). Lipid-related indicators: VLDL decreased ($P = 0.026$); triglyceride decreased ($P = 0.009$); and insulin resistance level improved: decreased Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) ($P = 0.019$).

Conclusion: Our analyses suggest that probiotics can improve cognitive and gastrointestinal symptoms in patients with AD, MCI, and PD, which is possibly through reducing inflammatory response and improving lipid metabolism. The safety has also been proven. However, more RCTs with rigorous study design are needed to support our findings.

Systematic Review Registration: PROSPERO, Identifier: CRD42021231502.

Keywords: probiotics, Alzheimer's disease, mild cognitive impairment, Parkinson's disease, cognitive, inflammation, meta-analysis

INTRODUCTION

Neurodegenerative diseases are a heterogeneous of disorders characterized by the progressive degeneration of the structure and function of the central nervous system (CNS) or peripheral nervous system (PNS). These age-related diseases are becoming more common, partly because the elderly population has grown in recent years (Heemels, 2016). Common neurodegenerative diseases include AD and PD (Fratiglioni and Qiu, 2009). MCI is considered a prodromal stage of clinical AD (Petersen, 2004). These diseases mainly affect aging individuals and progress steadily due to increased loss of specific neurons in the brain.

The intestinal microbiome refers to the symbiotic microorganisms such as bacteria, archaea, viruses and fungi that live in the human gut. For thousands of years, they have co-evolved with their hosts to form an intricate and mutually beneficial relationship (Guarner and Malagelada, 2003; Thursby and Juge, 2017). The gut-brain axis (GBA) is a bidirectional link between the CNS and the enteric nervous system (ENS) (Skonieczna-Zydecka et al., 2018). It involves direct and indirect pathways connecting cognitive and affective centers in the brain with peripheral intestinal function. Progressive loss of selectively vulnerable populations of neurons is a pathological manifestation of neurodegenerative diseases such as AD and PD (Dugger and Dickson, 2017). Although the etiology of neurodegenerative diseases is still unclear, numerous studies have implicated that inflammation may be involved in the process of these diseases (Dugger and Dickson, 2017). A growing recognition that the immune system is inextricably involved in shaping the brain during development, as well as mediating damage in neurodegenerative diseases, has prompted therapeutic approaches to the regulation of the immune system. But the regulatory mechanism of the immune system remains unclear (Stephenson et al., 2018). Studies have shown that in AD patients, there are intestinal microbes with decreased density and a decreased colony size, and there is a rich source of pro-inflammatory bacteria and lower anti-inflammatory bacteria (e.g., Bacillus fragilis, Eubacterium rectale, Eubacterium hallii, Faecalibacterium prausnitzii, and Bacteroides fragilis) in amyloid-positive patients as compared to healthy subjects (Kesika et al., 2021). MCI and AD patients shared similar alterations in gut microbiota (Li et al., 2019). In particular, the increase of gram-negative bacilli in patients with AD may lead to increased migration of Lipopolysaccharide (LPS) from the gut to the systemic circulation, which in turn promotes or aggravates the pathology of AD through neuroinflammation (Spielman et al., 2018). Clinical studies have shown a significant reduction in cellulose-degrading bacteria and a significant increase in hypothetical pathogens such as Escherichia coli, Streptococcus, Proteus and Enterococcus in fecal samples from PD patients compared to healthy controls (Cirstea et al., 2020). Notably, streptococci produce neurotoxins such as streptomycin and streptokinase, which can cause permanent nerve damage (Li et al., 2017). Therefore, if we can increase the number of anti-inflammatory bacteria in the gut of AD and PD patients and keep the gut bacteria stable, it may be possible to suppress inflammation and slow down the two diseases. Intestinal flora is a potential target for treatment of these diseases.

Probiotics are living microbes that are good for health when consumed in regular amounts, which may be explained by their anti-inflammatory or antioxidant properties (Lynch and Pedersen, 2016; Wallace and Milev, 2017; Chunchai et al., 2018). In recent years, several studies have found the anti-inflammatory effects of probiotics. A systematic review of 11 animal studies by Wang et al. showed that almost all the studies found significant effects on measured CNS functions, except for one testing effect of Bifidobacterium infantis on depression-like behavior (Wang et al., 2016). These preclinical results suggested that probiotics might be an effective dietary intervention to ameliorate age-related cognitive deficits. However, the results of previous clinical trials on the effects of probiotics on patients with PD, AD, or MCI were inconsistent (Akbari et al., 2016; Barichella et al., 2016; Georgescu et al., 2016; Agahi et al., 2018; Borzabadi et al., 2018; Hwang et al., 2019; Kobayashi et al., 2019; Tamtaji et al., 2019a,b; Xiao et al., 2020; Tan et al., 2021). Therefore, it is necessary to conduct a meta-analysis and systematic review of these RCT trials to further explore the effects of probiotics on various biochemical indicators and cognitive function, as well as explore the potential mechanisms.

MATERIALS AND METHODS

Literature Search

This meta-analysis complies with the systematic review and meta-analysis report program recommended by the PRISMA guidelines. The preliminary search was performed in October 2020 through the PubMed, Embase, and Cochrane Library without time restrictions. We searched the following terms in "all fields" in every electronic database: (probiotic OR yeast OR yogurt OR fermented product OR lactobacillus OR bifidobacterium OR fermented dairy product OR synbiotics OR cultured milk products) AND (Alzheimer's disease OR dementia

OR mild cognitive impairment OR cognitive dysfunction OR cognitive defect OR cognition OR memory OR mental capacity); (probiotic OR yeast OR yogurt OR fermented product OR lactobacillus OR bifidobacterium OR fermented dairy product OR synbiotics OR cultured milk products) AND (Parkinson OR Parkinson's disease OR parkinsonism). Articles were limited to randomized controlled trials (RCTs) in humans. The references of relevant articles were also checked to identify additional eligible studies. The titles and abstracts of articles were initially and independently screened for eligibility by two investigators. Duplicate and irrelevant papers were excluded. For the relevant candidates, the full articles were retrieved for review. And the references of each document were checked to identify potential candidates. Disagreements were resolved through discussion between the two researchers or with a third reviewer.

Inclusion and Exclusion Criteria

Included studies had to meet the following criteria: (1) The study was a randomized controlled trial (RCT); (2) Adult human participants who had a diagnosis of AD, MCI, or PD (aged over 18 y). Mild cognitive impairment (MCI) refers to a state of cognitive deterioration that precedes the clinical diagnosis of Alzheimer's disease (AD) and other dementias, which does not yet compromise daily functioning; (3) Pattern, taste, and smell of probiotics and placebo shouldn't have any significant difference at baseline; (4) Full English text; (5) Continuous data at baseline and post-intervention, or the change from baseline, and the number of participants at baseline and post-intervention were reported or could be calculated from the data reported in the article. Studies were excluded if they met any one of the following criteria: (1) Case reports or case series; (2) Abstracts, comments, reviews, letters, and conference speeches; (3) Nonhuman (*in vitro* and animal) research; (4) Changes in study indicators relative to baseline intervention were not reported, or information-based data could not be calculated. (5) The study reported on a sample that overlapped the sample in another study. In this case, only the study with the larger sample size was included.

Data Extraction

Data were extracted independently by two investigators using a predetermined format in accordance with the guidance of the Cochrane Handbook for Systematic Reviews of Interventions. Basic information of the RCTs was extracted from the included studies: (1) Name of the first author; (2) Date of publication; (3) Sample size; (4) Age and sex; (5) Species of strain, duration and dose; (6) Primary and secondary results; (7) Primary findings. For missing data, we sought missing information and essential clarification from the author. To perform the meta-analysis, we extracted the mean change score (standard deviation [SD] or standard error of the mean [SEM]) of the included variables. When change scores were not available, the scores (mean ± SD or mean ± SEM) and the numbers of participants at baseline and post-intervention were extracted.

Statistical Analysis

The primary outcomes of this study were the standardized mean differences (SMDs) of changes in MMSE, TYM, and RBANS from baseline between experimental group and control group. If the baseline standard deviation is not available, we calculated it using confidence interval (CI), standard error (SE), and T values according to the principles in the Cochrane Manual. The SMD was tested by a Z statistic, and a two-tailed $P < 0.05$ was regarded as statistically significant. The interstudy heterogeneity was examined by chi-square (χ^2) statistics and I^2 statistics. The heterogeneity among the different studies was considered high if $P < 0.1$ for the χ^2 statistic or $I^2 > 50\%$ (Cumpston et al., 2019). SMDs were calculated by fixed-effects or random-effects models. A sensitivity analysis was conducted to test the reliability of the findings using the leave-one-out method, and the publication bias was assessed by Egger's test and Begg's test. Forest plots, sensitivity analysis, Egger's test, and Begg's test were performed in STATA16 software, and Revman 5.3. was used to generate the summary of risk of bias assessment.

RESULTS

Literature Search and Screening

For AD or MCI, a total of 5,444 records were obtained after the initial search of the electronic databases. Of these, 2,395 trials were removed as duplicates, and 3,026 publications were excluded after screening the titles and abstracts. The remaining 23 articles were scrutinized by full text, of which 17 were excluded for reasons detailed in the PRISMA flow chart (**Figure 1**). Then, 6 studies were considered eligible and were eventually included in the quantitative meta-analysis. For PD, 1,920 articles were obtained after the initial search of the electronic databases and 3 studies were identified through a manual search of the reference lists of relevant published reviews. Of these, 832 trials were removed as duplicates, and 1,070 publications were excluded after screening the titles and abstracts. The remaining 18 articles were scrutinized by full text, of which 13 were excluded for reasons detailed in the PRISMA flow chart (**Figure 1**). Then, 5 qualified articles were included in the quantitative meta-analysis.

Study Characteristics

As shown in **Table 1**, the publication years of the 6 included studies on AD or MCI patients ranged from 2016 to 2020, with an aggregated sample of 499 individuals. All the 6 studies were randomized, double-blind, controlled trials, among which three studies (Akbari et al., 2016; Agahi et al., 2018; Tamtaji et al., 2019a) recruited subjects diagnosed with AD and the other three (Hwang et al., 2019; Kobayashi et al., 2019; Xiao et al., 2020) included people with mild cognitive impairment. Three studies had higher proportions of women; two (Kobayashi et al., 2019; Xiao et al., 2020) had balanced proportions; and the last one (Tamtaji et al., 2019a) did not report the sex ratio of the recruited subjects. The intervention duration of most included studies was 12 weeks except for one study (Xiao et al., 2020) whose duration was 16 weeks. Three studies recruited subjects diagnosed with AD using multiple strains of probiotics, and the other three included patients with MCI using a sole strain. All studies used probiotic-matched placebos which were indistinguishable from the probiotics in terms of packaging, shape, appearance, size, taste, smell, and so on. Of the three AD studies, two (Akbari

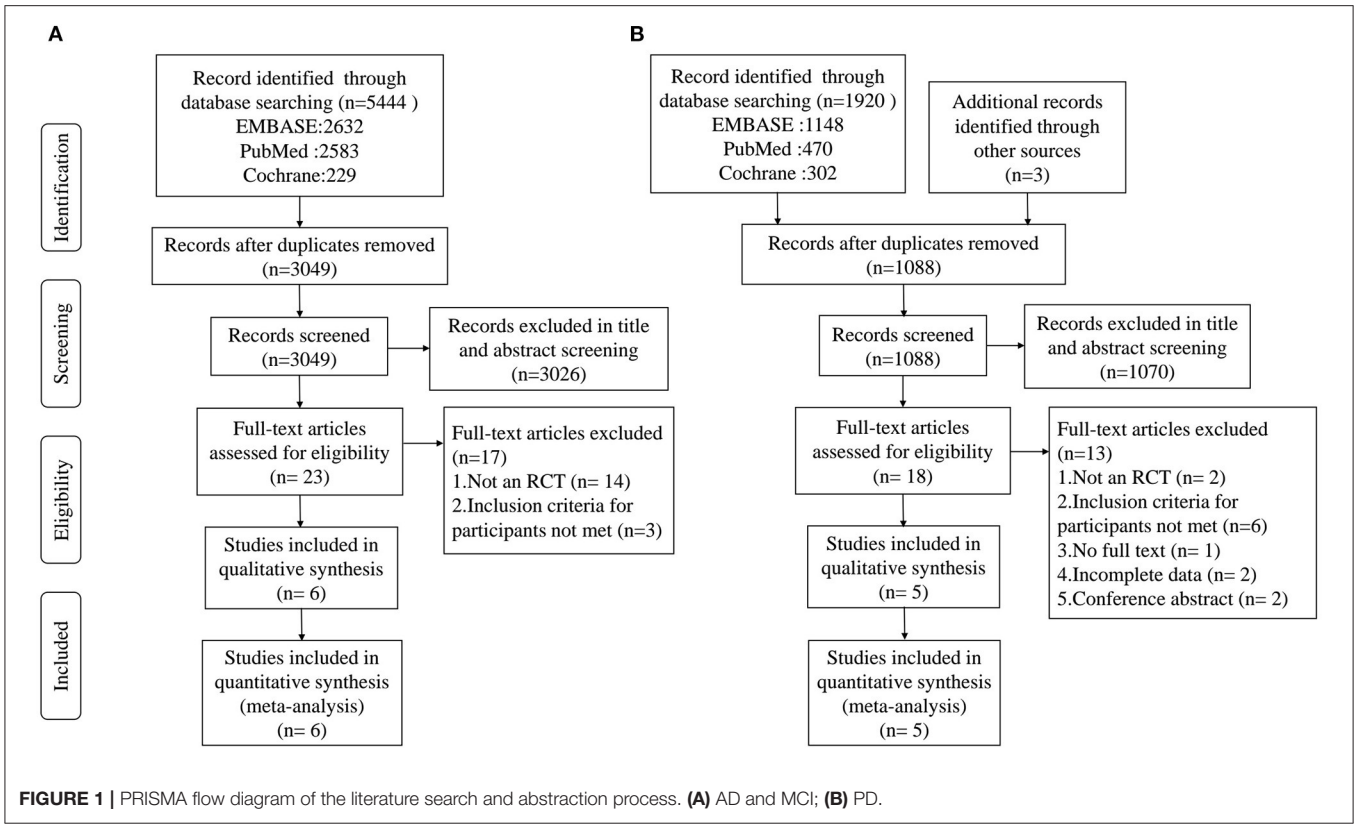

FIGURE 1 | PRISMA flow diagram of the literature search and abstraction process. **(A)** AD and MCI; **(B)** PD.

et al., 2016; Tamtaji et al., 2019a) found significant improvements in cognition in the probiotics group compared with the control group, while the third (Agahi et al., 2018) did not. One of the studies (Tamtaji et al., 2019a) divided subjects into three groups: a control group, a selenium group, and a probiotic-selenium group. In order to explore the influence of probiotics, we only included the experimental data of the latter two groups for the study. Of the three MCI studies, two (Hwang et al., 2019; Xiao et al., 2020) found significant differences, and one (Kobayashi et al., 2019) reported mixed findings. Similarly, the results of studies about the influences of probiotics on various inflammatory and oxidative metabolites have been inconsistent.

As shown in **Table 2**, five PD studies were published from 2016 to 2020 with a total sample size of 342 people. All these studies were randomized, double-blind, controlled trials. One (Georgescu et al., 2016) was based on the modified Hoehn-Yars scale; another (Tan et al., 2021) was based on the Queen Square Brain Bank; and the remaining three (Barichella et al., 2016; Borzabadi et al., 2018; Tamtaji et al., 2019b) were based on the UK Brain Bank. All but two studies (Georgescu et al., 2016; Tamtaji et al., 2019b) reported sex ratios, in which the participants included were mostly male. Multiple strains were used in all the studies. The intervention duration of two included studies (Barichella et al., 2016; Tan et al., 2021) was 4 weeks, and that of the other three (Georgescu et al., 2016; Borzabadi et al., 2018; Tamtaji et al., 2019b) was 12 weeks. All of the studies used probiotic-matched placebos which were indistinguishable from the probiotics in terms of packaging, shape, appearance, size, taste, smell, and so on. Regarding the main findings, three studies (Barichella et al., 2016; Georgescu et al., 2016; Tan et al., 2021) reported that the probiotics intervention group significantly improved the gastrointestinal symptoms of PD patients, such as abdominal pain, abdominal distension, constipation, and other gastrointestinal symptoms. Besides, two studies (Borzabadi et al., 2018; Tamtaji et al., 2019b) found that the probiotics intervention group reduced the gene expression of some inflammatory markers and improved cognition.

Risk of Bias Assessment

A total of 11 included studies were randomized controlled trials, but two studies (Georgescu et al., 2016; Agahi et al., 2018) did not provide information about random sequence generation and four studies (Georgescu et al., 2016; Agahi et al., 2018; Kobayashi et al., 2019; Tamtaji et al., 2019a) did not provide information about allocation concealment. All of the studies described the blindness of the participants and personnel, whereas only four studies (Akbari et al., 2016; Barichella et al., 2016; Georgescu et al., 2016; Kobayashi et al., 2019) reported the blindness of outcome assessments. No risk of incomplete data was found in any of the included studies. In general, the assessment of bias reported a low to moderate risk of bias across all areas. **Figure 2** summarizes the risks of bias assessment across the recruited studies.

Meta-Analysis: Main Results

Three AD studies included 85 patients in the probiotics group and 83 patients in the control group. In these three studies, due

TABLE 1 | Main characteristics of the included AD and MCI studies.

References	Study design	*N*	Diagnostic criteria	Age (M ± SD)		Sex ratio (M/F)		Type of Probiotics	Duration (weeks)	Dose	Primary outcome	Secondary outcome
				PRO	CON	PRO	CON					
Akbari et al. (2016)	Randomized Double-blind Placebo-Controlled Trial	60	AD(NINDS-ADRDA criteria)	77.67± 2.62	82.00 ± 1.69	6/24	6/24	Multiple (Lactobacillus acidophilus, Lactobacillus casei, Bifidobacterium bifidum, Lactobacillus fermentum)	12	8×10^9 (CFU/g)	MMSE	TAC GSH MDA hs-CRP NO
Agahi et al. (2018)	Randomized Double-blind Placebo-Controlled Trial	48	AD (NINDS-ADRDA criteria)	79.70 ± 1.72	80.57 ± 1.79	7/18	10/13	Multiple (*Lactobacillus fermentum, Lactobacillus plantarum*, *Bifidobacterium lactis Lactobacillus acidophilus, Bifidobacterium bifidum, Bifidobacterium longum*)	12	3×10^9 (CFU/d)	TYM	TAC GSH MDA NO
Tamtaji et al. (2019a)	Randomized Double-blind Placebo-Controlled Trial	90	AD (NINDS-ADRDA criteria)	76.2 ± 8.1	78.5 ± 8.0	/	/	Multiple (Lactobacillus acidophilus, Bifidobacterium bifidum, Bifidobacterium longum)	12	6×10^9 (CFU/d)	MMSE	TAC GSH MDA hs-CRP NO
Kobayashi et al. (2019)	Randomized Double-blind Placebo-Controlled Trial	121	Subjective memory complaints (MMSE, 22–27)	61.5 ± 6.83	61.6 ± 6.37	30/31	30/30	Sole (*Bifidobacterium breve* A1)	12	$>2.0 \times 10^{10}$ (CFU/d)	RBANS MMSE	hs-CRP
		44	MCI (RBANS <41)					Sole (*Bifidobacterium breve* A1)	12	$>2.0 \times 10^{10}$ (CFU/d)	RBANS MMSE	
Hwang et al. (2019)	Randomized Double-blind Placebo-Controlled Trial	100	MCI (DSM-5)	68.0 ±5.12	69.2 ± 7.00	20/30	14/36	Sole (*Lactobacillus plantarum* C29)	12	$>1.0 \times 10^{10}$ (CFU/d)	VLT ACPT DST	
Xiao et al. (2020)	Randomized Double-blind Placebo-Controlled Trial	80	MCI (MMSE ≥22)	61.3 ± 7.7	60.9 ± 6.9	19/21	20/20	Sole (*Bifidobacterium breve*A1)	16	$>2.0 \times 10^{10}$ (CFU/d)	RBANS	JMCIS

PRO, probiotics group; CON, control group; AD, Alzheimer's disease; MCI, mild cognitive impairment; NINCDS-ADRDA, National Institute of Neurological and Communicative Diseases and Stroke/Alzheimer's Disease and Related Disorders Association; DSM-5, Diagnostic and Statistical Manual of Mental Disorders, 5th edition; CFU, colony-forming units; MMSE, Mini-Mental State Examination; TAC, total anti-oxidant capacity; GSH, total glutathione; MDA, malondialdehyde; hs-CRP, high-sensitivity C-reactive protein; NO, nitric oxide; TYM, test your memory; RBANS, Repeatable Battery for the Assessment of Neuropsychological Status; VLT, verbal learning test; ACPT, auditory continuous performance test; DST, digit span test; JMCIS, Japanese version of the MCI Screen.

TABLE 2 | Main characteristics of the included PD studies.

Study	Study design	*N*	Diagnostic criteria	Age (M ± SD)		Sex ratio (M/F)		Type of Probiotics	Duration (weeks)	Dose	Primary outcome	Secondary outcome	Main findings
				PRO	CON	PRO	CON						
Georgescu et al. (2016)	Randomized Double-blind Placebo- Controlled Trial	40	PD (modified Hoehn–Yars scale)	69.80 ± 5.64	75.65 ± 9.66	/	/	Multiple (*Lactobacillus acidophilus, Bifidobacterium infantis*)	12	120 mg/d	Abdominal pain; Bloating; Constipation	Non-motor symptoms	Treatment with probiotics could improve abdominal pain and bloating as much as with trimebutine, but less for constipation with incomplete evacuation, where trimebutine showed better results.
Barichella et al. (2016)	Randomized Double-blind Placebo- Controlled Trial	120	PD (UK Brain Bank criteria and Rome III criteria)	71.8 ± 7.7	69.5 ± 10.3	41/39	24/16	Multiple (*Streptococcus salivarius subsp thermophilus, Enterococcus faecium, Lactobacillus rhamnosus, Lactobacillus acidophilus, Lactobacillus plantarum*)	4	2.5×10^{11} (CFU/d)	CBMs	3 or more CBMs; CBMs during weeks 3 and 4; stool frequency; stool consistency; the frequency of laxative use; satisfaction with treatment	The consumption of a fermented milk containing multiple probiotic strains and prebiotic fiber was superior to placebo in improving constipation in patients with PD.

(Continued)

TABLE 2 | Continued

Study	Study design	*N*	Diagnostic criteria	Age (M ± SD)		Sex ratio (M/F)		Type of Probiotics	Duration (weeks)	Dose	Primary outcome	Secondary outcome	Main findings
				PRO	CON	PRO	CON						
Borzabadi et al. (2018)	Randomized Double-blind Placebo- Controlled Trial	50	PD (the UK PD Society Brain Bank criteria)	66.9 ± 7.0	66.7 ± 10.7	17/8	16/9	Multiple (*Lactobacillus acidophilus, Bifidobacterium bifidum, L. reuteri, Lactobacillus fermentum*)	12	8×10^9 (CFU/d)	IL-1 IL-8 TNF-α TGF-β VEGF PPAR-γ	NO GSH GAPDH LDLR	Probiotics supplementation for 12 weeks in PD patients significantly improved gene expression of IL-1, IL-8, TNF-α, TGF-β, and PPAR-γ, but did not affect gene expression of VEGF and LDLR, and biomarkers of inflammation and oxidative stress.
Tamtaji et al. (2019b)	Randomized Double-blind Placebo- Controlled Trial	60	PD (the UK PD Society Brain Bank clinical diagnostic criteria)	68.2 ± 7.8	67.7 ± 10.2	/	/	Multiple (*Lactobacillus acidophilus, Bifidobacterium bifidum, Lactobacillus reuteri, Lactobacillus fermentum*)	12	8×10^9 (CFU/d)	MDS-UPDRS hs-CRP	TAC GSH MDA FPG LDL HDL VLDL HOMA-IR QUICKI Insulin Triglycerides Total cholesterol	Our study evidenced that 12 weeks of probiotic consumption by individuals with PD had useful impacts on MDS-UPDRS and few metabolic profiles
Tan et al. (2021)	Randomized Double-blind Placebo- Controlled Trial	72	PD (Queen Square Brain Bank Criteria and Rome IV criteria)	70.9 ± 6.6	68.6 ± 6.7	20/14	28/10	Multiple (*E. faecium, L. acidophilus, L. paracasei, L. rhamnosus, B. longum, B. bifidum, L. reuteri*)	4	1×10^{10} (CFU/d)	SBMs	Stool consistency; PAC-QOL; constipation severity score; laxative usage; satisfaction with treatment	SBM and secondary outcomes including stool consistency and quality of life related to constipation increased after treatment with probiotics.

PRO, probiotics group; CON, control group; PD, Parkinson's disease; CFU, colony-forming units; CBM, complete bowel movement; IL-1, Interleukin-1; IL-8, Interleukin-1; TNF-α, tumor necrosis factor-α; TGF-β, transforming growth factor-β; VEGF, vascular endothelial growth factor; PPAR-γ, peroxisome proliferator activated receptor gamma; NO, nitric oxide; GSH, total glutathione; GAPDH, glyceraldehyde-3-Phosphate dehydrogenase; LDLR, low-density lipoprotein receptor; MDS-UPDRS, Movement Disorders Society-Unified Parkinson's Disease Rating Scale; hs-CRP, high-sensitivity C-reactive protein; TAC, total anti-oxidant capacity; GSH, total glutathione; MDA, malondialdehyde; FPG, fasting plasma glucose; LDL, low density lipoprotein; HDL, high density lipoprotein; VLDL, very low density lipoprotein; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; QUICKI, quantitative insulin-sensitivity check index; SBM, spontaneous bowel movements; PAC-QOL, Patient Assessment of Constipation Quality of Life.

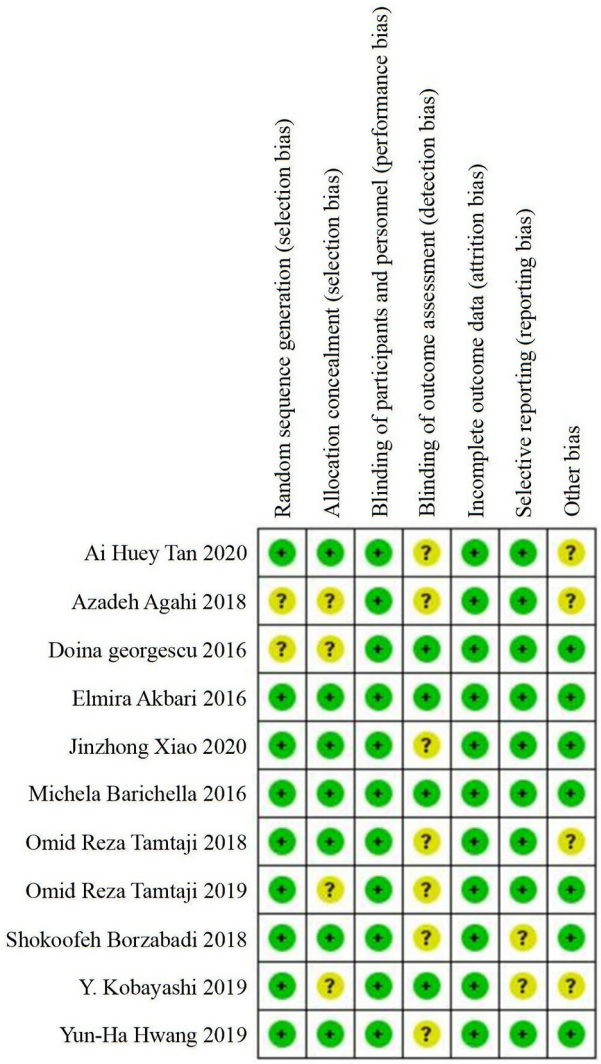

FIGURE 2 | Summary of risk of bias assessment: judgements of the review authors on each risk of bias item for the included studies ($n = 11$).

to acceptable heterogeneity, fixed effect models were selected for quantitative synthesis, and Mini-mental State Examination (MMSE) scores reflecting cognition were significantly different between the probiotics group and the control group (SMD = 0.36; 95% CI, 0.05–0.68; $P = 0.023$; $I^2 = 52.0\%$). In addition, there were significant differences in inflammation-related indicators: hs-CRP (SMD = −0.57; 95% CI, −0.95 to −0.2; $P = 0.003$; $I^2 = 0.0\%$), MDA (SMD = −0.57; 95% CI, −0.89 to −0.26; $P = 0.000$; $I^2 = 0.0\%$), and HOMA-IR (SMD = −0.45; 95% CI, −0.82 to −0.07; $P = 0.019$; $I^2 = 0.0\%$). Lipid-related indicators: VLDL (SMD = −0.42; 95% CI, −0.8 to −0.05; $P = 0.026$; $I^2 = 0.0\%$) and triglyceride (SMD = −0.5; 95% CI, −0.88 to −0.13; $P = 0.009$; $I^2 = 0.0\%$).

The three MCI studies had 117 participants in the probiotics group and 107 in the control group. The fixed effect models were selected for quantitative synthesis. There was a statistically significant difference between the experimental group and the control group in cognition (SMD = 0.86; 95% CI, 0.49–1.24; $P = 0.000$; $I^2 = 0.0\%$). However, there was no statistically significant difference in total cholesterol score (SMD = 0.02; 95% CI, −0.25 to 0.29; $P = 0.885$; $I^2 = 0.0\%$). Other hematological indicators, vital signs and blood biochemical indicators were not significantly different between the two groups, suggesting that probiotics were safe for the subjects. The forest plot of the meta-analysis is shown in **Figure 3**.

Five PD studies included 189 subjects in the intervention group and 153 subjects in the control group. We selected appropriate effect models to synthesize the quantitative data according to the magnitude of heterogeneity. Meta-analysis results revealed a significant difference in glutathione (GSH) between the probiotics group and the control group (SMD = 0.76; 95% CI, 0.37–1.15; $P = 0.001$; $I^2 = 0.0\%$). There was no significant difference in fecal viscosity (SMD = −0.07; 95% CI, −1.32–1.18; $P = 0.702$; $I^2 = 94.0\%$). **Figure 4** shows the remaining results that cannot be merged.

Assessment of Publication Bias and Sensitivity Analysis

We conducted quantitative evaluation of publication bias by Egger's test and Begg's test. The analysis of the cognitive function among AD patients showed no significant publication bias (Egger's test: $P = 0.993$, Begg's test: $P = 1.000$). Sensitivity analysis examined the reliability of the results of the meta-analysis by omitting each study in turn. The systematic removal of each trial did not significantly affect the overall effect of probiotics on the cognition of patients with AD (see **Supplementary Materials**). Therefore, the findings on improved cognitive function in AD patients between the probiotics and the control group were considered reliable.

DISCUSSION

Neurodegenerative diseases are increasingly serious health problems among the aging population. The most common neurodegenerative diseases are AD and PD, the incidences of which are also increasing year by year. Without effective prevention and treatment, the two diseases will impose an increasing socio-economic burden. Although these two diseases have different clinical manifestations, they share similar underlying mechanisms and both associate with normal aging. Age-related changes in the brain can be observed decades before neurological function begins to decline, and studies have repeatedly linked them to immune system activation (Bangen et al., 2017). Neurodegenerative diseases, such as AD and PD, are characterized by the gradual accumulation of abnormal proteins in the CNS (Jucker and Walker, 2018). Two thousand years ago, Hippocrates declared that "all disease begins in the gut," an interesting observation that influenced medical researchers (Cryan et al., 2019). At present, the influence of brain-gut axis on neurodegenerative diseases has attracted increasing attention. As a part of the brain-gut axis, gastrointestinal microbiota influences

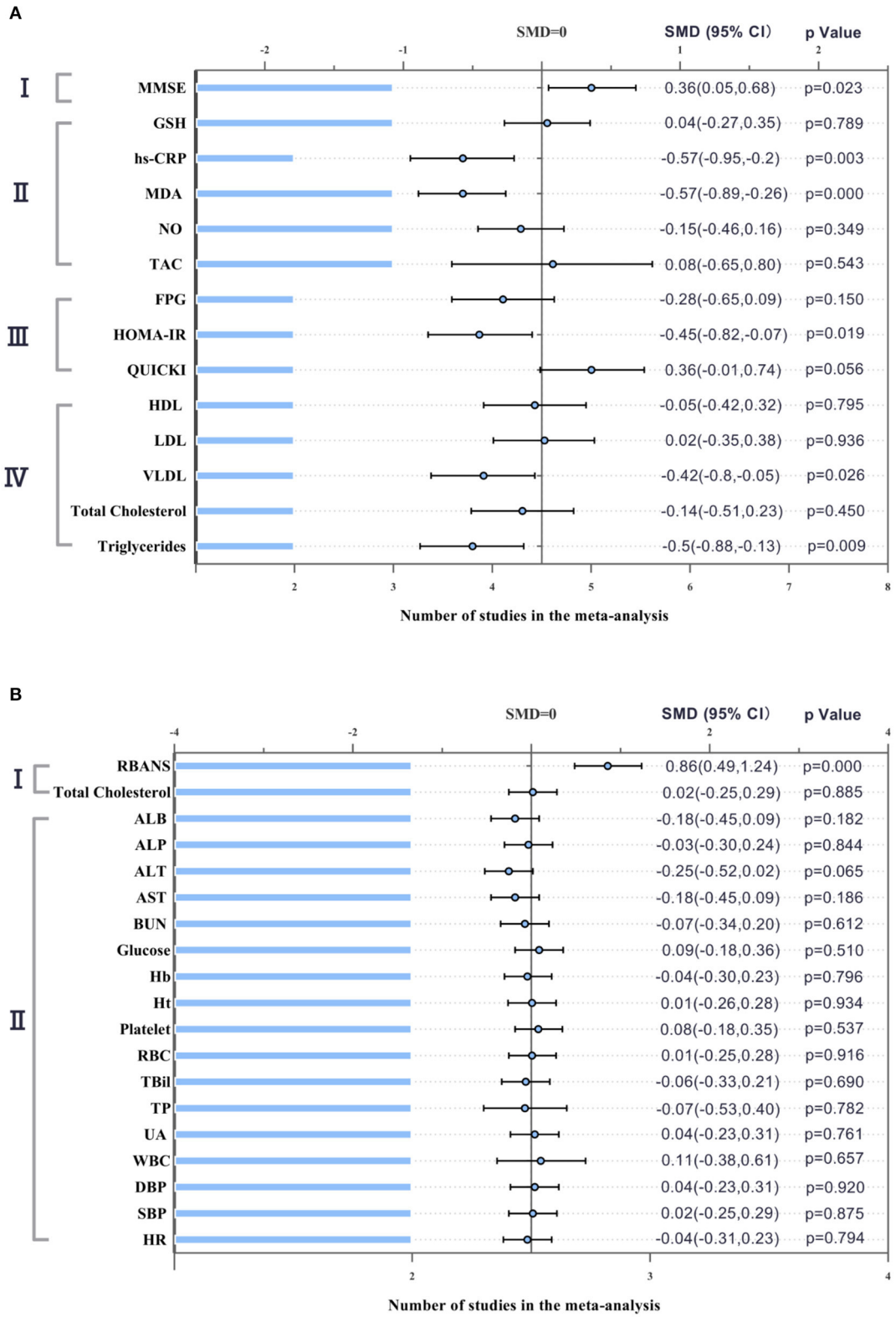

FIGURE 3 | Forest plot showing the effects in the probiotics group versus the control group on cognitive function and biochemical indicators. **(A)** Meta-analysis of the effects of probiotics on patients with AD. I. MMSE (Mini-Mental State Examination); II. Biomarkers of inflammation and oxidative stress. GSH (glutathione); hs-CRP (hypersensitive-CRP); MDA (Malondialdehyde); NO (Nitric Oxide); TAC (tricarboxylic acid cycle); III. Glucose related indicators. FPG (fasting plasma glucose);

(Continued)

FIGURE 3 | HOMA-IR (Homeostasis model assessment); QUICKI (Quantitative insulin sensitivity check index); IV. Lipid related indicators. HDL (high-density lipoprotein); LDL (low-density lipoprotein); VLDL (very low density lipoprotein). **(B)** Meta-analysis of the effects of probiotics on patients with MCI. I. RBANS (Repeatable Battery for the Assessment of Neuropsychological Status); II. Hematological and biological blood parameters and vital signs. ALB (Albumin); ALP (Alkaline phosphatase); ALT (Alanine transaminase); AST (Aspartate aminotransferase); BUN (Blood Urea Nitrogen); Hb (Hemoglobin); Ht (Hematocrit); RBC (red blood cell); TBil (total bilirubin); TP (total protein); UA (Uric acid); WBC (white blood cell); DBP (diastolic blood pressure); SBP (systolic blood pressure); HR (heart rate). The differences were significant if *P*-value was <0.05.

immunity, inflammation, and neuroregulation through the brain-gut axis. Therefore, maintaining a healthy and stable microbiome brings great benefits for immune defense, brain function, and dynamic balance (Westfall et al., 2017).

In the past decades, researchers have paid attention to the medication for AD and PD. Recently, aducanumab that targets the neurobiology of AD is approved for clinical use (Cummings and Salloway, 2021). However, the reported clinical benefits are limited and may not be apparent to individuals. In addition, the treatment has potential side effects, especially ARIA, which requires MRI monitoring for detection (Sperling et al., 2011). More recently, a growing number of research studies have shown that lifestyle changes, such as changes in diet, can ease cognitive decline (Morris et al., 2015). Probiotic intervention may be an effective measure to ameliorate age-related neurodegenerative diseases (Alkasir et al., 2017). Remarkably, our results suggest that low-cost, available, and safe probiotics could be potential candidates for the treatment of AD, MCI and PD, although the exact mechanisms remain unclear.

Oxidative stress and inflammation are two major causes of neurodegenerative diseases, and they get worse with age. Inflammation is an important risk factor for both morbidity and mortality in older adults, as most age-related diseases share the same inflammatory pathogenesis (Franceschi and Campisi, 2014). The mitochondria of cells constantly produce reactive oxygen species (ROS). In normal cells, the ROS generated can be neutralized by the antioxidant system without causing damage. However, with the aging of the organism, the defense function of cells is weakened and the cell membranes are damaged, which eventually leads to cell death (Mitsuma et al., 2008). The brain is particularly vulnerable to oxidative stress due to high oxygen consumption rates (OCRs), high polyunsaturated fatty acids, high iron content, and relatively low antioxidant capacity (Noseworthy and Bray, 1998), especially in the amygdala and hippocampal neurons (Wadhwa et al., 2018). The slow accumulation of ROS in neurons stimulates the release of cytokines, which in turn stimulates microglia activation and neuroinflammation.

For PD studies, the results of meta-analysis showed significantly higher levels of GSH in the probiotic group compared to the control group, which acts as an antioxidant, a free radical scavenger and a detoxifying agent. In addition, probiotic intake downregulated gene expression of interleukin-1 (IL-1), IL-8 and tumor necrosis factor alpha (TNF-α) but upregulated transforming growth factor beta (TGF-β) and peroxisome proliferator-activated receptor gamma (PPAR-γ). Probiotics can also improve abdominal symptoms, such as relieving abdominal pain, bloating and constipation, and increasing the number of SBMs and CBMs. For AD and MCI studies, the results showed that probiotics significantly improved cognitive function, insulin resistance, and lipid metabolism, and significantly reduced inflammatory markers such as hs-CRP and MDA.

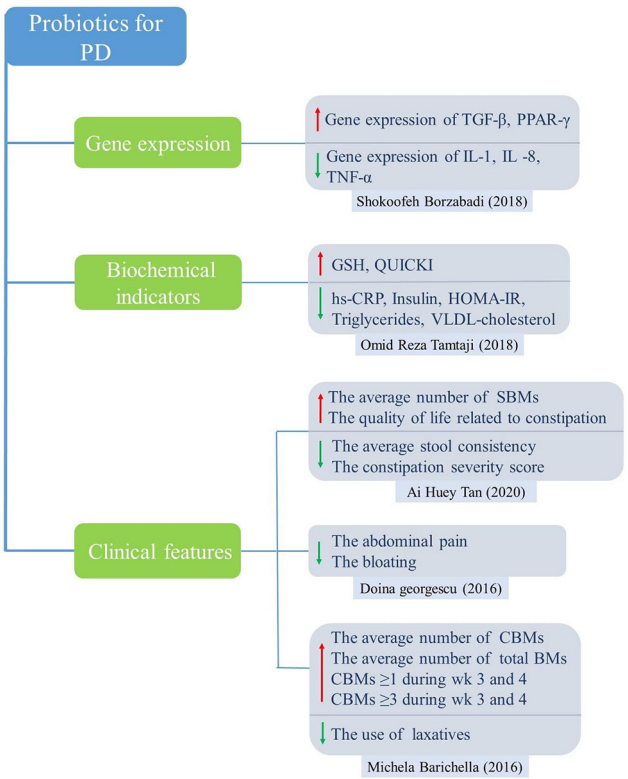

FIGURE 4 | Main findings of the effects of probiotics on patients with PD.

In the elderly, overstimulation of the immune system leads to a chronic low-grade inflammatory state, which may be related to a persistent inflammatory state of the gut microbiota characterized by reduced diversity and stability (Frasca and Blomberg, 2016). In the aging process, the number of pathogens increases, such as Enterobacteriaceae, which was positively correlated with difficulty in maintaining posture and balance (Scheperjans et al., 2015). However, the probiotics and neuroprotective molecules decrease (Lambert et al., 2009; Caracciolo et al., 2014). A study involving 72 PD patients and 72 healthy subjects found that the number of Prevotellaceae in the intestines of PD patients decreased by 77.6%. Mucin produced by this microbe can form a barrier along the intestinal wall to protect against pathogen invasion. In addition, a decrease in bacteria capable

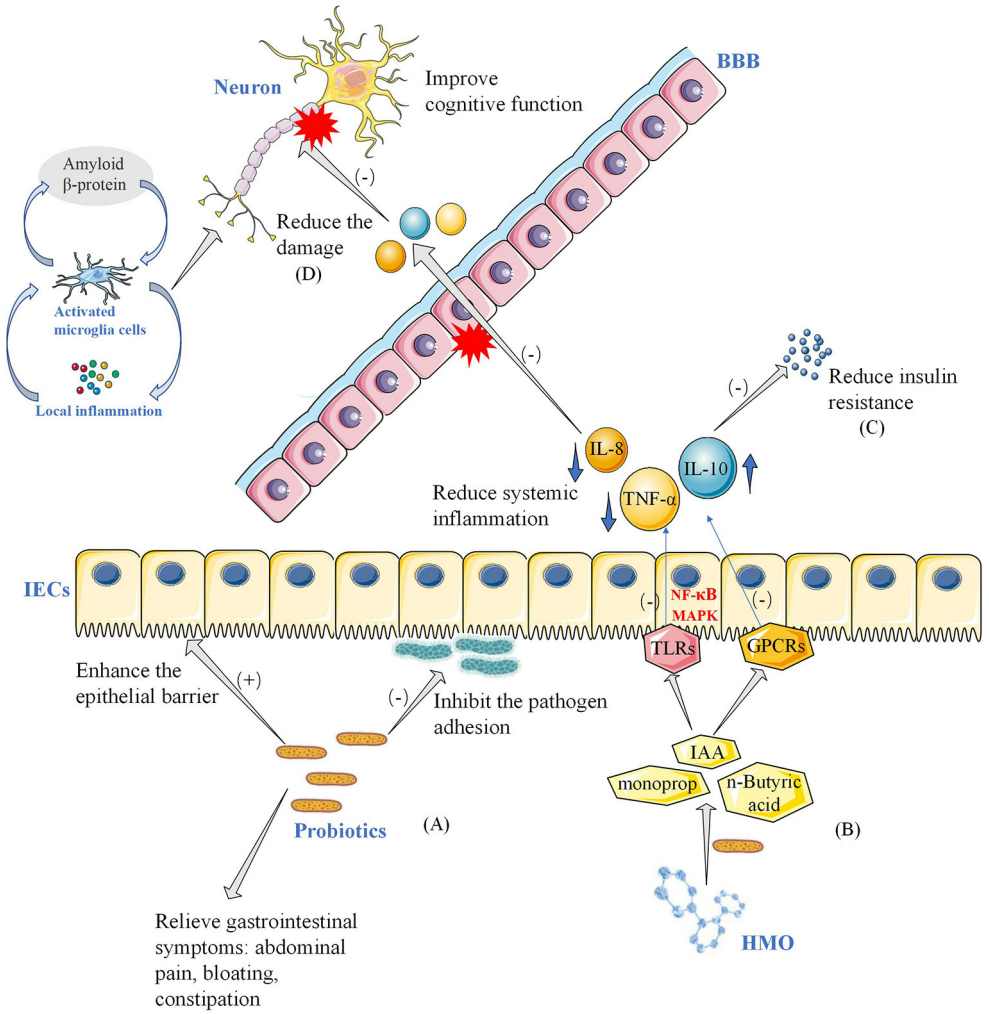

FIGURE 5 | Potential mechanisms of probiotics improving neurocognitive function and gastrointestinal symptoms. **(A)** In this model, probiotics in the intestinal tract strengthen the intestinal barrier and inhibit the attachment of pathogenic bacteria to the intestinal wall, thereby improving gastrointestinal symptoms such as abdominal pain, bloating, and constipation in patients with Parkinson's disease. **(B)** Probiotics take HMO as substrate and decompose it into metabolites such as acetic acid. SCFA and other bacterial metabolites act on APC and IECs through toll-like receptors and GPCRs. Reduced systemic inflammation, including pro-inflammatory cytokines and anti-inflammatory cytokines, through NF- B and MAPK pathways. **(C)** Reduction of inflammation reduces insulin resistance on the one hand, and on the other hand **(D)** reduction of damage to the blood-brain barrier, reducing damage to neurons, and thus improving neurocognitive function. **(E)** HMO, human milk oligosaccharides; SCFA, short-chain fatty acids; GPCRs, G Protein-Coupled Receptors; APC, antigen-presenting cells; IECs, intestinal epithelial cells; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; MAPK, Mitogen-activated protein kinase; IL-10, interleukin 10; IL-8, interleukin 8; TNF-α, tumor necrosis factor α; BBB, blood-brain barrier; IAA, auxin; TLRs, Toll-Like Receptors; BBB, blood–brain barrier.

of producing short-chain fatty acids (SCFA) was found in PD patients (Unger et al., 2016). The decrease in SCFA can lead to decreased expression of endothelial tight junction proteins, especially Occludin and Claudin-5, thus increasing blood-brain barrier (BBB) permeability (Braniste et al., 2014; Sampson and Mazmanian, 2015). A series of pro-inflammatory neurotoxins produced by intestinal microflora cross the BBB and impair the dynamic balance function of neurons in the CNS (Cryan et al., 2019). SCFA can also increase intestinal peristalsis by regulating the activity of ENS (Soret et al., 2010). Therefore, changes in SCFA concentration may lead to gastrointestinal motility disorders in patients with PD.

AD is considered a systemic disease because it is associated with neuroinflammation in the brain as well as peripheral inflammatory responses. Moreover, inflammation in the brain, which occurs many years before the appearance of plaques, is associated with Aβ production and antimicrobial responses (Bronzuoli et al., 2016; Le Page et al., 2018). Neuroinflammation is an inflammatory response of the CNS to injury or infection, accompanied by the aggregation of glial cells. During this process, chemokines, complements and pattern recognition receptors (PRRs) activate microglia and astrocytes to produce pro-inflammatory cytokines, especially IL-1β, IL-6, TNF-α (Morales et al., 2014; Calsolaro and Edison, 2016). The activated

microglia in turn activate other microglia and astrocytes. An acute inflammatory response caused by activated glial cells leads to repair of the damaged areas of the brain, while chronic inflammation is usually low-grade and persistent which causes tissue degeneration. Besides, in the aging brain, chronic inflammation impairs its function of clearing abnormal proteins, leading to amyloid precursor protein (APP) accumulation, Double Helix Filament formation, and synaptic dysfunction. All of these events lead to neurodegeneration and cognitive decline (Morales et al., 2014; Lim et al., 2015). In addition, the gut flora can produce a range of metabolites, such as γ-aminobutyric acid (GABA), 5-hydroxytryptamine (5-HT), histamine and dopamine, all of which act as neurotransmitters or neurotransmitter precursors to participate in a range of mood, behavior and cognitive functions (Clarke et al., 2014; Dinan and Cryan, 2017). Previous studies have also shown that hypercholesterolemia is an early risk factor for the pathological development of amyloidosis. There are more lipid granules (or fat inclusions) in glial cells in the brain of AD patients, suggesting abnormal lipid metabolism. Population-based longitudinal studies have shown the link between high cholesterol levels and AD (Kivipelto et al., 2001).

The World Health Organization defines probiotics as "live microorganisms, which when administered in adequate amounts, confer a health benefit on the host" (Hill et al., 2014). Although probiotic mechanisms of action are still being studied, several hypotheses have been proposed. Probiotics produce antimicrobial agents or metabolic compounds that suppress the growth of other microorganisms (Hemarajata and Versalovic, 2013). The metabolic activity of probiotics increases the amount of metabolites in the gut, such as SCFAs (Zartl et al., 2018), which represent the end products of bacterial activity in the gastrointestinal tract. These probiotics block the synthesis of hepatobiliary sterols, resulting in a decrease in the amount of lipids in the blood (Taylor and Williams, 1998; Byrne et al., 2015). They also regulate the transcription of genes involved in tight junction, improve intestinal epithelial barrier function (Anderson et al., 2010), reduce blood sugar levels and improve insulin resistance (Tabuchi et al., 2003; Bagarolli et al., 2017). The underlying mechanisms by which probiotics improve gastrointestinal function and reduce inflammatory response are shown in **Figure 5**. A growing body of evidence indicates that probiotics can help intestinal microflora proliferate, promote their composition shift toward a more balanced structure, and help fight pathogens by regulating the immune system (Pandey et al., 2015). The anti-inflammatory effects of probiotics in mouse models were preliminarily confirmed. Caroline Xie et al. showed that probiotics ameliorated hippocampal-dependent cognitive deficits in preclinical PD models (Xie and Prasad, 2020). Perez Visnuk et al. showed that decreased levels of inflammatory cytokines IL-6 and TNF-α in serum and increased levels of anti-inflammatory cytokines IL-10 in serum and brain tissue in the probiotic-treated PD mice (Perez Visñuk et al., 2020). A study by Athari Nik Azm et al. showed that probiotics reduced oxidative stress biomarkers and amyloid plaque formation in mice (Athari Nik Azm et al., 2018). Kobayashi et al. also showed that B. Breve A1 inhibited the expression of amyloid-β-induced hippocampal inflammation and immune response genes (Kobayashi et al., 2017). These results suggested that probiotics might play a role in health through anti-inflammatory and anti-oxidative stress. Our results showed that probiotics reduced the levels of inflammatory and oxidative stress markers (hs-CRP, MDA). In a randomized controlled trial, probiotics reduced the expression of pro-inflammatory factors (IL-1, TNF-α) genes, increased the expression of anti-inflammatory factors (TGF-β, PPAR-γ) genes, and reduced the expression of inflammatory and oxidative markers in PD patients (Borzabadi et al., 2018). It is worth noting that inflammatory and oxidative stress pathways are not unique to AD and PD, and they may play an important role in a variety of diseases, especially age-related diseases (Buford, 2017). After taking probiotics, the number of total bowel movements (CBM), the number of spontaneous bowel movements (SBM), and the Bristol Stool Scale in PD patients improved. VLDL, triglyceride and HOMA-IR were significantly improved.

Three studies about MCI showed that probiotics had no adverse effects on participants' hematological blood parameters, vital signs, and biological blood parameters, and no adverse reactions related to probiotics use were reported in the 11 included studies. Therefore, the Bifidobacterium and Lactobacillus strains used in involved studies were safe and well-tolerated.

In our study, three studies involving patients with AD used multiple strains (Akbari et al., 2016; Agahi et al., 2018; Tamtaji et al., 2019a), and the other three literatures with MCI applied a sole strain of probiotics (Hwang et al., 2019; Kobayashi et al., 2019; Xiao et al., 2020). There were significant differences between the experimental group and the control group in the cognitive function indicators MMSE and RBANS, but we could not confirm whether the single strain was effective for AD patients. In the study of Aghahi et al., AD patients were insensitive to a mixture of six strains (Agahi et al., 2018). It is suggested that the efficacy of probiotics may be related to the severity of the patient's disease, the time of intervention, the dose and the proportion of strains. Although the results showed that MMSE was statistically different between the probiotic group and placebo group, larger clinical data and longer follow-up are needed to validate its clinical value. The definition of probiotics requires an appropriate amount to obtain a health benefit, but what does is called an appropriate amount is not stated. Due to the limited information provided by the included studies, we were also unable to determine whether there was a dose-response relationship with the improvement of symptoms or determine the most appropriate dose of probiotics. Most of the included studies used a probiotic dose of 10^9 to 10^{10} CFU as a reference. More reliable evidence is needed in the future, especially beyond conventional doses (10^9 to 10^{10} CFU). Compared to the placebo groups, the probiotic groups all showed greater improvements in cognitive function in 12 weeks. Therefore, the present studies showed that a probiotic intervention of 12 weeks may improve cognitive function. But whether the effect can be achieved in a shorter time, or sustained over a longer period, remains to be verified.

There are also several limitations: (1) The strains, dosage and intervention time of probiotics are not the same, which will have a certain influence on the outcome. (2) The criteria for recruiting MCI subjects were different: (Kobayashi et al., 2019) used RBANS<41 as the diagnostic criteria for MCI patients; (Hwang et al., 2019) diagnosed MCI according to Diagnostic and The Statistical Manual of Mental Disorders, 5th Edition (DSM-5); (Xiao et al., 2020) took MMSE≥22 as the diagnostic criterion. (3) Among the 3 AD studies, two of them (Akbari et al., 2016; Tamtaji et al., 2019a) used MMSE as the evaluation criterion of cognition, and one (Agahi et al., 2018) evaluated cognition according to TYM, which may affect the results of the meta-analysis.

The included RCTs have some limitations. Therefore, larger RCTs with longer follow-up will be needed in the future to offer more reliable evidence. For the future RCTs, we have some suggestions as follows. Firstly, additional subgroups should be considered, including different probiotic doses, different intervention times and different severity of diseases, etc. Secondly, more indicators should be included, such as impaired cognitive related inflammatory biomarkers, S100A12, and neopterin. In addition, other cognitive assessments that may more sensitivity assess the impact of cognition rather than the global screening metric of MMSE should be conducted. At present, there are 3 ongoing RCTs on AD and 5 ongoing RCTs on PD, which will provide more evidence for future studies. The information of these RCTs is shown in the **Supplementary Material**.

CONCLUSION

This meta-analysis suggested that probiotics could enhance cognitive function in patients with AD and MCI and improve gastrointestinal symptoms in patients with PD. Probiotics may be involved in reducing biomarkers of inflammation and oxidative stress. However, the current RCTs still have some limitations, and larger RCTs with longer follow-up will be needed in the future to provide more reliable evidence.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

SX and C-CT: conceptualization and design of the study, collection and analysis of the data, drafting and revision of the manuscript, and prepared all the figures. J-LJ and SL: collection and analysis of the data, and revision of the manuscript. WX, X-PC, and LT: revision of the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnagi.2022.730036/full#supplementary-material

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Altered Brain Activity in Depression of Parkinson's Disease: A Meta-Analysis and Validation Study

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Background: The pathophysiology of depression in Parkinson's disease (PD) is not fully understood. Studies based upon functional MRI (fMRI) showed the alterations in the blood-oxygen-level-dependent (BOLD) fluctuations in multiple brain regions pertaining to depression in PD. However, large variance was observed across previous studies. Therefore, we conducted a meta-analysis to quantitatively evaluate the results in previous publications and completed an independent regions-of-interests (ROIs)-based analysis using our own data to validate the results of the meta-analysis.

Methods: We searched PubMed, Embase, and Web of Science to identify fMRI studies in PD patients with depression. Using signed differential mapping (SDM) method, we performed a voxel-based meta-analysis. Then, a validation study by using multiscale entropy (MSE) in 28 PD patients with depression and 25 PD patients without depression was conducted. The fMRI scan was completed in anti-depression-medication-off state. The ROIs of the MSE analysis were the regions identified by the meta-analysis.

Results: A total of 126 PD patients with depression and 153 PD patients without depression were included in meta-analysis. It was observed that the resting-state activities within the posterior cingulate gyrus, supplementary motor area (SMA), and cerebellum were altered in depressed patients. Then, in the validation study, these regions were used as ROIs. PD patients with depression had significantly lower MSE of the BOLD fluctuations in these regions (posterior cingulate gyrus: $F = 0.856$, $p = 0.049$; SMA: $F = 0.914$, $p = 0.039$; cerebellum: $F = 0.227$, $p = 0.043$).

Conclusion: Our study revealed that the altered BOLD activity in cingulate, SMA, and cerebellum of the brain were pertaining to depression in PD.

Keywords: Parkinson's disease, depression, functional magnetic resonance imaging, multiscale entropy, brain activities

INTRODUCTION

Parkinson's disease (PD) is the second most important age-related neurodegenerative disorder in older adults. PD patients suffer from multiple non-motor symptoms together with the motor issues. One of the most common non-motor symptoms in PD is depression, which impairs both mental and physical function and diminishes a person's quality of life (Aarsland et al., 2011; Skorvanek et al., 2015). Depression in PD is believed to be closely associated with pathological changes in neurotransmitter systems (Schapira et al., 2017). During the last decade, studies using positron emission tomography (PET) observed that dopamine transporter availability and noradrenergic innervation in the striatum and limbic brain regions were reduced in PD patients with depression compared to PD patients without depression (Burn et al., 2012; Vriend et al., 2014); and the alterations in dopamine transporter availability and noradrenergic innervation influence neural activity in cortical regions (Pa et al., 2013; Meyer et al., 2019). Depression is also characterized by deficits of excitatory glutamate neurons and inhibitory GABA interneurons (Duman et al., 2019). Evidence of associations between glutamate and/or GABA levels and fMRI signal was found recently (Delli Pizzi et al., 2020; Demartini et al., 2020; Kiemes et al., 2021). Therefore, characterization of the altered cortical neural activities in PD patients with depression is of great significance to help better understand the pathology underlying it, which can help optimize the design of therapeutic strategies and protocols for PD patients with depression.

Multiple studies have characterized resting-state brain activities in PD patients with depression by measuring the dynamics of functional magnetic resonance imaging (fMRI) blood oxygen level-dependent (BOLD) signals. Most results showed that alterations in PD patients with depression were predominantly in the prefrontal cortex and limbic system (Luo et al., 2014; Wang et al., 2020). However, the sample sizes of the studies were relatively small, and large variances in study protocol and data analytic techniques were observed between studies (Luo et al., 2014). For example, amplitude of low-frequency fluctuation (ALFF) is used to detect the regional intensity of spontaneous fluctuations in the BOLD signal (Cordes et al., 2001), Wen et al. (2013) and Hu et al. (2015) observed increased ALFF in the temporal lobe, while Luo et al. (2014) reported similar ALFF values in this area (Sheng et al., 2014; Wang et al., 2018). The regional homogeneity (ReHo) calculates the synchronization of low-frequency fluctuations between a given voxel with neighboring voxels (Zang et al., 2004), and the degree centrality (DC) value calculates the centrality of a node by adding the centrality of adjacent nodes (Wu et al., 2013). Sheng et al. (2014) and Wang et al. (2020) reported alterations in DC and ReHo in the lingual gyrus and supplementary motor area (SMA) in PD patients with depression; however, other studies did not find significant differences in the lingual gyrus and SMA between groups. These kinds of inconsistency across studies thus largely limit the understanding and characterization of the neural-physiologic mechanisms underlying depression in PD.

Here, we therefore performed a coordination-based meta-analysis with the goal of quantitatively and systematically examining the results from previous studies. To validate the results of meta-analysis, we performed a region-of-interest (ROI)-based study using fMRI data from 28 PD patients with depression and 25 PD patients without depression. The regions identified in our meta-analysis were used as ROIs of the analysis. The previous studies in our meta-analysis used various method of analysis, including ALFF, ReHo, and DC, which reflected the alteration of BOLD signals on a single scale. However, it is known that resting-state neural activities within the brain are regulated by multiple components across multiple scales of time, ranging from milliseconds (e.g., the time to transmit neural impulses) to hours or days (e.g., circadian rhythms). The multiscale dynamics of resting-state BOLD fluctuations are thus complex, which provides key information on neurophysiological regulations (Yang et al., 2015). Studies have emerged to characterize such complex dynamics by using multiscale entropy (MSE) and have demonstrated that resting-state BOLD complexity is closely associated with important functional performance (Liu et al., 2019; Zhou et al., 2020). Here, we also used MSE to characterize the complexity of BOLD fluctuation in each ROI. Our primary hypothesis is that the complexity of BOLD fluctuations within those ROIs would be lower in PD patients with depression as compared to PD patients without depression, confirming the findings in the meta-analysis.

MATERIALS AND METHODS

Meta-Analysis

Search Strategies

We searched the literature in PubMed, Embase, and Web of Science using the following free-text terms: ("Parkinson's disease" OR "PD") AND ("fMRI" OR "functional magnetic resonance imaging" OR "BOLD" OR "blood oxygen level dependent" OR "ALFF" OR "amplitude of low frequency fluctuation" OR "ReHo" OR "regional homogeneity" OR "DC" OR "degree centrality" OR "functional connectivity" OR "FC") AND ("depression" OR "depressed" OR "mood" OR "emotion" OR "emotional" OR "psychology" OR "psychological" OR "neuropsychological" OR "psychiatric" OR "neuropsychiatric"). Next, we examined the references of the included studies to identify additional eligible publications. The final search was completed in March 2021.

Study Selection

After the literature search, a total of 2,312 articles were retrieved. We first removed the duplicates from the search results. Then, full text reports were obtained and screened in detail. The inclusion criteria were: (1) resting state fMRI studies comparing a group of PD patients with depression with a sample of PD patients without depression; (2) studies using metrics for measuring local characteristics of resting state fMRI data (Such as ReHo, DC, or ALFF/fALFF) and based on the whole brain analysis; (3) studies reporting results with coordinates in Montreal Neurological Institute (MNI) or Talairach space. The exclusion criteria were as follows: (1) review articles, case reports and editorial letters; (2) conference proceedings without full report publication; (3) participates duplicate; (4) undefined PD patients with depression

or not enough information provided to determine whether depression was present; (5) no resting state fMRI. For all the articles that no whole brain results were reported in the papers, we contact the corresponding authors but there was no response we can receive. Any disagreement between the two researchers was resolved by discussion or consulting a third specialist. The study selection process is presented in a PRISMA flowchart.

Data Extraction

Two researchers independently extracted data, and discrepancies were resolved in a consensus meeting. When no consensus was reached, a third specialist was consulted. From all eligible studies, we extracted the following information: first author, year of publication, sample size, MRI type, analysis method, statistical threshold, standard stereotactic space and patient characteristics [age, Unified Parkinson's Disease Rating Scale (UPDRS) score, medication state, Hamilton Rating Scale for Depression (HRSD) score, and Mini-mental State Examination (MMSE) score et al.]. We also extracted peak coordinates and effect size measures of regions with a significant difference between the PD patients with depression and PD patients without depression.

Quality Assessment

We assessed the quality of fMRI studies by criteria derived from the guidelines for reporting an fMRI study described by Poldrack et al. (2008). These criteria were aimed at ensuring that detailed descriptions of the methods and results are included in fMRI studies. The criteria consisted of nine domains, and their specifications are provided as Supplementary Date in **Supplementary Material**. Each study domain was scored at 0.5 or 1 point, and the points of all the domains were totaled; studies that scored $\geq$7.5 were considered good, those that scored 4–7.5 were considered fair and those that scored $\leq$4 were considered poor quality. Two researchers performed the quality assessment independently. When the scores of assessments were different between these two reviewers, a third researcher was invited to join the discussion until all three reviewers agreed with the score.

Meta-Analysis

Our meta-analysis was conducted using signed differential mapping (SDM). SDM was a voxel-based meta-analysis that enables investigators to combine neuroimaging studies reporting peak coordinates. It uses peak coordinates to recreate a statistical parametric map of the effect size of the differences between PD patients with depression and PD patients without depression in each study and then performs a random-effects variance-weighted meta-analysis in each voxel (Wolters et al., 2019). In our meta-analysis, we used the default effect size version of signed differential mapping (ES-SDM) kernel size and thresholds (FWHM = 20 mm, voxel p = 0.005, peak height SDM-Z = 1, cluster extent = 10 voxels).

Robustness Analysis

To assess the robustness of the results, we first performed a heterogeneity analysis using a random effects model with Q statistics ($p < 0.005$) to determine whether there were significant unexplained variabilities between the study groups in the results. Then, we conducted jack-knife analysis ($p < 0.005$) by systematically repeating the meta-analyses after excluding one study at a time to test the replicability of the results in the meta-analysis.

Regions-of-Interests-Based Functional Magnetic Resonance Imaging Study

Participants

Twenty-eight PD patients with depression and 25 age- and sex-matched PD patients without depression were recruited via the Department of Neurology, Beijing Tiantan Hospital completed this study. Patients with a diagnosis of PD from three neurologists according to the 2015 Movement Disorder Society (MDS) criteria (Postuma et al., 2015) were included in both groups. Participants in both groups were excluded using the following criteria: moderate to severe head tremor, functional motor disorder (Tinazzi et al., 2021a,b), cerebrovascular disorders, antiparkinsonian treatment with dopamine agonists, antidepressant treatment or other psychiatric therapy, and cognitive impairment as defined by an MMSE score <24. For the PD patients with depression, depression was diagnosed based on the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-V) criteria by an experienced, board-certified psychiatrist trained to administer structured clinical interviews. This study was approved by the Medical Ethical Review Committee of Beijing Tiantan Hospital. All procedures conformed to the Declaration of Helsinki. Every subject signed informed consent forms prior to participation.

Data Acquisition

The severity of clinical symptoms was assessed according to the Hoehn and Yahr (H&Y) rating scale (Hoehn and Yahr, 1967), and the motor part of the UPDRS (Hamilton, 1960). H&Y score was calculated in anti-Parkinsonian medication OFF state. The severity of depression symptoms was assessed according to the HRSD (Hamilton, 1960). The demographic and clinical data of the included participants are shown in **Table 1**.

All the fMRI were completed in resting state, that is, no task was performed by the participants during the MRI scan. Patients

TABLE 1 | Demographic and clinical characteristics of all subjects.

	PD patients with depression	PD patients without no depression	*P*-value
Gender(female/total)	17/28	9/25	-
Age	63.61 ± 8.32	63.56 ± 8.79	0.984
Disease duration	8.19 ± 4.92	7.48 ± 4.68	0.600
MMSE	25.13 ± 3.66	26.54 ± 3.12	0.155
HRSD	15.79 ± 6.42	3.08 ± 1.96	<0.01
H&Y stage	2.64 ± 0.80	2.23 ± 0.67	0.063
UPDRS-III	39.93 ± 13.81	41.33 ± 13.70	0.776

Values are represented as the mean ± standard deviation (SD). P-values were obtained using two sample t-test (P < 0.05). MMSE, Mini-mental state examination; HRSD, Hamilton Rating Scale for Depression; HAMA, Hamilton Anxiety Scale; H&Y stage, Hoehn and Yahr stage; UPDRS-III, Unified Parkinson's disease rating scale part III.

underwent resting state fMRI after an overnight withdrawal of anti-Parkinsonian medication. Images were acquired on 3 T SIEMENS MAGNETOM Prisma scanner (Siemens Healthineers, Erlangen, Germany) using a 64-channel head coil. Structural 3D T1-weighted images were acquired with magnetization-prepared rapid gradient echo (MP-RAGE) sequence with the following parameters: repetition time = 2,300 ms, echo time = 2.26 ms, inversion time = 900 ms, flip angle = 8°, field of view = 256 mm × 256 mm, matrix = 256 × 256, number of slices = 186, voxel size = 1 mm × 1 mm × 1 mm with no gap. Resting state data were acquired using an echo-planar imaging sequence with repetition time = 750 ms, echo time = 30 ms, number of slices = 64, flip angle = 54°, field of view = 222 mm × 222 mm, matrix size = 74 × 74, voxel size = 3 mm × 3 mm × 3 mm with no gap. Resting state scans were carried out in a scanning run of 6 min and 11 s. Prior to scanning, foam padding and headphones were placed on the subjects to limit head motion and reduce scanner noise, respectively; also, patients were instructed to keep their eyes closed, relax but not fall asleep, and move as little as possible during scanning. Criteria for head motion correction are included as Supplementary Notes 1 in **Supplementary Material**.

Functional Magnetic Resonance Imaging Data Analysis

FMRI data were preprocessed using Resting-State fMRI Data Analysis Toolkit V1.24[1] (RESTplus V1.24) (Jia et al., 2019). Data were first transformed into the NIFTI format, and the first 10 volumes were excluded for magnetization stabilization. The following steps were then performed: slice-time correction, motion correction, spatial normalization using the EPI MNI template, 8-mm kernel smoothing, and scaling to a percentage change from the mean. Data were then bandpass filtered to no less than 0.01 Hz to reduce low-frequency drifts and entered into a general linear model to remove the effects of 24 degrees of motion and their derivatives, nuisance cerebrospinal fluid (CSF), white matter, and global signal. No data were excluded because of excessive head motion.

The residual time series from this deconvolution was then used to calculate the MSE within each ROI. The ROIs were defined according to the results of our meta-analysis. Then, the MSE was used to quantify the complexity of each BOLD time series by calculating the entropies across five temporal scales. Greater averaged entropies reflected greater complexity. Specific calculation of MSE values and validation of the MSE results are included as Supplementary Notes 2 in **Supplementary Material** (Costa et al., 2002, 2005; Yang et al., 2013; Lindquist et al., 2019).

Statistical Analysis

Statistical analysis was performed with SPSS version 25 software. Means, standard deviations (S.Ds.) and percentages of selected descriptive characteristics were calculated for the study sample. Independent two-sample t-tests were used to examine the differences in demographic and clinical characteristics and MSE values in each ROI between the PD patients with depression and PD patients without depression. The significance level was set to $p < 0.05$ for all analyses.

[1]http://www.restfmri.net/forum/sites/default/files/200902_1511_RESTplus_v1.24.tar.gz

RESULTS

Meta-Analysis

Study Selection and Quality Assessment

According to the search strategy, we found 2,312 results, and 1,789 articles remained after removing duplicates. Then, we excluded 1,783 articles on the basis of our inclusion and exclusion criteria. The details are shown in **Figure 1**. Finally, six studies involving 126 PD patients with depression and 153 PD patients without depression were included in the meta-analysis. In these studies, age and sex were matched in two groups. The demographic and clinical characteristics including UPDRS, HRDS, and MMSE as well as imaging information of the included and excluded studies are provided in **Table 2** and **Supplementary Table 1**.

During the quality assessment, three studies in our meta-analysis were classified as good, and the other three studies were deemed fair. The most common reasons for a deduction from the score were (1) the authors did not describe the inclusion or exclusion criteria of the study, (2) the authors did not specify how regions of interest were determined, (3) the authors did not describe detailed quality control measures, and (4) no slice coordinates were given for the figures. However, all of the studies in our meta-analysis scored above six, which meant that their quality reached an acceptable level. The specification of the quality assessment is presented Supplementary Date in **Supplementary Material**. We described the experimental design of the included studies in **Table 2**.

Differences of Neural Activity Between the Parkinson's Disease Patients With Depression and Parkinson's Disease Patients Without Depression

The meta-analysis for differences between activation in the PD patients with depression and PD patients without depression yielded significant convergence of activation in the left posterior cingulate gyrus (peak MNI: $X = 0$, $Y = -42$, $Z = 26$; voxels = 1,966) and right SMA (peak MNI: $X = 6$, $Y = 8$, $Z = 52$; voxels = 1,008) (PD patients with depression > PD patients without depression). On the other hand, a significant difference was also found in the left cerebellar hemispheric lobule (peak MNI: $X = -14$, $Y = -68$, $Z = -16$; voxels = 2,011) (PD patients with depression <PD patients without depression) (**Table 3** and **Figure 2**).

Robustness Analysis

Heterogeneity analysis from SDM showed that there was noteworthy between-study heterogeneity in Brodmann area (BA) 23, the SMA (BA 46), the middle frontal gyrus (BA 46), the cerebellum hemispheric lobule-VI (BA 19), the inferior frontal gyrus–orbital part (BA 47), the corpus callosum and the inferior frontal gyrus–opercular part (**Supplementary Table 2**). Jackknife sensitivity analysis revealed that abnormal activity in the cingulate gyrus was the most robust result. Other regions,

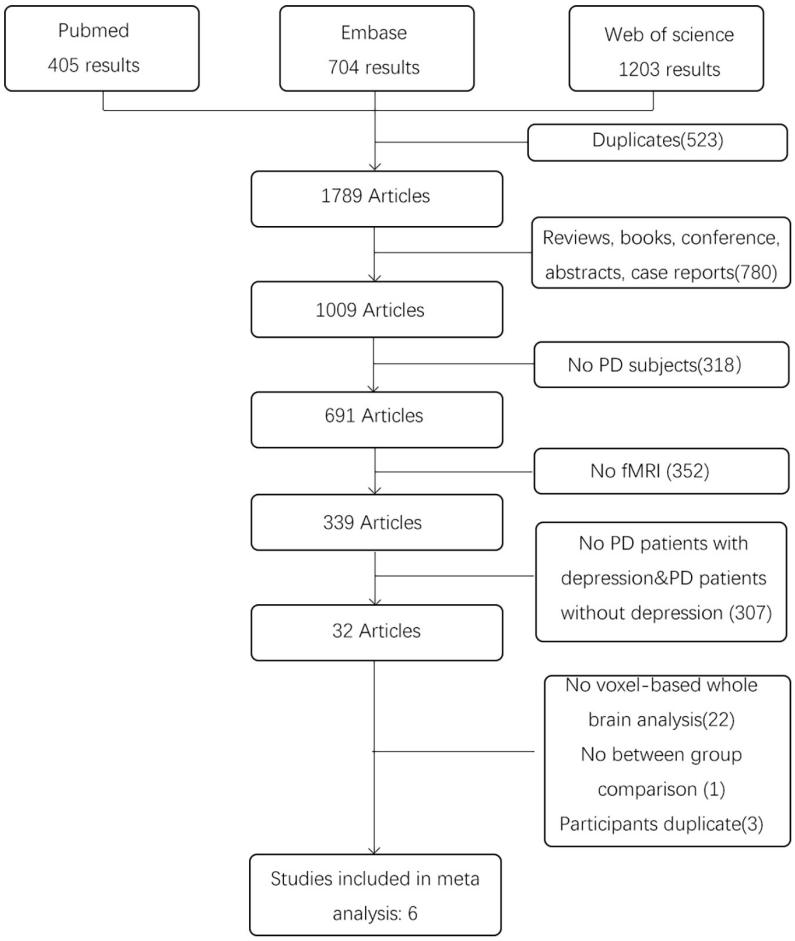

FIGURE 1 | Study selection flow chart, performed according to the PRISMA 2020 guidelines.

including the SMA and cerebellum, were not robust in the meta-analysis, as they showed poor replicability of the peak coordinate (**Supplementary Table 3**). Since the number of included studies was less than 10, Egger's test would not provide reliable results and was not performed.

Regions-of-Interests-Based Functional Magnetic Resonance Imaging Study

The demographic and clinical characteristics of the 53 participants (28 PD patients with depression and 25 PD patients without depression) are presented in **Table 1**. No significant differences in these characteristics were observed between the two groups. Based on the results of the meta-analysis, three clusters were identified as ROIs: the cingulate gyrus (peak MNI: $X = 0$, $Y = -42$, $Z = 26$; voxels = 1,966), SMA (peak MNI: $X = 6$, $Y = 8$, $Z = 52$; voxels = 1,008), and cerebellum (peak MNI: $X = -14$, $Y = -68$, $Z = -16$; voxels = 2,011). We observed that compared to the PD patients without depression, in the group of PD patients with depression, MSE of BOLD decreased significantly in the posterior cingulate gyrus (No depression vs. Depression = 1.16 ± 0.06 vs. 1.12 ± 0.07, $F = 0.856$, $p = 0.045$), SMA (No depression vs. Depression = 1.13 ± 0.05 vs. 1.10 ± 0.05, $F = 0.914$, $p = 0.039$), and cerebellum (No depression vs. Depression = 1.08 ± 0.06 vs. 1.04 ± 0.05, $F = 0.227$, $p = 0.043$) (**Supplementary Figure 1**), which was consistent with the results of previous meta-analyses. Except ROIs-based analysis, we also performed a whole-brain analysis, however, no positive result was found after false discovery rate (FDR) correction.

DISCUSSION

Our study aims to characterize the alterations in neural activity of brain regions in PD patients with depression. Using a coordination-based meta-analysis of publications in this field, we provide first-of-its-kind evidence that PD patients with depression had the most consistent abnormalities in the posterior cingulate gyrus, SMA, and cerebellum. Then, to provide an independent validation of the meta-analysis results, we conducted an ROI-based analysis to measure the multiscale dynamics of these ROIs in PD patients with depression. All the patients didn't take antidepressant medication. The results showed that compared to that in PD patients without depression,

TABLE 2 | Demographic characteristics and experimental design of the included studies.

References		Hu et al., 2015	Luo et al., 2014	Sheng et al., 2014	Wang et al., 2018	Wang et al., 2020	Wen et al., 2013
Sample size	**Depression**	20	29	20	27	13	17
	No depression	39	30	21	27	20	16
Gender (male/female)	**Depression**	9/11	14/15	13/8	17/10	6/7	7/10
	No depression	26/13	15/15	13/7	19/8	11/9	8/8
Disease duration	**Depression**	5.35 ± 2.82	1.98 ± 1.64	3.4 ± 1.7	-	29.0 ± 22.4	6.4 ± 5.4
	No depression	6.50 ± 3.84	2.12 ± 1.30	4.0 ± 2.4	-	28.35 ± 1.98	5.6 ± 7.4
	P	0.217	0.72	0.224	-	0.994	-
Age	**Depression**	58.05 ± 7.72	51.5 ± 8.2	55.9 ± 7.4	60.8 ± 9.53	63.9 ± 10.6	64.4 ± 13.4
	No depression	54.6 ± 1.05	53.6 ± 10	53.7 ± 6.1	59.3 ± 12.6	58.4 ± 7.0	60.7 ± 18.7
	P	0.305	0.59	0.25	0.94	0.246	0.105
MMSE	**Depression**	-	27.4 ± 2.5	26.9 ± 1.7	26.4 ± 1.2	22.1 ± 4.5	29.5 ± 0.5
	No depression	-	27.0 ± 2.7	27.6 ± 2.0	27.4 ± 1.4	27.3 ± 2.7	29.2 ± 2.2
	P	-	0.52	0.096	0.001	<0.001	0.495
UPDRS-III (Skorvanek et al., 2015)	**Depression**	27.7 ± 1.32	28.3 ± 16.9	39.4 ± 10.8	17.0 ± 3.8	50.4 ± 8.4	42 ± 46
	No depression	28.2 ± 13.2	26.8 ± 12.4	43.8 ± 8.2	16.3 ± 3.8	43.1 ± 5.0	33.8 ± 24.2
	P	0.879	0.70	0.078	0.50	0.004	0.110
HSD	**Depression**	15.0 ± 4.8	18.31 ± 6.14	19.3 ± 5.0	13.3 ± 4.63	30.0 ± 3.5	15.2 ± 7.8
	No depression	6.8 ± 3.1	4.4 ± 2.73	6.4 ± 2.1	2.5 ± 1.8	3.1 ± 1.7	4.4 ± 4.4
	P	0.0001	<0.01	<0.01	<0.001	<0.01	<0.01
Medication ON/OFF		ON	OFF	OFF	OFF	NR	OFF
Antidepressant medication		NO	NO	NO	NO	NR	NO
Criteria for		DSM-V	DSM-IV	DSM-IV	DSM-IV	-	DSM-IV
Scaner		3T	3T	3T	3T	3T	3T
Analysis		ALFF	ALFF	ReHo	DC	ReHo	ALFF
Software		SPM8	SPM8	SPM8	GRETNA	DPARSE	SPM8
Reported space		MNI	MNI	MNI	MNI	MNI	MNI
Minimum cluster size (voxel)		34	50	68	13	16	16
Corrected method		AlphaSim	AlphaSim	AlphaSim	AlphaSim	FEW	AlphaSim

Values are represented as the mean ± standard deviation (SD). P-values were obtained using two sample t-test (P < 0.05). Gender = male/female. MMSE, Mini-mental state examination; UPDRS-III, Unified Parkinson's disease rating scale part III; HRSD, Hamilton Rating Scale for Depression; FEW, Family-wise error correction; DSM-IV, Diagnostic and Statistical Manual of Mental Disorders version four; DSM-V, Diagnostic and Statistical Manual of Mental Disorders version five; ALFF, Amplitude of low frequency fluctuation; ReHo, Regional homogeneity; DC, Degree centrality; MNI, Montreal Neurological Institute; DPARSF, Data Processing Assistant for Resting-State fMRI; SPM, Statistical Parametric Mapping software; NR, Not reported.

TABLE 3 | Clusters of voxels with significant intergroup activation differences.

Neural region	Side	MNI coordinates			Voxels	*P*-value	SDM-Z
		X	*Y*	*Z*			
Depression > No depression							
Posterior cingulate gyrus	Left	0	–42	26	1966	0.00016	1.770
Supplementary motor area, BA 6	Right	6	8	52	1008	0.00076	1.557
No depression > Depression							
Left cerebellum, hemispheric lobule VI, BA 18	Left	–14	–68	–16	2011	0.00005	–1.601

Voxel threshold p < 0.005, peak height threshold: peak SDM-Z > 1.000, extent threshold: cluster size ≥ 10 voxels. BA, Brodmann area; MNI, Montreal Neurological Institute.

the complexity in ROIs was significantly lower in PD patients with depression, confirming the results of the meta-analysis that spontaneous activity of the posterior cingulate gyrus, SMA, and cerebellum pertain to the pathogenesis of depression in PD; these areas may serve as targets for the management and therapeutic strategies of PD patients with depression.

We observed alterations in resting-state neural activities within the cingulate gyrus, which is consistent with previous

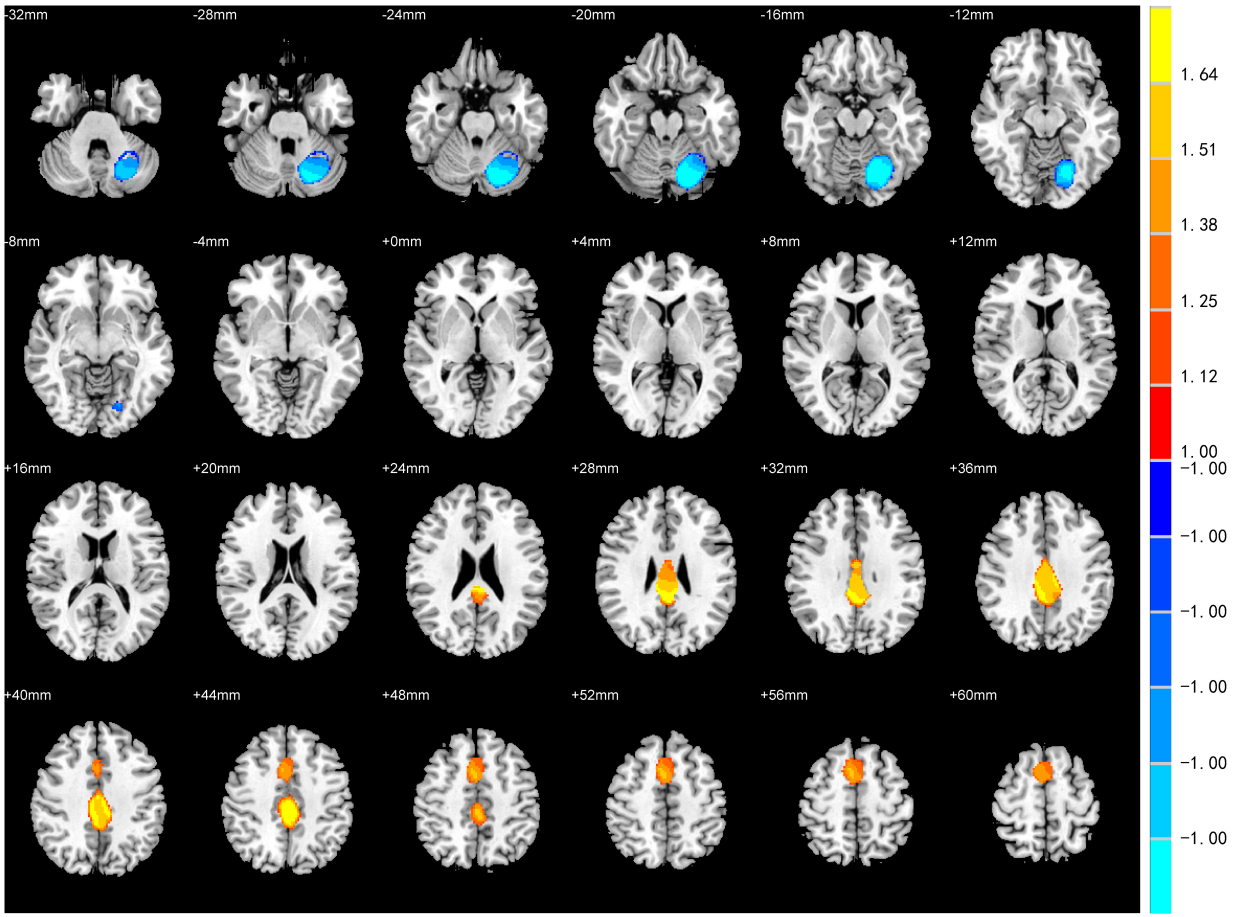

FIGURE 2 | Statistically significant effects of meta-analysis for Parkinson's disease (PD) patients with depression vs. PD patients without depression. Voxel threshold $p < 0.005$, peak height threshold: peak SDM-Z > 1.000, extent threshold: cluster size ≥ 10 voxels. Increased activity in PD patients with depression is indicated in yellow and decreased activity in blue.

studies (Cardoso et al., 2009; Wen et al., 2013; Sheng et al., 2014). The cingulate gyrus is a key region of the prefrontal cortex (PFC)-limbic circuit, which has been linked to depression in both non-PD and PD cohorts (Cardoso et al., 2009; Luo et al., 2014). Neuroimaging evidence suggests that functional connectivity (FC) in this circuit is negatively correlated with depression severity (Du et al., 2017; Pantazatos et al., 2020), and increased FC between the PFC and cingulate gyrus is associated with depression-related gene orthodenticle homeobox 2 (OTX2) (Gärtner et al., 2019). Taylor and Liberzon (2007) for example, showed a potential pathological mechanism in which the inhibitory effects of the PFC on the limbic system, an important function in the regulation of mood, were impaired in people with depression. Taken together, the results here provided confirmatory evidence that alteration of the cingulate gyrus is a contributor to the pathophysiologic changes of PD patients with depression.

Similarly, alterations in the activities of the cerebellum were also observed in PD patients with depression. The cerebellum may participate in the processing of depression in PD via the cerebellar-cerebral circuit, which is formed by separate cerebellar subregions connected to distinct cerebral regions (D'Angelo and Casali, 2012). Dynamic FC in cerebellar-cerebral circuits decreased in depression patients, characterized by decreased connections of the cerebellar subregions with the default-mode, executive and affective-limbic networks (Alalade et al., 2011; Zhu et al., 2020). Decreased cerebellar-cerebral FC was proven to be correlated with the severity of depressive symptoms in PD patients, and it increased after electroconvulsive therapy (ECT) (Wei et al., 2020). Moreover, Ma et al. (2013) demonstrated that this circuit could serve as a biomarker to distinguish depression patients from HCs, suggesting the important role of this circuit in depression. All these results support the important role of the cerebellum in the pathogenesis of depression in PD.

Furthermore, we found that PD patients with depression had abnormal neural activity in the SMA. The SMA is located in the medial posterior third of the superior frontal gyrus and is mainly concerned with the motor symptoms of PD, such as posture and gait (Shirota et al., 2013). Recently, converging evidence has suggested that the SMA also plays a critical role in the integration of affective and cognitive functions (Nachev et al., 2008). A PET study revealed that treatment-resistant depression

(TRD) patients were characterized by hypometabolism in the SMA (Li et al., 2015), and the symptoms of TRD patients were alleviated after the activity of SMA increased (Chen et al., 2018). However, the related studies are limited (Wang et al., 2018, 2020). Therefore, the mechanism by which SMA is involved in PD patients with depression still needs further research.

Of interest, all three regions indicated in our study showed altered function in depression patients as well (Liu et al., 2010; Bürger et al., 2017). Given the similarities between PD patients with depression and depression, most treatment strategies are the same. However, there are still some differences among treatment strategies. For example, the dopamine-releasing agent methylphenidate was not able to improve the depressive symptoms of PD patients due to degeneration of the dopaminergic innervation of the limbic system (Remy et al., 2005). In addition, most PD patients do not tolerate antipsychotics well because the dopamine-blocking action of these drugs contributes to PD symptoms (Ryan et al., 2019). For treatment-refractory depression, ECT leads to more adverse effects in PD patients than in purely depressed patients (Borisovskaya et al., 2016). Thus, for the better treatment of PD patients with depression, it is of significance for us to further study its pathophysiologic basis.

LIMITATIONS

Our study has several limitations. First, the meta-analysis was limited by the small number of studies and a relatively high level of heterogeneity in study characteristics. To get more data to support our study, we contacted all the corresponding authors of studies without whole brain results in papers. However, there was no response we can receive. As shown in **Table 2**, different statistical or analysis methods, software packages and threshold settings were used in the included studies, which may influence our meta-analysis results. Therefore, our meta-analysis results must be interpreted cautiously. It is necessary to perform further subgroup analysis for studies with different measures, such as ALFF, ReHo, and DC. Unfortunately, due to the limited number of included articles, further subgroup analysis was not able to be performed. We suggest the validation of our findings through future studies consisting of an independent, methodologically homogeneous data set. Second, our study put together studies in which PD patients underwent fMRI in anti-Parkinsonian medication ON state and anti-Parkinsonian medication OFF state, this might be a potential confound on resting state fMRI signal. A subgroup analysis is needed to explore the effect of L-dopa on resting state fMRI signal. Third, the sample size of the PD patients with depression and PD patients without depression in our ROI-based fMRI study was relatively small. Therefore, fMRI studies with larger sample sizes are needed to confirm our results of ROI-based analysis. Fourth, we performed the whole-brain analysis but no significant results were observed by passing FDR correction. This may be due to the relatively small sample size of participants. We totally agree that the whole-brain analysis in participants with much larger sample size will help confirm and expand the findings of the current ROI analysis, which are warranted in future studies. Finally, limited by the number of studies, we did not perform a meta-analysis on functional connectivity studies of PD patients with depression. Future studies are expected to provide a better understanding of the functional connectivity networks involved in PD patients with depression.

CONCLUSION

In conclusion, our meta-analysis revealed specific resting-state fMRI signal alterations in the cingulate gyrus, SMA and cerebellum in PD patients with depression. Our ROI-based fMRI analysis provided primary evidence that the complexity of neural activity was altered in the cingulate gyrus, SMA, and cerebellum. These findings are helpful in unraveling pathophysiology of depression in PD, and they also have the potential to serve as neuroimage biomarkers as well as provide novel targets for neuromodulation treatment for PD patients with depression.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Medical Ethical Review Committee of Beijing Tiantan Hospital. Number: KY 2018-080-02. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

DS contributed to the conception and design of the study. YC and DS contributed to the organization and execution of the research project and drafted the text, and prepared the figures. DS, YC, HC, JF, and HM contributed to acquisition, post-processing, and analysis of the data. JZ and TF revised the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnagi.2022.806054/full#supplementary-material

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The Comparative Efficacy of Non-ergot Dopamine Agonist and Potential Risk Factors for Motor Complications and Side Effects From NEDA Use in Early Parkinson's Disease: Evidence From Clinical Trials

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Background/Objectives: Non-ergot dopamine agonist (NEDA) are recommended as the first-line treatment for patients with early Parkinson's disease (PD) because of their efficacy in treating PD motor symptoms. However, systematic evaluations of the risk of motor complications induced by NEDA and risk factors potentially associated with motor complications are still lacking.

Methods: Medline, Embase, the Cochrane Central Register of Controlled Trials, and Web of Science were searched for potentially eligible randomized controlled trials. The incidence of motor complications (dyskinesia, motor fluctuations), impulsive-compulsive behaviors and adverse events and clinical disability rating scale (UPDRS) scores were evaluated using standard meta-analytic methods. Metaregression was conducted on the incidence of motor complications (dyskinesia) with treatment duration and NEDA dose as covariates.

Results: Patients treated with NEDA had significantly lower UPDRS total scores, motor scores and activity of daily living (ADL) scores than those receiving a placebo (weighted mean difference (WMD) −4.81, 95% CI −6.57 to −3.05; WMD −4.901, 95% CI −7.03 to −2.77; WMD −1.52, 95% CI −2.19 to −0.84, respectively). Patients in the NEDA and NEDA+open Levodopa (LD) groups had lower odds for dyskinesia than patients in the LD group (OR = 0.21, 95% CI: 0.15–0.29; OR = 0.31, 95% CI 0.24–0.42, respectively). Metaregressions indicated that the mean LD dose of the NEDA group increased, and the odds of developing dyskinesia increased ($p = 0.012$). However, the odds of developing dyskinesia in the NEDA group were not related to treatment duration ($p = 0.308$). PD patients treated with NEDA or NEDA+open LD had a lower risk of wearing-off implications than those treated with LD (all $p < 0.05$). No significant

difference was found between the NEDA and placebo groups in impulsive-compulsive behavior development ($p > 0.05$). Patients in the NEDA group were more likely to suffer somnolence, edema, constipation, dizziness, hallucinations, nausea and vomiting than those in the placebo or LD group.

Conclusion: NEDA therapy reduces motor symptoms and improves ADLs in early PD. The odds of developing motor complications were lower with NEDA than with LD, and dyskinesia increased with increasing LD equivalent dose and was not influenced by NEDA treatment duration. Therefore, long-term treatment with an appropriate dosage of NEDA might be more suitable than LD for early PD patients.

Registration: PROSPERO CRD42021287172.

Keywords: non-ergot dopamine agonist, motor complications, Parkinson's disease, risk factors, dose response

INTRODUCTION

Parkinson's disease (PD) is the most common neurodegenerative movement disorder and is characterized by a set of main motor symptoms, including bradykinesia, rigidity and tremor, and additional non-motor symptoms, such as depression, cognitive impairment, insomnia, and fatigue. According to the Global Burden of Disease Study 2016, in 2016, the PD population was almost 6,100,000, and PD had the fastest growth in prevalence, disability, and deaths, leading to poor quality of life in PD patients and an increasing burden on society and families (Tysnes and Storstein, 2017; Collaborators, 2018).

Dopaminergic anti-parkinsonism medicines, especially dopamine agonists (DAs) and levodopa (LD), are still recommended as the first-line treatment for patients with early PD based on the latest NICE guidelines (Rogers et al., 2017). DAs include ergot DAs and non-ergot agonist DAs, but ergot DAs are not recommended in PD treatment because of the risk of fibrotic reactions (Rasmussen et al., 2011). NEDA, mainly pramipexole, ropinirole, rotigotine and piribedil, can effectively reduce the symptoms of PD patients and potentially slow the progression of PD disease (Rogers et al., 2017). However, motor complications develop when using dopaminergic antiparkinsonism agents, which needs to receive more attention and has become one of the most challenging problems for movement disorder doctors. Moreover, comparisons of motor complications between NEDA and LD/placebo are still controversial and lacking. Previous meta-analyses have evaluated the efficacy and safety of DAs in PD and clarified that DAs, including NEDA, had a positive effect in PD (Baker et al., 2009; Zhou et al., 2014b). However, most of the meta-analyses did not assess motor complications (especially dyskinesia) in patients treated with NEDA vs. LD/placebo, so the use of NEDA could not comprehensively evaluated for early PD (Baker et al., 2009; Zhou et al., 2014b). One meta-analysis assessed motor complications in DA interventions. However, this analysis included ergot dopamine agonists that might increase the number of confounding factors in evaluations of NEDA in PD (Chondrogiorgi et al., 2014). Furthermore, no meta-analysis of NEDA has been conducted to examine the relation between the dose of NEDA or the duration of NEDA treatment and the incidence of motor complications (especially dyskinesia).

Therefore, in this study, we conducted a meta-analysis mainly to examine the risk of motor complications between NEDA monotherapy (or plus open-label LD) and either LD or a placebo in early PD. Meta-regression analyses were also performed to evaluate the contribution of the dose of NEDA and the duration of NEDA treatment to development of motor complications. Moreover, clinician-rated disability scales, impulsive-compulsive behaviors and adverse events were also used to fully evaluate the use of NEDA for PD treatment.

METHODS

Search Strategy

In an attempt to comprehensively identify all eligible studies, four electronic databases, Medline (Ovid), Embase (Ovid), the Cochrane Central Register of Controlled Trials, and Web of Science, were searched from inception through 13 August 2021. The search terms included medical subject headings and free terms regarding intervention treatments (NEDA), disease (PD), and research types (randomized clinical trial designs). The specific search strategies are listed in **Supplement 1**.

Inclusion and Exclusion Criteria

Eligible studies were included in the analysis if they met the following criteria: 1) randomized controlled trials (RCTs); 2) study population diagnosed with PD at the early stage and no history of motor complications; 3) intervention treatments contained NEDA vs. placebo/LD (open-label LD was also allowed during the treatment); 4) the absolute number or the incidence of motor complications (dyskinesia or wearing-off) was reported each group or the number or the incidence of impulsive-compulsive behaviors was reported for each group; or clinician-rated disability scale scores [Unified Parkinson's Disease Rating Scale (UPDRS) score] were reported.

Studies were excluded for providing insufficient data or if the outcome measures did not meet the inclusion criteria. Animal

trials, clinical protocols, conference abstracts and quasi-RCTs were also excluded.

Data Extraction and Quality Assessment

Data from and basic information on the eligible studies were extracted by two independent investigators (GHJ, XYS). The following information was extracted from each study: study characteristics (first author, year, design), population (disease at early stage), intervention and control (NEDA with or without open-label LD vs. LD/placebo, mean daily dose of NEDA, frequency, treatment duration), outcome measures (incidence of dyskinesia/wearing-off, impulsive-compulsive behaviors, changes in the UPDRS scores, and incidence of adverse events). The quality of the included studies was evaluated based on the standard criteria of the Cochrane Risk of Bias Tool (Savovic et al., 2014). Two reviewers (TCZ and CDF) conducted the quality assessment independently, and any disagreement was resolved by discussion with a senior investigator (ZML).

Outcome Measures

The outcome measures mainly included the incidence of motor complications (dyskinesia, motor fluctuations), incidence of impulsive-compulsive behaviors, mean daily dose of NEDA with dyskinesia, duration of treatment with NEDA with dyskinesia, UPDRS scores (II, III, and total) and incidence of adverse events.

Statistical Analysis

We conducted a meta-analysis of eligible studies by using Stata version 16. The incidences of dyskinesia/wearing-off, impulsive-compulsive behaviors and adverse events were regarded as dichotomous data and are presented as odds ratios (ORs) with 95% CIs. The mean change in the UPDRS scores from baseline was treated as continuous data and is reported as the weighted mean difference (WMD) with a 95% confidence interval (CI). Statistical heterogeneity was estimated using the I^2 statistic; if heterogeneity existed, a random effects model was used. Otherwise, the fixed-effects model was used to pool the results. If the pooled result exhibited clinically relevant heterogeneity, subgroup analysis was also conducted to identify the source of heterogeneity (Cumpston et al., 2019).

Meta-regression analyses were also performed to evaluate the relationship between LD equivalent doses (LEDs) of NEDA and dyskinesia. In addition, treatment duration was included independently as a covariate in the meta-regression to explore potential risk factors for motor complications (dyskinesia).

Finally, a sensitivity analysis was performed by removing one study at a time to estimate the stability of the results. The publication bias of the included studies was examined by Egger's test.

RESULTS

Study Identification and Selection

A total of 2,350 potentially eligible publications were identified in the four electronic databases, and 749 duplicate studies were eliminated. A total of 1,601 publications underwent further screening via review of their titles and abstracts. Then, 1,490 publications were excluded, and the remaining 111 studies underwent full-text review. Ultimately, 25 publications involving 6,427 patients met the inclusion criteria and were included in the quantitative meta-analysis [the specific PRISMA 2020 flow diagram (Page et al., 2021) is described in **Figure 1**].

Characteristics of the Included Studies

A total of 25 studies involving 6,427 participants who were diagnosed with early PD met the inclusion criteria. The NEDA treatments included in this meta-analysis were pramipexole, ropinirole, rotigotine, and piribedil. Placebo or LD treatment was adopted as the control in the included trials. The length of intervention varied from 9 to 480 weeks; the mean LED in the NEDA group ranged from 150 to 712 mg. The frequency of medicine use ranged from 1 to 3 times per day. Sixteen studies reported the occurrence of dyskinesia as an outcome; 7 studies reported the incidence of motor fluctuations; two studies reported the incidence of impulsive-compulsive behaviors; 12 trials recorded the mean daily dose of NEDA with dyskinesia; 17 studies used the UPDRS score (II, III, and total) as an outcome measure; and 22 studies recorded the incidence of adverse events (see **Supplementary Table 1**).

Quality of the Included Studies

Sixteen studies (64%) reported the methods of random sequence generation, and 68% had a low risk for allocation concealment. Nineteen studies (76%) used blinding methods for participants and personnel. Eighteen studies had a low risk bias for blinding the outcome assessment. Twenty-five trials had a low risk of attrition bias and reporting bias. Overall, nineteen studies (76%) were regarded as having a low risk of poor methodological quality (**Supplementary Figures 1**, **2**).

Analysis of Outcomes

Clinical Disability Rating Scales (UPDRS)

A total of 15 studies examined NEDA (NEDA+open LD) vs. a placebo/LD. In terms of the UPDRS motor subscale, patients who received NEDA had a significant reduction in motor scores compared with those receiving placebo (WMD −4.901, 95% CI −7.03 to −2.77; $p < 0.01$). However, no significant reduction in motor scores was seen between NEDA and LD or between NEDA+open LD and LD (WMD −0.171, 95% CI −4.50 to 4.16; WMD −1.42, 95% CI −6.18 to 3.35; all $p > 0.05$). The pooled results for the UPDRS motor scores exhibited significant heterogeneity among the three drug class subgroups ($p < 0.001$, $I^2 = 90.3\%$). The use of various drug interventions and control groups might be the sources of heterogeneity (**Figure 2A**).

A total of 10 trials assessed the UPDRS ADL subscale. Compared with a placebo, NEDA were associated with a significant improvement in the UPDRS-ADL score (WMD −1.52, 95% CI −2.19 to −0.84; $p < 0.01$). Patients who received NEDA + open LD had significantly reduced UPDRS-ADL scores compared with those receiving LD alone (WMD −1.94, 95% CI −2.68 to −1.19, $p < 0.05$). However, there was no significant difference in UPDRS-ADL score between NEDA and LD (WMD −0.60, 95% CI −1.44 to 0.23, $p > 0.05$). The heterogeneity

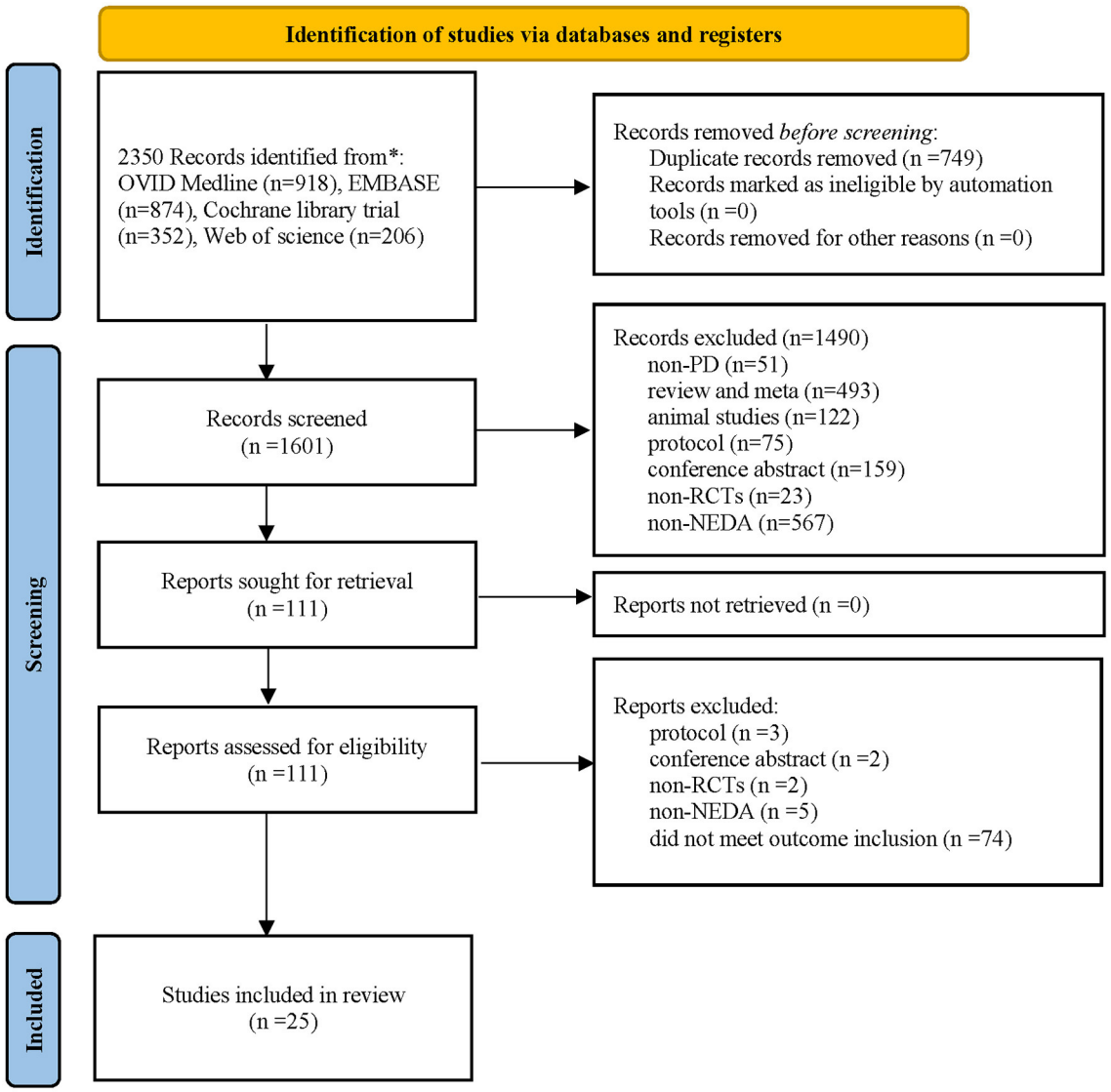

FIGURE 1 | Study flow diagram.

among subgroup drug classes was significantly different ($p < 0.01$, $I^2 = 56.1\%$) (**Figure 2B**).

Six studies reported the UPDRS total score, and the NEDA group had significantly reduced UPDRS total scores compared with the placebo group (WMD −4.81, 95% CI −6.57 to −3.05; $p < 0.01$). No significant differences in UPDRS-total were seen between NEDA and LD or between NEDA+open LD and LD (WMD −2.66, 95% CI −7.31 to 2; WMD −1.78, 95% CI −8.73 to 5.18; all $p > 0.05$). Subgroup heterogeneity also existed among different drug classes ($p = 0.035$, $I^2 = 58.3\%$) (**Figure 2C**).

Motor Complications

Dyskinesia

Incidence of Dyskinesia

A total of 16 studies reported the incidence of dyskinesia for NEDA compared with a placebo or LD. The results revealed that patients in the NEDA group did not have significantly greater odds of developing dyskinesia than patients in the placebo group (OR = 2.01, 95% CI 0.69–5.86). Patients in the NEDA and NEDA+open LD groups had lower odds (79 and 69%, respectively) than patients in the LD group (OR = 0.21, 95% CI 0.15–0.29; OR = 0.31, 95% CI 0.24–0.42, respectively). Each subgroup had low heterogeneity ($p = 0.589$; $p = 0.454$; $p = 0.056$, respectively), while heterogeneity existed among the various subgroups ($p = 0.016$, $I^2 = 47.3\%$) (**Figure 3A**).

Association Between Dyskinesia and the Mean LED of NEDA (Meta-Regression)

Twelve studies reported the dose of NEDA and converted them to LEDs of NEDA. To further explore the relationship between the mean LED of NEDA and dyskinesia, we used meta-regression, and the results showed that as the mean LD dose of NEDA

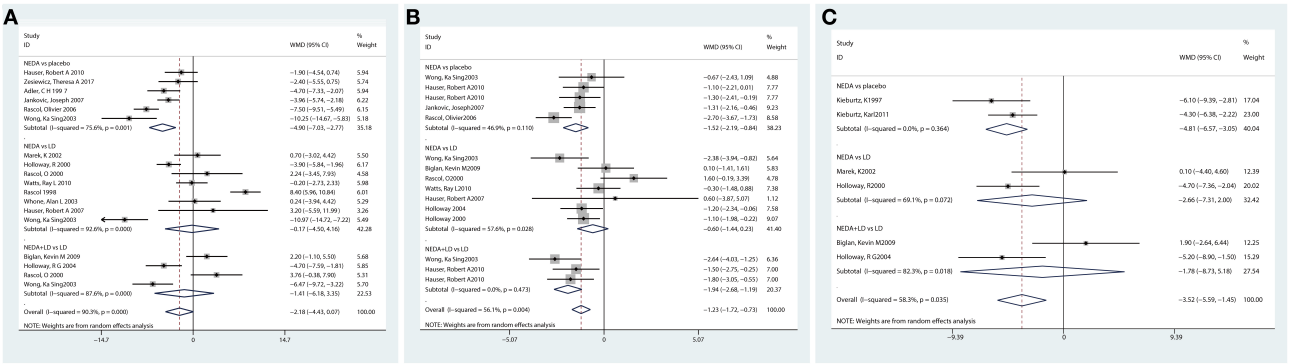

FIGURE 2 | Effect of NEDA on Unified Parkinson's Disease Rating Scale scores. **(A)** Effect of NEDA on Unified Parkinson's Disease Rating Scale motor score. **(B)** Effect of NEDA on Unified Parkinson's Disease Rating Scale Activities of Daily Living score. **(C)** Effect of NEDA on Unified Parkinson's Disease Rating Total Scale. CI, confidence interval; df, degrees of freedom; NEDA, non-ergot dopamine agonist; PD, Parkinson's disease; SD, standard deviation.

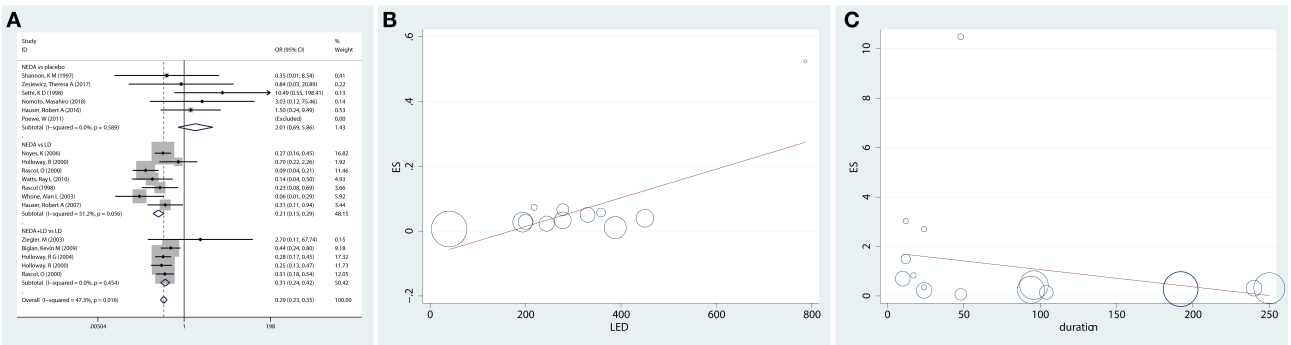

FIGURE 3 | Incidence of dyskinesia and potential risk factors for dyskinesia in trials of NEDA. **(A)** Forest plot for incidence of dyskinesia in trials of NEDA. **(B)** Meta-regression plot representing the association between dyskinesia and the mean LED of NEDA. **(C)** Meta-regression plot representing the association between dyskinesia and NEDA treatment duration. Circles represent individual trials. The size of the circles is proportional to the inverse of the variance in the incidence of dyskinesia found in that trial. OR, odds ratio; CI, confidence interval; LD, levodopa; NEDA, non-ergot dopamine agonist.

increased, the odds of developing dyskinesia increased [exp(b), SE = 1.00, 0.00014, $p = 0.012$) (**Figure 3B**).

Association Between Dyskinesia and NEDA Treatment Duration (Meta-Regression)

We also examined the association between dyskinesia and NEDA treatment duration, and the results indicated that the odds of developing dyskinesia in the NEDA group, compared with that in the placebo and LD groups, were not related to treatment duration [exp(b), SE = 0.99, 0.0066, $p = 0.308$] (**Figure 3C**).

Incidence of Wearing-Off

Seven trials reported motor implications (wearing-off) as an outcome measure. The results showed that PD patients who received NEDA or NEDA+open LD had (46%, 47%) lower odds of developing wearing-off implications than those in the LD group (OR = 0.54, 95% CI 0.41–0.73; OR = 0.53, 95% CI 0.38–0.72, respectively). However, there was no significant difference in the incidence of wearing-off outcomes between NEDA and a placebo (OR = 0.74, 95% CI 0.27–2.02). Each subgroup and all groups had low heterogeneity ($p = 0.952$; $p = 0.911$; respectively) (**Figure 4**).

Impulsive-Compulsive Behaviors

A total of two trials examined impulsive-compulsive behaviors with NEDA vs. a placebo. No significant difference was seen in the development of impulsive-compulsive behaviors in the NEDA group compared with the placebo group (OR = 1.986, 95% CI 0.495, 7.971). No heterogeneity existed among the two trials ($p = 0.123$, $I^2 = 57.9\%$) (**Figure 5**).

Adverse Events

A total of 22 studies that reported 18 types of adverse events (each event was reported in at least three studies) were analyzed in this study. The pooled incidences of eighteen types of adverse events, including vomiting, somnolence, nausea, and edema, are shown in **Table 1**. Patients who took NEDA were more likely to suffer somnolence, edema, constipation, dizziness, hallucinations, nausea, and vomiting than those in the placebo or LD group.

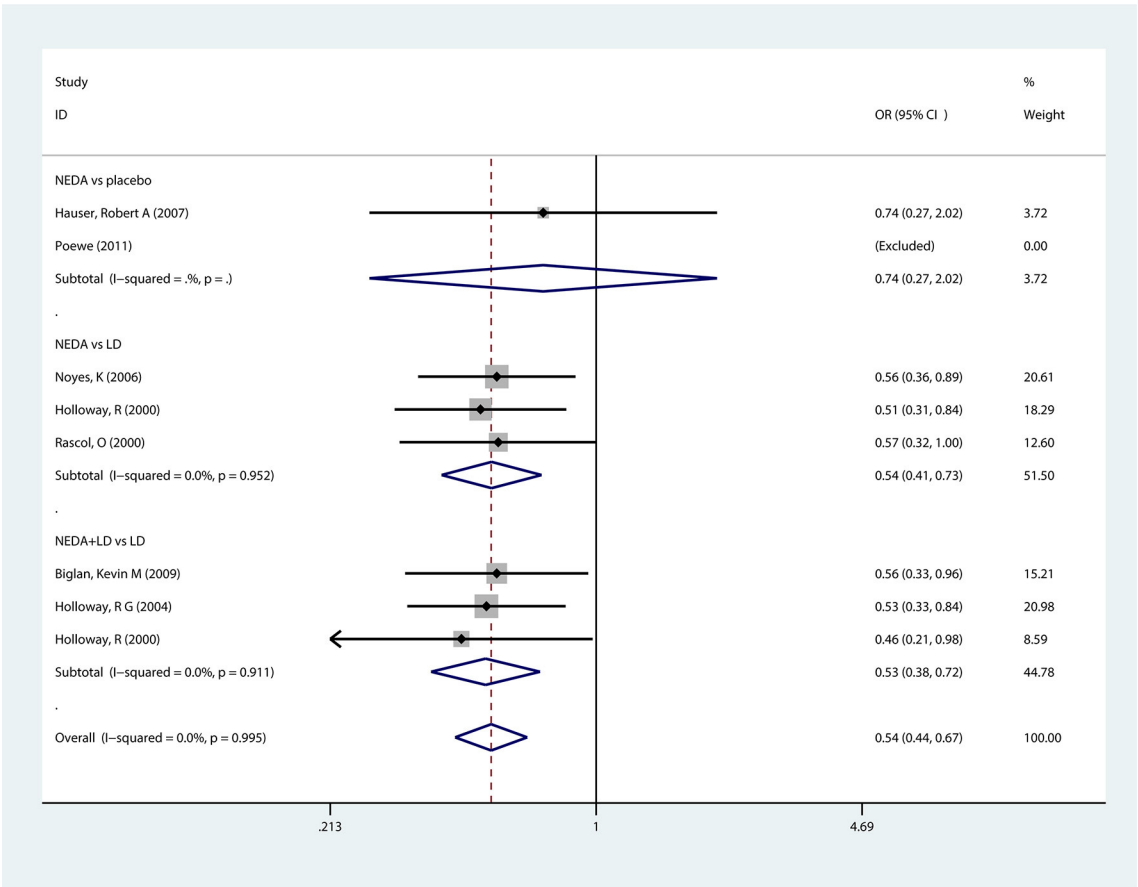

FIGURE 4 | Forest plot for incidence of wearing-off in trials of NEDA. OR, odds ratio; CI, confidence interval; LD, levodopa; NEDA, non-ergot dopamine agonist.

Sensitivity Analysis

We conducted a sensitivity analysis by excluding one study at a time to examine the robustness of the pooled results. The analysis showed that the pooled results for the incidence of dyskinesia/motor fluctuations, impulsive-compulsive behaviors and clinical disability rating scale outcomes were stable and not influenced by the individual data when excluding one study at a time (**Supplementary Figure 3**).

Publication Bias

No significant publication bias was seen in the included studies based on Egger's test or Begg's test for different outcome measures [incidence of dyskinesia, Egger's test $p = 0.161$; incidence of motor fluctuations, Egg's test $p = 0.471$; UPDRS-II (ADL), Egger's test $p = 0.218$; UPDRS-motor, Egger's test $p = 0.503$; UPDRS-total, Egger's test $p = 0.214$, **Supplementary Table 2**].

DISCUSSION

Summary of Findings

We conducted a meta-analysis of 25 trials with 6,427 participants. Motor complications (incidence of dyskinesia/wearing-off) and impulsive-compulsive behaviors were the primary outcomes used to assess the safety of the use of NEDA for PD. Moreover, clinical disability rating scales and adverse events related to NEDA use were also examined to comprehensively evaluate the effect of NEDA in patients with PD. After analyzing the pooled results, we arrived at several conclusions.

First, our pooled results indicated that NEDA were associated with significant improvement in the UPDRS-motor, UPDRS-ADL and UPDRS-total scale scores, compared with those for the placebo group. No significant differences were seen between the NEDA and LD groups on the UPDRS scales. These results demonstrated that NEDA could ameliorate motor function and ADLs in PD to some extent, and the treatment effect of NEDA was almost equal to that of LD. Previous studies reported similar results, i.e., that the use of NEDA reduced clinical disability rating scale (UPDRS) scores and improved motor ability and ADLs in PD patients (Zhou et al., 2014a; Cerri and Blandini, 2020; Poewe and Mahlknecht, 2020). However, most studies have demonstrated that LD is the most efficacious medication in the treatment of motor symptoms and might provide better symptom control than NEDA (Isaacson and Hauser, 2009; Sy and Fernandez, 2020).

NEDA were beneficial for ameliorating PD symptoms. The safety considerations and side effects of NEDA need to be

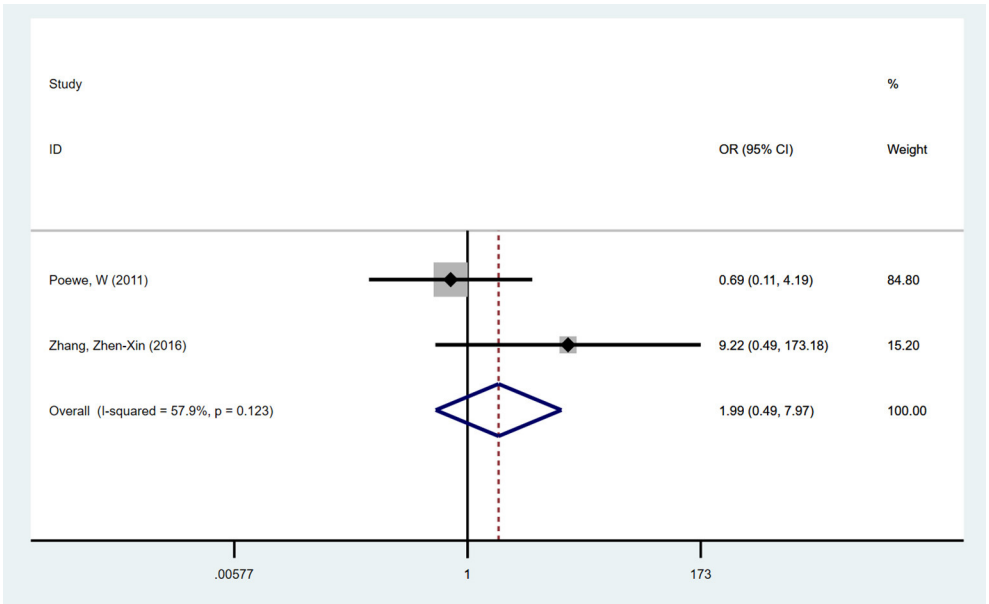

FIGURE 5 | Forest plot for incidence of impulse compulsive behaviors in trials of NEDA. OR, odds ratio; CI, confidence interval; LD, levodopa; NEDA, non-ergot dopamine agonist.

considered to fully understand the value of NEDA for PD patients and help doctors make optimal decisions. Our pooled results demonstrated that NEDA in general have a low risk of causing dyskinesia, which was similar to the placebo group. However, NEDA are associated with a lower incidence of dyskinesia than LD treatment. Dyskinesia is a common motor implication that occurs when taking dopaminergic antiparkinsonism medicine. Both NEDA and LD were beneficial for alleviating Parkinsonian motor symptoms, and LD was regarded as more efficacious than NEDA in symptom improvement; however, its long-term use was more strongly associated with the development of motor complications (Stowe et al., 2008; Isaacson and Hauser, 2009; Fox et al., 2018). Overall, NEDA might be the safe, optimal treatment option for PD patients in the early stage and possibly for the long term. The incidence of dyskinesia induced by NEDA is still controversial among current studies. However, most studies suggest that NEDA use is associated with a lower odds of developing dyskinesia than LD use, which is consistent with our results (Tambasco et al., 2012; Stathis et al., 2015; Poewe and Mahlknecht, 2020). In addition, we analyzed the relationship between the dose of NEDA and the incidence of dyskinesia using metaregression, and the results suggested that the incidence of dyskinesia significantly increased with increasing mean dose of NEDA, which meant that the mean LED was a potential risk factor for dyskinesia. Previous studies also indicated that increasing the LED would increase the odds of developing dyskinesia (Walker et al., 2011; Warren Olanow et al., 2013; Cabreira et al., 2019; Hong et al., 2020). Therefore, the dose of NEDA needs to balance the alleviation of PD symptoms and the potential risk of dyskinesia. However, we also found that the odds of dyskinesia did not increase with the duration of NEDA treatment, which was in contrast to findings on the long-term use of LD and supports that the cumulative dose of NEDA was safe (Kondo, 2002; Pilleri and Antonini, 2015; Guttler et al., 2021).

The results demonstrated that PD patients who received NEDA had a lower risk of wearing-off motor implications than patients who received LD. Previous studies drew a similar conclusion that PD patients who receive NEDA long-term have a potential risk of suffering wearing-off implications when maintaining symptomatic control but it is greater than that of patients who use LD (Schrag et al., 2007; Baker et al., 2009; Jenner, 2013).

A few studies reported impulsive-compulsive behaviors while taking NEDA, and the results indicated that early PD patients taking NEDA therapies did not have significantly different odds of impulsive-compulsive behaviors compared with those taking a placebo. However, the potential risk of developing impulsive-compulsive behaviors from NEDA use still exists and needs attention. Recent studies have also demonstrated that impulsive-compulsive behaviors might easily occur when patients use NEDA long term or at higher doses (Napier et al., 2020; Cao et al., 2021).

Furthermore, we examined the adverse events reported in at least three trials, and the results indicated that somnolence, edema, constipation, dizziness, hallucinations, nausea, and vomiting were the most frequent adverse events occurring in early PD patients treated with NEDA. However, none of these adverse events were severe, and they resolved after adjusting the dose of NEDA.

Regarding the quality of the included studies, 76% were regarded as high-quality studies, which indicated that the evidence that we analyzed was robust.

TABLE 1 | The adverse events of NEDA in Parkinson's disease.

Adverse events	Comparisons	OR (95%CI)	*P*	I^2%
Abdominal pain	NEDA vs. LD	0.853 (0.429, 1.693)	0.649	0.00
Arthralgia	NEDA vs. LD	1.027 (0.592, 1.784)	0.924	0.00
Back pain	NEDA vs. LD	1.370 (0.709, 2.646)	0.349	0.0
	NEDA vs. placebo	1.795 (0.559, 5.764)	0.325	0.0
Constipation	NEDA vs. placebo	2.038 (1.220, 3.406)	0.007	37.2
	NEDA vs. LD	4.607 (2.237, 9.488)	0.000	0.0
	NEDA+LD vs. LD	0.753 (0.265, 2.142)	0.595	82.5
	NEDA+LD vs. placebo	1.362 (0.463, 4.003)	0.575	53.8
Diarrhea	NEDA vs. placebo	1.121 (0.177, 7.113)	0.904	71.7
Dizziness	NEDA vs. LD	1.161 (0.834, 1.617)	0.377	0.0
	NEDA vs. placebo	1.636 (1.215, 2.204)	0.001	0.0
	NEDA+LD vs. LD	1.216 (0.818, 1.807)	0.334	4.6
	NEDA+LD vs. placebo	1.441 (0.721, 2.879)	0.301	0.0
Dyspepsia	NEDA vs. LD	1.447 (0.415, 5.041)	0.562	63.70
Edema	NEDA vs. LD	3.108 (1.360, 7.103)	0.007	0.00
	NEDA+LD vs. LD	0.973 (0.053, 17.771)	0.985	98.10
Fatigue	NEDA vs. placebo	1.097 (0.725, 1.660)	0.661	27.2
	NEDA vs. LD	1.885 (0.929, 3.827)	0.079	0.0
Hallucination	NEDA vs. placebo	4.671 (1.712, 12.748)	0.003	24.3
	NEDA vs. LD	2.269 (1.241, 4.148)	0.008	0.0
Headache	NEDA vs. LD	1.009 (0.629, 1.617)	0.972	0.0
	NEDA+LD vs. LD	1.083 (0.691, 1.697)	0.728	50.0
	NEDA vs. placebo	1.210 (0.835, 1.754)	0.315	3.9
Insomnia	NEDA vs. placebo	1.463 (0.823, 2.603)	0.195	46.2
	NEDA vs. LD	1.442 (0.955, 2.176)	0.082	0.0
	NEDA+LD vs. LD	0.929 (0.523, 1.653)	0.803	50.6
	NEDA+LD vs. placebo	1.867 (0.804, 4.339)	0.147	0.0
Nausea	NEDA vs. placebo	2.520 (1.992, 3.186)	0.000	36.5
	NEDA vs. LD	1.301 (1.004, 1.685)	0.046	47.4
Peripheral edema	NEDA vs. placebo	2.548 (1.062, 6.113)	0.036	0.0
	NEDA vs. LD	4.351 (2.169, 8.729)	0.000	0.0
	NEDA+LD vs. LD	1.487 (0.122, 18.098)	0.756	82.3
	NEDA+LD vs. placebo	1.515 (0.593, 3.870)	0.386	0.0
Somnolence	NEDA vs. placebo	4.214 (2.769, 6.412)	0.000	60.0
	NEDA vs. LD	2.190 (1.263, 3.800)	0.005	49.8
	NEDA+LD vs. LD	1.584 (0.735, 3.415)	0.240	87.1
Tremor	NEDA vs. placebo	0.624 (0.222, 1.752)	0.371	38.0
	NEDA vs. LD	1.342 (0.224, 8.030)	0.747	75.3
Vomiting	NEDA vs. placebo	3.848 (1.633, 9.066)	0.002	0.0
URTI	NEDA vs. placebo	0.882 (0.525, 1.482)	0.636	0.0

CI, confidence interval; NEDA, non-ergot dopamine agonist; URTI, upper respiratory tract infection; N/A, not available.

Findings in Relation to Previous Reviews

To our knowledge, this meta-analysis was the first study that comprehensively examined the efficacy and safety of NEDA in early stage PD patients. We focused mainly on the evaluation of motor implications, especially dyskinesia induced by NEDA. We also explored potential risk factors (dose of LED, duration of NEDA treatment) that might influence the incidence of motor complications. One previous study conducted a meta-analysis to examine the efficacy of NEDA in early and later PD patients. The pooled results regarding the effects of NEDA on motor function were consistent with our results. However, that study mainly focused on motor function improvements, and potential risk factors for motor complications were not analyzed (Zhou et al., 2014b). The side effects of NEDA are still essential for determining how NEDA are applied. Another study assessed the incidence of and risk factors for dyskinesia induced by DAs (including NEDA and EDAs) in PD treatment. This study indicated that DAs decreased the odds of developing

dyskinesia compared with LD treatment, which was similar to our result that indicates the positive effect of NEDA. However, this meta-analysis included most EDAs (a class of medicines that might easily cause fibroblasts and are not recommended for use as first-line medicines), which might weaken the strength of evidence and increase the number of confounding factors in analyzing the effect and safety of NEDA (Chondrogiorgi et al., 2014). Therefore, in this meta-analysis, we comprehensively assessed the incidence of motor implications for NEDA (or plus supplemental LD) and LD (or placebo) and determined whether potential risk factors, including dose response and dose accumulation, might influence the incidence of dyskinesia in PD patients.

Implications for Clinical Practice

Several implications for clinical practice were obtained from our analysis. First, clinical doctors could suggest that patients with early PD use NEDA as an evidence-based alternative for ameliorating motor symptoms and improving ADLs. Second, considering the low odds of developing motor implications, NEDA are recommended for long-term treatment for early PD patients, especially patients who do not urgently require significant alleviation of motor symptoms. Third, healthcare workers still need to control the dose of NEDA because the risk of dyskinesia increases with increasing dose of NEDA. However, the incidence of dyskinesia was not influenced by NEDA treatment duration, meaning that NEDA might be more suitable for long-term treatment. Last, doctors still need to pay attention to the side effects of NEDA, and alternative options are needed when serious side effects occur.

Limitations

There were several limitations in this meta-analysis, and the above findings should be interpreted with caution. Few studies have reported impulsive-compulsive behaviors during NEDA treatment, and it is difficult to provide comprehensive evidence to examine impulsive-compulsive behaviors associated with NEDA use. Moreover, a part of the DA monotherapy group received open LD during the trial or follow-up period, which might confound the analysis of the incidence of motor complications and side effects induced by NEDA monotherapy. Therefore, we excluded patients treated with NEDA + open LD from the NEDA monotherapy group and classified them as the NEDA + open LD group. However, excluding these studies from the NEDA monotherapy group might have removed some important data concerning NEDA monotherapy, which increased the incomplete reporting bias and weakened the strength of the evidence. Additionally, in this study, we focused mainly on exploring potential risk factors influencing the occurrence, rather than the severity, of motor complications. The factors that influence the severity of motor complications should be given more attention in future studies.

CONCLUSION

In conclusion, NEDA therapy effectively reduced motor symptoms and ameliorated ADLs in the early stage of PD. However, the incidence of motor implications, especially dyskinesia, was still influenced by NEDA use. Dyskinesia increased with increasing LED and was not influenced by the duration of NEDA treatment. Although a high dose of NEDA might increase the odds of developing motor complications, the odds were still lower than those associated with LD use. Considering the lower risk of motor complications and smaller dose accumulation effect, NEDA might be more suitable for early PD patients undergoing long-term treatment.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

MZ and DC designed the study and revised the manuscript for important intellectual content. YX, HG, LL, and XL acquired the data. CW and CT analyzed and interpreted the data. CW drafted the manuscript. All authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnagi.2022.831884/full#supplementary-material

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Parkinsonian Syndromes in Motor Neuron Disease: A Clinical Study

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Background: Parkinsonian syndromes may rarely occur in motor neuron disease (MND). However, previous studies are heterogeneous and mostly case reports or small case series. Therefore, we aimed to identify and characterize patients with concurrent parkinsonian syndromes extracted from a cohort of 1,042 consecutive cases diagnosed with MND at a tertiary Italian Center.

Methods: Diagnosis of Parkinson's disease (PD), progressive supranuclear palsy (PSP) and corticobasal syndrome (CBS) was made according to current criteria. Clinical characterization included: upper and lower motor neuron disease features, typical and atypical parkinsonian features, oculomotor disorders, cognitive testing, MRI features, and, when available molecular neuroimaging. Genetic testing was carried out for major MND and PD-associated genes.

Results: Parkinsonian syndromes were diagnosed in 18/1042 (1.7%) of MND patients (7 PD, 6 PSP, 3 CBS, 2 other parkinsonisms). Based on phenotype, patients could be categorized into amyotrophic lateral sclerosis (ALS)-parkinsonism and primary lateral sclerosis (PLS)-parkinsonism clusters. Across the whole database, parkinsonism was significantly more common in PLS than in other MND phenotypes (12.1 vs. 1.1%, $p = 5.0 \times 10^{-10}$). MND patients with parkinsonian features had older age of onset, higher frequency of oculomotor disorders, cognitive impairment, and family history of parkinsonism or dementia. Two patients showed pathogenic mutations in *TARDBP* and *C9orf72* genes.

Conclusion: Specific patterns in MND-parkinsonism were observed, with PLS patients often showing atypical parkinsonian syndromes and ALS patients more frequently showing typical PD. Systematic clinical, genetic, and neuropathologic characterization may provide a better understanding of these phenotypes.

Keywords: motor neuron disease (MND), parkinsonism, amyotrophic lateral sclerosis, primary lateral sclerosis (PLS), progressive supranuclear palsy

INTRODUCTION

Parkinsonian syndromes can occur in motor neuron diseases (MND), accompanying upper (UMN) and lower motor neuron (LMN) signs (amyotrophic lateral sclerosis, ALS-parkinsonism), UMN signs alone (primary lateral sclerosis, PLS-parkinsonism) and, less often, LMN signs alone (Qureshi et al., 1996; Sudo et al., 2002; Hideyama et al., 2006; Pradat et al., 2009; Gilbert et al., 2010; Calvo et al., 2019).

Some epidemiological studies showed a higher prevalence of Parkinson's disease (PD) among relatives of MND patients, suggesting a common clinicopathological spectrum rather than coincidental events (Eisen and Calne, 1992; Qureshi et al., 1996), and pathological evidence of nigrostriatal denervation was shown in MND-parkinsonism overlap syndromes (Sudo et al., 2002). The phenotypic variability of overlap syndromes is occasionally explained by the presence of pathogenic mutations in several genes, some recognized as causative genes for sporadic and familial ALS and Frontotemporal Dementia (FTD) such as *C9orf72* (Ticozzi et al., 2014). While the link between ALS and FTD has been well described in recent years, the focus of our study was to describe MND phenotypes with overlapping parkinsonian syndromes. Studies reporting parkinsonian syndromes overlapping MND are heterogeneous with regard to L-dopa responsiveness, molecular imaging evidence of nigrostriatal dysfunction, presence of cognitive impairment, and other additional features (e.g., oculomotor disorders) (Zoccolella et al., 2002; Mackenzie and Feldman, 2004; Pradat et al., 2009; Lim et al., 2013; Calvo et al., 2019). Therefore, the aims of this study were to: (i) report the frequency of parkinsonian syndromes in a large cohort of Italian MND patients that were evaluated at our tertiary ALS center; (ii) provide a clinical description and categorization of patients with MND and parkinsonian syndromes.

PATIENTS AND METHODS

Study Design and Patient Selection

This single center observational study was conducted at the Department of Neurology, Istituto Auxologico Italiano IRCCS, a tertiary MND center. All patients with a clinical diagnosis of ALS and other MNDs according to the El Escorial revised criteria were consecutively recorded in an electronic database (Brooks et al., 2000). The database contains structured demographic and clinical information, which is manually entered from each patient's clinical record. The database was built on January 2008 and accessed on January 10th 2022, when 1042 individual patients' records were present. Patients with a MND "plus" phenotype (defined as presence of prominent sensory, ocular, cerebellar, autonomic or parkinsonian sign and symptoms) were extracted, and those receiving a diagnosis of MND overlapping with parkinsonian syndromes [Parkinson's disease (PD), corticobasal syndrome (CBS), progressive supranuclear palsy (PSP) and multisystem atrophy (MSA)] at the time of evaluation were considered for this analysis (**Figure 1**). Causes of secondary parkinsonism were ruled out. Diagnoses of parkinsonism were formulated at the time of clinical evaluation. Based on the available criteria at the time of the evaluation, UK Parkinson's Disease Society (PDS) and Brain Bank criteria were used for the diagnosis of parkinsonian syndromes and idiopathic PD (Hughes et al., 1992). National Institute of Neurological Disorders and Stroke – Society for PSP (NINDS-SPSP) and Movement Disorders Society (MDS) criteria were considered when evaluating features of PSP (Litvan et al., 1996; Höglinger et al., 2017), while for CBS diagnosis the criteria proposed by Boeve and Armstrong were adopted (Boeve et al., 2003; Armstrong et al., 2013). Furthermore, to confirm the validity of the clinical data supporting the diagnosis, the original clinical records of the selected patients were also independently reviewed by clinicians experienced in the field of MND (NT) and movement disorders (JP).

Parkinsonian Features

The following features were recorded at the time of the evaluation in all MND-parkinsonism patients: rigidity with a clear cogwheeling phenomenon; rest or re-emergent tremor; bradykinesia with a clear slowing and decrement of amplitude during finger tapping; gait: short and shuffling steps, freezing of gait, trunk anteroflexion or camptocormia not attributed to muscle weakness, reduced arm swing not attributed to spasticity; postural instability; ocular movement disorders. Response to levodopa, molecular imaging evidence of dopamine transporter deficiency in the striatum, and genetic screening of genes associated to parkinsonian syndromes were also recorded when available (SNCA, PRKN, PINK1, DJ1, LRRK2, ATP13A2, DNAJC6, FXBO7, GBA, PARK7, RAB39B, VPS13C, VPS35, PLA2G6, DCTN1, GCH1, GRN, MAPT, PRNP, PSEN1, PSEN2).

Motor Neuron Disease Features

The following characteristics were considered in all MND patients: motor neuron disease phenotype, age and site of onset, revised ALS Functional Rating Scale (ALSFRS-r) (Cedarbaum, 1996), disease duration, cognitive and behavioral features evaluated at standard neuropsychological testing, presence of mutations in the *SOD1*, *TARDBP*, *FUS* and *C9orf72* genes. Additionally, when present, the following information were collected: genetic screening of other ALS-associated genes, brain MRI features, cerebrospinal fluid (CSF) analysis, other clinical and neuroimaging analyses (e.g. ^{18}F-FDG PET), pre-existing medical conditions.

Statistical Analysis

Comparisons between groups were performed with Mann-Whitney U test for quantitative variables and with chi-square (χ^2) test for categorical variables. Survival analysis (tracheotomy or death) was performed with the Kaplan-Meier method. The significance threshold for hypothesis testing was $p < 0.05$. Statistical analysis was carried out with Statistical Package for Social Sciences (IBM® SPSS) version 26. Pairwise deletion was used to handle missing data.

Ethical Standards and Data Availability

This study was approved by the ethical committee of Istituto Auxologico Italiano IRCCS (project DAMARE) and was

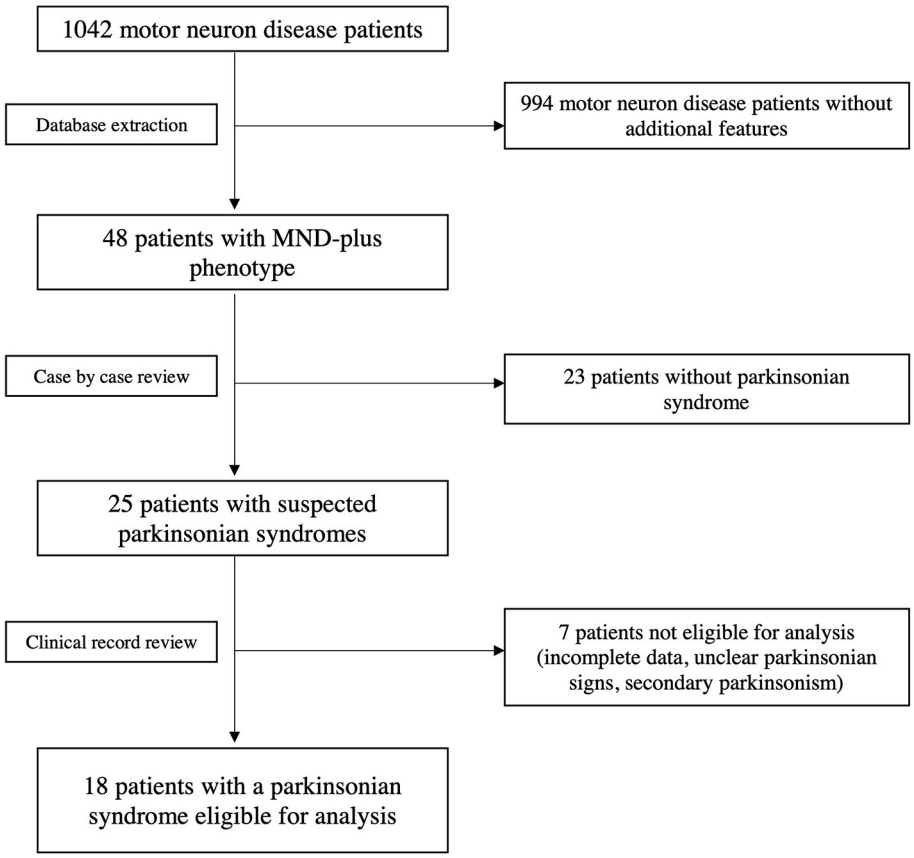

FIGURE 1 | Flow-chart showing database extraction and selection of patients with overlapping MND and primary parkinsonian syndromes.

performed in accordance with the 1964 Declaration of Helsinki and its later amendments. Written informed consent for using anonymized clinical data for research purposes was obtained at the time of evaluation from all participants. Pseudo-anonymized datasets analyzed for this study are archived on Zenodo (doi: 10.5281/zenodo.5647409) and will be shared upon reasonable request.

RESULTS

We identified in our cohort 18/1042 (1.7%, 8 female and 10 male) patients with MND-parkinsonism. Among these, seven patients (0.7%) received a diagnosis of concurrent PD, six of PSP (0.6%), three of CBS (0.3%). Two (0.2%) patients showed a progressive, akinetic-rigid, primary parkinsonian syndrome not responsive to levodopa treatment, but without elements suggestive of atypical parkinsonisms. No case of concurrent MSA and MND was observed. Based on the pattern of UMN and LMN signs, as well as parkinsonian features, MND-parkinsonism patients were grouped into two main phenotypic clusters. The first cluster was composed of 11 patients with ALS and parkinsonism: among these, seven patients had concurrent idiopathic PD, two received a diagnosis of CBS, while the remaining two manifested overt bradykinesia associated to cogwheel rigidity and/or rest tremor, not responsive to levodopa therapy, but without other signs or symptoms suggestive for atypical parkinsonian syndromes. Within this cluster, most patients (7/11) displayed a MND phenotype with prominent LMN signs (classic ALS, flail arm or flail leg syndromes), while bulbar and pyramidal ALS were each observed in two patients. The second phenotypic cluster includes seven patients with PLS and atypical parkinsonian syndromes characterized by axial rigidity, postural instability and oculomotor dysfunction (mainly slow saccades, vertical gaze palsy and ocular apraxia). Within this group, six individuals met the criteria for a diagnosis of concurrent PSP, while the remaining one had signs suggestive of CBS. Disease onset was in the bulbar segment in four patients and in the spinal muscles in the remaining three cases. Interestingly, in 5/7 patients with ALS and idiopathic PD, the PD diagnosis preceded the MND onset by several months or years. Conversely, in all PLS patients, atypical parkinsonism was present at the time of diagnosis or appeared later in the disease course. Demographic and clinical characteristics of patients with MND-parkinsonism are summarized in **Table 1** and **Supplemental Table 1**.

Considering all MND patients present in our cohort, extrapyramidal features were significantly more common in PLS than in other MND phenotypes (7/58, 12.1% vs. 11/984, 1.1%; $\chi^2 = 38.693$, $p = 5.0\text{x}10^{-10}$). Furthermore, compared

TABLE 1 | Detailed clinical characteristic of the 18 patients with a MND-parkinsonism phenotype.

ID	MND onset (y)	Park onset (y)	Sex	MND clinical phenotype	MND onset (site)	Time at first visit	Survival (months)	Parkinsonism clinical phenotype	Rigidity	Tremor	Brady kinesia	Posture and gait	Arm swing	Postural instability	Eye movements	Other	LD response	Cognitive decline	DaT imaging
1	55.2	58.8	F	PLS	B	27.3	78.5†	PSP	UL asymmetric	N	Y	Freezing and festination	N	Y	Fragmented smooth pursuit	Hyposmia; hypomimia	Y	Mild memory impairment	NA
2	49.1	49.2	M	PLS	LL	18.0	35.3	CBS	UL asymmetric	N	Y	Camptocormia	N	Y	N	Left hand and foot dystonia, hypomimia	N	Frontal cognitive decline, left limb apraxia	Normal
3	61.4	63.0	F	PLS	B	19.1	47.1	PSP	UL asymmetric	Postural tremor	N	N	N	N	Upgaze and lateral limitation	Hypomimia	NA	Frontal cognitive decline	NA
4	77.6	77.6	F	PLS	LL	12.2	36.0†	PSP	UL asymmetric	N	N	Unstable	Reduced	Y	Upgaze limitation; slow saccades	N	NA	Frontal cognitive decline	NA
5	76.4	76.4	M	PLS	B	28.8	76.9†	PSP	UL asymmetric	Rest tremor	Y	Wide based	Reduced	Y	Vertical and horizontal gaze limitation; slow saccades; fragmented smooth pursuit	N	NA	Frontal cognitive decline	NA
6	43.2	43.3	M	PLS	UL	8.4	25.4†	PSP	UL symmetric	N	Y	Trunk anteroflexion	Reduced	N	Very slow saccades; gaze apraxia	Hypomimia	Poor	Frontal dysexecutive syndrome, apathy	Bilateral asymmetric reduction
7	66.6	69.6	F	PLS	B	12.4	102.7†	PSP	UL asymmetric	N	Y	N	N	Y	Slow horizontal saccades; upgaze limitation	Hypomimia	Y	Frontal cognitive decline	Baseline: normal, 5y FU: mild reduction right putamen
8	80.1	80.1	M	ALS-classic	LL	18.0	38.0	PD	UL and LL contralaterally	N	Y	Trunk anteroflexion	Reduced	N	N	Hypomimia	Y	Subclinical frontal cognitive impairment	NA
9	70.7	71.0	M	ALS-classic	UL	6.4	18.3	PD	All limbs asymmetric	N	Y	Trunk anteroflexion; festination	N	N	Fragmented smooth pursuit	N	Y	Subclinical frontal cognitive impairment	Bilateral asymmetric reduction
10	68.1	62.0	M	ALS-classic	LL	5.8	15.9	PD	UL asymmetric	Rest tremor	Y	Trunk anteroflexion	Reduced	Y	Fragmented smooth pursuit; upgaze limitation; downward slow saccades	Hypomimia	Y	N	NA

(Continued)

TABLE 1 | Continued

ID	MND onset (y)	Park onset (y)	Sex	MND clinical phenotype	MND onset (site)	Time at first visit	Survival (months)	Parkinsonism clinical phenotype	Rigidity	Tremor	Brady kinesia	Posture and gait	Arm swing	Postural instability	Eye movements	Other	LD response	Cognitive decline	DaT imaging
11	68.3	67.9	M	ALS-flail leg	LL	39.9	61.0†	PD	UL asymmetric	Rest tremor	Y	Unable to stand	Unable to stand	Unable to stand	N	N	Partial	N	Bilateral asymmetric reduction
12	64.2	61.1	M	ALS-flail arm	UL	12.4	17.9†	PD	All limbs asymmetric	Rest remor	Y	Trunk anteroflexion	N	N	N	N	Y	N	Abnormal
13	78.7	77.6	M	ALS-bulbar	B	9.8	36.6	PD	UL asymmetric	Rest tremor, asymmetric; kinetic tremor	Y	N	Reduced	Y	Upgaze limitation	Micrographia, hypomimia	Y	N	NA
14	67.2	64.4	F	ALS-classic	LL	10.7	10.7†	PD	UL asymmetric	Postural and kinetic	Y	Unable to stand unaided	Unable to stand unaided	Unable to stand unaided	N	Hypomimia	Partial	Mild memory deficit	Bilateral asymmetric reduction
15	60.8	66.5	M	ALS-bulbar	B	45.9	104.3†	Parkinsonism	UL symmetric	N	Y	N	Reduced	Y	Fragmented smooth pursuit; slow horizontal saccades.	N	N	Mild memory and frontal deficits	NA
16	75.9	75.9	F	ALS-UMNp	LL	4.7	6.7	Parkinsonism	All limbs asymmetric	N	Y	N	N	N	Fragmented smooth pursuit; slow saccades	N	N	N	NA
17	61.8	61.9	F	ALS-UMNp	UL	7.4	25.3	CBS	All limbs	Intermittent right hand rest tremor	Y	N	Reduced	N	Slow saccades in all directions	Hypomimia; left hand dystonia and apraxia	N	Mild memory deficit and frontal dysexecutive syndrome buccofacial apraxia	Normal
18	65.7	65.1	F	ALS-classic	UL	24.6	62.4	CBS	LL asymmetric	N	Y	Trunk anteroflexion; festination	Reduced	Y	Ocular apraxia	Hypomimia, left hand apraxia	Partial	Frontal cognitive decline, ideomotor and buccofacial apraxia	Bilateral asymmetric reduction

The table has been subdivided in two parts according to the following grouping: PLS-parkinsonism (IDs 1-7) and ALS-parkinsonism (IDs 8-18). Rigidity was considered to have parkinsonian characteristics when showing a clear cogwheeling phenomenon. Tremor was considered to have parkinsonian characteristics when showing a clear rest component. The presence of postural and kinetic is also reported. Bradykinesia was considered to have parkinsonian characteristics when showing a clear slowing and reduction of amplitude during finger tapping. Time at first visit is the disease duration expressed in months between onset of MND signs and symptoms and first evaluation at our Center. ALS, amyotrophic lateral sclerosis; B, bulbar; CBS, corticobasal syndrome; F, female; LL, lower limbs; M, male; N, no; NA, not available; Park, parkinsonism PD, Parkinson's disease; PLS, Primary lateral sclerosis; PSP, progressive supranuclear palsy; UL, upper limbs; UMNp, upper motor neuron-predominant; Y, yes; y, years; †, alive or lost to follow-up.

to "pure" MND cases, the 18 patients with extrapyramidal features had a significantly older age of MND onset (65.9 ± 10.2 vs. 60.0 ± 12.0; $p = 0.036$), higher frequency of cognitive and/or behavioral impairment (9/18, 50.0% vs. 165/863, 19.1%; $\chi^2 = 10.584$, $p = 0.001$), of extraocular movement disorders (13/18, 72.2% vs. 80/910, 8.8%; $\chi^2 = 78.758$, $p = 7.0 \times 10^{-19}$), and of a family history of neurodegenerative disorders (dementia and/or parkinsonism; 8/18, 44.4% vs. 193/835, 23.1%; $\chi^2 = 4.451$, $p = 0.035$).

Conversely, no significant differences in sex, survival, site of onset, ALSFRS-R score, progression of disability (delta ALSFRS-R) and MND family history were found.

Genetic screening of major ALS-associated genes was performed in all patients and revealed a p.A383T *TARDBP* mutation in a sporadic ALS-parkinsonism case (ID: 15) and a $(G_4C_2)_n$ *C9orf72* repeat expansion in a PLS patient with neuropsychological features of CBS and family history of Alzheimer's disease-type dementia (ID: 2) (**Supplementary Figure 1**). Interestingly, Southern blot on DNA extracted from peripheral blood revealed a large expansion (>3000 repeats), possibly explaining the extrapyramidal and cognitive features observed in our patient. Conversely, genetic screening of major PD-associated genes was performed in 10/18 patients and did not reveal any pathogenic variant (**Supplementary Table 1**).

DISCUSSION

In this study we described the clinical characteristics, including genetic testing for major ALS- and PD-associated mutations, in a group of 18 patients with MND and overlapping parkinsonian syndromes. Based on clinical features, we identified two main disease patterns: 1) PLS-parkinsonism and 2) ALS-parkinsonism. PLS patients showed mostly atypical parkinsonian signs, while ALS patients showed mostly overlapping PD (Brait-Fahn disease) (Brait et al., 1973). Among the first group, 6/7 patients met the criteria for a diagnosis of PSP-PLS syndrome (Joseph et al., 2006; Nagao et al., 2012; Höglinger et al., 2017), while the remaining case had a PLS-CBS phenotype (Murakami et al., 2022). The onset of extrapyramidal signs was variable in the different phenotypic groups, often preceding MND diagnosis in ALS-PD patients, while being simultaneous or subsequent in PLS- and ALS-parkinsonism cases. It should also be noted that 3/9 patients with available DaT imaging did not show dopaminergic nigrostriatal degeneration. This is not completely unexpected, since it has been shown that lesions localizing to wide motor networks can cause clinical parkinsonism (Joutsa et al., 2018). Furthermore, as expected levodopa responsiveness was more consistent in ALS-PD cases, while it was documented in only 3/11 participants with MND and atypical parkinsonism. Compared to previous similar studies, our analysis involved a very large, single center cohort of MND patients thus identifying a comparably greater number of patients showing overlapping parkinsonian syndromes (Brait et al., 1973; Qureshi et al., 1996; Mackenzie and Feldman, 2004; Lim et al., 2013). It has been previously reported that up to 69% of ALS patients may show parkinsonian stiffness and 28% may show a parkinsonian syndrome (Pradat et al., 2009; Calvo et al., 2019). However, lower percentages have also been reported, with only 6.8% showing at least two parkinsonian cardinal signs at the time of MND diagnosis (Pupillo et al., 2015). Conflicting percentages are probably due to different study designs and patient characteristics at the time of recruitment. As an example, in a prospective case-control study, the number of participants with certain pre-specified characteristics (e.g. parkinsonian features in MND) may be inadvertently inflated in the recruitment process. Furthermore, MND disease duration at the time of evaluation is also critical, as mild parkinsonian signs may develop in late-stage MND. Therefore, by including only individuals with overt parkinsonian syndromes in our analysis, it is very likely that we missed those patients with very mild symptoms, thus resulting in a lower frequency of MND-parkinsonism cases compared to other reports. On the other hand, however, we can be confident that signs and symptoms detected in our patients, specifically bradykinesia, stiffness and postural instability, are indeed due to a concurrent parkinsonian syndrome, rather than to the underlying MND.

It has been suggested that overlap syndromes involving MND and parkinsonism are more frequent than expected based on population data of the two diseases (Eisen and Calne, 1992). Therefore, it is likely that a shared etiopathogenetic process occurs in these patients, as suggested by the recent observation of a genetic correlation between ALS and PSP (van Rheenen et al., 2016; Chen et al., 2018). Indeed, the finding of an increased frequency of family history for dementia and parkinsonism in our cohort indicates that a common genetic background may exist. Interestingly, if such genetic overlap exists, it is due to mutations in ALS- rather than PD-associated genes. Indeed, two of the 18 patients showed disease-causing mutations in *TARDBP* and *C9orf72*, while we did not observe any variant in genes associated to parkinsonian syndromes. Large *C9orf72* expansions like in our patient are known to be associated with more severe pathological burden in extramotor areas and more complex phenotypes (Brettschneider et al., 2013), while *TARDBP* mutations have been rarely associated to familial PD (Cannas et al., 2013). Notably, in this study the four major ALS-associated genes were systematically tested in MND-parkinsonism patients, whereas genetic analysis has been limited in previous studies.

Neuropathological reports of patients with MND-parkinsonism are conflicting, describing various combinations of Lewy bodies, ubiquitinated cytoplasmic inclusions, tau pathology and atrophy in disease-associated brain regions, as well as a loss of dopaminergic neurons in the substantia nigra (Uitti et al., 1995; Mackenzie and Feldman, 2004; Liang et al., 2005). Since postmortem examination was not performed, we do not have neuropathological information about our patients. However, based on the available clinical, genetic and nuclear medicine data, and on previously mentioned neuropathological studies, we can speculate that patients with ALS and PD may have concurrent TDP-43 and a-synuclein pathology. Conversely, MND-CBS cases may be associated with predominant TDP-43 pathology as suggested by recent reports (Murakami et al., 2022; Seibert et al.,

2022), while PLS-PSP cases could be explained by tau, TDP-43, or a combination of both pathologies (Liu et al., 2000).

Limitations of this study include the observational nature of the data with a lack of systematicity in terms of the clinical exams available for each patient (available findings are reported in **Table 1** and **Supplementary Table 1**); the difficulty in distinguishing motor slowing due to UMN impairment from extrapyramidal bradykinesia with decremental amplitude of movements; (Norlinah et al., 2007) the possible random co-occurrence of ALS and PD in the same patient; the unavailability of neuropathological examinations; the lack of systematic screening for other genes responsible for mixed phenotypes, such as *TFG*, (Yoo et al., 2022) or for spinocerebellar ataxias associated with MND and parkinsonian signs, such as *ATXN2*. In the context of MND, the difference between mild spasticity and mild plastic rigidity might not be easily recognizable even by experienced clinicians. Therefore, only the presence of cogwheeling phenomenon was classified as parkinsonian rigidity. Finally, a control cohort was not included in this study. However, the strict inclusion of patients with complex MND-parkinsonian syndromes warrants a comparison with classic MND rather than with elderly healthy controls.

In conclusion, we observed a clear parkinsonian syndrome in 1.7% of patients affected by MND. Although individual phenotypes were heterogeneous, two disease patterns were identified (PLS with atypical parkinsonian syndromes and ALS with typical parkinsonian syndromes resembling PD). Interestingly, these phenotypes may be associated to genetic defects, such as mutations in the *C9orf72* and *TARDBP* genes. It is likely that further investigations into the neuropathological and genetic aspects of these overlap syndromes may yield further insights into the etiopathogenesis of these and other neurodegenerative disorders, and, therefore, on their potential treatments.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: doi: 10.5281/zenodo.5647409.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Istituto Auxologico Italiano IRCCS. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JP, FT, and NT: study design and writing of first draft. JP, FT, CM, BP, AC, VS, and NT: data collection. SP, AB, and AR: genetic analysis. JP, FT, FG, and NT: data analysis. NT: supervision. All authors revised the manuscript for intellectual content. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnagi.2022.917706/full#supplementary-material

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Multi-predictor modeling for predicting early Parkinson's disease and non-motor symptoms progression

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Background: Identifying individuals with high-risk Parkinson's disease (PD) at earlier stages is an urgent priority to delay disease onset and progression. In the present study, we aimed to develop and validate clinical risk models using non-motor predictors to distinguish between early PD and healthy individuals. In addition, we constructed prognostic models for predicting the progression of non-motor symptoms [cognitive impairment, Rapid-eye-movement sleep Behavior Disorder (RBD), and depression] in *de novo* PD patients at 5 years of follow-up.

Methods: We retrieved the data from the Parkinson's Progression Markers Initiative (PPMI) database. After a backward variable selection approach to identify predictors, logistic regression analyses were applied for diagnosis model construction, and cox proportional-hazards models were used to predict non-motor symptom progression. The predictive models were internally validated by correcting measures of predictive performance for "optimism" or overfitting with the bootstrap resampling approach.

Results: For constructing diagnostic models, the final model reached a high accuracy with an area under the curve (AUC) of 0.93 (95% CI: 0.91–0.96), which included eight variables (age, gender, family history, University of Pennsylvania Smell Inventory Test score, Montreal Cognitive Assessment score, RBD Screening Questionnaire score, levels of cerebrospinal fluid α-synuclein, and *SNCA* rs356181 polymorphism). For the construction of prognostic models, our results showed that the AUC of the three prognostic models improved slightly with increasing follow-up time. The overall AUCs fluctuated around 0.70. The model validation established good discrimination and calibration for predicting PD onset and progression of non-motor symptoms.

Conclusion: The findings of our study facilitate predicting the individual risk at an early stage based on the predictors derived from these models. These

predictive models provide relatively reliable information to prevent PD onset and progression. However, future validation analysis is still needed to clarify these findings and provide more insight into the predictive models over more extended periods of disease progression in more diverse samples.

KEYWORDS

Parkinson's disease, predictive model, diagnosis, non-motor symptoms, progression

Introduction

Parkinson's disease (PD, OMIM 168600), the most common motoric neurodegenerative disease, affects approximately 1–2% of people older than 60 years (Wirdefeldt et al., 2011; Ascherio and Schwarzschild, 2016). When a classical feature of bradykinesia is present and combined with other features such as rigidity, tremor, and postural instability, a clear clinical diagnosis of PD can be made (Postuma et al., 2015). Although PD is categorized as a movement disorder, non-motor symptoms (for instance, olfactory disorders, constipation, and sleep disorders) indeed frequently occur in the early stage of the disease, and may even precede the emergence of motor dysfunction (Postuma et al., 2012; Schapira et al., 2017). Non-motor symptoms are almost inevitably linked to the later development of diseases and a lower quality of life (Schrag et al., 2000; Santos García et al., 2021). With the increasing understanding of PD as a multi-system heterogeneous disorder, the modern scientific diagnosis should include the assessment and management of non-motor symptoms (Chaudhuri et al., 2006). There is inconsistency in the manifestation of these symptoms and their rate of progression in PD patients, which presents a challenge for researchers developing drugs to modify the disease process (Olanow et al., 2009; Smedinga et al., 2021). Therefore, evaluating non-motor symptoms, especially in the prodromal and early stages of the disease, can help determine whether individuals are at risk for developing PD or later complications.

In the past decades, researchers have made many efforts to identify patients with PD during the prodromal and early periods of the disease, such as the Parkinson Progression Marker Initiative (2011). A few recent diagnostic and prognostic models have been generated that are devoted to predicting different aspects of PD symptomatology specifically (Ma et al., 2020; Ren et al., 2021). Most previous diagnostic models focused on various clinical motor scores as predictors (Nalls et al., 2015; Searles Nielsen et al., 2017; Schrag et al., 2019). However, given the heterogeneous nature of motor symptoms, the predictive accuracy of many existing risk models is only moderate or even low (Fereshtehnejad et al., 2015; Searles Nielsen et al., 2017). Considering the potential value of non-motor symptoms for early identification, developing the predictive models utilizing non-motor predictors may identify high-risk PD groups. Olfactory dysfunction and Rapid-eye-movement sleep Behavior Disorder (RBD) are the most common non-motor symptoms. The findings from a prospective study suggested that 10% of individuals with olfactory dysfunction developed PD during a 2-year follow-up, and thus idiopathic hyposmia can be as a preclinical sign of PD (Ponsen et al., 2004). Many candidate biomarkers have been tested in different study cohorts, such as cerebrospinal fluid (CSF) α-synuclein (α-syn), amyloid-β_{42} ($A\beta_{42}$), and total tau (t-tau) (Parnetti et al., 2013). Recently, CSF or serum neurofilament light chain (NFL) as one promising candidate biomarker has added diagnostic value to biomarker panels (Oosterveld et al., 2020).

From observational and longitudinal studies, several non-motor symptoms affecting cognition, sleep, and psychosis appeared to be correlated with the later progression of PD (Schrag et al., 2015; Galtier et al., 2019). Dementia is one of the most common non-motor symptoms of PD, with a prevalence of about 30% (Aarsland et al., 2005). The cumulative incidence of dementia steadily increases with age and duration of PD, increasing to 80% by age 90 (Buter et al., 2008). RBD is another common non-motor symptom reported in all stages of PD, with a combined prevalence of 42% (Zhang et al., 2017). In total, 10–45% of PD patients will become depressed as the disease progresses (Lemke, 2008). The progression of non-motor symptoms has dominant-negative effects on health-related quality of life, thus identifying prognostic factors of non-motor symptoms is vital for minimizing impairments and proposing the personalization of PD management (Gómez-Esteban et al., 2011; Duncan et al., 2014). Yet lack of longitudinal assessments in a few previous studies, there was no fuller insight into the potential role of developing comprehensive multivariable prognostic models.

Our goals in the present study were to: (i) establish and validate the most explanatory model on a baseline dataset to diagnose early PD groups with non-motor clinical characteristics, biomarkers and genetic information; (ii) construct prognostic models for predicting the progression of non-motor symptoms in *de novo* PD patients, for instance, cognitive impairment, RBD and depression at 5 years of follow-up; and (iii) identify the predictors in order to better predict individual prognosis and guide the prevention.

Materials and methods

Parkinson's Progression Markers Initiative database and participants

In the present study, we retrieved the data from the PPMI database. The PPMI is a global, multicenter, prospective research with the design goal of investigating and verifying biomarkers that may slow the disease progression. As described previously, the study investigated drug-naive, *de novo* PD patients and age- and gender-matched healthy controls (HCs) between June 2010 and May 2013 (2011). The participants with PD were recruited if they met the following requirements: (1) age older than 30 years; (2) existence of two symptoms as below: bradykinesia, rigidity, resting tremor, or asymmetric resting tremor, or asymmetric bradykinesia; (3) diagnosis recently made within the last 2 years; (4) PD drug naivety; and (5) dopamine transporter deficit in the putamen on the DaTscan by central reading. HCs were required to meet the following criteria: no significant neurological dysfunction, no first-degree relatives with PD and Montreal Cognitive Assessment (MoCA) score above 26. In this article, the baseline dataset used for diagnostic models and the 5-year follow-up dataset used for constructing progression models were downloaded on 5 March 2022. More detailed information could be sought at http://ppmi-info.org/.

Standard protocol approvals, registrations, and patient consent

The study was approved in all participating sites, respectively, by each local ethical standards committee on human experimentation as described (Lee et al., 2019). Written informed consent for research was obtained from all study participants. The PPMI study is registered with clinicaltrials.gov_(identifier: NCT01141023).

Cerebrospinal fluid and blood biomarker assessments

Biomarker analyses have been previously described and based on the PPMI biologics manual (Marek et al., 2018). CSF was collected by standardized lumbar puncture procedures. Measurements of $A\beta_{42}$ and t-tau were analyzed by using the xMAP-Luminex platform with INNOBIA AlzBio3 immunoassay kit–based reagents (Fujirebio-Innogenetics, Ghent, Belgium) at Penn (Kang et al., 2013). Additionally, CSF total α-syn levels were measured by BioLegend (San Diego, CA, United States) by means of a commercially accessible and previously described sandwich immunoassay. Serum NFL was quantified by the Simoa Human NF-light Advantage Kit (Quanterix, Lexington, MA, United States) using the Single Molecule Array technology in a fully automated SIMOA HD-1 analyzer. Biochemical analyses of uric acid have been carried out in Covance laboratories in a uniform fashion, as per the study protocol.

Genetic assessments

We analyzed genetic data for *MAPT*, and single-nucleotide polymorphisms related to *SNCA* (rs3910105 and rs356181), provided from the PPMI Genetics Core. SNPs of *SNCA* and *MAPT* genes were determined using Illumina NeuroX array on whole-blood extracted DNA per manufacturer's protocol (Illumina Inc., San Diego, CA, United States) (Nalls et al., 2016).

Predictor variables

Predictive variables included demographic data (age, gender, years of education, family history, age at onset, and ethnicity), disease duration, risk gene (*SNCA*_rs356181, *SNCA*_rs3910105, and *MAPT* status) and measures of non-motor function. For non-motor symptoms evaluation, sleep quality and disturbances of patients were measured by the Epworth Sleepiness Scale score (ESS) and RBD Screening Questionnaire score (RBDSQ). MoCA was the most common screening instrument for cognitive function. The University of Pennsylvania Smell Inventory Test (UPSIT) score was applied to assess olfactory function. The Questionnaire for Impulsive-Compulsive Disorders in Parkinson's Disease (QUIP) score was a rating scale designed to evaluate impulse control disorders. The Geriatric Depression Scale (GDS) score was applied to assess depression. The global motor impairment was assessed using total score and section III of the Movement Disorder Society-Sponsored Revision of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS). In addition, we assessed whether biomarkers including CSF $A\beta_{42}$, t-tau and CSF α-syn, serum uric acid, and NFL had impacts on the risk models in this study.

Longitudinal assessments

All the above predictive variables are remeasured annually. We constructed 5-year longitudinal models to evaluate the progression of non-motor symptoms (cognitive impairment, RBD, and depression) in early PD. Patients with less than 1-year follow-up were excluded from the longitudinal analyses. For constructing three prognostic models, we excluded PD patients who had corresponding outcome status at baseline. In the present study, defining the presence of non-motor symptoms was determined by several neuropsychological assessments. Cognitive status was defined based on the MDS task force

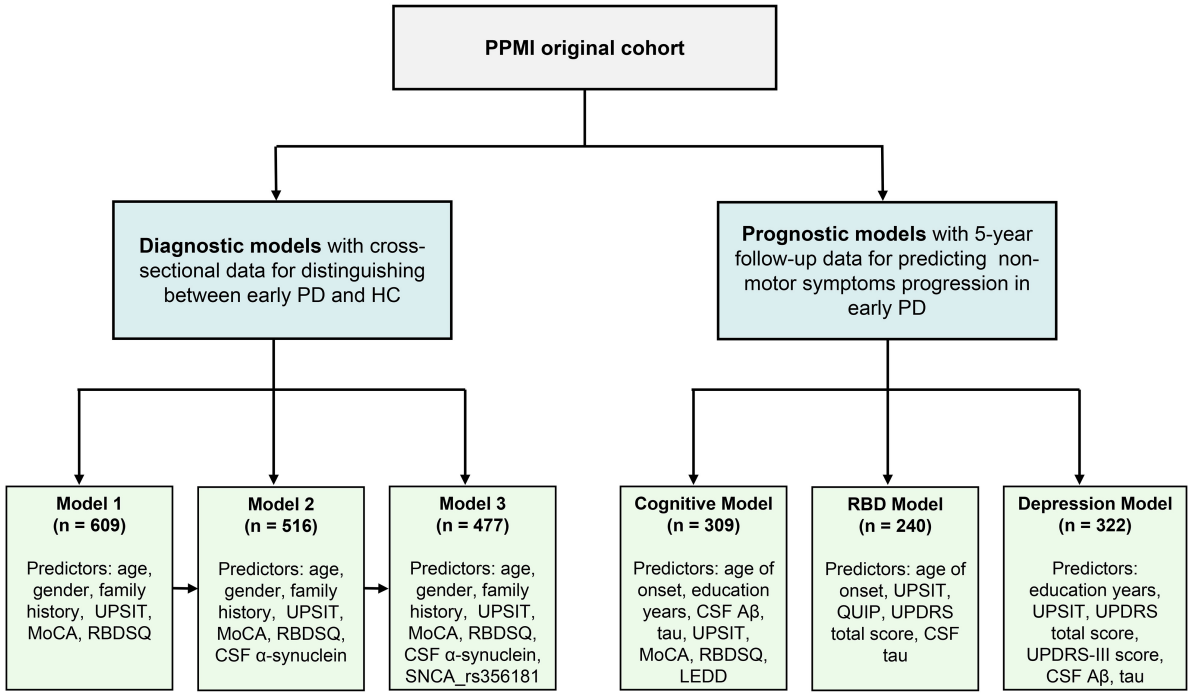

FIGURE 1
Flowchart of data analysis. PPMI, Parkinson's Progression Markers Initiative; PD, Parkinson's disease; MoCA, Montreal Cognitive Assessment; UPSIT, University of Pennsylvania Smell Inventory Test; RBM, Rapid-eye-movement sleep Behavior Disorder Screening Questionnaire; MDS-UPDRS, Movement Disorder Society-Sponsored Revision of the Unified Parkinson's Disease Rating Scale; CSF, cerebrospinal fluid; Aβ, amyloid-β.

level II guideline criteria: PD with normal cognition (PD-NC) if MoCA > 26, PD with mild cognitive impairment (PD-MCI) if MoCA between 23 and 26, and PD with dementia (PD-D) if MoCA < 23 (Litvan et al., 2012). During the longitudinal follow-up, PD-NC patients who progressed to PD-MCI or PD-D were classified as "cognitive impairment progression." The RBDSQ consists of 10 questions with 13 items overall, and RBD positive was defined if RBDSQ was above 6-points. RBD-negative patients who progressed to RBD positive were considered to have "RBD progression." The GDS is a self-report questionnaire for rating depressive symptoms, with a score of ≥5 indicating clinically significant depression in PD. Progression from no depressive symptoms at baseline to depressive symptoms was considered "depressive progression."

In the PPMI cohort, individuals were evaluated at baseline and followed for 5 years, during which some subjects started dopamine replacement therapy (DRT) such as levodopa, dopamine agonist, and others. The prescribed dose of DRT at the 5th year follow-up was expressed as levodopa equivalent daily dose (LEDD) in milligrams (mg). Therefore, we added LEDD as a candidate predictor in the prognostic model to examine the effect of DRT on the progression of non-motor symptoms.

Statistical analyses

All statistical analyses were performed by R software (version 4.1.3). Baseline characteristics were presented as mean ± standard deviation (SD) or number (percentages, %), as appropriate. Differences cross groups were evaluated by the Mann–Whitney–Wilcoxon test for continuous variables and the Chi-square test for categorical variables. Missing values were excluded from the analysis. For constructing diagnostic models, all candidate risk factors were entered into the logistic regression analysis and the assumption of proportional hazards was confirmed. Additionally, we used multivariate cox proportional-hazards models to predict non-motor symptom progression. A backward variable selection approach with a cut-off value at the $P < 0.05$ was used to identify the set of independent predictors. Final variables were tested by the Spearman rank correlation analysis to ensure that Spearman's correlation coefficients of no more than 0.5. Receiver operating characteristics analysis (ROC), the area under the ROC curve (AUC), sensitivity and specificity at each optimal cut-off value were applied to assess the model performance. The statistical analyses of ROC curves were carried out using the "pROC" packages in R. Model calibration was evaluated by generating a smooth curve in the

calibration plot between the observed and predicted outcomes. The calibration plot would equal 1 (optimization criteria) if the observed and predicted probabilities represent perfect agreement. The predictive models were internally validated by correcting measures of predictive performance for "optimism" or overfitting with bootstrap resampling approach (with 1,000 replicas) in the *rms* packages in R.

Results

Demographic and clinical characteristics of included participants

Figure 1 presents a flowchart outlining the main analysis in this study. In the risk model for distinguishing between early PD and healthy normal, a total of 194 HC individuals and 415 *de novo* PD patients were included. The baseline characteristics of participants are shown in Table 1. For clinical characteristics, no significant differences in age ($P = 0.65$), gender ($P = 0.66$), education level ($P = 0.08$), ethnicity ($P = 0.61$), ESS score ($P = 0.28$), and QUIP score ($P = 0.62$) were found between the two diagnostic groups. While the family history of PD, Hoehn and Yahr, MoCA score, UPSIT score, GDS score, RBDSQ score, and UPDRS score differed between groups ($P < 0.05$). For CSF and blood biomarkers, CSF α-syn ($P = 0.01$), $A\beta_{42}$ ($P = 0.05$), and t-tau ($P = 0.003$) were lower in the PD group compared to control group, but serum NFL levels were higher in the *de novo* PD group than control. For genetic characteristics, only *SNCA* rs356181 status differed between two diagnostic groups ($P = 0.005$). Using longitudinal data to predict non-motor symptoms progression in *de novo* PD patients, three prognostic models were performed separately to estimate the risk of cognition, RBD, and depression. The three models included 309, 240, and 322 *de novo* PD patients, respectively. The baseline characteristics of three prognostic models are summarized in Supplementary Table 1.

Predictive modeling for distinguishing between early Parkinson's disease and healthy normal

The first model was constructed with demographics, neuropsychological tests and health variables which can be easily available from primary clinical assessments. After stepwise logistic regression, we retained six PD risk factors in Model 1: age, gender, family history of PD, total UPSIT score, the MoCA score and the RBDSQ score (see Supplementary Table 2). None of these risk factors were used as part of the PD diagnosis criteria. The model has acceptable accuracy for predicting whether subsets of healthy individuals with abnormal baseline linical characteristics will develop *de novo* PD; the AUC was 0.91 (95% CI: 0.89–0.94, sensitivity 86.6% and specificity 84.4%, Figure 2).

Cerebrospinal fluid biomarkers (α-syn, $A\beta_{42}$, and t-tau) and blood biomarkers (serum uric acid and NFL) were included as possible variables besides the easily available variables used in the construction of Model 2. After variable selection by backward stepwise logistic regression, Model 2 included age, gender, family history of PD, total UPSIT score, the MoCA score, RBDSQ score, and CSF α-syn (Supplementary Table 2). The AUC was improved to 0.92 (95% CI: 0.89–0.94, sensitivity 79.7% and specificity 92.2%, Figure 2) with the inclusion of new variables.

Model 3 evaluated the genetic status (*MAPT* status, *SNCA* rs356181, and *SNCA* rs3910105) of PD adjusted for the covariates included in Model 2 to acquire more accurate calculation in predicting risk individuals. Age, gender, family history of PD, total UPSIT score, the MoCA score, RBDSQ score, CSF α-syn, and *SNCA* rs356181 polymorphism were selected as final variables in Model 3, and Model 3 as the final diagnostic model predicted early PD in this study. In this analysis, the ROC curves demonstrated an AUC of 0.93 (95% CI: 0.91–0.96, Figure 2) with a sensitivity of 88.1% and a specificity of 87.3%. Figure 3A described the heatmap of the Spearman correlation coefficients between the final variables, and there was no strong correlation between the final eight included variables (Supplementary Table 3). All variables were superior predictive indicators in the multifactorial analyses ($P < 0.05$, Figure 3B).

Internal validation and calibration of the diagnostic model

Finally, Model 3 was selected as the prediction model for distinguishing early PD from healthy normal, and we conducted model-fitting analysis and internal validation based on Model 3. This model showed calibration (calibration slope, 1; Brier score, 0.10; Hosmer–Lemeshow $\chi^2 = 15.17$; $P = 0.06$, Supplementary Figure 1). Internal validation showed minimal mean optimism of 0.008 with bootstrap optimism corrected AUC of 0.92 based on 1000 resamplings.

Prognostic modeling for predicting non-motor symptoms progression in *de novo* Parkinson's disease patients

Candidate variables used in the construction of cognitive decline, depression and RBD model included age, gender, education years, ethnicity, family history, age at symptom onset, disease duration, UPSIT score, GDS score, QUIP score, MoCA score, CSF Aβ, α-syn, t-tau, MDS-UPDRS total score, MDS-UPDRS Part III score, ESS score, RBDSQ score, and level of LEDD.

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TABLE 1 Baseline demographic and disease characteristics of included participants.

Characteristics	PD subjects (n = 415)	HC subjects (n = 194)	*P-value*
Demographic and clinical characteristics			
Age (mean, SD)	61.7 (9.7)	60.9 (11.2)	0.65
Gender (male/female)	272/143	124/70	0.66
Education years (mean, SD)	15.6 (3.0)	16.1 (2.9)	0.08
Family history of PD (any family with PD/no family with PD)	102/313	10/184	<0.0001
Ethnicity (Hispanic or Latino/not Hispanic or Latino)	9/406	3/191	0.61
Age of PD onset	59.5 (10.0)	NA	NA
Hoehn and Yahr			<0.0001
Stage 0	0	192	
Stage 1	182	2	
Stage 2	231	0	
Stage 3–5	2	0	
MoCA score (mean, SD)	27.1 (2.3)	28.2 (1.1)	<0.0001
UPSIT score (mean, SD)	22.3 (8.3)	34.0 (4.9)	<0.0001
RBDSQ score (no RBD/RBD)	258/157	156/38	<0.0001
GDS score (not depressed/depressed)	356/59	194/0	0.01
ESS score (not sleepy/sleepy)	350/65	156/38	0.28
QUIP score (mean, SD)	0.3 (0.6)	0.3 (0.7)	0.62
MDS-UPDRS Part I score (mean, SD)	5.6 (4.1)	1.2 (2.2)	<0.0001
MDS-UPDRS Part II score (mean, SD)	5.9 (4.2)	0.5 (1.0)	<0.0001
MDS-UPDRS Part III score (mean, SD)	20.8 (8.8)	2.9 (3.0)	<0.0001
MDS-UPDRS total score (mean, SD)	32.2 (13.1)	4.6 (4.5)	<0.0001
CSF and blood markers			
α-Synuclein (pg/ml, mean, SD)	1,550.7 (687.2)	1,703.8 (731.8)	0.01
$A\beta_{42}$ (pg/ml, mean, SD)	931.8 (420.5)	1,030.8 (504.0)	0.05
Total tau (pg/ml, mean, SD)	171.1 (59.0)	193.8 (80.1)	0.003
Urate (pg/ml, mean, SD)	313.8 (75.6)	322.7 (78.4)	0.18
NFL (pg/ml, mean, SD)	13.1 (7.2)	11.9 (6.7)	0.03
Genetic characteristics			
*SNCA*_rs356181			0.005
C/C	114	32	
C/T	183	95	
T/T	86	51	
Missing	32	16	
*SNCA*_rs356105			0.09
C/C	63	44	
C/T	197	82	
T/T	123	52	
Missing	32	16	
MAPT			0.77
H1/H1	240	114	
H1/H2	126	56	
H2/H2	17	8	
Missing	32	16	

PD, Parkinson's disease; HC, healthy control; SD, standard deviation; MoCA, Montreal Cognitive Assessment; UPSIT, University of Pennsylvania Smell Inventory Test; RBDSQ, Rapid-eye-movement sleep Behavior Disorder Screening Questionnaire; ESS, Epworth Sleeping Scale; GDS, Geriatric Depression Scale; MDS-UPDRS, Movement Disorder Society-Sponsored Revision of the Unified Parkinson's Disease Rating Scale; NFL, neurofilament light; CSF, cerebrospinal fluid; $A\beta_{42}$, amyloid-β_{42}.

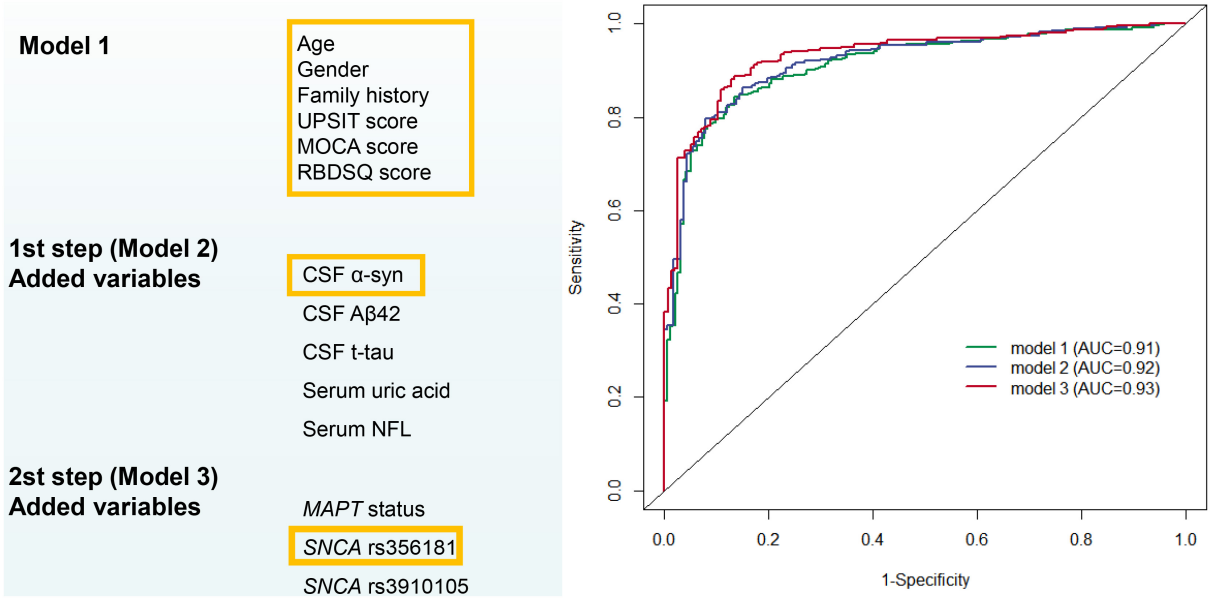

FIGURE 2
Predictive modeling for distinguishing between early Parkinson's disease and healthy normal. AUC, area under receiver operating characteristic curves.

Of the 309 early PD patients with cognitive normal at baseline were included in the cognitive decline model with eight variables (age at symptom onset, education years, MoCA score, UPSIT score, RBDSQ score, LEDD and levels of CSF $A\beta_{42}$, and t-tau, **Supplementary Table 4**). As shown in **Figure 4**, the model predicted the incident of cognitive decline within 1, 3, and 5 years with moderate accuracy (AUC of 0.73, 0.77, and 0.78, respectively, **Figures 4A–C**).

A subcohort of 240 individuals with normal RBD score at baseline were included to develop the RBD prognostic model. One hundred and twenty-six subjects (52.5%) converted to RBD over the follow-up period while others remained negative. Cox proportional-hazards models demonstrated individuals with baseline abnormal UPSIT score, MDS-UPDRS total score, QUIP score and CSF t-tau levels had a higher risk of conversion from RBD-negativity to RBD-positivity (AUC 0.65 with 1 year; AUC 0.66 within 3 years; AUC 0.68 within 5 years; **Figures 4D–F** and **Supplementary Table 4**). Given the small sample size, the results of the RBD prognostic model should be interpreted with caution.

Furthermore, we explored the depression prognostic model in a subgroup of 322 subjects without depression at baseline. The predictive accuracy performed moderate using the combined variables (education years, MDS-UPDRS total score, MDS-UPDRS Part III score, UPSIT score, CSF $A\beta_{42}$ and t-tau levels, **Supplementary Table 4**), and the ROC curves demonstrated an AUC of 0.79 within 1 year, 0.70 within 3 years and 0.70 within 5 years (**Figures 4G–I**).

Internal validation and calibration of the prognostic models

Calibration plots of three prognostic models indicated a good agreement between predicted and observed probabilities (**Supplementary Figure 2**). Internal validation using the bootstrapping technique with 1,000 repetitions resulted in optimism corrected AUC within 5 years of 0.75 (cognitive decline model), 0.60 (RBD prognostic model), and 0.62 (depression prognostic model).

Discussion

In the present study, we aimed to develop and validate clinical risk models using non-motor predictors to distinguish *de novo* PD from individuals. In addition, we constructed prognostic models for predicting the progression of non-motor symptoms (cognitive impairment, RBD, and depression) in *de novo* PD patients at 5 years of follow-up. The model validation established good discrimination and calibration for predicting PD onset and progression of non-motor symptoms.

Parkinson's disease is a heterogeneous disorder, especially in the early disease course (Marras and Chaudhuri, 2016). Non-motor symptoms are prominent factors that influence fatality rate and mutilation rate in PD. It has long been recognized that many of them precede the motor features in many patients (Hely et al., 2005). In the models for distinguishing between early PD and HCs, we identified three general categories of predictors:

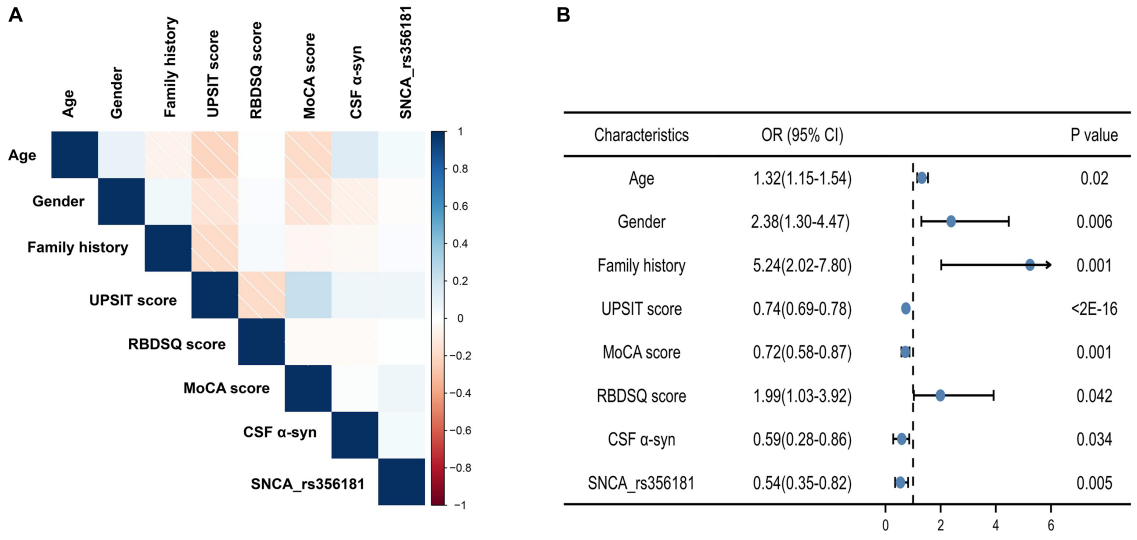

FIGURE 3
The eight predictors in the final diagnostic model. **(A)** Correlation heatmap between the eight variables; **(B)** multivariate analysis of eight variables in the diagnostic model. MoCA, Montreal Cognitive Assessment; UPSIT, University of Pennsylvania Smell Inventory Test; RBM, Rapid-eye-movement sleep Behavior Disorder Screening Questionnaire; CSF α-syn, cerebrospinal fluid α-synuclein.

clinical-related predictors, biomarker-related predictors and genetic-related predictors. Model 1 was developed with easily available and low-cost variables like demographics, health factors and functional assessments that can be widely used for screening PD risk in primary care settings. Model 2 added CSF and blood biomarkers to Model 1, and the final model (Model 3) reached a high accuracy with an AUC of 0.93 (95% CI: 0.91–0.96), which included three categories of predictors. The diagnostic performance in this study was similar to that of Nalls et al. (2015), with high accuracy and sensitivity (AUC 0.92, sensitivity 83.4%). Moreover, compared with their study, our analysis reported the CSF biomarkers' influence on the disease risk models. Despite diagnostic decisions still relying on clinical features in practice, encouragingly, breakthroughs have been made recently in PD biomarker discovery (Parnetti et al., 2019). CSF biomarkers in PD (such as α-syn, $A\beta_{42}$, tau, and NFL) have been suggested to possess the potential diagnostic and prognostic value of PD (Parnetti et al., 2019; Kwon et al., 2022). Tracking pathophysiological processes of PD, abnormal deposition of α-syn plays a critical role, which should become the foundation of composite biomarker panels (Majbour et al., 2016). In addition, biomarker-related factors, missense mutations as well as duplications in the α-syn protein-encoding *SNCA* gene are associated with *SNCA*-related parkinsonism, providing further support for a central neuropathological role of α-syn in PD (Kay et al., 2008; Rosborough et al., 2017). As the results showed, the value of logistic regression AUC improved slightly (0.02) after adding the CSF α-syn and *SNCA* rs356181 polymorphism, suggesting a predictive link of PD with α-syn levels. Approximately 10% of patients clinically diagnosed as PD have normal dopamine transporter (DAT) single-photon emission computed tomography (SPECT) imaging (Marek et al., 2014). This subgroup is referred to as having scans without evidence of dopaminergic deficit (SWEDD). In the present study, the exclusion of SWEDD participants from the PD model allows us to focus our efforts on more etiologically typical PD as defined by the clinical diagnosis and DAT scanning data. We also attempted an extended analysis to validate whether our diagnostic model could discriminate SWEDD from etiologically typical PD. The results suggested this model only achieved an AUC of 0.59 (95% CI: 0.52–0.66), with a low diagnostic value. Therefore, clinical features and non-motor symptoms cannot accurately distinguish between SWEDD and etiologically typical PD. DAT-SPECT imaging is a valuable diagnostic tool to help differentiate between PD and SWEDD, and imaging features will be taken into account to optimize our model in the future studies.

Longitudinal data provided the most substantial evidence on prognostic modeling, whereas relatively few previous studies accounted for longitudinal measurements when constructing NMS progression models. Our findings indicated that the AUC of prognostic models improved slightly with follow-up time. The overall AUCs fluctuated around 0.70. The present findings proved that participants with abnormal accumulation of amyloid, tau, older age at onset, higher level of LEDD, a lower level of education, abnormal measurements of UPSIT, MoCA, and RBDSQ had a significantly higher likelihood of cognitive decline. Similar results were suggested in a previous study which tested the five variables (age, UPSIT score, RBDSQ,

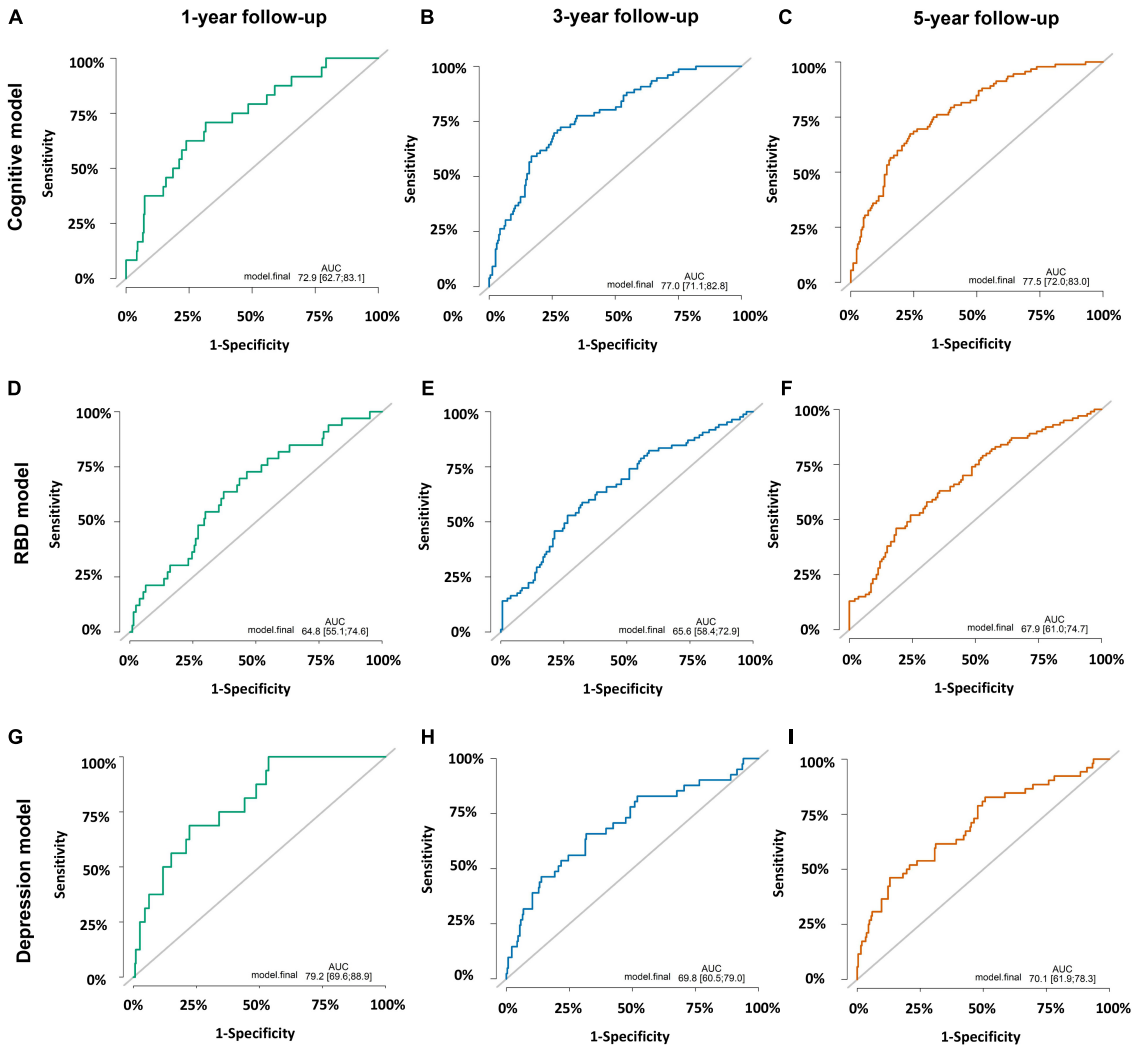

FIGURE 4
Prediction accuracies of three prognostic models. **(A)** Cognitive decline model within 1 year with area under receiver operating characteristic curves (AUC) of 0.73 (95% CI: 0.63–0.83). **(B)** Cognitive decline model within 3 years with AUC of 0.77 (95% CI: 0.71–0.83). **(C)** Cognitive decline model within 5 years with AUC of 0.78 (95% CI: 0.72–0.83). **(D)** Rapid-eye-movement sleep Behavior Disorder (RBD) prognostic model within 1 year with AUC of 0.65 (95% CI: 0.55–0.75). **(E)** RBD prognostic model within 3 years with AUC of 0.66 (95% CI: 0.58–0.73). **(F)** RBD prognostic model within 5 years with AUC of 0.68 (95% CI: 0.61–0.75). **(G)** Depression prognostic model within 1 year with AUC of 0.79 (95% CI: 0.70–0.89). **(H)** Depression prognostic model within 3 years with AUC of 0.70 (95% CI: 0.61–0.79). **(I)** Depression prognostic model within 5 years with AUC of 0.70 (95% CI: 0.62–0.78).

CSF $A\beta_{42}$, and mean caudate uptake) by logistic regression analysis and generated the AUC of 0.80 (95% CI: 0.74–0.87) (Schrag et al., 2017). Differently, our models possessed apparent advantages in predicting prognostic risk in multiple time dimensions. Besides, the early identification of patients at risk for depression and depression-related predictors as soon as possible is necessary to improve the quality of life (Reijnders et al., 2008). Previous studies also reported the association of several clinical information and CSF biomarkers with development of depression in PD using machine learning algorithm methods (Byeon, 2020a; Gu et al., 2020). Compared with our cohort, Gu et al. (2020) reported a slightly higher predictive value (AUC 0.94, 95% CI: 0.89–0.99). Similarly, the findings suggested that RBD and education levels were associated with depression in PD in previous studies, which supported our results (Byeon, 2020a). Sleep behavior disorders could serve as prodromal markers with a high risk for predicting neurodegeneration, and there has been a strong correlation with depression (Postuma et al., 2013). The presence of RBD in early PD patients may be a key determinant of increased risk of functional dependency, which indicated that RBD portended an unfavorable prognosis in Parkinson's processes (Kim et al., 2019). We constructed the RBD prognostic model that could detect conversion from RBD-negativity to RBD-positivity with

moderate accuracy. This finding was in line with the recent study, which developed a model for predicting the high-risk groups of RBD using random forest model with the prediction accuracy of 71.5% (Byeon, 2020b). Together, the UPSIT score was selected as a final predictor in three prognostic models. Our findings suggested that olfactory impairment may be a significant predictor predicting the occurrence of non-motor symptoms in PD, particularly cognitive decline. Considering the low cost and ease of assessment, olfactory impairment has become an attractive biomarker and also correlated with other non-motor features that may present later in the disease course (Fullard et al., 2017).

Our research possesses some strengths. Firstly, in our cohort, we constructed clinical risk models using non-motor predictors to distinguish between early PD and healthy individuals. In addition, we developed prognostic models for predicting the progression of non-motor symptoms among *de novo*, untreated PD. This study is the most comprehensive analysis of predictive models available in PD diagnosis and progression, keeping with overall assessments of the list of risks proposed in current clinical guidelines (Lennaerts et al., 2017). Additionally, this study provided relatively convenient methods, with low-cost and easily available clinical information as model features, which made the models feasible for practical application. Furthermore, in order to predict non-motor symptoms progression based on the patient's baseline clinical data, clinicians can embed the predictive models in the electronic medical record system. The PPMI database collected data from multiple hospitals, which can improve the accuracy of our predictive models. The database describes a dynamic process of repeating measurements of clinical data annually with a higher degree of practical clinical application value.

There are also several potential limitations. First, the sample size is limited, especially a few participants were excluded from this study for missing records for CSF biomarkers and genetic assessments, which may cause bias in the final results. Future, more comprehensive research in larger cohorts is required to define prediction accuracy of models. Although the models were validated internally, developing risk model is still a work in progress that requires continuous refinement and revalidation in different cohorts. Besides, the data of this study were obtained from the PPMI database, which is not particularly appropriate to represent the general PD population, as it is an early study within 2 years of diagnosis. Further studies with more extended follow-up periods may enable long-term predictions.

Conclusion

In total, the findings of our study facilitate predicting the individual risk at an early stage based on the predictors derived from these models. These predictive models provide reliable information to prevent PD onset and progression and further establish management strategies. Further research in large cohorts should explore how the clinical measurements and biomarkers combinations would present the best value for clinical and research purposes. Finally, future validation analysis is still needed to clarify these findings and provide more insight into the prognostic models over more extended periods of disease progression in more diverse samples.

Data availability statement

The original contributions presented in this study are included in the article/**Supplementary material**, further inquiries can be directed to the corresponding author.

Ethics statement

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

KD participated in the design of the study, drafted the manuscript, and carried out the conceptualization of the study. JM, XZ, WS, and MT performed the data analysis and drafted the manuscript. AX carried out the conceptualization of the study, reviewing, and critiquing the article at the same time. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnagi.2022.977985/full#supplementary-material

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Nilotinib in Parkinson's disease: A systematic review and meta-analysis

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Background: Nilotinib, which inhibits cellular Abelson tyrosine kinase, may be an effective treatment for patients with Parkinson's disease (PD). The purpose of this study is to evaluate the outcomes of different doses of nilotinib in patients with PD.

Methods: We searched PubMed, Embase, Web of Science, and Cochrane Central Register of Controlled Clinical Trials from inception to 7 March 2022 to identify all randomized controlled trials (RCTs) of nilotinib reporting outcomes of interest in patients with PD. Outcomes included tolerability, efficacy, safety, and CSF biomarker levels. Review manager 5.4 software was used to analyze all data.

Results: Three RCTs with a total of 163 patients were included. No significant difference was found between 150 mg nilotinib or 300 mg nilotinib and placebo in terms of tolerability, adverse events, or HVA levels. 300 mg nilotinib showed significantly higher Movement Disorder Society Unified Parkinson's Disease Rating Scale III (MDS-UPDRS III) scores [SMD = 0.52, 95%CI = (0.12, 0.92), P = 0.01] and 3,4-dihydroxyphenylacetic acid (DOPAC) levels [SMD = 0.52, 95%CI = (0.12, 0.92), P = 0.01], and lower α-synuclein levels [SMD = −2.16, 95%CI = (−3.38, −1.84), $P < 0.00001$] compared with placebo. And compared with 150 mg nilotinib, 300 mg nilotinib showed significantly lower α-synuclein levels [SMD = −1.16, 95%CI = (−1.70, −0.61), $P < 0.0001$].

Conclusions: Although our study demonstrated favorable tolerability and safety of different doses of nilotinib, and improvement in part of CSF biomarker levels of 300 mg nilotinib, the poor efficacy on motor outcomes indicated that nilotinib had no advantages in the clinic.

KEYWORDS

nilotinib, Parkinson's disease, tolerability, MDS-UPDRS, safety, CSF biomarker levels

Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease, of which the prevalence and disability have more than doubled over the past two decades, affecting more than 6 million individuals worldwide (Bloem et al., 2021; Tolosa et al., 2021). PD is an age-related progressive disorder, pathologically characterized by the loss of dopaminergic neurons in the pars compacta of the substantia nigra and by the accumulation of α-synuclein in Lewy bodies and Lewy neuritis (Bloem et al., 2021). Parkin also plays a pivotal role in PD pathogenesis, and its inactivation can aggravate the accumulation of α-synuclein and accelerate the progression of PD (Ganguly et al., 2020; Madsen et al., 2021). Primary motor symptoms of PD include tremor, rigidity, bradykinesia, gait and posture alterations. Current therapies are symptomatic and primarily focus on dopamine replacement strategies and effective relief of motor dysfunctions (Balestrino and Schapira, 2020). Although dopamine replacement strategies, including levodopa, dopamine agonists and monoamine oxidase type B (MAO-B) inhibitors, are beneficial in the early stages of disease, they can't slow or stop disease progression (Balestrino and Schapira, 2020; Bloem et al., 2021). Moreover, long-term levodopa treatment relates to the development of motor complications such as fluctuations, dyskinesia and freezing, as well as other non-motor side-effects due to decreased tolerance (Balestrino and Schapira, 2020; Werner and Olanow, 2022). Slowing and stopping PD progression pathologically and reducing relevant clinical manifestations remain a major unmet need in the treatment of PD.

Nilotinib, an oral cellular Abelson tyrosine kinase (c-Abl) inhibitor, was approved for the treatment of chronic myeloid leukemia (CML) at dosages of 300 mg twice daily by the U.S FDA in 2007 (Deremer et al., 2008; Sacha and Saglio, 2019). Some studies found increased activation of c-Abl in PD models and in brain tissues of PD patients (Imam et al., 2011; Brahmachari et al., 2016, 2019; Karim et al., 2020; Ghosh et al., 2021). suggesting that c-Abl might be associated with PD progression and that its inhibitor nillotinib might have a potential benefit in treating PD. Subsequent studies found that nilotinib (1–10 mg/kg) could protect animal models of PD from neurodegeneration in the brain via inhibiting c-Abl, degrading α-synuclein and blocking inactivation of parkin (Hebron et al., 2013a, 2014; Karuppagounder et al., 2014; Lonskaya et al., 2014; Wu et al., 2021; Werner and Olanow, 2022). Researchers also found that nilotinib could improve motor behavior in PD models (Hebron et al., 2013a). These findings sparked interest in whether nilotinib can slow PD progression clinically. In 2016, comparing 150 mg nilotinib with 300 mg nilotinib, a small clinical trial of 12 patients with advanced PD and dementia with Lewy bodies firstly demonstrated that nilotinib could effectively improve cerebrospinal fluid (CSF) biomarker levels and had a beneficial effect on motor and cognition outcomes (Pagan et al., 2016). Subsequently, two clinical trials on tolerance, efficacy, safety and biomarkers of multi-dose nilotinib were conducted (Pagan et al., 2020; Simuni et al., 2021). However, there is still a lack of a systematic review to synthesize existing evidence for a definitive conclusion about whether nilotinib is a clinically effective treatment and the appropriate dose of nilotinib for clinical use.

Therefore, based on the available clinical evidence, we aimed to evaluate differences in tolerability, efficacy, safety and CSF biomarker levels in different doses of nilotinib by this systematic review and meta-analysis, in order to guide the development of more multi-center, large-sample and high-quality clinical trials and broaden its indications.

Methods

This study followed the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines (Moher et al., 2009).

Search strategy

We searched PubMed, Embase, Web of science, and the Cochrane Central Register of Controlled Clinical Trials for studies published from database inception to March 7, 2022, using medical subject headings (MeSH) and free words combined with *nilotinib* and *Parkinson's disease*. The full search strategy for Pubmed is included in the Supplementary material.

Inclusion and exclusion criteria

Studies that fulfilled the following inclusion criteria were included: (1) only randomized controlled trials (RCTs); (2) patients were diagnosed with PD; (3) nilotinib was used in at least 1 treatment arm; (4) the studies reported at least 1 outcome of interest. Studies were excluded as follows: (1) duplicates from the same clinical trial; (2) full text unavailable; (3) unable to extract data.

Outcomes

There were four outcomes, which included tolerability, efficacy and safety of nilotinib, and CSF biomarker levels in PD patients. Tolerability was defined as the proportion of patients who had the ability to complete the study while receiving the assigned dose. Efficacy was defined as the improvement of motor behavior of patients who had lower Movement Disorder Society Unified Parkinson's Disease Rating Scale III (MDS-UPDRS III) scores and Unified Parkinson's Disease

Rating Scale III (UPDRS III) scores. Safety was represented by adverse events (AEs) reported in the included studies, including non-serious adverse events (non-SAEs) and serious adverse events (SAEs). Common non-SAEs included fall, musculoskeletal disorders, skin and subcutaneous disorders, and gastrointestinal disorders. Common SAEs included serious cardiac disorders and serious gastrointestinal disorders. CSF biomarker levels included the concentration of α-synuclein, the dopamine metabolites homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC) in the CSF. A decrease in α-synuclein concentration, and an increase in HVA and DOPAC concentrations indicate that nilotinib can improve PD-related pathological features (Hebron et al., 2013a; Pagan et al., 2020).

Study selection and data extraction

After removing duplications by Endnote X9, two reviewers (XX and KL) independently screened the title and abstract according to the inclusion and exclusion criteria, and then read the full text to determine the final inclusions. For articles from the same clinical trial, we included only the most comprehensive data. We extracted the following data: study characteristics (first author, publication year, study design, intervention and control, and follow-up), baseline demographics of participants (age, sex, diagnostic criteria, Hoehn-Yahr [H/Y] stage, and disease duration), and outcomes of interest. Discrepancies were resolved by a third reviewer (CX).

Quality assessment

Two reviewers (XX and KL) independently assessed the risk of bias of RCTs with the Cochrane Collaboration's tool, (Higgins et al., 2011) and discrepancies were resolved by a third reviewer (CX).

Statistical analysis

Review manager software (version 5.4; the Cochrane Collaboration) was used to analysis all data. For dichotomous data (eg, tolerability, AEs), odds ratio (OR) and 95% Confidence interval (CI) were estimated for each study. For continuous data (eg, MDS-UPDRS III, UPDRS III, CSF biomarker levels), standard mean difference (SMD) and 95% CI were calculated as effect indexes. Heterogeneity among individual studies was judged by I^2 values. We used a fixed effects model when I^2 <50%; otherwise, we used a random effects model. Publication bias was assessed by an Egger test and a Begg rank correlation (Egger et al., 1997). $P < 0.05$ was considered statistically significant.

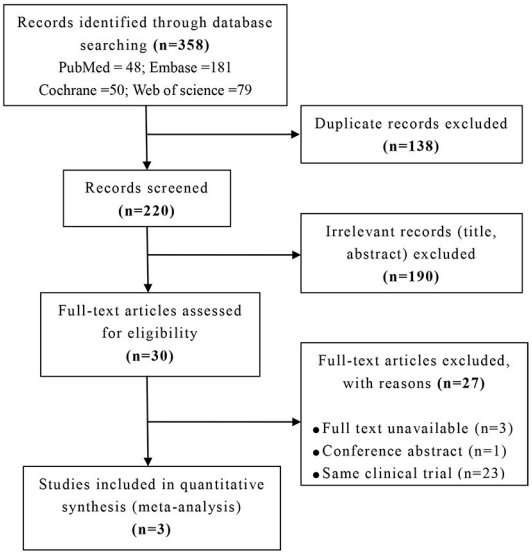

FIGURE 1
Flow chart of literature searching.

Results

Study selection

Overall, the retrieval identified 358 studies, 138 duplicates removed, and 220 studies were screened title and abstract. After excluding 190 irrelevant studies, 30 studies were screened full text. Of those, 27 were excluded. Of the excluded studies, 3 were not available, 1 was conference abstract, and 23 were same clinical trials. Ultimately, 3 studies were included for analysis with 163 patients (Pagan et al., 2016, 2020; Simuni et al., 2021). The full selection strategy was presented in Figure 1.

Study characteristics

All three studies were conducted in the United States. Two studies were single center studies, (Pagan et al., 2016, 2020) and 1 study was multicenter study (Simuni et al., 2021). Two studies were phase 2, double blind, placebo controlled trials, (Pagan et al., 2020; Simuni et al., 2021) and 1 study was phase 1, open label trial without placebo (Pagan et al., 2016). All three studies adopted the UK Brain Bank diagnostic criteria for PD. One study contained patients diagnosed with dementia with lewy bodies (Pagan et al., 2016). All included studies compared 150 mg nilotinib with 300 mg nilotinib. The general characteristics of the included studies were in Table 1.

TABLE 1 General characteristics of the included studies.

Reference	Study design	No. of patients	Characteristics of study participants	Intervention	Follow up	Outcomes
Pagan et al. (2016)	Single center, RCT, open label	12	Mean age, y: 150 mg nilotinib (72.4); 300 mg nilotinib (71.8) Male (%): 150 mg nilotinib (80); 300 mg nilotinib (71) H/Y stage: 3–5 Mean disease duration, y: 150 mg nilotinib (10.6); 300 mg nilotinib (12.4)	150 mg or 300 mg nilotinib once daily for 6 months	3 months	Tolerability, UPDRS III, α-synuclein, HVA, AEs
Pagan et al. (2020)	Single center, RCT, double blind, placebo controlled	75	Mean age, y: palcebo (68.6); 150 mg nilotinib (66.6); 300 mg nilotinib (70.0) Male (%): palcebo (84); 150 mg nilotinib (56); 300 mg nilotinib (80) H/Y stage: 2.5–3 Mean disease duration, y: palcebo (10.0); 150 mg nilotinib (12.3); 300 mg nilotinib (10.0)	150 mg or 300 mg nilotinib once daily for 12 months	3 months	Tolerability, MDS-UPDRS III, α-synuclein, HVA, DOPAC, AEs
Simuni et al. (2021)	Multicenter, RCT, double blind, placebo controlled	76	Mean age, y: palcebo (65.5); 150 mg nilotinib (61.2); 300 mg nilotinib (66.9) Male (%): palcebo (64); 150 mg nilotinib (60); 300 mg nilotinib (81) H/Y stage: 2–3 Mean disease duration, y: palcebo (9.4); 150 mg nilotinib (8.5); 300 mg nilotinib (11.7)	150 mg or 300 mg nilotinib once daily for 6 months	2 months	Tolerability, MDS-UPDRS III, HVA, DOPAC, AEs

Risk of bias assessment

Of the 3 included studies assessed for risk of bias, 2 were assessed at low risk on all assessed items. One study was assessed at high risk on performance and detection bias due to open label without blinding, and at unclear risk on selection bias without reporting the method of allocation concealment. The risk of bias was summarized in Figure 2.

Outcomes

Tolerability

Tolerability was reported in three studies (Pagan et al., 2016, 2020; Simuni et al., 2021). Compared with placebo, there were no significant differences in the 150 mg nilotinib group [OR = 0.62, 95%CI = (0.20, 1.90), $P > 0.05$] and in the 300 mg nilotinib group [OR = 0.56, 95%CI = (0.19, 1.69), $P > 0.05$] and all low heterogeneity ($I^2 = 0\%$). There were also no significant differences between the 300 mg nilotinib group and 150 mg nilotinib group [OR = 0.84, 95%CI = (0.32, 2.19), $P > 0.05$] and low heterogeneity ($I^2 = 0\%$) (Figure 3).

Efficacy

MDS-UPDRS III

Two studies reported MDS-UPDRS III scores (Pagan et al., 2020; Simuni et al., 2021). Compared with placebo, there were significantly higher MDS-UPDRS III scores in the 300 mg nilotinib group [SMD = 0.52, 95%CI = (0.12, 0.92), $P = 0.01$] with low heterogeneity ($I^2 = 0\%$). There were no significant differences between the 150 mg nilotinib group and placebo [SMD = 0.19, 95%CI = (−0.20, 0.58), $P > 0.05$] with low heterogeneity ($I^2 = 0\%$); between the 300 mg nilotinib group and 150 mg nilotinib group [SMD = 0.26, 95%CI = (−0.13, 0.65), $P > 0.05$] with low heterogeneity ($I^2 = 0\%$) (Figure 4).

UPDRS III

Only 1 study reported UPDRS III scores in the 300 mg nilotinib group and 150 mg nilotinib group (Pagan et al., 2016). And no significant differences were found between the two groups [SMD = −0.45, 95%CI = (−1.62, 0.72), $P > 0.05$] (Supplementary Figure 1).

CSF biomarker levels

α-synuclein

Two studies reported α-synuclein levels (Pagan et al., 2016, 2020). We found lower α-synuclein levels in the 300 mg nilotinib group [SMD = −1.16, 95%CI = (−1.70, −0.61), $P < 0.0001$] when compared with the 150 mg nilotinib group, and low heterogeneity ($I^2 = 37\%$). And one study showed that the 300 mg nilotinib group had significantly lower α-synuclein levels

FIGURE 2
Risk of bias assessment.

than placebo [SMD = −2.16, 95%CI = (−3.38, −1.84), $P <$ 0.00001] and that there were no significant differences between the 150 mg nilotinib group and placebo [SMD = 0.14, 95%CI = (−0.42, 0.69), $P > 0.05$] (Supplementary Figure 2).

HVA

All studies reported HVA levels. A pooled analysis showed no significant differences in the 150 mg nilotinib group [SMD = −0.08, 95%CI = (−1.26, 1.11), $P > 0.05$] with high heterogeneity (I^2 = 88%) and in the 300 mg nilotinib group [SMD = −0.10, 95%CI = (−0.77, 0.57), $P > 0.05$] with high heterogeneity (I^2 = 66%) when compared with placebo. There were also no significant differences between two nilotinib groups [SMD = −0.06, 95%CI = (−0.64, 0.51), $P > 0.05$] with high heterogeneity (I^2 =51%) (Supplementary Figure 3).

DOPAC

Two studies reported DOPAC levels (Pagan et al., 2020; Simuni et al., 2021). Compared with placebo, there were significantly higher DOPAC levels in the 300 mg nilotinib group [SMD = 0.52, 95%CI = (0.12, 0.92), P = 0.01] with low heterogeneity (I^2 = 32%). There were no significant differences between the 150 mg nilotinib group and placebo [SMD = 0.18, 95%CI = (−0.92, 1.27), $P > 0.05$] with high heterogeneity (I^2 = 87%) and between two doses of nilotinib [SMD = 0.39, 95%CI = (0, 0.78), P = 0.05] with low heterogeneity (I^2 = 0%) (Supplementary Figure 4).

Safety

Our pooled results showed no significant increase or decrease in the incidence of non-SAEs or SAEs of interest when comparing 150 mg nilotinib with 300 mg nilotinib and when comparing different doses of nilotinib with placebo (Supplementary Figures 5–10). Among non-SAEs, the most common were fall [17 of 50 (34%) in the 150 mg nilotinib group, 13 of 51 (25.5%) in the 300 mg nilotinib group], followed by musculoskeletal disorders [17 of 55 (30.9%) in the 150 mg nilotinib group, 10 of 58 (17.2%) in the 300 mg nilotinib group]. The incidences of skin and subcutaneous disorders, and gastrointestinal disorders were summarized in Supplementary Table 1. Among SAEs, the incidences of

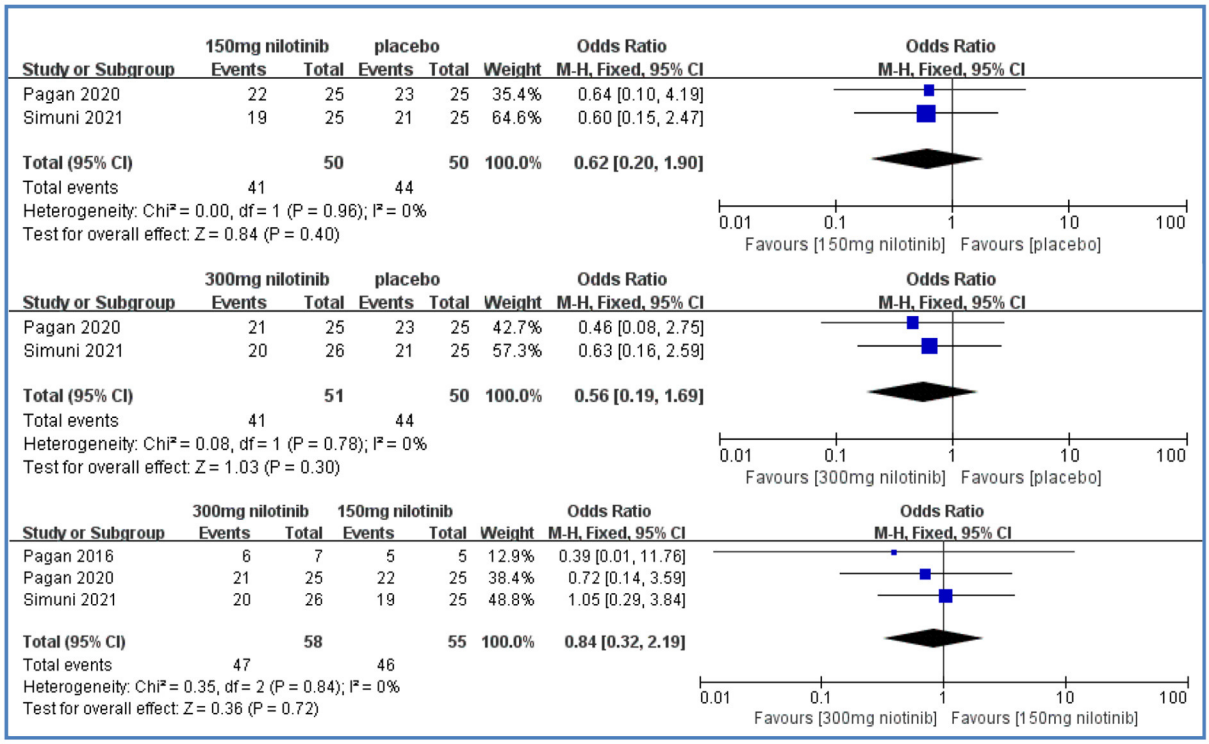

FIGURE 3
Forest plot of tolerability.

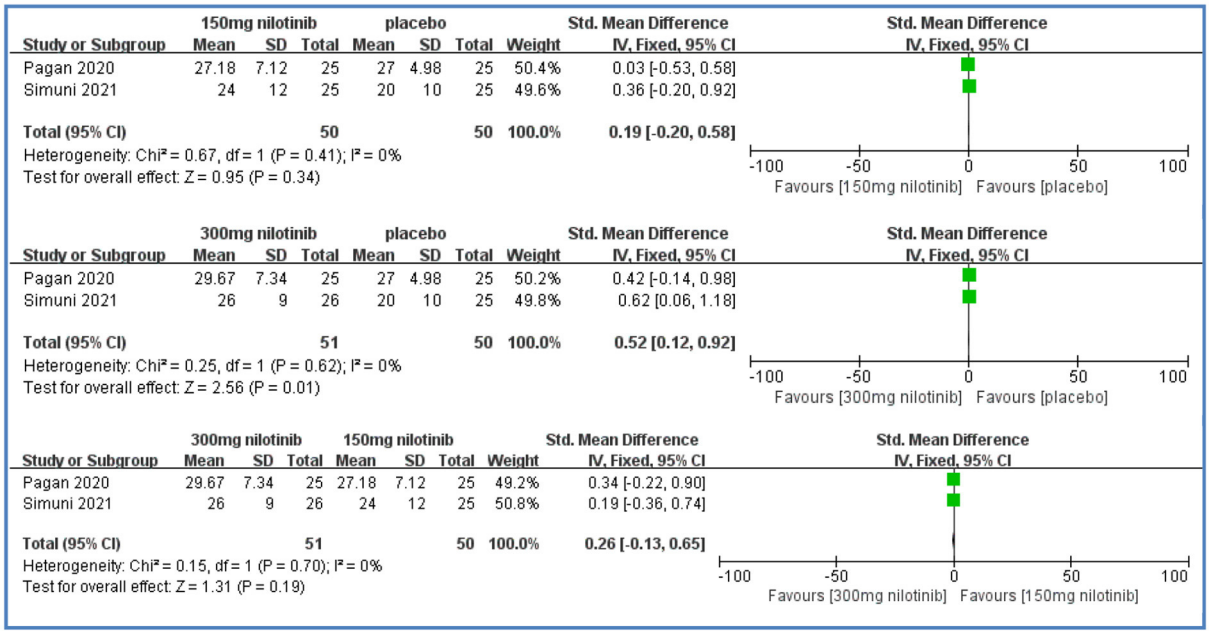

FIGURE 4
Forest plot of MDS-UPDRS III scores.

serious cardiac disorders and serious gastrointestinal disorders increased with higher dose of nilotinib, and were summarized in Supplementary Table 1.

Discussion

This is the first meta-analysis comparing tolerability, efficacy, safety, and CSF biomarker levels at different doses of nilotinib in PD patients. Our study showed that neither 150 mg nor 300 mg nilotinib had beneficial clinical effects in the treatment of PD patients, except that 300 mg nilotinib could improve α-synuclein and DOPAC levels. Moreover, nilotinib had acceptable safety and tolerability with no significant differences in any comparison, which was consistent with the original studies. Our study showed that the incidence of serious cardiac disorders was correspondingly doubled when treated with double doses of nilotinib. Nilotinib has been warned with an increased risk of dose-related cardiac disorders, and the incidences are 9.9 and 15.9% among patients treated with nilotinib 300 mg twice daily, nilotinib 400 mg twice daily in the treatment of CML patients (Jabbour and Kantarjian, 2018). Therefore, this dose-related toxicity should be considered when conducting clinical trials with enlarged doses.

When treated with 150 mg nilotinib, it failed to provide an improvement in motor ability and CSF biomarker levels, possibly because the low concentration of nilotinib accumulated in the brain is not sufficient to inhibit c-Abl. Previous preclinical studies found that low doses of nilotinib had the ability to inhibit c-Abl, improve motor outcomes and CSF biomarker levels (Hebron et al., 2013a,b, 2014; Pagan et al., 2016). However, nilotinib does not appear these effects clinically because only a maximum of 10% of the concentration thought to be adequate to inhibit c-Abl was detected in the brain of PD patients (Pagan et al., 2019; Werner and Olanow, 2022). This may be interpreted by ATP-binding cassette (ABC) transporters which facilitate nilotinib removal from brain, therefore, nilotinib hardly achieves effective concentration to inhibit c-Abl. In addition, the duration of nilotinib to inhibit c-Abl in the brain is 6 hours, (Pagan et al., 2016) once-daily administration can not sustain the effect throughout the day.

When treated with 300 mg nilotinib, we found a conflict result that nilotinib could significantly worsen motor ability but significantly decrease α-synuclein levels and increase DOPAC levels, which was inconsistent with previous studies. These studies had demonstrated that nilotinib could accelerate autophagic clearance to degrade α-synuclein accumulated in the cells, protect dopaminergic neurons, increase dopamine and its metability DOPAC levels, and result in a motor improvement (Hebron et al., 2013a, 2014; Karuppagounder et al., 2014; Lonskaya et al., 2014; Wu et al., 2021). This confounding result between dose-dependent motor disability and improvement in CSF biomarker levels in our study may be due to the fact that all patients in three included studies were diagnosed with PD over 10 years with least H/Y stage 2 and all of them were treated with the concurrent chronic levodopa therapy. The ELLDOPA study, which aimed to assess the effect of levodopa on the progression of PD for a period of 42 weeks, found that levodopa significantly improved UPDRS scores in a dose-dependent manner, but this effect gradually diminished, eventually, UPDRS III worsened compared with baseline (Fahn et al., 2004). One of the potential mechanisms related to this variable effect may be the dopamine neurotoxicity caused by dopamine metabolites 2,4,5trihydroxyphenylalanine (TOPA) and TOPA-quinone, (LeWitt, 2015) which may counterbalance the neuroprotective effect of nilotinib. Nilotinib enters the brain through the blood-brain barrier in a dose-independent manner, and its inhibition of c-Abl is equivalent to that of 150 mg (Pagan et al., 2019). It is possible that the detrimental effects of chronic levodopa therapy on motor outcomes may conceal the minor clinical benefits of nlotinib on the inhibition of c-Abl, which may be an interpretation for this conflict.

In terms of HVA levels, we found nilotinib could nonsignificantly decrease HVA levels with high heterogeneity in any comparison. This result is consistent with the original study conducted by Simuni et al. (2021). However, in the open label study conducted by Pagan et al. (2016) CSF HVA levels significantly increased in the 150 mg nilotinib group at 2 months but not at 6 months, and it only significantly increased in the 300 mg nilotinib group at 6 months. In the double blind, placebo controlled study conducted by Pagan et al. (2020) CSF HVA levels significantly increased in the 150 mg nilotinib group at 12 months but not in the 300 mg nilotinib group. The variable HVA levels and differences between three original studies may be because of concurrent treatment with PD dopaminergic therapies, especially MAO-B inhibitors in the study conducted by Simuni et al., affecting dopamine metabolites and confounding results. High heterogeneity may result in different analytical methods and course of treatment. Study published by Pagan et al. (2016) used ELISA analysis. Pagan et al. (2020) and by Simuni et al. (2021) used LC-MS/MS analysis. Therefore, the results should be viewed with caution given the high heterogeneity.

Strengths of this systematic review and meta-analysis include that we performed a comprehensive literature search about this topic and this is the first meta-analysis based on all published RCTs. Apart from one study lack of blinding, other studies were of high quality. All outcomes had low heterogeneity except for HVA levels. And no publication bias was detected in our study. There are also several limitations in this meta-analysis. Firstly, only 3 relatively small RCTs with 163 patients were included. Secondly, one study included the patients with dementia with lewy bodies, which could impact the accuracy of results comparing different doses of nilotinib. Thirdly, some outcomes were reported

in only 1 study, the pooled results should be carefully considered. Finally, the pooled results may be affected by data selected in different stages of treatment due to variable courses of treatment and length of follow-up. In our study, except for MDS-UPDRS scores selected in June, data of the last time node reported in the included studies were used for pooling.

Given the potential effects of chronic levodopa treatment on motor function and CSF biomarkers, further clinical trials should be conducted in patients with early PD who are not treated with levodopa to determine whether nilotinib stabilizes PD symptoms and/or its association with levodopa. Although nilotinib has shown well tolerability and safety, we still recommend low doses of nilotinib in further trials because of dose-related cardiac disorders. Pagan et al. (2020) found that 150 mg nilotinib significantly improved UPDRS III motor score at 15 months compared with baseline (−2.82 points), which was greater than that of the ELLDOPA study (1.4, 1.4, and −1.4 points for 150 mg, 300 mg, and 600 mg/ day, respectively) (Fahn et al., 2004). Due to the variable symptoms of PD, different PD management and care in multi-center studies can minimize its impact on results compared with single center studies. Pagan et al. (2020) and Simuni et al. (2021) both identified limitations of open-label studies on symptomatic results in PD. Taken together, further clinical trials should be conducted in strict accordance with protocols and criteria of randomized, double-blind, placebo-controlled, multicenter, large-sample trials over 15 months to investigate the effects of 150 mg or 300 mg nilotinib in early PD patients without levodopa use.

Conclusion

Although our study demonstrated favorable tolerability and safety of different doses of nilotinib, and improvement in part of CSF biomarker levels of 300 mg nilotinib, the bad efficacy on motor outcomes indicated that nilotinib had no advantages in the clinic. These findings from three small sample-size trials should not be applied to a larger population. And stronger evidence from large-sample, well-designed trials in patients without chronic levodopa treatment is needed in the future.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding authors.

Author contributions

XX, PY, LK, XC, JL, and YL designed the study. XX, LK, and XC identified included studies, assessed risk of bias, extracted data, and performed data analysis. XX, PY, JL, and YL wrote and revised the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnagi.2022.996217/full#supplementary-material

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Interactions between gut microbiota and Parkinson's disease: The role of microbiota-derived amino acid metabolism

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Non-motor symptoms (NMS) of Parkinson's disease (PD), such as constipation, sleep disorders, and olfactory deficits, may emerge up to 20 years earlier than motor symptoms. A series of evidence indicates that the pathology of PD may occur from the gastrointestinal tract to the brain. Numerous studies support that the gut microbiota communicates with the brain through the immune system, special amino acid metabolism, and the nervous system in PD. Recently, there is growing recognition that the gut microbiota plays a vital role in the modulation of multiple neurochemical pathways *via* the "gut microbiota-brain axis" (GMBA). Many gut microbiota metabolites, such as fatty acids, amino acids, and bile acids, convey signaling functions as they mediate the crosstalk between gut microbiota and host physiology. Amino acids' abundance and species alteration, including glutamate and tryptophan, may disturb the signaling transmission between nerve cells and disrupt the normal basal ganglia function in PD. Specific amino acids and their receptors are considered new potential targets for ameliorating PD. The present study aimed to systematically summarize all available evidence on the gut microbiota-derived amino acid metabolism alterations associated with PD.

KEYWORDS

Parkinson's disease, gut microbiota, amino acid metabolism, microbiota-host interaction, gut microbiota-brain axis

Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease and affects an estimated 3 million people worldwide. The typical feature of PD is the accumulation of intracellular aggregates called Lewy bodies, composed largely of the presynaptic protein alpha-synuclein (Collier et al., 2017). In clinical practice, individuals with PD benefit from drugs developed to act on a single molecular target (Figure 1; Sarkar et al., 2016; Tambasco et al., 2018). Levodopa (L-DOPA) is still the gold standard therapy for patients with PD. However, long-term L-DOPA treatment is associated with motor fluctuations and dyskinesias. As the disease progresses, amino acid decarboxylase inhibitors (AADC inhibitors), catechol-O-methyl transferase inhibitors (COMT inhibitors), monoamine oxidase B inhibitors (MAO-B inhibitors), and dopamine receptor agonists are also used. The surgical treatment of deep brain stimulation can be used for patients with PD who have severe limitations in functioning despite optimal pharmacological treatment (Weiner, 2002; Malek, 2019). However, there is not currently a medication available to cure or halt the progression of the disease.

The intestine is a complex and dynamic ecosystem caused by various microbial communities known as the gut microbiota (Zhao and Liu, 2020). The human intestinal tract contains more than 10-100 trillion microorganisms (Duttaroy, 2021; Zhang J. et al., 2021), which contribute to food digestion, nutrient provision, and combat pathogen invasion. Numerous studies have demonstrated that the gut microbiota plays a vital role in maintaining human health and adjusting numerous physiological functions, such as the nervous system, cardiovascular system, and immune system (Izco et al., 2021). Considering the importance of host-microbiota interactions for human health, access to yet unexplored resources will lead to the discovery of new actors regulating this crosstalk and therefore new potential therapeutic targets (Alam and Neish, 2018; Chang and Kao, 2019). Although genetic, societal, environmental, and other influencing factors are closely involved in disease etiology and progression, the host-microbiota interaction is increasingly recognized for its influence in a variety of diseases (Duttaroy, 2021), such as obesity (Arnoriaga-Rodríguez et al., 2020), inflammatory bowel disease (Sankarasubramanian et al., 2020), non-alcoholic fatty liver disease (Rom et al., 2020), and Parkinson's disease (Vascellari et al., 2020). More importantly, a better understanding of the host-microbiota interaction will improve an understanding of the relationship between gut microbiota and health. Interestingly, the change in microbial composition, intestinal dysfunction, bacterial metabolites, and endocrine function in the intestine is associated with PD (Sun et al., 2017). In addition, the abundance of *Prevotellaceae* presented a declining trend in PD patients compared with healthy individuals. Simultaneously, the abundance of *Enterobacteriaceae* has been found to be associated with the severity of postural instability and gait difficulty of PD patients (Scheperjans et al., 2015). Hence, the interaction between the gut microbiota and the occurrence of PD will provide a bright future for intervention, especially for the diagnosis and therapy of PD. Growing evidence suggests that the modulation of gut microbiota could be a novel therapeutic target in PD patients.

Amino acids are fundamental elements for protein and peptide synthesis. More importantly, a series of evidence indicates that amino acids are also important bioactive molecules that play key roles in signaling pathways and metabolic regulation (Sankarasubramanian et al., 2020). The gut microbiota composition related to protein metabolism in the intestinal tract is similar to that in feces and important in amino acid homeostasis and health (Bishu, 2016; Lin et al., 2017; Zhao et al., 2019). The result showed that the primary bacteria, such as *Klebsiella* spp., *Escherichia coli,* and *Anaerovibrio lipolytica,* are associated with protein metabolism. In addition, amino acids could be directly metabolized by some of these bacteria which own the ability to excrete numerous proteases and peptidases (Fan et al., 2017). Evidence shows that amino acids are exchanged between gut microbiota and the host (Metges, 2000). Thus, there is no doubt that a profound grasp of the significance of gut microbiota-derived amino acid metabolism has become imperative. Notwithstanding, the effects of amino acid metabolism on PD pathogenesis have not been comprehensively reviewed at present. In this review, detailed descriptions of the relationship between gut microbiota-derived amino acid metabolism and PD are summarized, and dysbiosis of microbial metabolite and microbiota-targeted interventions in PD disease are also discussed (Figure 2).

Gut microbiota and Parkinson's disease

The hypothesis of PD in the gut

Few researchers investigated the pathogenesis of PD prior to 1980. The mechanism remained elusive until the description of Lewy bodies first appeared. The main hypotheses involved in the pathogenesis of PD were as follows: oxidative stress (Trist et al., 2019), mitochondrial dysfunction (Navarro and Boveris, 2009), excitotoxicity (Ambrosi et al., 2014), inflammation, neurotrophic factor-deficiency (Lindahl et al., 2020), and the theory of gut origin (Postuma, 2015). Among them, oxidative stress and PD originating from the gut have attracted more attention. Accumulating evidence illustrated that oxidative stress may be the cause of many neurodegenerative diseases, including PD (Trist et al., 2019). Low levels of oxidative

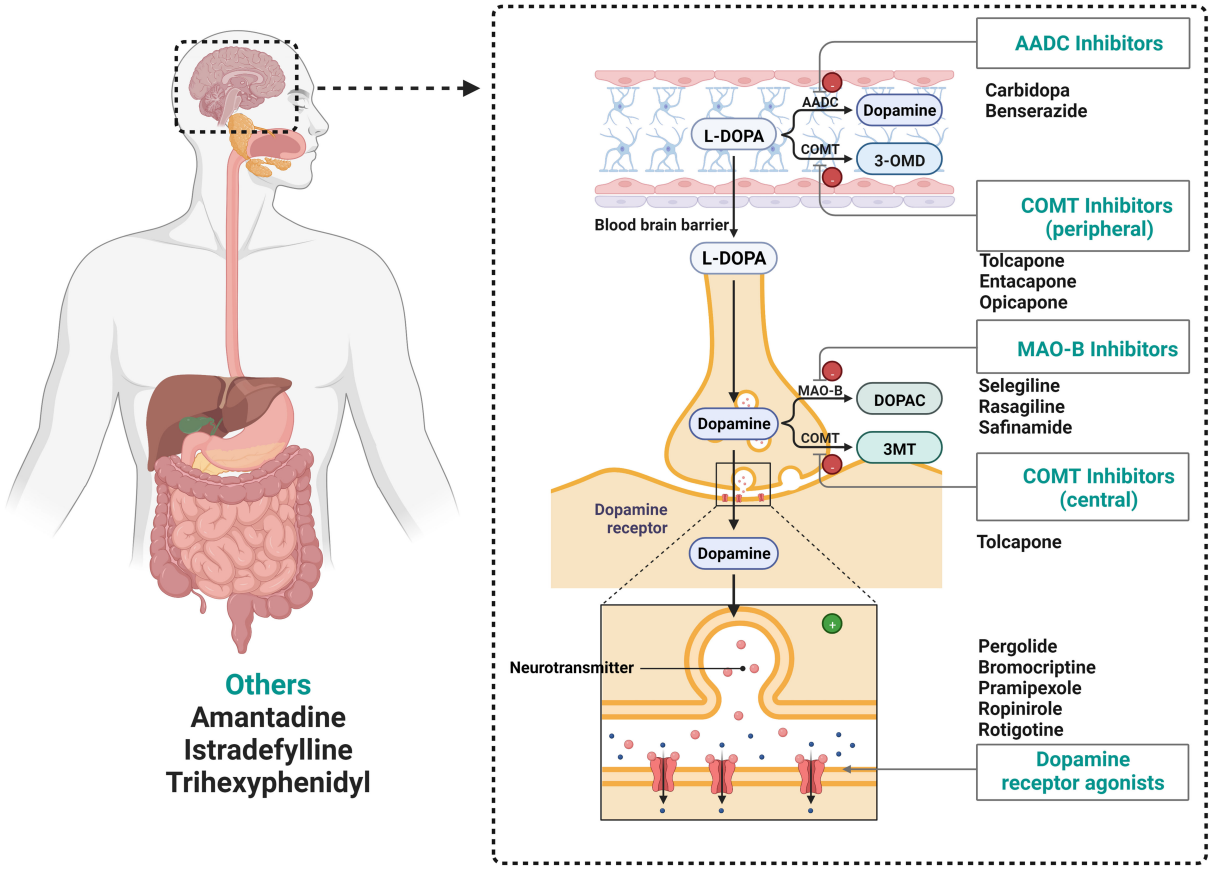

FIGURE 1
Drugs utilized in the treatment of Parkinson's disease. MAO-B, Monoamine oxidase-B inhibitors; COMT, catechol-O-methyl transferase inhibitors; L-DOPA, L-3,4-dihydroxyphenylalanine; AADC, amino acid decarboxylase (AADC, Dopa decarboxylase); DOPAC, 3,4-dihydroxyphenylacetic acid; 3-OMD, 3-O-methyl-dopa; 3-MT, 3-methoxytyramine.

stress could promote mitochondria elimination and protect their biological function, while opposite effects appear in high-level oxidative stress beyond the cellular capacity by affecting the mitochondrial membrane's potential and protein synthesis in cytoplasm (Lee and Wei, 2005; Barodia et al., 2017). Previous studies mainly focused on the abnormal deposition of α-syn in the central nervous system in PD pathogenesis. Recently, multitudes of studies have elucidated that gut microbiota homeostasis and metabolites are correlated with the pathogenesis of PD. Simultaneously, Yang et al. (2019) revealed the existence of a bidirectional network called GMBA. The GMBA could affect human behavior and brain neurochemistry by regulating neurotransmitters and their receptors in PD patients. Taken altogether, the gut origin hypothesis implicates the gut as a potential origin of PD pathogenesis, offering fresh insights into the mechanisms underlying PD.

A bidirectional link between intestinal disorders and neurodegeneration in the pathogenesis of Parkinson's disease

Relevant preclinical and epidemiological studies illustrated that the occurrence of common intestinal disorders, such as colorectal cancer (CRC), constipation, irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), and small intestinal bacterial overgrowth syndrome (SIBO), were associated with PD by affecting the central nervous system (Barboza et al., 2015). Interestingly, a bidirectional link between the brain and gut plays an essential role in the association between intestinal dysfunction and PD. The association between PD and intestinal disorders is described in Table 1.

PD and CRC are different diseases with diverse pathogenic mechanisms. Gut microbiota diversity (Xie et al., 2017),

TABLE 1 The association between Parkinson's disease and intestinal disorders.

Intestinal disorders	Connection with Parkinson's disease (PD)	Publication trends on web of science (accessed on 7 May 2022)	References
Colorectal cancer (CRC)	1. PD patients had a reduced risk of CRC 2. CRC occurrence was significantly lower in patients with PD	"Parkinson's disease" and "Colorectal cancer" (176)	Xie et al., 2017; Fang et al., 2021
Constipation	1. Constipation patients are at a 2.27-fold increased risk of developing PD compared to the control group, and this phenomenon emerges up to 20 years before diagnosis 2. Compared with people without constipation, regional neural activity and functional connectivity in the brain show much difference in PD patients with constipation 3. Constipation is associated with a sustained increased risk of a PD diagnosis and progression of neurodegenerative pathology, and there was a higher incidence for men than women 4. Constipation is associated with cognitive decline in PD patients 5. Constipation is associated with the increased severity of motor symptoms and decreased dopamine levels in PD patients in a dose-dependent manner. Simultaneously, the different constipation-loading times could lead to different clinical characteristics, especially in motor symptoms	Parkinson's disease and "Constipation" (1,016)	Kaye et al., 2006; Adams-Carr et al., 2016; Svensson et al., 2016; Gan et al., 2018; Zhou et al., 2019; Camacho et al., 2021; Kang et al., 2022; Santos García et al., 2022; Zheng et al., 2022
Irritable bowel syndrome (IBS)	1. Patients with IBS are at an increased risk of developing PD in Taiwan in both genders in an age-dependent manner 2. IBS increased PD risk only in individuals $\geq$ 65 years 3. PD patients with IBS-like symptoms had more non-motor symptoms	"Parkinson's disease" and "Irritable bowel syndrome" (134)	Lai et al., 2014; Mertsalmi et al., 2017; Liu B. et al., 2021; Zhang J. et al., 2021; Lu et al., 2022; Yoon et al., 2022; Zhang X. et al., 2021

(Continued)

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TABLE 1 (Continued)

Intestinal disorders	Connection with Parkinson's disease (PD)	Publication trends on web of science (accessed on 7 May 2022)	References
Inflammatory bowel disease (IBD)	1. The overall risk of PD in IBD, both Crohn's disease and ulcerative colitis is significantly higher than in controls 2. IBD is associated with increased PD risk regardless of sex, especially in patients over 65 years of age. Furthermore, the therapies for IBD using corticosteroids, anti-TNF and early anti-inflammatory methods may decrease the risk of PD 3. The risk of neurodegenerative diseases is higher in IBD patients than in the non-IBD population 4. Abnormal changes in the intestinal environment trigger the onset of PD *via* the brain-gut axis 5. Gut inflammation and higher LRRK2 levels in Crohn's disease (IBD) may be a biomarker of increased risk for sporadic PD	Parkinson's disease and "Inflammatory bowel disease" (302)	Lin et al., 2016; Killinger et al., 2018; Peter et al., 2018; Park et al., 2019; Villumsen et al., 2019; Zhu et al., 2019, 2022; Fu et al., 2020; Noh et al., 2020; Herrick and Tansey, 2021; Kim et al., 2022
Small intestinal bacterial overgrowth syndrome (SIBO)	1. The risk of SIBO is higher in PD patients than in non-PD patients, and SIBO could influence the progression of PD using negative and positive manners 2. SIBO may lead to fluctuation in the absorption of medications utilized to therapy PD, which could further influence the treatment of PD 3. SIBO is associated with increased motor fluctuations present in PD patients compared with individuals without SIBO	"Parkinson's disease" and "Small intestinal bacterial overgrowth syndrome" (128)	Marrinan et al., 2014; Tan et al., 2014; Niu et al., 2016; Dănău et al., 2021

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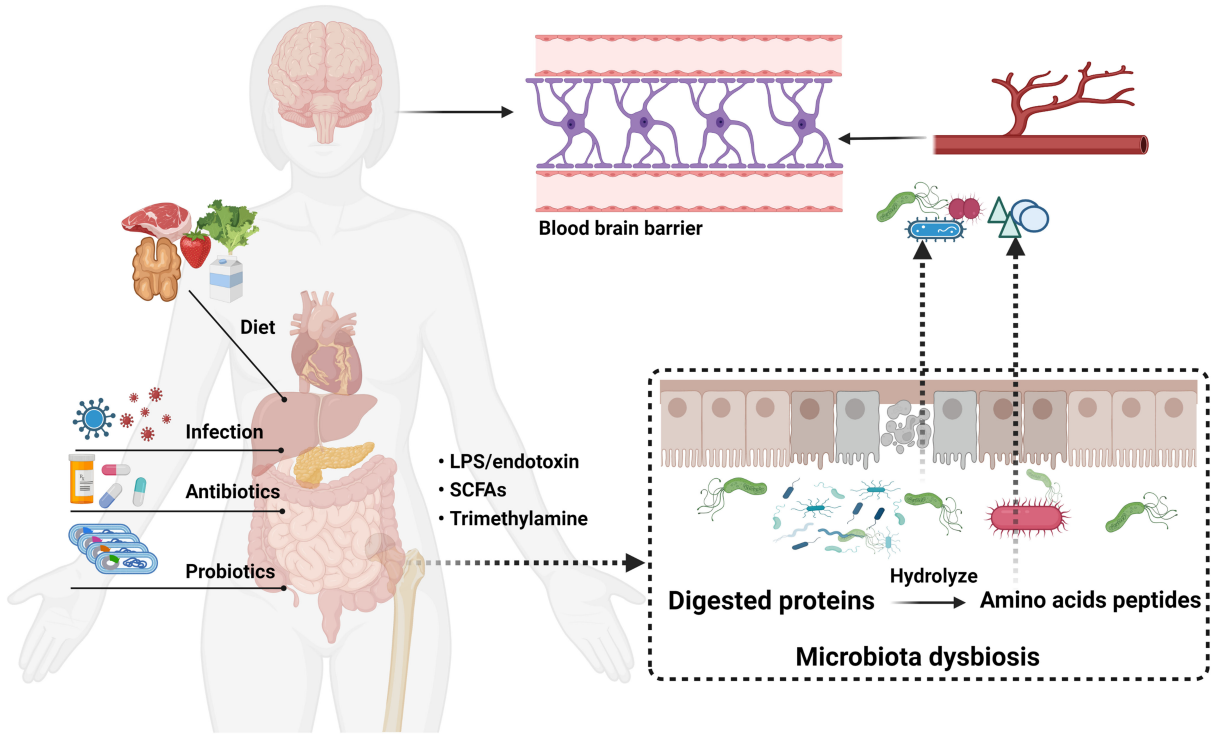

FIGURE 2
Microbiota-gut-brain axis communication pathway linking gut microbial dysbiosis with brain function in PD.

melatonin (Schernhammer et al., 2006), dopamine (Sarkar et al., 2015), and smoking (Hernán et al., 2002) may account for the bidirectional link between PD and CRC. The disorders of the ubiquitin-proteasome system may lead to the formation and accumulation of Lewy bodies including α-syn (Sherman and Goldberg, 2001). More importantly, the level of ubiquitin proteasome system is usually up-regulated in CRC patients (Manasanch and Orlowski, 2017). Notwithstanding, there is a clear need for more prospective studies to validate these hypotheses. Interestingly, phosphatidylinositol-3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/Akt/mTOR) are over-expressed in CRCs (Bahrami et al., 2018), while the PI3K/Akt/mTOR activation could prevent PD *via* reducing the dopaminergic neuron apoptosis (Leikas et al., 2017).

Constipation, the second most common non-motor symptom of PD after anosmia, is characterized by infrequent stools, difficult stool passage, or both. Previous studies indicated that the main cause of constipation was slowed colonic transit. Furthermore, constipation is one of the indicators and occurs more than 20 years earlier than the diagnosis of PD (Frazzitta et al., 2019). Simultaneously, gastrointestinal dysfunction is a common feature of PD and could lead to the impaired absorption of L-DOPA, which also causes the motor fluctuations of PD (Svensson et al., 2016). Despite copious research, the exact mechanism of IBS remains unclear. Genetic variation, altered gut microbiota, increased gut permeability, and low-grade inflammation are risk factors for IBS (Ohman and Simrén, 2010). Compared with those without IBS, an individual with IBS is at a 48% increased risk of PD based on the meta-analysis and systematic review methods (Zhang J. et al., 2021). The risk factors of IBS can make the gastrointestinal tract more vulnerable to bacterial endotoxin and pathogens which may cause an increase in α-syn expression and aggregation (Mertsalmi et al., 2021). Furthermore, the previous research about the correlation between the gut microbiota and the occurrence and development of IBS has been observed and the gut-brain axis plays a vital role, which was consistent with the hypothesis of "PD originates in the gut" (Braak et al., 2006; Raskov et al., 2016; Canakis et al., 2020). The altered gut microbiota and increased gut permeability can activate systemic inflammation and enteric neuroglial cells, thereby initiating the development of α-syn pathology (Klingelhoefer and Reichmann, 2015). An increasing number of clinical studies revealed that IBD could cause neuroinflammation through the gut-brain axis which was consistent with the elevated stool calprotectin levels in PD patients (Schwiertz et al., 2018). Intestinal inflammation could accelerate the aggregation of α-syn in the gut and then spread to the brain, finally leading to PD (Stokholm et al., 2016).

SIBO is characterized by the excessive levels of bacteria colonized in the small intestine causing inflammation and malabsorption. The pathogenesis, clinical manifestation, and progression of sporadic PD might be affected by SIBO (Dobbs et al., 2012; DiBaise et al., 2018; Manole et al., 2021). Several studies found that H_2-predominant vs. methane-predominant SIBO shows various effects on PD progression. In addition, because of different drug bioavailability and absorption, SIBO has conflicting effects on intestinal symptoms of PD with different pharmacological interventions (Gibson and Barrett, 2010; Maini Rekdal et al., 2019; van Kessel et al., 2019). Among the pathogenesis of PD, SIBO may cause local and systemic reactions that would further destroy the integrity of the intestinal barrier by influencing tight junctions and intestinal permeability. Many studies illustrated that the intestinal disorder may serve as a warning sign for PD.

The association pathogenesis of PD and gastrointestinal disorders was mainly focused on genetic factors, diet, environmental toxins, and gut microbiota, etc. Interestingly, PD is characterized by severe motor and NMS that can result in debilitating gastrointestinal (GI) symptoms. Specifically, the association between intestinal disorders and PD pathogenesis attracting many researchers' attention may be due to the existence of the gut-brain axis. Overall, the PD risk was higher in IBS patients than others, indicating that the intestinal disorder may serve as a warning sign for PD. Further research is warranted to explore the underlying mechanisms of this correlation.

Roles of gut microbial dysbiosis and microbial products in PD

The key pathological characteristics of PD are the accumulation of α-syn and cell death in the brain's basal ganglia, affecting an estimated three million people (more than 60 years of age) (Wolters and Braak, 2006; Sulzer, 2007). This damage to dopaminergic neurons is responsible for the distinctive movement disorder and vagal nerve dysfunction associated with PD. Until recently, the NMS including constipation, dysphagia, disrupted sleep architecture, impaired olfaction, and depression were presented in PD patients (Raval et al., 2020). A great quantity of studies has shown that constipation is an early manifestation of the neurodegenerative process underlying PD. More importantly, the NMS of GI dysfunction (i.e., constipation) often appears much earlier in PD patients, predating the onset of motor symptoms by as many as 20 years (Abbott et al., 2001; Savica et al., 2009). Many studies found that gut microbiota composition alters in various PD-related NMS, possibly pre-dating motor symptoms (Heintz-Buschart et al., 2018). Interestingly, current research studies have illustrated that gut microbial disturbance can damage the intestinal barrier and raise chronic inflammation both in the gut (de La Serre et al., 2010; de Theije et al., 2014) and brain (Sampson and Mazmanian, 2015; Sampson et al., 2016).

The intestinal barrier

The role of the intestinal barrier as an interface between the external and internal environments of the host is an obvious focus. The intestinal barrier facilitates the absorption of nutrients and prevents the entry of harmful intraluminal components into the blood circulation, such as endotoxins, and deleterious rhizobacteria (Furness et al., 1999; Sun and Shen, 2018). The tight junction complexes in epithelial cells, the mucosal surface, and the immune system are vital for the intact intestinal barrier and function, of which the first line of defense is the intestinal lumen itself (Figure 3; Schoultz and Keita, 2020; Gharib-Naseri et al., 2021).

Commensal luminal bacteria inhibit the colonization of pathogens by, for example, the production of bacteriocins, pH modification of the luminal content, and competition for nutrients required for the growth of pathogens (Bharadia et al., 2020). Otherwise, the commensal microbiota resides in the intestinal epithelium enhanced physiological paracellular permeability, and the mucus layer (Schoultz and Keita, 2019).

The single cell layer of epithelium consists of plentiful cell types and could divide the body and the outside luminal milieu. The tight barrier of the intestinal luminal milieu is established by a large quantity of cells and their functions (Schoultz and Keita, 2019). It is shown that antimicrobial peptides could be produced by Paneth cells by eliminating pathogenic bacteria (Wang et al., 2018). Simultaneously, innate and adaptive immune cells, such as neutrophils, T cells, monocytes, and natural killer cells, respond to the intrusion of xenobiotics and suppress the inflammation. Neutrophils, a kind of immunological cells, are among the first cells to reach inflamed areas and eliminate microorganisms through phagocytosis (Rosales et al., 2016). T-regulatory cells, a specific subpopulation of lymphocytes, are critical in the maintenance of immune homeostasis in suppressing the activation of various immune cells related to inflammation (Corthay, 2009). The crosstalk established between the gut microbiota and the intestinal epithelium is key to maintaining homeostasis in the gut. More importantly, the bi-directional communications between the innate immune system and pattern recognition receptors, such as Toll-like receptors (TLRs), scavenger receptors, and nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs), are crucial to the intestinal barrier homeostasis in some conditions. The intestinal epithelial and immune cells are constantly in contact with foreign material and then respond appropriately to their intrusions, protecting the host (Wells et al., 2011). This interactivity ability makes the identification of external ingredients possible based on the antigen presenting cells. Even more interesting is that these cells can migrate to the

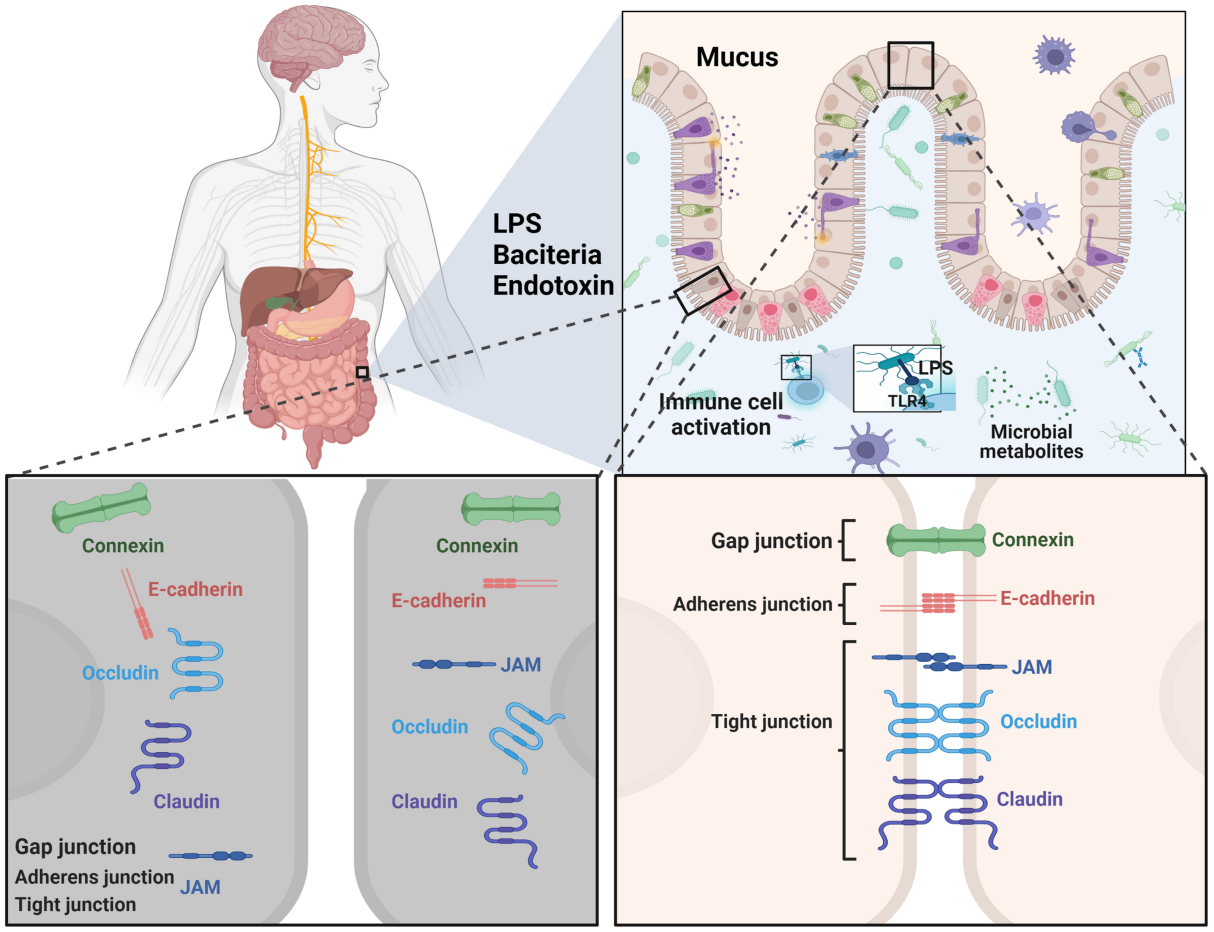

FIGURE 3
A schematic overview of the main cell types and physical defense mechanisms as targets related to intestinal barrier function for therapeutic strategies in PD.

peripheral site and transmit antigens to T-cells, leading to increased secretion of pro-inflammatory cytokines, IFN-γ for example, to improve the intestinal barrier (Wallace et al., 2014). Simultaneously, the enzyme of indoleamine 2,3-dooxygenase (Bessede et al., 2014), owning the effect of regulating the inflammatory reaction and the ability of protective effect against IBD, is responsible for the transformation of tryptophan to kynurenine (Arsenescu et al., 2011). TLRs are pro-inflammatory and conserved transmembrane receptors that can identify conserved molecular structures to detect microorganisms (Kubinak and Round, 2012). Pathogen triggered TLRs are generally understood to cause an inflammatory immune response, supporting the subsequent clearance of pathogenic microorganisms (Mayne et al., 2020). da Silva et al. (2016) found that these receptors were regulated by peripheral leukocyte responses in PD patients.

Intestinal permeability (between intestinal epithelial cells) is mainly characterized by medium-sized hydrophilic molecules transporting along their concentration gradient in a system without the assistance of a carrier system (Schoultz and Keita, 2020). A vital function of the intestinal epithelium is the maintenance of normal intestinal barrier function, which allows the selective permeability of essential nutrients, water, and ions without the entry of bacterial toxins and pathogens (Tabler et al., 2020). The increase in intestinal permeability represents intestinal barrier function disorders. A number of studies have found that increased permeability is correlated with the development of several diseases. Higher intestinal permeability induces the translocation of gut bacteria and microbial components that initiate inflammation and oxidative stress in the enteric nervous system, resulting in enteric α-synucleinopathy in PD (Bjarnason et al., 2004). Simultaneously, these findings also have important clinical applications. Barrier integrity in IBD patients, whose endoscopy findings are normal, may be detected using confocal laser endomicroscopy, which may predict the relapses (Galipeau and Verdu, 2016).

The development of reliable and sensitive methods to measure intestinal permeability could contribute to identifying patients who are at high risk of suffering from PD and assist in the development of preventative therapies (Galipeau and Verdu, 2016). PD is characterized by an altered gut microbiota composition, the intestinal barrier, and the enteric neuroimmune system, therefore it is possible that intestinal barrier dysfunction may play a key role in PD development and/or progression. Meanwhile, the significant role of intestinal permeability has been highlighted in PD, which lays the groundwork for novel PD therapy targeting the restoration of the intestinal barrier.

Inflammation of the gut and neuroinflammation of the brain

The maturation and function of microglia are affected by the altered cell proportions, while immature phenotypes are based on the circulating levels of pro-inflammatory and anti-inflammatory cytokines contributing to host homeostasis (Erny et al., 2015). The astrocytes and microglia can produce pro-inflammatory cytokines *via* TLRs activation (Lucas and Maes, 2013). TLRs can recognize microbial-associated molecular patterns (MAMPs), such as lipopolysaccharides (LPS), and are activated by changes in the composition of gut microbiota and gut permeability (Bowman et al., 2003). LPS can activate immune cells (such as macrophages, neutrophils, and dendritic) and promote the inflammation and permeability of the gut (Rhee, 2014). In particular, the activated immune cells can release various pro-inflammatory cytokines, binding with receptors expressed in neurons and glial cells, and then spread to the brain *via* the blood-brain-barrier and can result in the neuroinflammation and death of neurons in the brain (Dantzer et al., 2000). The new research illustrated that gut leakiness and endotoxemia owing to the alteration of microbiota led to the co-occurrence of intestinal inflammation and neuroinflammation *via* the activation of the TLR4 (Alhasson et al., 2017).

Amino acids and gut microbiota-brain axis in PD

Source and composition of amino acid in dietary proteins

In the gastrointestinal tract, enterocyte peptidases further hydrolyze resultant peptide products into amino acids, dipeptides, and tripeptides for enterocyte transport into the portal venous system. At the same time, amino acids serve as substrates for protein synthesis that are utilized for energy production under extreme conditions, such as disease for example. Amino acids are classified as essential amino acids, non-essential amino acids, and conditionally essential amino acids based on the species, age, disease, and condition dependence (Thalacker-Mercer et al., 2020). Essential amino acids, including tryptophan, valine, lysine, leucine, isoleucine, phenylalanine, threonine, and methionine, must be provided by dietary protein (Church et al., 2020; Verzola et al., 2020) and non-essential amino acids can be synthesized through other pathways.

Recently, amino acids, including total amino acid and free amino acid, have been found to exist in mushrooms, corn grain, peanut, soybean, and white rice, etc. Research has found that the average total free amino acid content of the mushroom was 4.35% (Sun et al., 2017). The relative amount of total amino acid is 8.76% in coffee silverskin (Machado et al., 2020), 10.67% in corn grain, 29.29% in peanut, 9.08% potato, and 8.49% in white rice (Hou et al., 2019). It should be noted that amino acids are substrates in polyamine synthesis by numerous enzymes in humans and microbes (Krzystek-Korpacka et al., 2020).

The absorption and metabolization of amino acids in the intestine

Amino acids perform several crucial functions in the human body, either directly or indirectly. They are the main constituents of protein in bioactive substances and adjust the balance of energy and immunity in organisms. In addition, amino acids can be catalyzed through oxidized, reduced, fissioned, or coupled pathways (Davila et al., 2013). Several reactions including transamination, deamination, and decarboxylation are required steps in this process. Lots of work demonstrated that glycine, proline, and arginine usually act as the acceptors of hydrogen, and alanine, leucine, isoleucine, and histidine are hydrogen donors in the metabolic pathways (Rist et al., 2013). The metabolisms of relevant amino acids are summarized in Figure 4. The microorganisms are mixed with endogenous proteins, such as mucous proteins, chemicals secreted by the pancreas and gastric gland in the alimentary canal (Hayashi et al., 2005). The nitrogen-containing compounds are broken down into amino acids and peptides and related enzymes are excreted *via* the exocrine pancreas. The amino acids and peptides are absorbed by bacteria or spread from the intestinal lumen to the portal vein.

Amino acids are absorbed and digested in the intestinal epithelium (Bos et al., 2005; Hu and Guo, 2021). During the process, a tremendous amount of nitrogen-containing compounds passes by the ileocecal junction and gets into the posterior part of the intestine (Blachier et al., 2007). However, some proteins are still difficult to absorb and digest completely in the small intestinal. The remaining nitrogen-containing compounds without digestion are transported to the large intestine. Microorganisms play a vital role in protein hydrolysis to provide free amino acids for both host and

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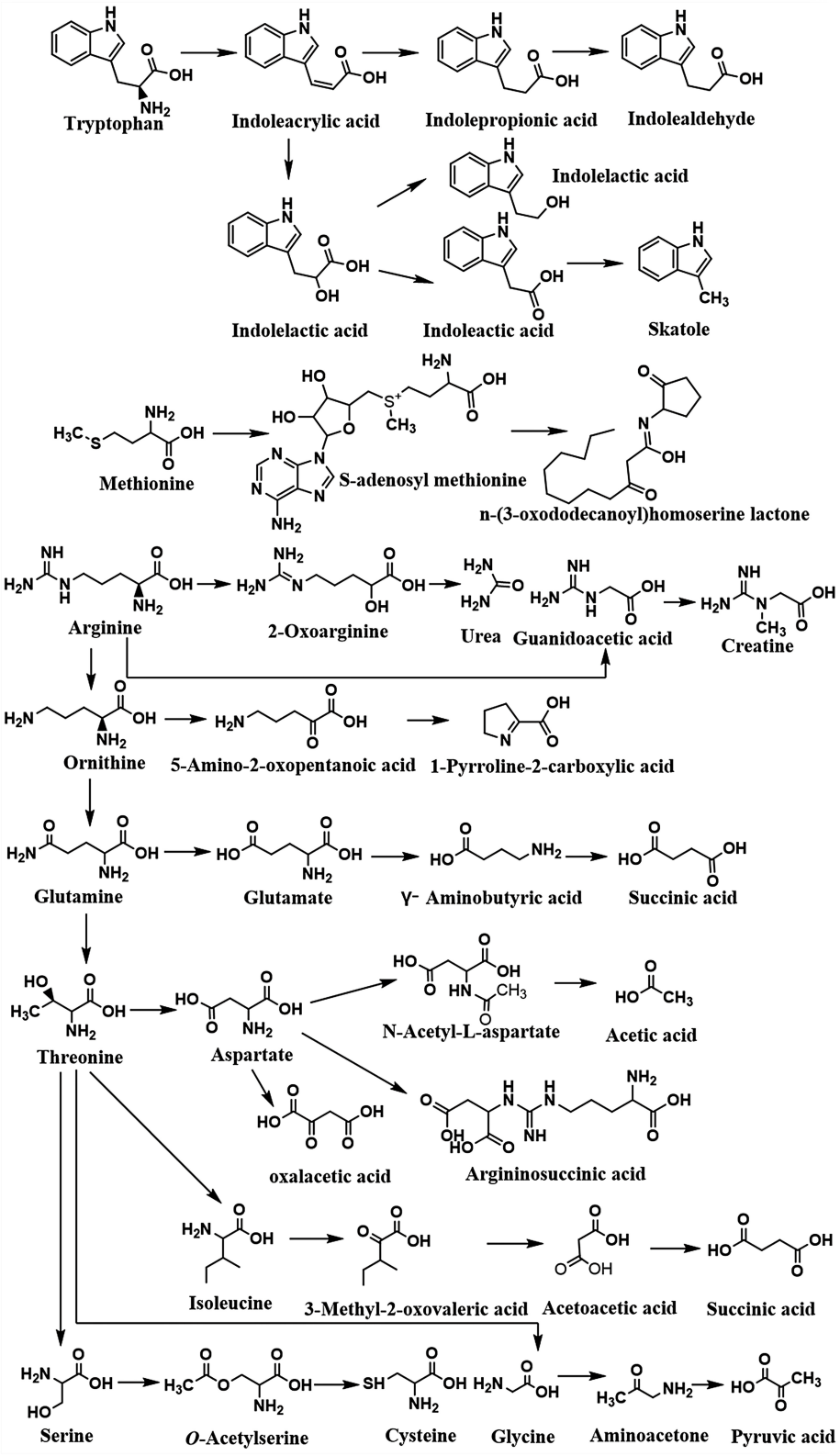

FIGURE 4
The summary of microbiota-derived amino acid metabolism profile.

microbial metabolism functions. Furthermore, most amino acids, synthesized by microorganisms, can be absorbed by the intestinal epithelial cells of the small and large intestines to implement a sequence of functions, like synthesis and catabolism (Laparra and Sanzm, 2010; Ma and Ma, 2019). The proteolysis and recycling of nitrogen-containing compounds are performed by proteolytic bacteria residing in the gastrointestinal tract (Macfarlane and Macfarlane, 2007). A large amount of amino acid maintains the immense and widespread metabolism via the function of microbiota. This diversity of amino acid metabolism in microorganisms may impart either active or passive effects on the host. Hence, adjusting protein or amino acid absorption and metabolization in the intestine may provide a strategy for shaping the amino acid fermenting bacteria and their metabolic pathways, thereby potentially affecting host metabolism.

Amino acids metabolism and gut microbiota

The roles of gut microbiota on amino acid metabolism have been well investigated (Zhao et al., 2019). The great majority of digestive products, such as small peptides, amino acids, and tripeptides, are utilized by certain microbes or transported into systemic circulation by the small intestine. The functions of amino acids between the gut microbiota and host are bi-directional (Zhao et al., 2019). The gut microbiota plays an essential role in dietary protein and nitrogen recycling. In addition, the microbial activity induced by indigestible amino acids promotes the production of metabolic end products including short-chain fatty acids (SCFA; acetate, butyrate, propionate), branched-chain fatty acids, ammines, indoles, and ammonia, etc. (Macfarlane et al., 1988). Previous work revealed that bacterial metabolites appear to have beneficial or opposite effects on the host depending on their concentrations. As the major nutrients in the diet, amino acids are the basic components of proteins and peptides, and are essential for the emergence of organism-active molecules involved in the signaling pathways and metabolism modulation (Dai et al., 2015). Additionally, the gut microbiota is crucial for the metabolism and recycling of amino acids. The amino acids, deriving from the diet or the host, are utilized as the fundamental element by gut microbiota for the synthesis of proteins or produce a large number of metabolites by the nutritional metabolism, such as polyamines, phenolic, nitric oxide (NO), and hydrogen sulfide (H_2S) (Dai et al., 2015). In addition, the "essential" amino acids, as a regulator for amino acid homeostasis in humans, can be synthesized by intestinal bacteria. For instance, research has illustrated that a large number of bacteria, like *Selenomonas ruminantium,* perform de novo synthesis of amino acids in the existence of peptides using experiments *in vitro* (Atasoglu et al., 1998; Neis et al., 2015). These results suggest that amino acid metabolism provides nutrition for gut microbiota and supports physiological action for the host (Dai et al., 2011; Collins et al., 2012). The intestinal bacterial communities are influenced by constituents of dietary protein, especially protein sources and concentrations. Proteins derived from plants are generally utilized in humans and animals. Nevertheless, crude proteins are characterized by low digestibility because of the anti-nutritional elements (He et al., 2015). By contrast, proteins originating from animals are characterized by high digestibility by aerobes in the large intestine. The homeostasis of gut microbiota (mainly in the large intestine) can be disorganized, leading to intestinal disorders (Fan et al., 2015). Consequently, it is essential to explore the connection between gut microbiota and amino acids (which are necessary for protein metabolism). In addition, comprehending the functions of different constituents of dietary protein on gut microbiota is crucial.

Regulation of gut microbiota on amino acid metabolism and synthesis

An enormous number of metabolic pathways, such as glucose, lipid, and amino acid metabolism, are regulated by gut microbiota. The composition of gut microbiota-derived metabolites in the intestinal tract is very complex. The nutrition can be provided by amino acids acquired in the diet or synthesized by the host for the gut microbiota for protein production. Amino acids can be integrated into bacterial cells as amino acid residues in proteins, and further incorporated into the metabolic pathway. Amino acids and peptides are obtained by the hydrolysis of proteins through proteases and peptidases secreted by gut microbiota. Ultimately, amino acids and peptides could be absorbed by microbiota through specific transporters. However, the small bioactive molecules will encounter various consequences under different physiological conditions of humans (Davila et al., 2013). Transamination or deamination is the first stage of amino acid catabolism, followed by oxidation or reduction. Biogenic amines are alkaline organic compounds mainly produced by decarboxylation reactions of free amino acids present by the enzyme decarboxylase (Wu et al., 2021).

Recent studies demonstrated convincingly that a large number of amino acid-fermenting bacteria are inhabited the intestinal tract (up to 1,011 per gram of dry feces) (Atasoglu et al., 1998). The "essential" amino acid of lysine, produced by bacteria, is absorbed and integrated as a whole host protein *in vivo* studies (Metges et al., 1996, 1999). The research illustrated that the 15N-lysine originated from microbial lysine with the comparison of the integration of ^{15}N from $^{15}NH^4Cl$ into the inside body system (Torrallardona et al., 1996; Gill et al., 2006).

Additionally, gut microbiota-derived lysine and threonine were shown to be crucial for free lysine and threonine in a study on nitrogen adequate diets in humans (Metges et al., 1999). Furthermore, this research found that the abundance of gut microbiota was associated with the precursors generated by humans related to "essential" amino acids (Metges et al., 1999).

A genome-wide analysis illustrates that a number of *clostridium* species, *Clostridium acetobutylicum* for example, have comprehensive genes for the biosynthesis of amino acids, whereas *Clostridium perfringens* lack the genes for amino acid biosynthesis such as lysine, serine, glutamic acid, and aromatic amino acids (Kevin et al., 2016). Hence, in order to elucidate the functions of amino acids from the perspective of bacterial species, it is necessary to understand the biosynthesis of amino acids in microorganisms. However, nitrogen and amino acids, required for the growth and fermentation of glycolytic bacteria, could be provided by the residual dietary proteins in the large intestine (Mafra et al., 2013). Various *Lactobacillus* and *Bifidobacterium* strains can produce γ-aminobutyric acid which is a neural active substance (Barrett et al., 2012). It is acknowledged that imidazole propionate can be transformed into histidine-derived metabolite by gut microbiota (Koh et al., 2018). Moreover, gut microbiota can produce tryptophan owing to the existing of tryptophan decarboxylase in the intestinal tract (van de Wouw et al., 2017). Given the above, amino acids can be utilized for the synthesis of bacterial cell components and catabolized. Major amino acid-fermenting bacteria in the intestinal tract are concluded in Table 2.

Amino acids as precursors for microbially derived short-chain fatty acids

The literature reports that short-chain fatty acids (SCFAs) constitute a major class of fermentation products. The microbiota-derived carbohydrates of SCFAs are cardinal examples of beneficial metabolites (Sonnenburg and Sonnenburg, 2014). Acetate (accounts for ∼50–70%), propionate (produced by the species of *Phylum Firmicutes*), and butyrate (produced by a small quantity of *Phylum Firmicutes*, such as *Faecalibacterium prausnitzii* and *Roseburia hominis*) (Sokol et al., 2008; Machiels et al., 2014) are SCFAs. These SCFAs supply key metabolic signaling that underlies homeostasis and the function of colonic Tregs by different molecular mechanisms (Mu et al., 2017; Yang, 2022). In faith, a wide variety of amino acids generated from microbial protein fermentation in the large intestine can be presented as precursors for short-chain fatty acid synthesis (Ciarlo et al., 2016). The great majority of amino acids such as glutamate, ornithine, and glycine can be metabolized by anaerobic bacteria and utilized for the synthesis of butyrate (Neis et al., 2015). Zou et al. (2021) reported that phenylalanine, tyrosine, and tryptophan could be metabolized to indolic and phenolic compounds *via* the gut microbiota. However, indolic and phenolic compounds can be utilized to mitigate inflammatory responses. Concurrently, it plays a significant role to promote the comprehension of the pathophysiology of diseases.

SCFAs have many ameliorative effects on gut health. First, SCFAs can maintain the integrity of the intestinal barrier and exert therapeutic potential for intestinal inflammation (Lewis et al., 2010). For instance, butyrate can activate adenosine monophosphate kinase (AMPK) or down-regulate the expression of claudin 2 to regulate the expression of tight junction proteins, eventually enhancing intestinal barrier function (Daly and Shirazi-Beechey, 2006; Peng et al., 2009). Second, SCFAs contribute to the mucous production in the gastrointestinal tract (Dalile et al., 2019). The research (Gaudier et al., 2009) illustrated that butyrate regulated MUC2 gene expression to regulate the production of mucin. Additionally, SCFAs can affect gastrointestinal motility by inhibiting histone deacetylases (Cherbut et al., 1998). Ultimately, SCFAs can interact with vagal afferents by influencing inflammation and hormonal regulation (Dalile et al., 2019).

To summarize, amino acids can alter the gut microbiota composition by forming an intestinal microenvironment favorable to the survival and proliferation of certain microbes (Libao-Mercado et al., 2009). Conversely, gut microbiota delivers energy and reduces co-factors *via* providing amino acids to meet the needs of the host (Metges, 2000). Gut microbiota owns the substitutable role (both the host nutrition and physiology) in amino acid metabolism, and the phenomenon shows the bidirectional process between amino acids and gut microbiota (Macfarlane and Macfarlane, 2012). Briefly, SCFAs play numerous biological roles: (1) constitute an energy source for muscles; (2) implicate in the transportation and metabolism of epithelial cells; and (3) influence epithelial cell growth and differentiation.

Amino acid transporters in the large intestine

It is widely known that several amino acids are likely absorbed in the large intestine. The significance of the balance between whole-body proteins and amino acids is still unclear, especially in humans. The amino acids and peptide transporters in the intestinal tract contribute to amino acid absorption and metabolism in the large intestine (Hendriks et al., 2012).

A tremendous amount of solute carrier (SLC) transporters, which contain a large number of families of distinct transporters, are adopted to transport amino acids and peptides (including di- and tripeptides). Many above-mentioned transporters are expressed in the large intestine. There is evidence for transporter

TABLE 2 Major amino acid-fermenting bacteria in the digestive tract.

Amino acids	Intestinal bacteria	Metabolite	Related mechanisms in PD	Trends in PD	Niches	References
Phenylalanine	*Subdoligranulum*	Phenylpropionate	Phenylalanine is the precursor of dopamine and could participate in protein sequences in all tissues	Ascend	Rumen	Hirayama et al., 2016; Dodd et al., 2017
	Lactobacillus	Phenyl lactate			Small intestine	
	Clostridium	Phenylacetate			Cecum	
	Pepto streptococcus spp.	Phenyl pyruvic acid			Colon	
		4-OH-phenylpyruvic acid				
		Ammonia				
		CO_2				
Asparagine	*Fusobacterium nucleatum*	Aspartic acid	Related to oxidative stress and dopamine cell degeneration in PD	Descend	Small intestine	Nagaraja et al., 2021
	Escherichia coli	Ammonia			Large intestine	
	Klebsiella pneumoniae	CO_2			Colon	
	Campylobacter jejuni				Feces	
	Bacteroides fragilis					
Tryptophan	*Lactobacillus* spp.	Indole	Associated with psychiatric problems in advanced PD	Descend	Stomach	Hatano et al., 2016; Dehhaghi et al., 2019; Liu B. et al., 2021; Liu J. R. et al., 2021
	Bifidobacterium spp.	Indole ethanol			Small intestine	
	Clostridium spp.	Indolepropionic acid			Colon	
	Pepto streptococcus spp.	Indolelactic acid				
	Bacteroides spp.	Indoleacetic acid			Feces	
	Clostridium sporogenes	Skatole				
	Ruminococcus gnavus	Tryptamine				
		Indolealdehyde				
		Indoleacrylic acid				
Lysine	*Clostridium sticklandii*	Lysine-5,6-aminomutase	Acylation of lysine neutralizes may change the conformation of proteins	Ascend	Stomach	Potrykus et al., 2008; Wan et al., 2019
					Small intestine	
	Porphyromonas gingivalis	Lysine			Colon	
					Feces	
		2,3-aminomutase				
		2,5-diaminohexanoate				
		Dehydrogenase				
		3,5-diaminohexanoate				
		Dehydrogenase				
		3,6-diaminohexanoic acid				

(Continued)

10.3389/fnagi.2022.976316

TABLE 2 (Continued)

Amino acids	Intestinal bacteria	Metabolite	Related mechanisms in PD	Trends in PD	Niches	References
Serine	Fusobacterium	Acetate	Signaling molecule to regulate the growth, repair, and maintenance of brain functions	Ascend	Stomach	Campbell et al., 1967; Schierack et al., 2007; Donatti et al., 2020
	Varium	Lactate			Small intestine	
	Campylobacter jejuni	Pyruvate			Colon	
	Bacteroides fragilis	Ammonia				
	Acidaminococcus fermentans	Malate			Feces	
		CO_2			Rumen	
	Clostridium aminophilum				Cecum	
	Clostridium perfringens				Stomach	
	Clostridium sticklandii				Large intestine	
	Pepto streptococcus spp.					
Aspartic acid	*Fusobacterium nucleatum*	Asparagine	Related to oxidative stress, and dopamine cell degeneration in PD	Descend	Small intestine	Sugihara et al., 1974; Guccione et al., 2008
	Escherichia coli	Fumarate			Colon	
		Oxaloacetate				
	Campylobacter jejuni				Feces	
	Acidaminococcus					
	Fermentans					
	Bacteroides fragilis					
Glutamine	*Clostridium aminophilum*	Pyroglutamate	Involved in the glutamate-glutamine cycle	Ascend	Mouth	Donatti et al., 2020; Kumari et al., 2020
	Selenomonas ruminantium	Acetate			Small intestine	
	Acidaminococcus fermentans	Butyrate			Large intestine	
		Ammonia			Stomach	
	Clostridium perfringens	CO_2				
					Colon	
	Pepto streptococcus spp.				Feces	
	Streptococcus bovis				Rumen	

(Continued)

10.3389/fnagi.2022.976316

TABLE 2 (Continued)

Amino acids	Intestinal bacteria	Metabolite	Related mechanisms in PD	Trends in PD	Niches	References
	Campylobacter jejuni *Klebsiella pneumoniae* *Escherichia coli* *Fusobacterium nucleatum*				Cecum	
Glutamate	*Clostridium aminophilum*	2-Oxaloacetate	Participating in the disruption of the normal basal ganglia function, thus leading to neuronal death	Descend	Mouth	Donatti et al., 2020; Jiménez-Jiménez et al., 2020; Kumari et al., 2020
	Selenomonas ruminantium *Acidaminococcus fermentans*	Ammonia GABA CO_2			Small intestine Large intestine	
	Clostridium perfringens *Campylobacter jejuni* *Klebsiella pneumoniae* *Escherichia coli* *Fusobacterium varium* *Fusobacterium nucleatum*				Stomach Colon Feces Cecum	
Glutamic acid	*Fusobacterium nucleatum*	Glutamate	Glutamic acid is a precursor of glutathione and may reflect an increase in oxidative stress in the disease progression	Descend	Small intestine	Rychlik and Russell, 2002; Whitehead and Cotta, 2004; Anderson et al., 2009; Vascellari et al., 2020
	Fusobacterium varium	Ammonia Acetate			Large intestine	
	Escherichia coli *Selenomonas ruminantium* *Acidaminococcus fermentans* *Clostridium aminophilum*	Butyrate GABA CO_2 2-Oxaloacetate 2-ketoglutarate			Stomach	

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10.3389/fnagi.2022.976316

TABLE 2 (Continued)

Amino acids	Intestinal bacteria	Metabolite	Related mechanisms in PD	Trends in PD	Niches	References
Histidine	*Fusobacterium nucleatum*	Urocanate	Suppressive neurotransmitter effects and hormone secretion	Ascend	Mouth	Attwood et al., 1998; Kumari et al., 2020; Sylte et al., 2020
					Small intestine	
	Fusobacterium varium	Ammonia			Large intestine	
	Klebsiella pneumoniae	Glutamic acid				
		Histamine			Colon	
		CO_2			Feces	
					Rumen	
Glycine	*Fusobacterium nucleatum*	Acetate	Glycine could stimulate the release of dopamine and acetylcholine	Ascend	Mouth	Luan et al., 2015; Kumari et al., 2020; Sánchez-Andrea et al., 2020
	Clostridium perfringens	Pyruvate			Small intestine	
	Bacteroides fragilis	Serine			Large intestine	
					Stomach	
	Escherichia coli				Colon	
					Feces	
Threonine	*Fusobacterium nucleatum*	Acetate	Signaling molecule to regulate the growth, repair, and maintenance of brain functions	Ascend	Mouth	Donatti et al., 2020; Wu and Deng, 2020; Karkache et al., 2021
	Escherichia coli	Ammonia			Small intestine	
	Pepto streptococcus spp.	Propionate			Large intestine	
	Clostridium sporogenes	n-butyrate				
	Clostridium sticklandii	Ketobutyrate			Stomach	
	Clostridium difficile	Lactate			Colon	
	Clostridium perfringens	CO_2			Feces	
	Megasphaera elsdenii					
	Acidaminococcus fermentans					
	Bacteroides fragilis					
Arginine	*Fusobacterium nucleatum*	2-Oxoarginine	Serve as a helpful clinical diagnostic biomarker for PD	Ascend	Mouth	Amano et al., 2020; Donatti et al., 2020; Martí I Líndez and Reith, 2021

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10.3389/fnagi.2022.976316

TABLE 2 (Continued)

Amino acids	Intestinal bacteria	Metabolite	Related mechanisms in PD	Trends in PD	Niches	References
	Escherichia coli	Guanidinoacetic acid			Small intestine	
	Klebsiella pneumoniae	Creatine			Large intestine	
	Clostridium sticklandii	Urea			Stomach	
	Clostridium perfringens	Putrescine			Colon	
	Selenomonas ruminantium	Spermine			Feces	
		Agmatine			Cecum	
		Ammonia			Rumen	
		CO_2				
Alanine	*Staphylococcus aureus*	Isovalerate	Alanine may point to mitochondrial dysfunction, oxidative stress, and inflammation markers in PD	Ascend	Small intestine	Nielsen et al., 2020
	Streptococcus spp.	Isocaproate			Large intestine	
		Ammonia			Feces	
	Gram negative bacteria					
	Escherichia coli					
	Selenomonas ruminantium					
	Prevotella spp.					
	Bacteroides spp.					
	Clostridium spp.					
	Ruminal bacteria					
Valine	*Staphylococcus aureus*	Ammonia	Related to myelination dysfunction of the neurons.	Descend	Large intestine	Toczylowska et al., 2020
	Streptococcus spp.	CO_2				
		Acetate				
	Escherichia coli	Isobutyrate				
	Klebsiella spp.					
	Selenomonas ruminantium					
	Megasphaera elsdenii					
Methionine	*Klebsiella pneumoniae*	S-adenosyl-L-methionine	Associated with hyperhomocysteinemia	Ascend	Small intestine	Toczylowska et al., 2020
		n-(3-oxododecanoyl) homoserine lactone			Colon	
					Feces	
Leucine	*Pepto streptococcus* spp.	Isovalerate	Contribute to muscle wasting, twitching, and tremors.	Descend	Rumen	Luan et al., 2015; Ma et al., 2020; Vascellari et al., 2020
		Isocaproate			Small intestine	

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10.3389/fnagi.2022.976316

TABLE 2 (Continued)

Amino acids	Intestinal bacteria	Metabolite	Related mechanisms in PD	Trends in PD	Niches	References
	Clostridium bifermentans	Ammonia			Cecum	
		CO2				
	Clostridium sporogenes	Acetate			Colon	
		Isobutyrate			Large intestine	
	Clostridium sticklandii					
	Clostridium difficile					
	Prosthecobacter					
Isoleucine	*Pepto streptococcus* spp.	Isovalerate	Contribute to muscle wasting, twitching, and tremors	Descend	Rumen	Kumari et al., 2020; Vascellari et al., 2020
	Clostridium bifermentans	Isocaproate			Small intestine	
	Clostridium sporogenes	Ammonia			Cecum	
	Clostridium sticklandii	CO_2				
		Acetate			Colon	
	Clostridium difficile	Isobutyrate			Large intestine	
Linoleic acid	Bacteroidaceae	9-,13-oxoODEs	Associated with protective effects and may reflect an excess of oxidative stress	Descend	Small intestine	Vascellari et al., 2020
	Streptomyces griseorubens	Ammonia			Colon	
		CO_2				
		Acetate			Feces	
Tyrosine	*Clostridium*	Ferulic acid	Tyrosine is then further hydroxylated to produce Dopa by tyrosine hydroxylase	Ascend	Stomach	Kumari et al., 2020
	Bacteroides	4-Hydroxycinnamic acid			Small intestine	
	Bifidobacterium	4-Hydroxyphenylacetic acid			Colon	
		4-Hydroxyphenylpropionic acid			Feces	
	Faecalibacterium	3-Hydroxybenzoic acid				
Pyruvate	*Faecalibacterium*	Acetate	Reduced pyruvate may be related to impairment in energetic and repair functions	Descend	Rumen	Toczylowska et al., 2020; Shen et al., 2021
	Clostridium bifermentans	Formate			Small intestine	
	Clostridium sporogenes	Lactate			Large intestine	
		Lactoyl-CoA				
		Acryloyl-CoA			Cecum	
		Propionyl-CoA			Colon	
		Propionate				

10.3389/fnagi.2022.976316

expression at different levels, such as the protein level and the gene expression level. Peptide transporter 1 (PEPT1), encoded by SLC family 15 member 1 (SLC15A1), is involved in the intestinal uptake of oligopeptides and peptide-mimetic drugs (Liu et al., 2019). As we have seen previously, SLC15A1 is the unique transporter that has been verified at the protein level in the human large intestine. The host relies on the system L transporters to obtain essential amino acids, such as SLC7A5, also known as L-type amino acid transporter (LAT)1, SLC7A8, also known as LAT2, SLC43A1, also known as LAT3, and SLC43A2, also known as LAT4. Both SLC7A5 and SLC7A82 are amino acid exchangers, while SLC43A1 and SLC43A2 facilitate amino acid proliferation (Wang and Holst, 2015). Some of the amino acids, such as tryptophan, tyrosine, isoleucine, leucine, valine, and phenylalanine, can be transported into cells via LAT1 and LAT2. Meanwhile, the other amino acids, such as Leucine, isoleucine, valine, phenylalanine, and methionine can be transported into cells by LAT3 and LAT4 (Wang and Holst, 2015). Furthermore, SLC6A14, coupled with a Na^+ gradient, a Cl^- gradient, and membrane potential, shows higher expression in the colon than in the ileum and presents special characteristics with much a better sense of amino acids than the other three transporters (Anderson et al., 2009). Simultaneously, SLC36A1, also called proton-assisted amino acid transporter 1 (PAT1), shows a similar ability to SLC6A14. In addition, other transporter genes expressed in the large intestine discussed in current research also attract our attention. Furthermore, the SLC transporter functions of amino acids and peptides in the large intestine require further study.

Amino acids regulate the intestinal epithelial barrier functions

The elementary mechanism of intestinal homeostasis relies on complex molecular crosstalk between host and gut microbiota. It is demonstrated that the catabolites of tryptophan by bacteria are various, such as tryptamine, skatole, indoleacrylic acid, indoleacetic acid, indolelactic acid, indolepropionic acid, and indolealdehyde, which are the ligands of the aryl hydrocarbon receptor. A recent study indicated that the catabolite of indole could improve intestinal epithelial barrier functions by regulating certain gene expressions (Roager and Licht, 2018). Simultaneously, the catabolite of indolepropionic acid acted as a ligand of the pregnane X receptor and showed the effect of adjusting the intestinal barrier function at the existence of indoles (Venkatesh et al., 2014). In addition, catabolite indoleacrylic acid was found to promote goblet cell differentiation and mucus production to regulate intestinal barrier function. In summary, the catabolites of tryptophan have the effect on improving intestinal barrier function by regulating the pregnane X receptor and aryl hydrocarbon receptor. The study demonstrated that glutamine improved IL-13-induced barrier dysfunction by up-regulating the expression of the tight junction protein claudin-1, through preventing the PI3K-Akt signaling pathway (Li et al., 2021), and consequently damaged the intestinal barrier function and increased intestinal permeability. At the same time, glutamate can up-regulate the expression of tight junction proteins and protect the diquat-induced oxidative stress to promote intestinal epithelial cell growth and health based on the new biochemical.

The role of amino acids in Parkinson's disease

There is increasing evidence that dopamine dysfunction plays an important role in the pathogenesis of PD. There is also increasing evidence to suggest that the other amino acids, such as glutamate, γ-aminobutyric acid, homocysteine, and large neutral amino acids in the brain were involved in the pathogenesis of PD (Yuan et al., 2013; Figura et al., 2018). According to the research, dopamine treatment is associated with elevated homocysteine in Parkinson's disease (Kuhn et al., 1998). The three amino acids of glutamate, cysteine, and glycine are involved in the synthesis of glutathione. The ratio of the two forms of glutathione (reduced and oxidized forms) can affect the cellular redox status. Mitochondrial complex I has been considered central to the pathogenesis of PD. However, low glutathione concentrations may inhibit mitochondrial complex I (Müller et al., 2016). Meanwhile, the finding suggests that branched-chain amino acids (leucine, isoleucine, and valine) can serve as nitrogen donors to maintain the balance of glutamate-glutamine in astrocytes and neurons (Zhang et al., 2022). Branched-chain amino acids could promote the catabolism of glutamate which may exert beneficial effects on PD. Interestingly, tyrosine and phenylalanine are the key substrates for the production of dopamine in PD. The elevation of phenylacetyl-L-glutamine was positively correlated with firmer stool and constipation severity among PD patients. Simultaneously, gut microbiota-derived tryptophan catabolites could adjust the inflammatory response *via* affecting pro-inflammatory cytokines and lipogenesis in macrophages and hepatocytes (Shao et al., 2021). To compound the issue, glutamic acid and γ-aminobutyric acid are related to the clinical heterogeneity of PD, the symptoms of Parkinson's disease, and especially to the parkinsonian tremor (Yuan et al., 2013). Promisingly, the detection of amino acid neurotransmitter levels may offer new insight into the pathogenesis and early diagnosis of Parkinson's disease. Therefore, more stringent studies with a larger sample size are needed to explore the changes

in amino acid neurotransmitter levels during Parkinson's disease progression.

Conclusions

Researchers have found that amino acids provide powerful support for human health. In recent years, the gut microbiota has received great attention, putting it in the spotlight of biomedical research. The balance of intestinal microbes and the host play a vital role in several disorders, including the impairment of the nervous system. Notably, gut microbiota, a major determinant of bidirectional communication between the gut and brain, could be regulated by a number of mechanisms, and it is illustrated that the microbiota and its produced biochemical messengers are major facets of maintaining a balanced intestinal microenvironment. Microbiota-derived amino acid molecules contribute to host health through modulating immune function, gastrointestinal physiology function, and microbiota composition. Striatal dopamine depletion in PD contributes to the hyperactivity of subthalamic nucleus output pathways, resulting in an abnormal increase in specific microbiota-derived amino acid molecules (glutamate for example), which are involved in the degeneration of the nigrostriatal system based on the excitotoxicity mechanisms. All the studies have shown that GMBA may be significantly correlated with PD pathogenesis.

Although microbiota-host interaction has drawn much attention both from clinicians and researchers in recent years, it still calls for a deeper understanding of the complex GMBA communication. Furthermore, microbiota-derived amino acid metabolism is a crucial factor suggesting major perturbations of the microbiome. These studies illustrated that amino acids metabolized by gut microbiota might affect the degeneration of the nigrostriatal system in PD patients. Gut microbiota and amino acids may be potential therapeutic targets for PD.

Author contributions

GZ and YZ contributed to the conceptualization. WW and GZ contributed to the investigation and funding. WW, GZ, SJ, CX, YL, and LT contributed to the writing and final approval. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Stability of motor-nonmotor subtype in early-stage Parkinson's disease

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Background: The different clinical characteristics and prognostic values of the motor-nonmotor subtypes of Parkinson's disease (PD) have been established by previous studies. However, the consistency of motor-nonmotor subtypes in patients with early-stage Parkinson's disease required further investigation. The present study aimed to evaluate the consistency of motor-nonmotor subtypes across five years of follow-up in a longitudinal cohort.

Materials and methods: Patients were classified into different subtypes (mild-motor–predominant, intermediate, diffuse malignant; or tremor-dominant, indeterminate, postural instability and gait difficulty) according to previously verified motor-nonmotor and motor subtyping methods at baseline and at every year of follow-up. The agreement between subtypes was examined using Cohen's kappa and total agreement. The determinants of having the diffuse malignant subtype as of the fifth-year visit were explored using logistic regression.

Results: A total of 421 patients were included. There was a fair degree of agreement between the baseline motor-nonmotor subtype and the subtype recorded at the one-year follow-up visit ($\kappa = 0.30 \pm 0.09$; total agreement, 60.6%) and at following years' visits. The motor-nonmotor subtype had a lower agreement between baseline and follow-up than did the motor subtype. The baseline motor-nonmotor subtype was the determinant of diffuse malignant subtype at the fifth-year visit.

Conclusion: Many patients experienced a change in their motor-nonmotor subtype during follow-up. Further studies of consistency in PD subtyping methods should be conducted in the future.

KEYWORDS

follow-up studies (MeSH), Parkinson's disease, prognosis, subtype, stability

Introduction

Parkinson's disease (PD) is a multisystem neurodegenerative disease that features predominant motor and nonmotor symptoms. Patients with PD exhibit huge individual differences in their motor and nonmotor characteristics. Previously, efforts have been made to distinguish the clinical subtypes of PD and the prognosis associated with the different subtypes (Hendricks and Khasawneh, 2021). In terms of motor symptoms, patients can be classified into tremor-dominant (TD), postural instability and gait difficulty (PIGD), and indeterminate subtypes (Stebbins et al., 2013). However, patients may transfer from one subtype to another as the disease progresses (Eisinger et al., 2020). By two years after baseline, around half of patients have experienced a change in their motor subtype (Erro et al., 2019). von Coelln et al. (2021) found that 50% of patients with the TD subtype and 60% of patients with the indeterminate subtype had shifted to the PIGD subtype by five years after baseline, indicating that most transfers are from a milder to a more severe subtype. However, inconsistencies in the motor subtypes, as observed via cross-sectional evaluation, have raised the question that these subtypes might simply reflect longitudinal disease progression (Simuni et al., 2016; Erro et al., 2019; Luo et al., 2019; von Coelln et al., 2021).

Recent evidence has shown that nonmotor PD symptoms are multidimensional, and that the prevalence and severity of these symptoms are diverse among patients (Ou et al., 2021). Classification methods based on both motor and nonmotor symptoms have been developed (Fereshtehnejad et al., 2015, 2017). In this context, patients can be categorized into mild-motor–predominant, intermediate, or diffuse malignant subtypes according to the severity of motor symptoms, the presence of rapid eye movement sleep behavior disorder (RBD), and the status of autonomic function and cognition (Fereshtehnejad et al., 2017). This kind of classification has been widely used in subtype studies (Fan et al., 2021; Mestre et al., 2021). Patients with the diffuse malignant subtype have been found to experience a faster deterioration of motor and cognitive symptoms and a shorter progression time to disease milestones (e.g., regular falls, dementia, wheelchair dependence, and placement in residential or nursing home care) and death (Fereshtehnejad et al., 2017; De Pablo-Fernandez et al., 2019). However, a recent study found that patients with advanced disease had a higher prevalence of the diffuse-malignant subtype compared to those who had shorter disease duration or lower disease staging (Erro et al., 2020). In addition, no significant differences were found between Lewy pathology staging and Alzheimer's disease–related pathology staging among the different subtypes (De Pablo-Fernandez et al., 2019). Similar to the motor subtypes, the motor-nonmotor classification method has also made use of cross-sectional data, and the consistency of the subtypes in patients with early-stage PD still requires further investigation.

The previous review pointed out that, in order to support the hypothesis that the subtype accurately represents the underlying pathological characteristics, subtypes must exhibit consistency (Fereshtehnejad and Postuma, 2017). In the present study, we evaluate the consistency of the previously established subtypes in a large multicenter longitudinal cohort. We also explore the association between the subtype at baseline and the subtype at follow-up, as well as the potential determinants of change in subtype.

Materials and methods

Study design and patient inclusion

The Parkinson Progression Marker Initiative (PPMI) study was a prospective, multicenter observational cohort study whose cohort was used to develop the motor-nonmotor subtyping method. We therefore used the same cohort to evaluate the consistency of the motor-nonmotor subtypes (Fereshtehnejad et al., 2017). The details of the PPMI study design have been described elsewhere (Parkinson Progression Marker, 2011).

The inclusion criteria for the present study were: (i) patients diagnosed with PD and having dopaminergic deficit, as confirmed by dopamine transporter imaging; (ii) patients with a disease duration ≤ 3 years and Hoehn and Yahr stage < 3 at baseline. Patients with missing data at baseline in the scales used for the classification were excluded.

The PPMI study was approved by the institutional review boards at each participating PPMI site. Written informed consent was obtained from all participants in the study.

Measurements

Patients were scheduled to be followed up for five years, so we used data from baseline as well as from all five years of follow-up. Face-to-face evaluations were conducted by experienced neurological doctors. Demographic features including age, age of onset, sex, and disease duration were recorded. Motor and nonmotor symptoms were evaluated by the following: the Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS); the Hoehn and Yahr scale; the Rapid Eye Movement Sleep Behavior Disorder Screening Questionnaire (RBDSQ); the Geriatric Depression Scale; the State-Trait Anxiety Inventory; the Questionnaire for Impulsive-Compulsive Disorders; the Scales for Outcomes in Parkinson's Disease–Autonomic; and the Epworth Sleepiness Scale. Activities of daily living were assessed by the modified Schwab and England Activities of Daily Living scale. Cognitive evaluation was conducted using the following: the Montreal Cognitive Assessment; the 15-item version of Benton Judgment of Line Orientation (BJLO) test; the Hopkins

Verbal Learning Test–Revised (HVLT-R); the Letter-Number Sequencing (LNS) task; a modified Semantic Fluency (SF) test; and the Symbol Digit Modalities Test (SDMT).

Subtyping

Patients were classified into different subtypes at baseline, and the classification was repeated during follow-up, according to the motor and nonmotor symptoms. Because the motor-nonmotor subtyping method depends on the quartile level of disease severity of the whole group, as well as on cross-sectional data, we included all the patients available at baseline in the subtyping process. The subtyping process during follow-up also used all available patients, and those without baseline subtype information were excluded.

The method of motor-nonmotor subtype classification is described in the previous review (Fereshtehnejad et al., 2017). In brief, patients with motor scores >75th percentile and ≥1 of 3 nonmotor scores >75th percentile, or with all 3 nonmotor scores >75th percentile, were classified into the diffuse malignant subtype, and patients with motor and all three nonmotor scores <75th percentile were classified into the mild-motor–predominant subtype. Those who did not meet the aforementioned criteria were classified into the intermediate subtype. Motor scores (off-state) were calculated via the means of the Z-scores of the MDS-UPDRS part II, MDS-UPDRS part III, and PIGD score. The PIGD score was calculated as the mean of the scores of items 2.12, 2.13, 3.10, 3.11, and 3.12 of the MDS-UPDRS (Stebbins et al., 2013). The three nonmotor scores were the Z-scores of the RBDSQ, SCOPA-AUT, and cognitive tests. The cognition score was the mean of the Z-scores of the BJLO, HVLT-R (i.e., the mean score of the total recall, delayed recall, retention, and recognition discrimination index), SDMT, and combined scores (i.e., the mean score of the SF and LNS). In addition, we also divided patients into different motor subtypes according to the ratio of TD and PIGD scores (Stebbins et al., 2013). Patients with TD/PIGD score ratios ≥1.15 were classified as TD, those with ratios ≤0.90 were classified as PIGD, and the others were classified as indeterminate.

Statistical analysis

Data were shown as median (quartile) for the continuous variables and as number (percent) for the categorized variables. The distribution was tested with Shapiro–Wilk tests. The Mann–Whitney U test and Chi-square test (or Fisher's exact test) were used to compare the baseline characteristics of patients between groups, as appropriate, since the data were abnormally distributed. Cohen's kappa (κ) and proportion of observed agreement were used to evaluate the agreement of the subtypes between baseline and follow-up. Cohen's kappa value can be interpreted as following: $\kappa < 0$, no agreement; $\kappa = 0$–0.20, poor agreement; $\kappa = 0.21$–0.40, fair agreement; $\kappa = 0.4$–0.60, moderate agreement; $\kappa = 0.61$– 0.80, substantial agreement; and $\kappa = 0.81$–1, excellent agreement. Binary logistic regression was used to evaluate the determinants of the diffuse malignant subtype at follow-up.

Patients were divided into two groups according to whether they were in the diffuse malignant subtype at the fifth year of visit. Baseline motor-nonmotor subtype and characteristics that were not used for the classification were set as the dependent variables. Statistical significance was set as $p < 0.05$, and all tests were two-sided. Statistical Product and Service Solutions (SPSS) software, version 22.0, was used to conduct all the analyses.

Results

A total of 421 patients were included in the present study. Their baseline demographics are presented in **Table 1**. About two-thirds (66.5%) of the patients were male. The median age at baseline and age of onset was 62.4 years and 61.2 years, respectively. At baseline, 213 patients (50.6%) were mild-motor–predominant, 162 patients (38.5%) were intermediate, and 46 patients (10.9%) were diffuse malignant. There were 318, 259, 241, 234, and 208 patients available for re-subtyping at each year's follow-up, respectively. Subtype change between baseline and the one-year visit is displayed in **Figure 1**. At year five, 102 patients (49.0%) were classified as mild-motor–predominant, 88 patients (42.3%) were intermediate, and 18 patients (8.7%) were diffuse malignant. Only 120 patients (57.7%) had the same subtype at year five as at baseline. Specifically, 68 patients with mild-motor subtype at baseline (63.5%), 46 patients with intermediate subtype (52.9%), and 6 patients with diffuse malignant subtype (42.8%) had stable subtypes at year five. For the patients with mild-motor subtype at baseline, 33.6% and 2.8% of them, respectively, transferred to intermediate and diffuse malignant subtypes. For the intermediate patients, 36.8% and 10.3% of them, respectively, transferred to mild-motor–predominant and diffuse malignant. And 14.3% and 42.8% of diffuse malignant patients, respectively, transferred to the mild-motor–predominant and intermediate subtypes.

The kappa value indicated a fair agreement between the baseline and follow-up subtypes (**Table 2**). The kappa values of the baseline and follow-up motor subtypes are also listed for comparison. With disease progression, the kappa value of the motor-nonmotor subtype showed a mild decrease, but the kappa value of the motor subtype fell from a moderate agreement to a fair agreement. In addition, the total agreement of the motor-nonmotor subtype was lower than that of the motor subtype during follow-up.

Patients completing their fifth-year visit were younger and had lower ages of onset, lower MDS-UPDRS part II and III scores, and greater BJLO and SDMT scores compared to those

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TABLE 1 Baseline characteristics of all patients.

	All patients (*n* = 421)	Mild–motor (*n* = 213)	Intermediate (*n* = 162)	Diffuse malignant (*n* = 46)	*P*-value
Age (median, quartile)	62.4 (55.2–69)	62.3 (54.8–69)	62.1 (54.8–68.9)	64.4 (59.2–70.2)	0.402
Age of onset (median, quartile)	61.2 (53.7–67.5)	61.2 (53.5–67.3)	60.6 (53.4–67.1)	62.4 (57.5–68.2)	0.548
Male (*n*, %)	280 (66.5%)	133 (62.4%)	114 (70.4%)	33 (70.7%)	0.199
Disease duration, years (median, quartile)	15 (10–24)	14 (9–22)	15.5 (9.8–24.3)	22 (11–28.5)	0.007*
MDS-UPDRS part I score (median, quartile)	5 (3–8)	4 (2–6)	5 (3–8)	9.5 (5.8–13)	<0.001*
MDS-UPDRS part II score (median, quartile)	5 (3–8)	3 (2–6)	5.5 (3–9)	11.5 (7.8–14)	<0.001*
MDS-UPDRS part III score (median, quartile)	19 (14–26)	17 (12–23)	20 (14–26)	31 (26–38)	<0.001*
Hoehn and Yahr stage (*n*, %)					<0.001*
1	182 (43.2%)	105 (49.3%)	75 (46.3%)	2 (4.3%)	
2	239 (56.8%)	108 (50.7%)	87 (53.7%)	44 (93.7%)	
SE-ADL score (median, quartile)	90 (90–100)	95 (90–100)	90 (90–100)	90 (83.8–90)	
RBDSQ score (median, quartile)	5 (3–7)	4 (3–5)	6 (4–9)	8 (5–10)	<0.001*
SCOPA-AUT score (median, quartile)	8 (5–12)	7 (5–9)	11 (6–16)	14 (11–18)	<0.001*
GDS score (median, quartile)	2 (1–3)	2 (0–3)	2 (1–3)	2 (1–5)	0.004*
STAI score (median, quartile)	63 (52–76)	61 (51–74.5)	62 (52–77)	73.5 (58.8–97)	<0.001*
QUIP score (median, quartile)	0 (0–0)	0 (0–0)	0 (0–1)	0 (0–0)	0.003*
ESS score (median, quartile)	5 (3–8)	5 (3–8)	5 (3–8)	7 (3–10)	0.091
MoCA score (median, quartile)	27 (25–29)	27 (25–29)	27.5 (25–29)	27 (25–29)	0.891
BJLO score (median, quartile)	13 (11–14)	13 (11–14)	13 (12–15)	13 (11–14)	0.073
HVLT score: Total recall (median, quartile)	24 (21–27)	24 (21–27)	25 (20–28)	24 (20.8–26.3)	0.154
HVLT score: Delayed recall (median, quartile)	8 (7–10)	8 (7–10)	9 (6.8–11)	8 (6–10)	0.230
HVLT score: Retention (median, quartile)	0.9 (0.7–1)	0.9 (0.7–1)	0.9 (0.7–1)	0.9 (0.7–1)	0.418
HVLT score: Discrimination recognition (median, quartile)	10 (9–11)	10 (9–11)	10 (9–11)	10 (8–11)	0.143
LNS score (median, quartile)	11 (9–12)	11 (8–12)	11 (9–13)	11 (10–12)	0.088
SF score (median, quartile)	47 (40–56)	47 (40–56)	48 (41–56)	47 (37.5–54)	0.614
SDMT score (median, quartile)	42 (34–48)	42 (34.5–47.5)	42.5 (34–48)	39.5 (31–45.3)	0.180

*Significant at 0.05.

MDS-UPDRS, Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale; RBDSQ: Rapid Eye Movement Sleep Behavior Disorder Screening Questionnaire; GDS: Geriatric Depression Scale; STAI: State-Trait Anxiety Inventory; QUIP: Questionnaire for Impulsive-Compulsive Disorders; SCOPA-AUT: Scales for Outcomes in Parkinson's Disease–Autonomic; ESS: Epworth Sleepiness Scale; SE-ADL: modified Schwab and England Activities of Daily Living scale; MoCA: Montreal Cognitive Assessment; BJLO: Benton Judgment of Line Orientation, 15-item version; HVLT: Hopkins Verbal Learning Test–Revised; LNS: Letter-Number Sequencing task; SF: modified Semantic Fluency scale; SDMT: Symbol Digit Modalities Test.

without a fifth-year visit. To minimize the influence of the loss of follow-up, we performed a sensitivity analysis that included only the 208 patients who completed a fifth-year visit. The 208 patients were reclassified into a new motor-nonmotor subtypes at baseline and during the follow-up based on the data of these 208 patients. The agreements between the old and new motor-nonmotor subtypes were compared and the kappa agreement indicated a substantial to excellent agreement (data not shown). The results of the agreement between the new baseline and follow-up subtypes were also similar to the previous results (**Supplementary Table 1**).

Logistic regression analysis showed that only baseline subtypes were the determinants of having the diffuse malignant subtype by the fifth-year visit. Having the diffuse malignant (OR = 26.00; 95% CI, 5.46–123.90; $p < 0.001$) or intermediate (OR = 4.00; 95% CI, 1.05–15.26; $p = 0.042$) subtype at baseline was associated with a higher risk of being classified as diffuse malignant by the fifth-year visit, compared to mild-motor–predominant patients.

Discussion

In the present study, we evaluated the agreement of clinical subtypes from baseline through five years of follow-up in a large multicenter cohort with early-stage PD. A fair degree of agreement was observed, and only 120 patients (57.7%) had the same subtype at baseline and at the fifth-year visit. The mild-motor–predominant subtype had the highest proportion of stable patients, followed by the intermediate and diffuse malignant subtypes. Although the motor-nonmotor subtype was inconsistent, having the intermediate or diffuse malignant

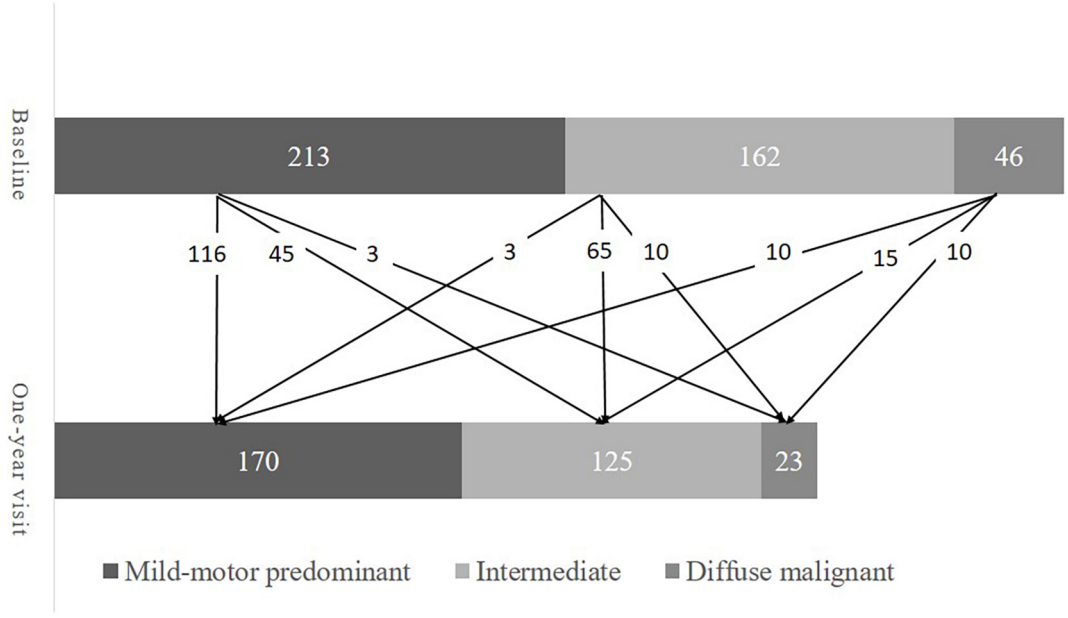

FIGURE 1
Subtype change from baseline to one-year visit.

TABLE 2 Consistency of subtypes during follow-up compared to baseline.

	Number	Motor-nonmotor subtype		Motor subtype	
		Kappa value ± SD	Total agreement	Kappa value ± SD	Total agreement
1-year visit	318	0.30 ± 0.09	192 (60.6%)	0.46 ± 0.09	229 (72.0%)
2-year visit	259	0.26 ± 0.10	148 (57.1%)	0.40 ± 0.10	181 (69.9%)
3-year visit	241	0.24 ± 0.11	136 (56.4%)	0.32 ± 0.10	160 (66.4%)
4-year visit	234	0.29 ± 0.11	139 (59.4%)	0.23 ± 0.10	143 (61.1%)
5-year visit	208	0.25 ± 0.12	120 (57.7%)	0.23 ± 0.10	122 (58.6%)

subtype at baseline was related to an increased risk of having the diffuse malignant subtype five years later.

The total agreement of motor-nonmotor subtype between baseline and follow-up (57.7%) was higher than the agreement of motor-nonmotor subtypes in a cohort with late-stage PD (35.3%) (Ygland Rodstrom and Puschmann, 2021). This study also had longer intervals between the baseline and re-subtyping, and only 38% of baseline patients were included in the re-subtyping (Ygland Rodstrom and Puschmann, 2021).

In the present study, the overall agreement of the motor subtypes decreased with disease progression, which is consistent with previous studies of motor subtypes (Kohat et al., 2021). Shifts in both directions (i.e., from less to more severe, and vice versa) were observed, and shifts in subtype were influenced by the degree of disease progression during follow-up. Those with faster deterioration tended to transfer from the mild subtype to the severe subtype, and those with lesser deterioration tended to transfer from the severe subtype to the mild subtype. Such bidirectional transfer has also been observed among the motor subtypes (Erro et al., 2019; Lee et al., 2019; Luo et al., 2019). Although patients tended to fall into the PIGD type in the advanced disease stage in the previous studies, some patients with the PIGD subtype at baseline were re-classified into the TD or indeterminate subtypes during follow-up because of increased tremor scores (Erro et al., 2019; von Coelln et al., 2021). In addition, compared to the motor subtype, the motor-nonmotor subtype showed a lower degree of consistency, even in the first year of follow-up, and had lower total agreements with baseline through the five years of follow-up.

In developing the subtyping method, one of the most important clinical considerations for us was that it could predict individual prognosis in the early stages of the disease. The prognostic value of the current motor-nonmotor subtype has been proven in patients with early- and late-stage PD (Fereshtehnejad et al., 2017; De Pablo-Fernandez et al., 2019; Ygland Rodstrom and Puschmann, 2021). Patients in these

studies with the intermediate or diffuse malignant subtype had a shorter progression time to dementia and death compared to mild-motor–predominant patients (De Pablo-Fernandez et al., 2019). These patients also had varying disease duration. The results of the present study indicate that there is a need to re-subtype and update the prognosis after even one year from baseline. In addition, the results of subtyping studies should be compared carefully, as they used different inclusion criteria and the characteristics of patients classified in each subtype varied between studies.

Another important aim of subtyping is to identify distinct disease entities (Fereshtehnejad and Postuma, 2017). In this regard, consistency in subtyping can be an important indicator of disease entities, but it has rarely been examined in subtyping studies (Hendricks and Khasawneh, 2021). Our results revealed that motor-nonmotor subtypes change from one to another during follow-up. Future studies using predefined methods or data-driven methods for clustering should confirm the consistency of these subtypes.

Internal and external limitations can explain the inconsistencies of the motor-nonmotor subtype. First, the definition of the motor-nonmotor subtype did not include the effects of treatment on the nonmotor symptoms. We used off-state data to eliminate the effects of dopamine replacement therapy on patients' motor symptoms. However, several nonmotor symptoms such as rapid eye movement sleep behavior disorder (RBD) and constipation can also be improved by drugs or non-medicinal treatments (Grimes et al., 2019). Treatment can decrease the severity reported by patients and decrease the differences between patients.

Moreover, patients who completed the fifth-year visit had lower MDS-UPDRS part II and part III scores compared to those without the fifth-year visit, which added to the inconsistency of the subtypes between baseline and follow-up. To test the influence of the loss of follow-up on baseline–follow-up consistency, we performed a sensitivity analysis that included only patients with a fifth-year visit. The result of this analysis showed similar results, i.e., that the consistency between the baseline and follow-up subtypes was not good. This indicated that the loss of follow-up was not the reason for the inconsistency.

Another important limitation was that the current motor-nonmotor subtyping criteria was based on an individual's position within the group. Individual subtype at follow-up was determined not only by individual progression, but also by the speed of deterioration of other patients in the group. This added difficulty in interpreting the shifts between the subtypes and reduced the applicability of motor-nonmotor subtyping methods in the follow-up. This limitation has also been found in other subtyping criteria using data-driven methods and cross-sectional data (Erro et al., 2016; Lawton et al., 2018; Zhang et al., 2019). Methods based on absolute values or ratios (such as the motor subtypes) would not be influenced by the progression of others in the group and are therefore more suitable for analyzing the consistency of subtypes in follow-up (Stebbins et al., 2013). We plan to develop a new subtyping method based on the absolute values or ratios of motor and nonmotor symptom scale scores and establish its applicability in the longitudinal cohort in future studies.

Our study also had several strengths. We used data from the same cohort that was used to develop the motor-nonmotor subtyping criteria. This is a high-quality longitudinal cohort, and its use therefore has the potential to increase the reliability of our results. We also adopted comprehensive assessments for the classification, which increased the accuracy of subtyping (Ygland Rodstrom and Puschmann, 2021).

In conclusion, we found that motor-nonmotor subtypes were not fixed, but rather changed during follow-up in patients with early-stage PD, and that the agreement of the motor-nonmotor subtypes was lower than that of the motor subtypes. The inconsistency of motor-nonmotor subtypes suggests that they are not distinct disease entities. Future clustering studies should devote more attention to the consistency of subtypes.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: All the data was available from the PPMI database with the consent of the PPMI Data and Publications Committee.

Ethics statement

The PPMI study was approved by the institutional review boards at each participating site. The patients/participants provided their written informed consent to participate in this study.

Author contributions

YX: statistical analysis, design, execution, review and critique, manuscript writing of the first draft, and review. QW, RO, YH, LZ, KL, JL, TY, and QJ: manuscript review. HS: statistical analysis review and critique and manuscript review. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnagi.2022.1040405/full#supplementary-material

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Proteomics analysis of the p.G849D variant in neurexin 2 alpha may reveal insight into Parkinson's disease pathobiology

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Parkinson's disease (PD), the fastest-growing neurological disorder globally, has a complex etiology. A previous study by our group identified the p.G849D variant in *neurexin 2* (*NRXN2*), encoding the synaptic protein, NRXN2α, as a possible causal variant of PD. Therefore, we aimed to perform functional studies using proteomics in an attempt to understand the biological pathways affected by the variant. We hypothesized that this may reveal insight into the pathobiology of PD. Wild-type and mutant NRXN2α plasmids were transfected into SH-SY5Y cells. Thereafter, total protein was extracted and prepared for mass spectrometry using a Thermo Scientific Fusion mass spectrometer equipped with a Nanospray Flex ionization source. The data were then interrogated against the UniProt *H. sapiens* database and afterward, pathway and enrichment analyses were performed using *in silico* tools. Overexpression of the wild-type protein led to the enrichment of proteins involved in neurodegenerative diseases, while overexpression of the mutant protein led to the decline of proteins involved in ribosomal functioning. Thus, we concluded that the wild-type NRXN2α may be involved in pathways related to the development of neurodegenerative disorders, and that biological processes related to the ribosome, transcription, and tRNA, specifically at the synapse, could be an important mechanism in PD. Future studies targeting translation at the synapse in PD could therefore provide further information on the pathobiology of the disease.

KEYWORDS

neurexin 2α (*NRXN2*), Parkinson's disease, proteomics, mass spectrometry, p.G849D, synaptic translation, mitochondrial dysfunction, ribosomal functioning

Introduction

Parkinson's disease (PD) is an incurable neurodegenerative disorder which primarily affects movement, resulting in bradykinesia, rigidity, postural instability, resting tremor, and a range of neuropsychiatric symptoms. Notably, it has been reported to be the fastest-growing neurological disorder globally (Feigin et al., 2017), affecting over 6 million people (Dorsey et al., 2018). Over the past two and a half decades, several genetic causes of PD have been identified, implicating various biological processes including mitochondrial dysfunction, toxic protein accumulation, dysfunctional vesicle recycling, and synaptic dysfunction in PD development (Panicker et al., 2021).

Recently, we reported the finding of a p.G849D variant in the neurexin 2 gene (*NRXN2*) in a South African multiplex PD family (Sebate et al., 2021). The translated protein, NRXN2α, is a synaptic regulation protein involved in processes such as calcium channel regulation, neuronal cell adhesion, and transmembrane signaling (Craig and Kang, 2007). There have been a limited number of studies on NRXN2α in disease, but a few have implicated the protein in neuronal and synaptic disorders (Missler et al., 2003; Dachtler et al., 2015). In addition, it has been shown that neurexins and their common binding partners, neuroligins, link synaptic dysfunction to cognitive disease (Südhof, 2008).

Here, we aimed to further investigate the effect of the p.G849D variant on biological pathways by using a proteomics approach in an SH-SY5Y cellular model of PD, transfected with wild-type and mutant NRXN2α plasmids. To this end, we examined the total proteome of the different treatment groups in an attempt to understand the changes in biological pathways. We hypothesized that overexpression of the wild-type NRXN2α would provide an indication of the pathways related to NRXN2α's function, while overexpression of the mutant NRXN2α could give an indication of the method of action by which it potentially leads to neurodegeneration. Together, these findings may provide a better understanding of the function of both the wild-type and mutant NRXN2α, and its possible involvement in PD.

Materials and methods

Ethical considerations

Ethical approval was obtained from the Health Research Ethics Committee (Protocol numbers 2002/C059 and S20/01/005 PhD) and the Research Ethics Committee: Biological and Environmental Safety (Protocol number BEE-2021-13149). Both committees are located at Stellenbosch University, Cape Town, South Africa.

Cell culture

SH-SY5Y cells were cultured in DMEM with high glucose (4.5 g/l) and 4 mM L-Glutamine (Lonza). In addition, the media was supplemented with 15% FBS (Gibco) and 1% penicillin/streptomycin (Sigma Aldrich). Cells were maintained at 37°C and 5% CO_2 in a humidified incubator (ESCO Technologies).

Plasmids

NRXN2α wild-type

The NRXN2α-ECFP-N1 plasmid is a kind gift from Prof. Ann Marie Craig (University of British Columbia, Canada). This plasmid expresses wild-type mouse NRXN2α-CFP and was generated as per Kang et al. (2008). The pECFP-N1 plasmid without an insert (empty vector) was a kind gift from Prof. Harald Sitte (Medical University of Vienna, Austria).

Site-directed mutagenesis

In order to generate the p.G849D mutant plasmid [p.G882D in our mouse model, mouse genomic position: 540693_chr19 (GRCm39)], site-directed mutagenesis was performed on the wild-type NRXN2α-ECFP-N1 plasmid using the Q5 Site-Directed Mutagenesis kit (New England Biolabs), as per the manufacturer's instructions. More information on the primers used and PCR conditions can be found in the Supplementary material.

Treatment groups

A total of four treatment groups were used for the analysis: (1) non-transfected cells (NT), (2) cells transfected with the wild-type plasmid (WT), (3) cells transfected with the mutant plasmid (MUT), and (4) cells transfected with the empty vector (EV). All treatments were performed in triplicate.

Transfection

SH-SY5Y cells were grown in sterile 25 cm^3 flasks until 70% confluent and transfected using Lipofectamine3000 (Invitrogen) as per the manufacturer's instructions. The transfection efficiency was determined by examining the cells under an Oxion Inverso Fluo E4 fluorescent microscope (Euromex) at 100x magnification for the presence of cyan fluorescent protein (CFP).

NRXN2α levels

Prior to proteomics analysis, NRXN2α protein levels were determined using immunofluorescent flow cytometry and measured with the Guava® Muse® Cell Analyzer (Luminex) to confirm overexpression of NRXN2α. Please see Supplementary material for more details.

Proteomics analysis

Protein extraction and clean-up

Cells were detached using Trypsin–EDTA and centrifuged at 2739 ×*g* for 5 min to collect cell pellets. Cell pellets were stored at −80°C until required. The pellets were then thawed in 100 mM Tris buffer pH 8 containing 0.5% sodium dodecyl sulfate (SDS, Sigma), 100 mM NaCl (Sigma), 5 mM triscarboxyethyl phosphine (TCEP, Sigma), protease inhibitor cocktail (Thermo Fisher), and 2 mM EDTA (Thermo Fisher). Once thawed, the pellets were submerged in an ice-cold sonic bath for 30 s prior to vortexing for 30 s. This cycle was repeated three times and the pellets were completely dissolved. Extraction reagents were then removed using a chloroform-methanol–water liquid–liquid extraction method. More information on the protein extraction, on-bead digest and liquid chromatography performed in preparation for mass spectrometry, can be found in the Supplementary material.

Mass spectrometry

Mass spectrometry was performed by Stellenbosch University's Central Analytical Facilities (CAF) using a Thermo Scientific Fusion mass spectrometer equipped with a Nanospray Flex ionization source. The sample was introduced through a stainless-steel emitter. Data were collected in positive mode with spray voltage set to 1.8 kV and ion transfer capillary set to 280°C. Spectra were internally calibrated using polysiloxane ions at m/z = 445.12003 and 371.10024. MS1 scans were performed using the Orbitrap detector set at 120,000 resolution over the scan range 350–1,650 with automatic gain control (AGC) target at 3 E5 and maximum injection time of 40 milliseconds. Data were acquired in profile mode.

MS2 acquisitions were performed using monoisotopic precursor selection for ion with charges +2 − +7 with error tolerance set to ± 10 ppm. Precursor ions were excluded from fragmentation once for a period of 60 s. Precursor ions were selected for fragmentation in higher-energy C-trap dissociation (HCD) mode using the quadrupole mass analyzer with HCD energy set to 32.5%. Fragment ions were detected in the Orbitrap mass analyzer set to 30,000 resolution. The AGC target was set to 5E4 and the maximum injection time to 80 milliseconds. The data were acquired in centroid mode.

Data analysis

The raw files generated by the mass spectrometer were imported into Proteome Discoverer v1.4 (Thermo Fisher) and processed using the SequestHT algorithm. Database interrogation was performed against the UniProt *H. Sapiens* database concatenated with the cRAP contaminant protein database.[1] Semi-tryptic cleavage with 2 missed cleavages was allowed for. Precursor mass tolerance was set to 10 ppm and fragment mass tolerance set to 0.02 Da. Deamidation (NQ) and oxidation (M) were allowed as dynamic modifications and thiomethyl of C as static modification. Peptide validation was performed using the Target-Decoy PSM validator node. The results files were imported into Scaffold 1.4.4 (Searle, 2010) and identified peptides validated with X!Tandem and the Peptide and Protein Prophet algorithms included in Scaffold. Quantitation was performed by Scaffold after one-way ANOVA and Student's *t*-test were performed.

Pathway analysis and enrichment analysis

First, the data for the separate treatment groups were combined into a Venn diagram using Venny 2.1[2] (Oliveros, 2015) to identify proteins unique to each treatment group. Thereafter, in order to identify differentially abundant proteins, the data were compared as follows: empty vector transfected cells vs non-transfected cells (EV vs NT), wild-type transfected cells vs non-transfected cells (WT vs NT), mutant transfected cells vs non-transfected cells (MUT vs NT), and mutant transfected cells vs wild-type transfected cells (MUT vs WT). Functional information for unique and differentially abundant proteins was obtained from UniProt[3] (The UniProt Consortium, 2021). Pathway analysis was conducted using the KEGG[4] (Kanehisa et al., 2021) and STRING[5] (Szklarczyk et al., 2019) databases. Each protein set was then uploaded to WebGestalt[6] (Liao et al., 2019) for enrichment analysis as per the default parameters.

Results

Overexpression of NRXN2α

Transfection efficiency, determined by evaluating CFP microscopically, was 68% for the wild-type construct, 65% for the mutant construct, and 67% for the empty vector construct. In addition, overexpression of NRXN2α was confirmed using the Guava® Muse® Cell Analyzer (Luminex). There was a 26 and 21% increase in NRXN2α levels in the wild-type and mutant samples, respectively, with no change in the empty vector sample when compared to non-transfected cells (Supplementary Figure S1).

Total proteins identified

The total ion chromatograms in Supplementary Figure S2 show the successful digestion of peptides in each sample as well

1 https://www.thegpm.org/crap

2 https://bioinfogp.cnb.csic.es/tools/venny
3 https://www.uniprot.org
4 https://www.kegg.jp
5 https://string-db.org
6 http://www.webgestalt.org

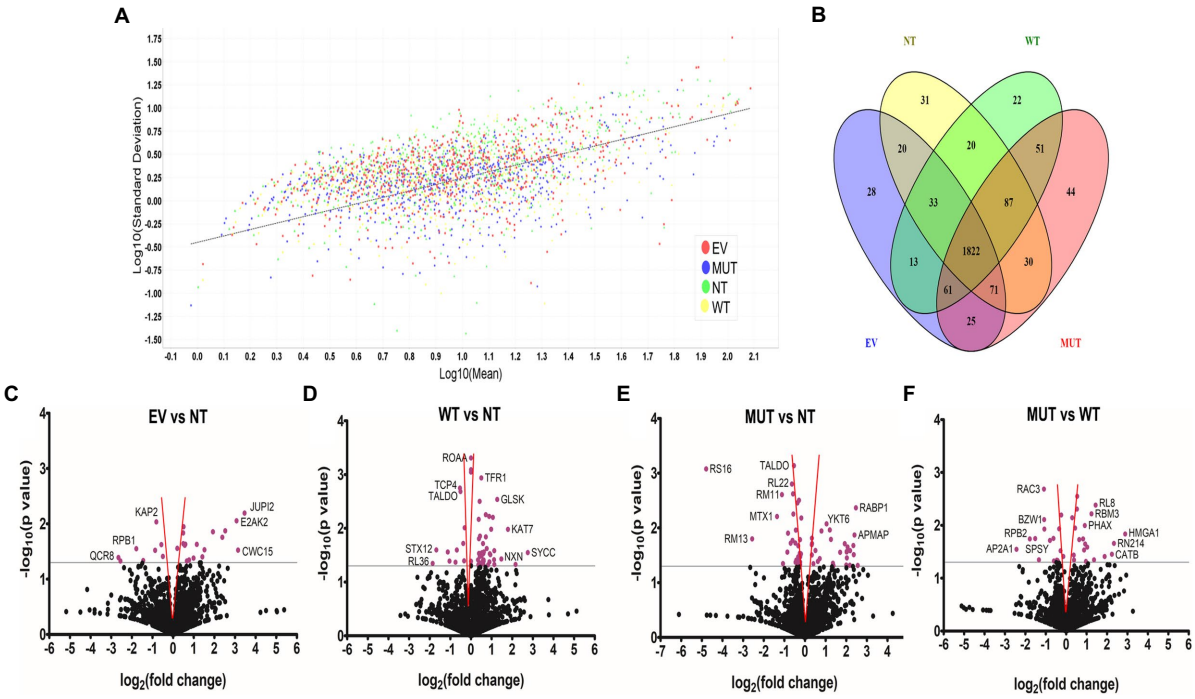

FIGURE 1
Unique and differentially abundant proteins identified in this experiment. **(A)** Scatterplot of the standard deviation (log_{10}) vs mean (log_{10}) shows good clustering of all samples along the linear regression line. Graph generated by Scaffold 1.4.4. **(B)** Venn diagram of the total proteins identified in each treatment group shows the distribution of shared proteins and numbers of proteins unique to each group. Diagram generated with Venny 2.1 (https://bioinfogp.cnb.csic.es/tools/venny; Oliveros, 2015). Volcano plots showing differentially abundant proteins when comparing EV vs NT **(C)**, WT vs NT **(D)**, MUT vs NT **(E)**, and MUT vs WT **(F)**. The gray line indicates the significance threshold. Significant proteins ($p \leq 0.05$, Students *t*-test) are colored purple. Where possible, individual proteins have been labelled. Proteins found within the red funnel may become statistically insignificant with increased sample sizes. Graphs generated with GraphPad Prism® 5.02. Abbreviations: EV: empty vector transfected cells, MUT: mutant transfected cells; NT: non-transfected cells; WT: wild-type transfected cells.

as their protein profile. Quantitation and regression analysis performed in Scaffold showed that most data points clustered within one standard deviation from the mean and that all the sample sets showed the same grouping (Figure 1A), indicating a successful experiment. The total number of proteins detected was 2,667, 2,630, 2,691, and 2,646 for the non-transfected cells (NT), wild-type transfected cells (WT), mutant transfected cells (MUT), and empty vector transfected cells (EV), respectively. Since all treatments were performed in triplicate, a protein had to be present in a minimum of 2 replicates in order to be considered an identified protein.

Unique proteins in each group

The Venn diagram in Figure 1B shows that 1822 proteins were shared by all four treatment groups. NT cells had 31 unique proteins, while WT, MUT, and EV cells had 22, 44, and 28 unique proteins, respectively. Functional information for all of these proteins can be found in Supplementary Tables S1–S4. Each protein set was then uploaded to STRING[7] (Szklarczyk et al., 2019) for pathway analysis.

Enrichment terms obtained from STRING for the unique proteins in each treatment group are shown in Table 1. There were no enriched terms for the EV cells, so they were excluded from the table. The NT cells showed enrichment terms for "compound binding." "Acetylation" was the only term enriched for in the WT cells. STRING analysis of the proteins involved in acetylation shows that they are not predicted to interact with each other and do not form part of the same networks. Therefore, enrichment of "acetylation" in these cells is likely to be a chance finding, showing that the cells are undergoing modification upon transfection with the WT plasmid. However, since acetylation of proteins is also potentially implicated in neurodegenerative disorders, such as PD (Yakhine-Diop et al., 2019), it could also be an important mechanism of action for the WT NRXN2α. "Metabolic processes" were enriched in the MUT cells, which is also possibly a result of introducing the MUT NRXN2α into the

7 https://string-db.org

TABLE 1 Enrichment terms obtained from the STRING online tool for the unique proteins in each treatment group.

NT (no. of proteins)	WT (no. of proteins)	MUT (no. of proteins)
Heterocyclic compound binding (24)	Acetylation (12)	Cellular metabolic process (31)
Organic compound binding (24)		Macromolecule metabolic process (28)
		RNA processing (12)
		Metabolism of RNA (9)

STRING: https://string-db.org (Szklarczyk et al., 2019). NT: non-transfected cells; MUT: mutant transfected cells; and WT: wild-type transfected cells.

TABLE 2 The number of differentially abundant proteins found for each comparison.

Treatment Comparison	Total differentially abundant proteins (No.)	More abundant proteins (No.)	Less abundant proteins (No.)
EV vs NT	28	8	20
WT vs NT	52	11	41
MUT vs NT	61	31	30
MUT vs WT	37	16	21

EV: empty vector transfected cells; NT: non-transfected cells; MUT: mutant transfected cells; WT: wild-type transfected cells.

cells. Interestingly, terms related to RNA processes were also enriched in the MUT cells. These cells contain several unique proteins which are involved in RNA metabolism and processing, thus showing that there may be changes in transcription in these cells. Therefore, it is possible that the MUT protein is somehow disrupting RNA processing.

Differentially abundant proteins between groups

For downstream analyses, all proteins identified in the EV cells were then compared to those in the NT cells as a control since we speculate that the vector backbone should not cause significant changes to the cellular proteome. The proteins in the WT cells and MUT cells were then each compared to the NT to better understand their individual contributions to the proteome. Finally, the main analysis involved comparison of the MUT cells to the WT cells in an attempt to understand the effect of the p.G849D variant. Table 2 shows the number of differentially abundant proteins found for each comparison group, divided into those that are less abundant and those that are more abundant. Volcano plots representing the differentially abundant proteins in each analysis are shown in Figures 1C–F. Functional information for all of these proteins can be found in Supplementary Tables S5–S8.

Each protein set was uploaded to both KEGG[8] (Kanehisa et al., 2021) and STRING[9] (Szklarczyk et al., 2019) for pathway analysis. KEGG examines which pathways the proteins in each set are involved in, whereas STRING identifies the pathways that both the proteins and their immediate interactors are involved in.

STRING interaction diagrams are shown in Figure 2. As can be seen in the EV vs NT analysis (Figure 2A), there are not many interactions and most consist of only a few nodes. This shows that these protein networks are likely enriched by chance. The MUT vs NT analysis (Figure 2C) has the largest number of interactions, showing the potential of the MUT to influence cellular pathways. In the main analysis (MUT vs WT; Figure 2D), multiple proteins are shown to interact in succession, hinting that there is a single mode of action for the MUT NRXN2α. Each analysis using STRING also provided a set of enrichment terms which show which molecular mechanisms the generated protein network is involved in.

Enrichment terms from STRING were combined with those from KEGG and are shown in Table 3. "Metabolic pathways" are enriched across all analyses, suggesting that any treatment could have an effect on the general cellular metabolic pathways. In the EV vs NT analysis, "Alzheimer disease," "Huntington disease," and "Parkinson disease" were enriched. However, the same three proteins (QCR8, ATPD, and SNCA) were present in each group, showing that this may be a chance finding. "Alzheimer disease," "Amyotrophic lateral sclerosis," "Huntington disease," "prion disease," and "Parkinson disease" were enriched in both the WT vs NT and MUT vs NT analyses. When examining the MUT vs WT, the only enrichment term related to neurodegenerative disorders was "Huntington disease." However, "ribosome," "spliceosome," "mRNA surveillance pathway," and "nucleocytoplasmic transport" were also enriched in the MUT vs WT analysis. This may hint toward a mode of action of the mutant protein whereby protein translation and transport are affected by its overexpression.

In the MUT vs WT analysis, the only enrichment term related to neurodegenerative disorders was "Huntington disease." The three proteins involved in this pathway were AP2A1, RPB2, and SDHB. STRING analysis shows that these proteins are not predicted to interact with each other and do not form part of the same networks. Therefore, this enrichment is more likely to be a random result of introducing cDNA to the cells. However, "ribosome," "spliceosome," "mRNA surveillance pathway," and "nucleocytoplasmic transport" were also enriched in this analysis. RPL8, RPS6, RPS21, and RPS25 are shown to be part of the "ribosome." XPO2, PHAX, and PNN are involved in "nucleocytoplasmic transport." FUS, PNN, and PP1B are involved in the "mRNA surveillance pathway." FUS, RBMX, and PRP4 are involved in the "spliceosome." RPL8, RPS6, RPS21, and RPS25 are

8 https://www.kegg.jp

9 https://string-db.org

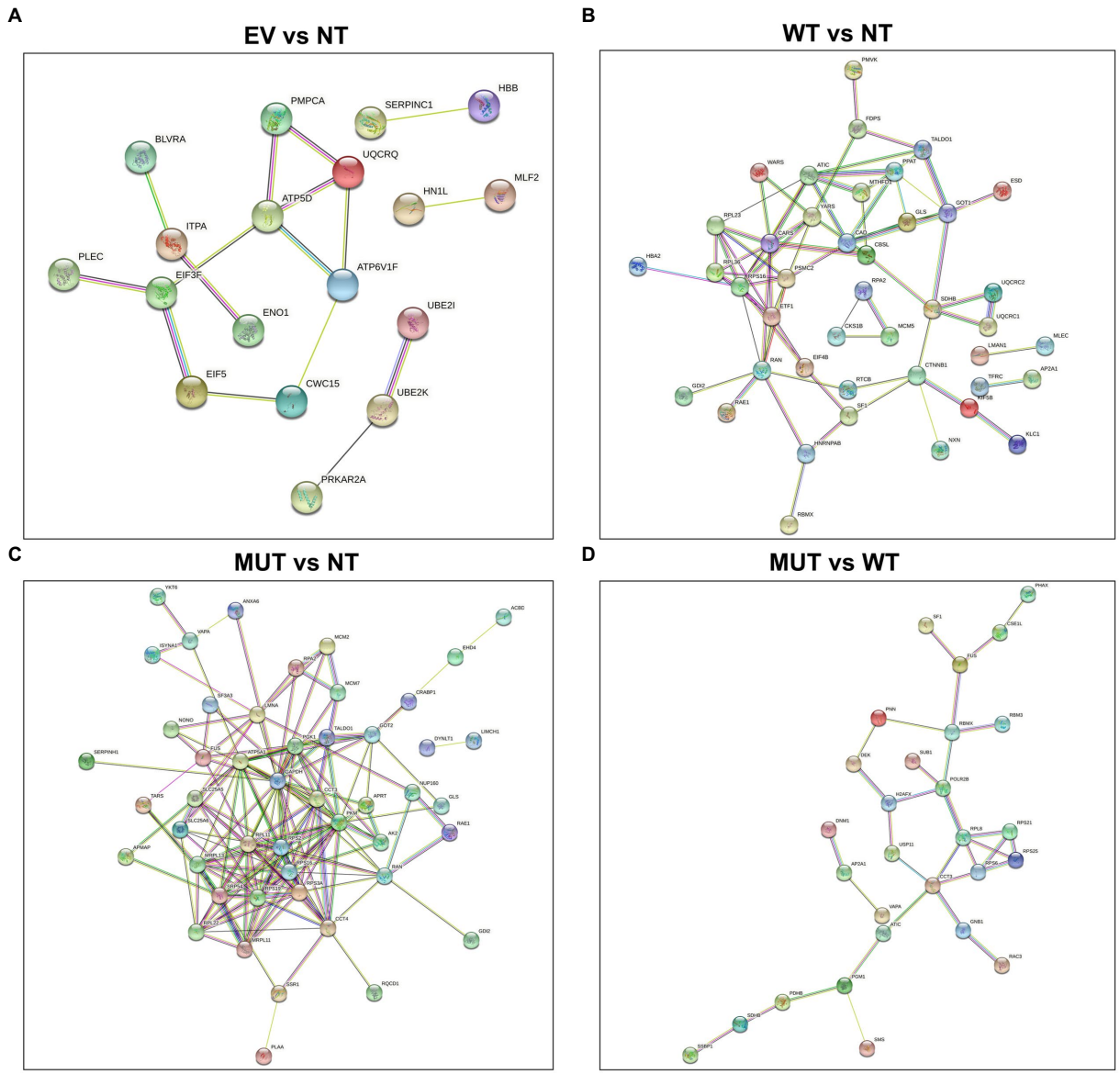

FIGURE 2
STRING protein–protein interaction diagrams of the differentially abundant proteins. **(A)** EV vs NT, **(B)** WT vs NT, **(C)** MUT vs NT, **(D)** MUT vs WT. Proteins which do not form part of an interaction network have been excluded. STRING: https://string-db.org (Szklarczyk et al., 2019). EV: empty vector transfected cells, MUT: mutant transfected cells; NT: non-transfected cells; WT: wild-type transfected cells.

all ribosomal subunits, involved in ribosomal and mRNA pathways.

STRING analysis shows that PHAX, XPO2, FUS, RBMX, and PNN potentially interact in sequence. PHAX is a phosphoprotein adaptor involved in RNA export and XPO2 plays a role in protein import/export within the nucleus. FUS and RBMX are both RNA-binding proteins. FUS plays a role in processes such as transcription regulation, RNA splicing, RNA transport, DNA repair, and damage responses, while RBMX plays several roles in the regulation of pre- and post-transcriptional processes. PNN is a transcriptional activator for the E-cadherin promoter gene, but it is also involved in RNA binding and mRNA splicing via the spliceosome. PRP4 is a U4/U6 small nuclear ribonucleoprotein which participates in pre-mRNA splicing. PP1B is a serine/threonine-protein phosphatase. Protein phosphatases are essential for cell division, and PP1B participates in the regulation of glycogen metabolism, muscle contractility, and protein synthesis. It has also been shown to be involved in the mRNA surveillance pathway. Taken together, the pathways of these proteins all relate back to the regulation of transcription, strongly suggesting that this process is being affected in the MUT cells.

TABLE 3 Enrichment terms obtained from the KEGG and STRING online tools for each protein set.

EV vs NT (no. of proteins)	WT vs NT (no. of proteins)	MUT vs NT (no. of proteins)	MUT vs WT (no. of proteins)
Metabolic pathways (7)	Metabolic pathways (15)	Metabolic pathways (12)	Metabolic pathways (5)
Alzheimer's disease (4)	Alzheimer's disease (7)	Ribosome (8)	Coronavirus disease—COVID-19 (4)
Pathways of neurodegeneration—multiple diseases (3)	Amyotrophic lateral sclerosis (7)	Amyotrophic lateral sclerosis (5)	Ribosome (4)
Huntington's disease (3)	Huntington's disease (7)	Alzheimer's disease (5)	Alcoholism (3)
Oxidative phosphorylation (3)	Parkinson's disease (6)	Biosynthesis of amino acids (5)	Diabetic cardiomyopathy (3)
Parkinson's disease (3)	Prion disease (6)	Carbon metabolism (5)	Huntington's disease (3)
	Alanine, aspartate and glutamate metabolism (4)	Pathways of neurodegeneration— multiple diseases (5)	mRNA Surveillance Pathway (3)
	Aminoacyl-tRNA biosynthesis (3)	Salmonella infection (5)	Nucleocytoplasmic transport (3)
	Cysteine and methionine metabolism (3)	Diabetic cardiomyopathy (4)	Spliceosome (3)
	Carbon metabolism (3)	DNA replication (4)	
		Huntington's disease (4)	
		Parkinson's disease (4)	
		Prion disease (4)	

KEGG: https://www.kegg.jp (Kanehisa et al., 2021), STRING: https://string-db.org (Szklarczyk et al., 2019). EV: empty vector transfected cells; NT: non-transfected cells, MUT: mutant transfected cells; and WT: wild-type transfected cells.

Enrichment analysis

WebGestalt facilitates the uploading of a gene or protein set and the corresponding fold changes and then provides enriched Gene Ontology (GO) terms. The graphical results of this analysis are shown in Supplementary Figure S3. "Negative enrichment" scores refer to GO terms that are downregulated or suppressed, while "positive enrichment" scores refer to GO terms that are upregulated or promoted. No specific enrichment was observed for the EV vs NT analysis, suggesting that transfecting the cells with the empty vector had a minimal effect.

In the WT vs NT analysis, the highest positive enrichment was for "aminoacyl-tRNA biosynthesis." Interestingly, "oxidative phosphorylation," "Alzheimer disease" and "Parkinson disease" also showed positive enrichment scores. The mitochondrial proteins QCR1, QCR2, and SDHB were the ones enriched in all these pathways, again showing that the mitochondrial dysfunction may be affected in the WT cells.

In the MUT vs NT analysis, "metabolic pathways" was positively enriched with the increased abundance of NADC, a protein involved in the catabolism of quinolinic acid. In the MUT vs WT analysis "Huntington disease" and "metabolic pathways" were negatively enriched and had false discovery rates (FDRs) lower than 0.05. RPB2, SPSY, SDHB, PGM1, PUR9, and ODPB were negatively enriched for "metabolic pathways." All these proteins, except RPB2, are involved in carboxylic acid metabolism. AP2A1 and RPB2 were negatively enriched in "Huntington disease." AP2A1 is part of the adaptor protein complex 2 which functions in protein transport via transport vesicles. It is also involved in endolysosomal trafficking and is thus implicated in several neurodegenerative disorders (Müller, 2014; Heaton et al., 2020; Srinivasan et al., 2022). RPB2 is a subunit of DNA-dependent RNA polymerase II, and therefore, its main function is in RNA transcription.

Findings from our study suggest that transfecting cells with the plasmids is having an effect on ribosomal processes. Therefore, enrichment scores for the GO term "ribosome" across each analysis have been summarized in Supplementary Figure S4. In the WT vs NT analysis, only this GO term had an FDR $\leq$ 0.05, showing that it highly likely that it has been negatively enriched in this protein set. In the MUT vs NT analysis, "ribosome" was again negatively enriched and had an FDR $\leq$ 0.05. However, in the MUT vs WT analysis, "ribosome" was positively enriched, but its FDR was above 0.05. Still, dysregulated ribosomal functioning seems to be common among the analyses and may be an important biological process related to the NRXN2α protein.

Discussion

This exploratory analysis has revealed that wild-type NRXN2α may play a role in pathways related to neurodegenerative disorders. Since the transfection efficiency and NRXN2α levels between the WT and MUT were similar, we can be relatively confident that the proteomics analysis showed differences caused by the overexpressed proteins and not by other technical differences between the two groups. In addition, while overexpression of the empty vector plasmid did show similar enrichment terms to the other analyses (Table 2), when performing enrichment analysis for the EV transfected cells vs NT

cells, it can be seen that none of these terms were significantly enriched (Supplementary Figure S3A). Therefore, we postulate that the EV only had a minimal effect on the cells and the majority of changes in the other analyses are in fact due to the NRXN2α cDNA insert (WT or MUT).

Overexpression of the WT protein in SH-SY5Y cells led to the enrichment of proteins involved in neurodegenerative diseases, such as Alzheimer's disease, Amyotrophic lateral sclerosis, and Parkinson's disease. In particular, the enriched proteins were involved in mitochondrial and lysosomal functioning, which are known to be dysregulated in PD and other neurodegenerative disorders (Rego and Oliveira, 2003; Wang et al., 2018). Thus, the wild-type protein may be involved in pathways related to the development of neurodegenerative disorders, such as PD. This provides further evidence potentially implicating synaptic proteins in the pathobiology of PD. Overexpression of the MUT NRXN2α-CFP protein showed similar results. Proteins unique to the MUT cells were enriched for terms related to "ribosome." In addition, when directly comparing the WT transfected cells with the MUT transfected cells, terms related to "ribosome" were enriched. This may thus hint at a mode of action for the p.G849D mutant protein. Since the main function of the ribosome is translation of mRNA into protein, dysregulated translation could be implicated as a biological process involved in neurodegeneration. Furthermore, both cytoplasmic and mitochondrial ribosomal proteins were enriched. Indeed, it has been shown that if synaptic translation is dysregulated, mitochondrial physiology can be altered (Kuzniewska et al., 2020). In addition, EIF4G1, another protein implicated in PD, is known to be involved in protein translation processes (Chartier-Harlin et al., 2011). Furthermore, the DJ-1 and SYNJ1 proteins implicated in PD (Bonifati et al., 2003; Krebs et al., 2013) also have RNA-binding functions. DJ-1 acts to protect cells from oxidative stress and cell death by acting as an oxidative stress sensor and redox-sensitive chaperone and protease, while SYNJ1 is a phosphatase involved in synaptic vesicle endocytosis and neurotransmitter transport. A few studies have additionally identified mitochondrial ribosomal proteins in PD. Gaare et al. (2018) identified *MRPL4*, which encodes a component of the large mitochondrial ribosome subunit, in an analysis of two PD cohorts, while Billingsley et al. (2019) identified *MRPL43* and *MRPS34*, encoding components of the large and small mitochondrial ribosome subunits, using data from a PD genome-wide association study (GWAS). Both these studies thus link mtDNA translation to PD risk. Dysregulated mRNA translation can therefore be considered to play a role in PD pathogenesis (Martin, 2016). In addition, a recent RNA-sequencing analysis showed that there was differential expression of ribosomal-related pathways in their PD cohort (Hemmings et al., 2022). Therefore, it is plausible that synaptic translation could also be important in PD pathogenesis. Here, changes in translation could affect oxidative stress and the transport of neurotransmitters, thereby causing cells to be more susceptible to cell damage and death. In addition, a study on lymphoblasts generated from PD patients showed an overall downregulation of genes involved in protein synthesis (Annesley et al., 2022). Thus, recent literature has shown that dysregulated synaptic translation and mitochondrial dysfunction are linked (Kuzniewska et al., 2020), mitochondrial ribosomal proteins have been linked in a PD GWAS (Billingsley et al., 2019), pathways related to ribosomes are enriched in an RNA-sequencing analysis of a PD cohort (Hemmings et al., 2022), and that lymphoblasts generated from PD patients have dysregulated expression of genes involved in protein synthesis (Annesley et al., 2022). In addition, some of the known PD-associated proteins are also shown to have RNA or protein translation roles. This link between mRNA translation is poorly understood, but a few reviews have highlighted that restoring translation and proteostasis might be a useful target for new therapeutics (Correddu and Leung, 2019; Zhou et al., 2019). Impaired proteostasis at the synapse could also be important for PD (Nachman and Verstreken, 2022) while reduced synaptic activity and dysregulated extracellular matrix pathways have recently been reported in midbrain neurons from PD patients, providing evidence that synaptopathy is a general phenotype in PD (Stern et al., 2022). Thus, biological processes related to the ribosome, translation, and tRNA, specifically at the synapse, could possibly be an important molecular mechanism in PD pathobiology.

The strength of this study is that we examined the effect of overexpression of both the wild-type and mutant protein using a hypothesis-free approach. In this way, we were able to show that potential mode of action of the mutant protein but were also able to conclude that the wild-type protein is also involved in pathways related to neurodegeneration. Therefore, it is possible that any dysregulation of NRXN2α could potentially lead to neurodegeneration.

However, we also acknowledge several limitations, including the use of a commercial cell line for this study. While SH-SY5Y cells are good *in vitro* model for PD as they display a catecholaminergic phenotype, producing both dopamine and noradrenaline (Xicoy et al., 2017), there are always limitations when using cell lines to study a complex human disorder. Unfortunately, we were not able to obtain dermal fibroblast samples from the individuals harboring the *NRXN2* variant as an *ex-vivo* model for this study. We also acknowledge the limitations of overexpressing a murine gene in a human cell line. Therefore, in the future it would be important to repeat these experiments in fibroblasts from the patients or in animal models. Another limitation is the use of shotgun proteomics. Since this study is explorative, we investigated the total proteome to determine which biological pathways were being affected. However, it may be important to do more targeted proteomics work in future, such as looking into post-translational modifications as well as investigating phospho-proteomics to determine signaling changes. Indeed, several kinases and phosphatases were observed in the different analyses, therefore phospho-proteomics would be required to better understand the effect of these protein changes.

In conclusion, findings from this exploratory study possibly implicate the NRXN2α protein in neurodegenerative processes and show that synaptic ribosomal and translation processes may

be important in PD and/ or other neurodegenerative disorders. However, further validation of NRXN2α and the proteins implicated in synaptic ribosomal and translation processes in other models of PD or neurodegenerative disorders would be required to prove or disprove this hypothesis.

Data availability statement

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD036636 and 10.6019/PXD036636.

Ethics statement

Ethical approval was obtained from the Health Research Ethics Committee (Protocol numbers 2002/C059 and S20/01/005 PhD) and the Research Ethics Committee: Biological and Environmental Safety (Protocol number BEE-2021-13149). Both committees are located at Stellenbosch University, Cape Town, South Africa.

Author contributions

KC conducted all experiments, performed all analyses, and wrote the first draft of the manuscript. SF assisted with analysis of the mass spectrometry data. AM-N assisted with data processing. MV performed the protein extraction and mass spectrometry. RC assisted with writing and editing of the manuscript. KC and SB conceptualized the study and acquired funding. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

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Multifactorial assessment of Parkinson's disease course and outcomes using trajectory modeling in a multiethnic, multisite cohort – extension of the LONG-PD study

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Background: The severity, progression, and outcomes of motor and non-motor symptoms in Parkinson's disease (PD) are quite variable. Following PD cohorts holds promise for identifying predictors of disease severity and progression.

Methods: PD patients ($N = 871$) were enrolled at five sites. Enrollment occurred within 5 years of initial motor symptom onset. Disease progression was assessed annually for 2-to-10 years after onset. Group-based trajectory modeling was used to identify groups differing in disease progression. Models were developed for UPDRS-III scores, UPDRS-III tremor and bradykinesia-rigidity subscores, Hoehn & Yahr (H&Y) stage, Mini-Mental Status Exam (MMSE) scores, and UPDRS-III, H&Y and MMSE scores considered together. Predictors of trajectory-group membership were modeled simultaneously with the trajectories. Kaplan–Meier survival analysis evaluated survival free of PD outcomes.

Results: The best fitting models identified three groups. One showed a relatively benign, slowly progressing trajectory (Group 1), a second showed a moderate, intermediately progressing trajectory (Group 2), and a third showed a more severe, rapidly progressing trajectory (Group 3). Stable trajectory-group membership occurred relatively early in the disease course, 5 years after initial motor symptom. Predictors of intermediate and more severe trajectory-group membership varied across the single variable models and the multivariable model jointly considering UPDRS-III, H&Y and MMSE scores. In the multivariable model, membership in Group 2 (28.4% of patients), relative to Group 1 (50.5%), was associated with male sex, younger age-at-onset, fewer education-years, pesticide exposure,

absence of reported head injury, and akinetic/rigid subtype at initial presentation. Membership in Group 3 (21.3%), relative to Group 1, was associated with older age-at-onset, fewer education-years, pesticide exposure, and the absence of a tremor-predominant subtype at initial presentation. Persistent freezing, persistent falls, and cognitive impairment occurred earliest and more frequently in Group 3, later and less frequently in Group 2, and latest and least frequently in Group 1. Furthermore, autonomic complications, dysphagia, and psychosis occurred more frequently in Groups 2 and 3 than in Group 1.

Conclusion: Modeling disease course using multiple objective assessments over an extended follow-up duration identified groups that more accurately reflect differences in PD course, prognosis, and outcomes than assessing single parameters over shorter intervals.

KEYWORDS

longitudinal monitoring, Parkinson's disease, group-based-trajectory model, motor symptoms, non-motor symptoms, disease outcomes

Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disease with an insidious onset and a long pre-symptomatic and symptomatic course. Disease onset is currently defined by the appearance of the cardinal motor symptoms, i.e., resting tremor, bradykinesia, rigidity, and postural instability. Manifestations of bradykinesia include hypokinetic dysarthria, oropharyngeal dysphagia, micrographia, hypomimia, reduced dexterity, stooped posture, difficulty arising from a chair/bed, shuffling gait, and general slowness of movement. Different motor disease subtypes have been described including tremor-predominant, akinetic/rigid-predominant and mixed subtypes (Schiess et al., 2000; Thenganatt and Jankovic, 2014). In addition to the motor symptoms, non-motor features, including cognitive dysfunction, anosmia, anxiety, depression, sleep disorders, and autonomic dysfunction may be present either alone or in varying combinations, and a non-motor dominant subtype of PD has been identified (Pavelka et al., 2022).

The symptomatic phase is preceded by a long prodromal phase (Mahlknecht et al., 2022; Borghammer, 2023; Elliott et al., 2023; Joza et al., 2023; Leite Silva et al., 2023). The combination and severity of prodromal symptoms can affect disease severity and progression after motor symptom onset.

The temporal profile of motor and non-motor symptom appearance and progression is rather variable. As discussed below, different cohorts have been followed longitudinally for varying lengths of time to identify predictors of disease progression. In the Primary Progression Markers Initiative (PPMI) cohort, higher baseline (Movement Disorders Society-Unified Parkinson's Disease Rating Scale [MDS-UPDRS]) motor score, male sex, and increased age, as well as a novel PD-specific epistatic interaction, were indicative of faster motor progression (Latourelle et al., 2017). In the same cohort, higher baseline non-motor scores were associated with female sex and a more severe motor phenotype. Longitudinal increase in non-motor score severity was associated with older age and lower cerebrospinal fluid (CSF) levels of Aβ1–42 at baseline (Schrag et al., 2017; Simuni et al., 2018). In addition, the postural instability gait disorder (PIGD) subtype was characterized by more severe disease manifestations at diagnosis, greater cognitive progression, and more frequent psychosis than in tremor-predominant patients (Aleksovski et al., 2018).

Analysis of the Tracking Parkinson's and Discovery cohorts reported four clusters: one with fast motor progression and symmetrical motor disease, poor olfaction, cognitive impairment and postural hypotension; a second with mild motor and non-motor disease and intermediate motor progression; a third with severe motor disease, poor psychological well-being and poor sleep with an intermediate motor progression; and a fourth with slow motor progression with tremor-dominant, unilateral disease (Lawton et al., 2018).

In the *De Novo* Parkinson cohort (DeNoPa; Mollenhauer et al., 2019), baseline predictors of more rapid progression of motor symptoms included male sex, orthostatic blood pressure drop, diagnosis of coronary artery disease, arterial hypertension, elevated serum uric acid, and elevated CSF neurofilament light chain. Predictors of cognitive decline included previous heavy alcohol use, diabetes mellitus, arterial hypertension, elevated periodic limb movement index during sleep, decreased hippocampal volume measured by magnetic resonance imaging (MRI), and higher serum uric acid, C-reactive protein, high-density-lipoprotein (HDL) cholesterol, and glucose levels at baseline. In this cohort, faster disease progression was associated with cardiovascular risk factors, poor diabetes control, higher serum uric acid levels, and inflammation.

A more recent comparison of the DeNoPa cohort and PPMI cohorts showed similar slopes of progression in both. Faster progression from baseline was associated with higher activities of daily living (ADL) scores and rigidity/bradykinesia subscores. In addition, freezing, and rigidity were predictors of faster deterioration in both cohorts (Bartl et al., 2022).

Comparing predictors of disease progression identified in the previously reported longitudinal cohorts reveals partial overlap but also differences. The lack of uniformity of predictors identified in the different cohorts may reflect differences in patient population characteristics, including genetic variation, contributing to the development and progression of PD, but also variability in how PD was assessed and different duration of follow-up. To improve our understanding of patterns of disease progression, identify predictors

associated with these patterns, and determine how these patterns are related to clinically significant milestones of disease progression, we extended our previous longitudinal analysis of PD patients (Markopoulou et al., 2020). In this analysis, we included an additional cohort, the LuxPark cohort, which was assessed using the same protocol as in the original study, and analyzed annual follow-up data obtained over a period of 2-to-10 years from the initial motor symptom. Three groups showing different patterns of disease progression were identified in group-based trajectory models (GBTMs) using UPDRS-III total score, tremor- and bradykinesia/rigidity subscores, Hoehn & Yahr (H&Y) stage, Mini-Mental Status Exam (MMSE) score, and UPDRS-III, H&Y and MMSE scores considered jointly. Assignment to a trajectory group remained stable 5 years after the initial motor symptom, at which time misclassification was low. In addition, we performed survival analysis to determine how the appearance of debilitating symptoms including dyskinesias, motor fluctuations, persistent falls, persistent freezing, dysphagia, persistent orthostatism, persistent urinary incontinence, cognitive impairment, psychosis, REM sleep behavior disorder (RBD), and impulse control disorder (ICD) were associated with different group trajectories.

Materials and methods

Patients and clinical data collection

Five sites participating within the Genetic Epidemiology of Parkinson's Disease (GEoPD) consortium (Farrer et al., 2021) contributed longitudinal data on patients with clinically probable or clinically definite PD (Bower criteria, Bower et al., 2000). These were: (1) Department of Neurology, St. Olav's Hospital, The Norwegian University of Science and Technology, Trondheim, Norway (NUST, $N = 77$); (2) Asan Medical Center, University of Ulsan College of Medicine, Seoul, South Korea (ASAN, $N = 270$); (3) Department of Neurology, University Hospital of Larissa, University of Thessaly, Larissa, Greece (UT, $N = 13$); (4) *The Luxembourg Parkinson's Study*, Luxembourg Institute of Health, Laboratoire National de Santé, Centre Hospitalier de Luxembourg, and Luxembourg Centre for Systems Biomedicine, University of Luxembourg, Belval, Luxembourg (LuxPark, $N = 103$); and (5) *The DodoNA Project: DNA Predictions to Improve Neurological Health*, Department of Neurology, NorthShore University HealthSystem, Evanston, IL USA (DodoNA, $N = 408$). Four sites (NUST, ASAN, UT, and DodoNA) had contributed data to previous analyses of LONG-PD data presented in Markopoulou et al., (2020). The current analyses included previously obtained data from the NUST site, new data from the LuxPark site and additional data collected from 2019 to 2022 by the ASAN, UT, and DodoNA sites.

For details of the study protocol, see Markopoulou et al. (2020). Salient features are summarized below. Investigators at each site entered initial- and annual-follow-up visit data into REDCap, a web-based database, or, due to data governance requirements, securely transmitted a spreadsheet containing their data. This cohort included both previously diagnosed and treatment naïve PD patients. Patients were enrolled at an initial clinical encounter if their first motor symptom occurred within 5 years of that encounter and the encounter resulted in a diagnosis of clinically probable or definite PD (Bower et al., 2000). In this study, we analyzed annual follow-up data through 10 years after initial motor symptom onset. Although patients were retained if their diagnosis at a subsequent annual visit changed to PD with dementia (PDD), they were excluded if their diagnosis changed to Lewy body dementia, drug-induced parkinsonism, multiple system atrophy, post-encephalitic parkinsonism, cerebrovascular disease with parkinsonism features, progressive supranuclear palsy, cortical basal syndrome, or parkinsonism-unspecified.

A family history of PD, dementia, or tremor was defined as having at least one first- or second-degree relative with the disease. Pesticide exposure, including any past or present, hobby, and/or occupational use, was self-reported. Head injury was self-reported and documented if the injury resulted in loss of consciousness or required medical attention. Cognitive status was assessed at the initial visit and annually thereafter. If a patient was cognitively impaired, a legally authorized representative provided relevant information. As previously described (Markopoulou et al., 2020), "MMSE score" reflects actual MMSE scores or Montreal Cognitive Assessment (MoCA) or Short Test of Mental Status (STMS) scores converted to MMSE scores using published normograms that allow interconversion (Roalf et al., 2013; van Steenoven et al., 2014; Townley et al., 2019), and UPDRS-III refers to MDS-UPDRS-III or UPDRS-III scores.

Disease trajectories

We used group-based trajectory modeling, a semi-parametric, model-based clustering method, to identify latent groups with similar longitudinal progression of PD (Jones and Nagin, 2007; Nagin and Odgers, 2010). As done previously (Markopoulou et al., 2020), trajectories were calculated starting from time point zero, defined as year of initial motor symptom appearance, reported at the initial clinical encounter. Trajectories assessing progression of motor impairment were generated for total UPDRS-III (motor) scores ($N = 871$), Hoehn & Yahr (H&Y) stage ($N = 871$), and using individual-item scores on the UPDRS-III (available only at the ASAN and DodoNA sites) ($N = 678$): UPDRS-III tremor subscores (the sum of questions 3–9) and bradykinesia-rigidity subscores (the sum of questions 1–2, 10–25, 27). Trajectories assessing cognitive function impairment were generated using Mini-Mental Status Exam (MMSE, $N = 870$, data missing for one patient) scores. To determine whether multiple assessment measures considered jointly would provide a more accurate characterization of disease progression and reliable association with disease outcomes, latent groups of patients were also identified by simultaneously assessing the trajectories of three variables: UPDRS-III, H&Y, and MMSE ($N = 870$).

We report the trajectories obtained when the *traj* plug-in in Stata/BE 17.0 (Jones and Nagin, 2013) was used to fit longitudinal data from the above six assessment measures to finite-mixture models. Models fit longitudinal data from patients who had between two and nine annual visits (maximum $N = 871$). The link function between the time and the assessment variable was censored-normal (a tobit model). Dropout was modeled for single-measure outcomes, but could not be included in the three-variable model jointly assessing UPDRS-III, H&Y, and MMSE.

In a basic trajectory model, the probability of trajectory group membership follows the multinomial logistic function. To understand which baseline characteristics were associated with trajectory-group membership, we generated models with predictors of group-membership probability relative to a reference group. In all analyses, the reference group was defined as the group with the most benign disease course, so that the odds ratios (OR) and 95% confidence intervals (95% CIs)

we report are relative to that group. In these models, the parameters measuring the association of the predictor variables with trajectory-group membership were estimated jointly with the parameters specifying the shapes of the trajectory. Continuous predictors were age at first motor symptom onset (AAO) and, in models utilizing MMSE score, years of education. The evaluated binomial predictors were restricted to variables present in at least 5% of patients. These included sex; history of pesticide exposure, head-injury, or diabetes; the presence of RBD (at the initial encounter); family history of PD, tremor, dementia; tremor-predominant subtype at initial presentation; and akinetic/rigid subtype at initial presentation. Since cohort sizes, demographic and clinical characteristics (Table 1), and outcomes (Supplementary Table S12) varied by study site, and genetic background may differ by study site, we also report results for trajectory models where study site, weighted by cohort size, was used as an additional predictor (covariate). Compared to models where study site was weighted by cohort size, models "dummy coding" each study site and evaluating it relative to the largest cohort, DodoNA gave identical trajectories, trajectory groups, and for predictors other than individual study sites, effect sizes and p values. In the latter models, some study sites were significant covariates due to the differences in cohort size. Years of education was always used as a predictor in these models.

Eight patients had undergone functional surgery for PD, specifically subthalamic nucleus deep-brain stimulation (DBS), at [median (MD)(range(R)): 7(5–10)] years after motor symptom onset. Since the number of patients with DBS was <1% of the total, and the inclusion of DBS as a time-varying covariate in all group-based trajectory models (GBTMs) was not significant, DBS therapy is not reported in this analysis.

Best-fitting models were identified following an iterative process utilizing a fit-criteria assessment plot (Klijn et al., 2017). Initially, the optimal number of latent trajectories, k, was identified based on the fit indices Bayesian Information Criterion (BIC), Aikake Information Criterion (AIC) and model-maximized likelihood. Here, the trajectory variable was fit to the same-order polynomial, typically cubic, with dropout modeled based on the prior two data entries, in models with $k = 2$-to-5. We required the smallest group to have >5% of patients to retain clinical relevance. With this restriction, the optimal k was chosen by identifying the model with fit indices, particularly BIC, nearest zero, and for each group, average posterior probability of group assignment ≥0.85 and odds of correct classification ≥5.0. Then, optimal polynomial terms were determined by evaluating the significance of zero-, first-, second- or third-order polynomials in modeling each trajectory. Finally, the significance and effect on model-fit of covariates were evaluated, and polynomial-fit was re-optimized. For each set of trajectories, we report each trajectory's convergence (number of patients assigned to their final trajectory placement) and misclassification (percent of patients not assigned to their final trajectory placement) over the follow-up period.

Associations between trajectories and PD outcomes

Kaplan–Meier survival analysis was used to evaluate whether the time from initial motor symptom onset to the first occurrence of one of 12 clinical outcomes differed across each set of trajectory-groups.

TABLE 1 Demographic and clinical characteristics of the LONG–PD cohort.

Characteristic[a]	Study site					All
	Norwegian University of Science and Technology	ASAN LONG–PD	University of Thessaly	LuxPark	DodoNA	
N (% of all)	77 (8.8)	270 (31.0)	13 (1.5)	103 (11.8)	408 (46.8)	871 (100)
Female, *N* (%)	28 (36.4)	138 (51.1)	6 (46.2)	37 (35.9)	131 (32.1)	340 (39.0)
Education–years, median (range)	12 (7–20)	12 (2–22)	6 (4–14)	13 (5–25)	16 (4–24)	14 (2–25)
Age at initial motor symptom onset, median (range)	66 (27–82)	62 (29–103)	67 (38–80)	63 (27–88)	70 (38–94)	66 (27–103)
Years from initial motor symptom onset at initial visit, median (range)	2 (0–5)	1 (0–4)	2 (0–5)	2 (0–5)	2 (0–5)	2 (0–5)
Follow-up interval in years, median (range)	6 (2–10)	5 (1–9)	4 (2–8)	7 (5–10)	6 (1–10)	6 (1–10)
Self-reported pesticide exposure, *N* (%)	3 (3.9)	64 (23.7)	4 (30.8)	69 (67.0)	76 (18.6)	216 (24.8)
Self-reported head injury, *N* (%)	7 (9.1)	22 (8.2)	0 (0)	22 (21.4)	154 (37.8)	205 (23.5)
Diabetes, *N* (%)	1 (1.3)	35 (13.0)	1 (7.7)	11 (10.7)	71 (17.4)	119 (13.7)
REM sleep behavior disorder at initial visit, *N* (%)	2 (2.6)	20 (7.4)	0 (0)	16 (15.5)	14 (3.4)	52 (6.0)
Family history of PD, *N* (%)	19 (24.7)	23 (8.3)	2 (15.4)	33 (32.0)	100 (24.5)	177 (20.3)
Family history of dementia, *N* (%)	4 (5.2)	29 (10.7)	2 (15.4)	29 (28.2)	114 (27.9)	178 (20.4)
Family history of tremor, *N* (%)	9 (11.7)	23 (8.5)	0 (0)	28 (27.2)	58 (14.2)	118 (13.6)
Tremor-predominant subtype (initial visit), *N* (%)	43 (55.8)	48 (17.8)	3 (23.1)	32 (31.1)	108 (26.5)	234 (26.9)
Akinetic/rigid subtype (initial visit), *N* (%)	21 (27.3)	205 (75.9)	7 (53.8)	45 (43.7)	117 (28.7)	395 (45.4)
Mixed subtype (initial visit), *N* (%)	13 (16.9)	17 (6.3)	3 (23.1)	26 (25.2)	183 (44.8)	242 (27.8)

[a]Except where noted, percent refers to the percent of patients at each site or the percent of all patients.

With the exception of ICD, we restricted these evaluations to clinical outcomes that occurred in at least 5% of all patients: motor fluctuations, dyskinesia, dysphagia, cognitive impairment, psychosis (hallucinations, paranoid ideations, and delusions), RBD (excluding patients with RBD at their initial encounter), persistent freezing, persistent falls, persistent orthostatism, and persistent urinary incontinence. In the LuxPark cohort, ICD was documented in 26% of participants (3.1% of all patients) during the 10-year follow-up reported here, but was documented later at other sites. We chose to analyze persistent freezing, falls, orthostatism, and urinary incontinence, with persistence defined as being present in ≥2 annual follow-ups, as persistent occurrence is more likely to reflect disease progression rather than a treatment effect. For each outcome, Kaplan–Meier survival curves are reported for each set of trajectory groups as well as the χ^2 and p values for a log-rank test evaluating differences across that set of trajectory groups. For log-rank tests significant at $p < 0.05$, we also report pairwise differences between trajectories that remained significant following a Bonferroni correction for multiple tests.

Results

Demographic and clinical characteristics

Figure 1 and Table 1 show demographic and clinical characteristics of our cohort. Table 1 also summarizes data on follow-up and variables used as predictors of group membership in trajectory models. Trajectories modeled in this analysis consider data from patients whose initial clinical encounter was within 5 years of their initial motor symptom [MD(R): 2(0–5)] and who were followed for up to 10 years [MD(R): 6(1–10) (Figures 1A,B, Table 1)]. The cohort displayed a wide range of AAO [MD(R): 66(27–103)]. About 10% had an AAO ≤ 50 yr., while nearly 70% had an AAO > 60 yr (Figure 1A). The distribution of patients at study enrollment by year from the initial motor symptom (duration), and their initial-visit UPDRS-III scores, H&Y stage and MMSE scores are shown in Figures 1C–F. As documented there, UPDRS-III score and H&Y stage differed by disease duration at the initial visit. More specifically, UPDRS-III score differed between patients with <1-yr and 3-yr duration (Figure 1E), while H&Y stage differed between patients having either <1 or 1-yr and 3-, 4-, or 5-yr duration (Figure 1D). At the initial visit, 26.9% of the cohort displayed a tremor-predominant subtype, 45.4% an akinetic/rigid subtype, and 27.8% a mixed subtype (Table 1).

Disease trajectories

With the goal of developing an accurate measure of PD progression that is related to PD outcomes, we first modeled the trajectories of individual assessment measures, and then compared these results to models where multiple assessment measures (UPDRS-III, H&Y, and MMSE) were considered jointly. Model-fit statistics supported the assignment of patients to three groups for both single and multiple assessment measures (Table 2, Supplementary Tables S1–S5). While model-fit criteria for some assessment measures identified a better fit to a greater number of groups, model fits with more than three groups violated the specification that, to retain clinical relevance, all groups must have membership >5%. Similar trajectory patterns and trajectory-group assignments were obtained when longitudinal data from 3-to-9 annual visits (maximum $N = 675$) were used, and when longitudinal data collected through disease-duration year eight, instead of year 10, were used. Compared to models using data through disease-duration year 10, trajectory-group assignments in models using data through disease-duration year eight were different for 4.3% (mean (M) of six models, R: 2.5%–6.2%) of patients.

Single-variable trajectory groups

In each motor (UPDRS-III total score, UPDRS-III tremor subscore, UPDRS-III bradykinesia-rigidity subscore, H&Y stage) and cognitive (MMSE score) GBTM, three trajectory-groups differed in disease severity and rate of progression (Supplementary Figures S1, S2). The trajectory of Group 1 was more benign, that of Group 2 was intermediate, and that of Group 3 was both more severe and more rapidly progressing. All but one of the trajectories converged (i.e., patients were assigned to their final trajectories) with <5% misclassification (i.e., <5% of patients reassigned to a different final trajectory) by 5 years after the initial motor symptom; the intermediate UPDRS-III score trajectory reached this point at 6 years. Very similar trajectories were identified when *study site* and *years of education* (for motor scores) were added as predictors (*cf.* Supplementary Figures S1–S17 and S2–S18). The trajectory-group assignments in the models with these additional predictors were different for 4.6% (M of five models, R: 2.1%–8.7%) of patients.

For each objective assessment, predictors of Group 2 or Group 3 membership relative to Group 1 (= reference group), which followed the more benign trajectory, were modeled jointly with the trajectories (Supplementary Tables S1–S5). Individuals with the tremor-predominant subtype at initial presentation were more likely to be in Group 1 for UPDRS-III total score, UPDRS-III bradykinesia-rigidity subscore, and H&Y stage, but not more likely to be in any Group for UPDRS-III tremor-predominant subscore. In contrast, individuals with the akinetic/rigid subtype at initial presentation were more likely to be in Group 2 or 3 for UPDRS-III total score and Group 3 for UPDRS-III bradykinesia-rigidity subscore, but Group 1 for UPDRS-III tremor subscore, as tremor is not a manifestation of the akinetic/rigid subtype.

Sex was predictive only for MMSE-score trajectory membership, with females more likely to be in Group 1. Older AAO was associated with membership in Group 2 or 3 for H&Y stage and for MMSE-score, and with membership in Group 3 for UPDRS-III bradykinesia-rigidity subscore. Individuals with fewer years of education were more likely to be in Group 2, and even more likely to be in Group 3, for MMSE score.

Individuals with self-reported pesticide exposure were more likely to be in Group 2, and even more likely to be in Group 3, for UPDRS-III total score. Individuals with self-reported prior head injury were more likely to be in Group 1 for UPDRS-III-total score, but more likely to be in Group 2 or 3 for MMSE score. Individuals with diabetes were more likely to be in Group 3 for UPDRS-III-total score, UPDRS-III-tremor subscore, and H&Y stage, and in Group 2 or 3 for MMSE score. Individuals with RBD at their initial encounter were more likely to be in Group 3 for UPDRS-III-total score. Interestingly, individuals

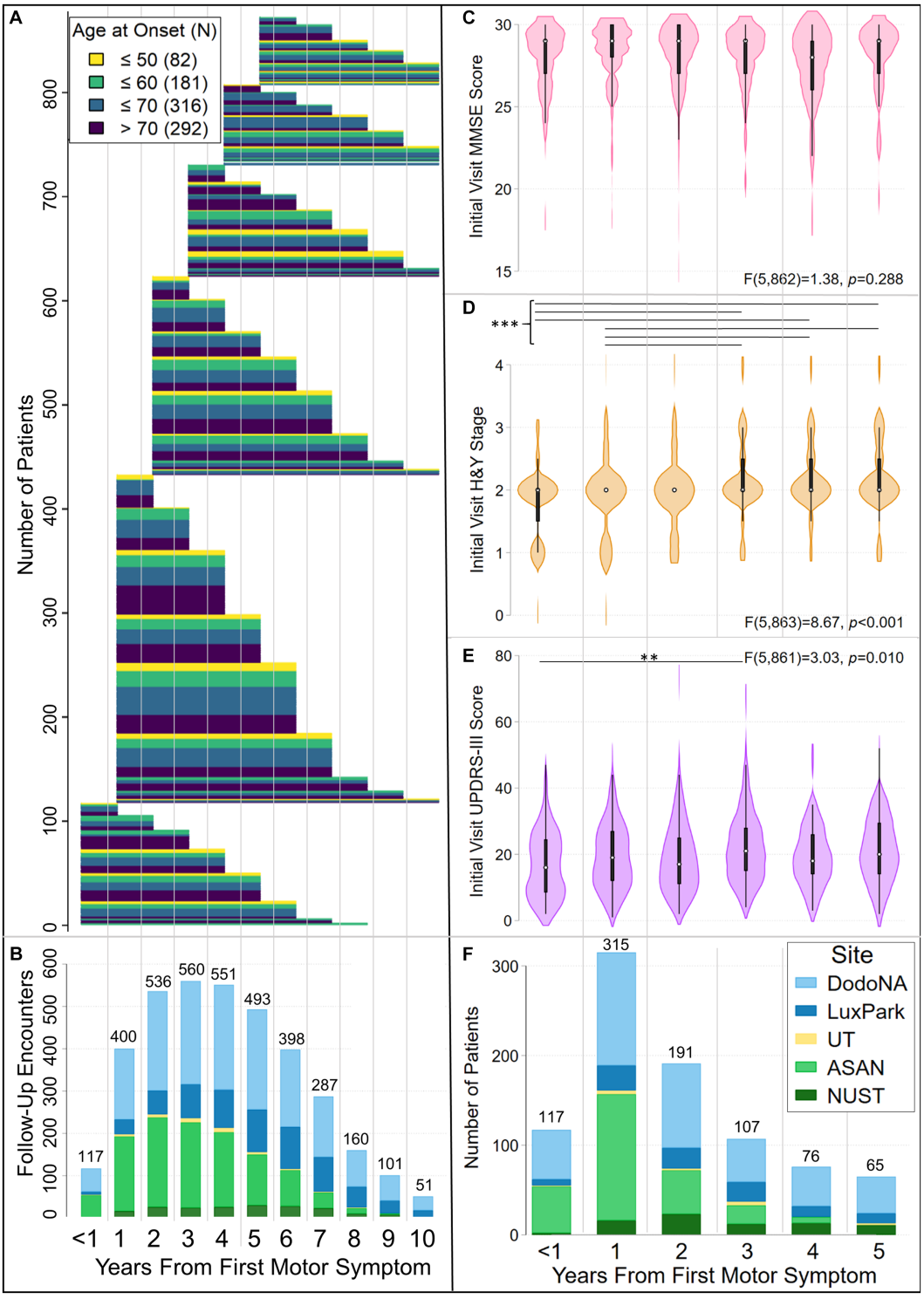

FIGURE 1
Characteristics of the LONG-PD cohort. Panels **(A)** and **(B)** show follow-up of LONG-PD participants by disease duration (years from initial motor symptom) and age at first motor symptom. Patients assessed in this analysis had motor symptom onset within 5 years of the initial clinical encounter and were followed for up to 10 years after the onset of the initial motor symptom. Panel **(A)** shows the duration of follow-up, color coded by age at onset. Panel **(B)** shows the total number of encounters per year, color coded by site. The <1 coordinate on the *x*-axis, which is jointly used by panels **(A)** and **(B)**, corresponds to initial clinical encounters that occurred within a year of the appearance of the initial motor symptom. To facilitate comparison of data in panels **(A)** and **(B)**, thin gray vertical lines demarcate clinical encounters that occurred in subsequent years. Each patient's follow-up is depicted by a thin, horizontal line extending from when the initial encounter occurred relative to the initial motor symptom (left end) to the last year of follow-up (right end). Lines are colored by age group at onset (legend top left). Thick bars result from the merged lines of patients in the same age groups who were followed for the same length of time. **(B)** Bar chart illustrating the total number of patient encounters at each year of disease duration, color-coded by site [legend in panel **(F)**]. Violin plots (box plots modified with overlaid plots of the estimated kernel density) show the distribution of **(C)** MMSE scores, **(D)** Hoehn and Yahr (H&Y) stages and **(E)** UPDRS-III scores at the initial clinical encounter, by disease duration. In each

(Continued)

FIGURE 1 (Continued)
violin plot, the white dot identifies the median, the black rectangle the interquartile range, and the spikes extend to the upper- and lower-adjacent values. The results of one-way ANOVAs for MMSE scores, H&Y stages, and UPDRS-III scores by disease duration are noted. Horizontal lines identify differences between disease-duration groups that were significant after Bonferroni correction for multiple testing. Hoehn & Yahr stage differed at the initial clinical encounter between patients with durations of either <1 yr. or 1 yr. and 3-, 4-, or 5- yrs (***$p \leq 0.002$). UPDRS-III scores differed at the initial clinical encounter between patients with <1-yr and 3-yr duration (**$p = 0.005$). Panel **(F)** shows the total number of initial encounters by year from the initial motor symptom, color-coded by site. Panels **(C)**–**(F)** use the same X-axis. Thin gray vertical lines demarcate clinical encounters for each year.

TABLE 2 Groups identified in trajectory models jointly considering UPDRS-III, Hoehn & Yahr, and MMSE scores.

Group	1 (Reference)	2		3	
N (% of 870)	440 (50.5)	247 (28.4)		183 (21.0)	
Fit[a]					
UPDRS-III Hoehn & Yahr MMSE	Linear Linear Linear	Quadratic Linear Quadratic		Linear Linear Linear	
Average posterior probability	0.969	0.935		0.950	
Odds of correct classification[b]	31.20	37.08		69.90	
Observed probability[c]	0.505	0.281		0.213	
Baseline characteristics associated with trajectory membership[d]		**OR [95% CI][d]**	***p*[d]**	**OR [95% CI][d]**	***p*[d]**
Female	–	**0.63 [0.41–0.98]**	**0.042**	1.18 [0.74–1.87]	0.483
Age at motor-symptom onset	–	**0.97 [0.95–0.99]**	**0.004**	**1.10 [1.07–1.13]**	**<0.001**
Years of education	–	**0.83 [0.78–0.88]**	**<0.001**	**0.88 [0.83–0.93]**	**<0.001**
Medical history					
Pesticide exposure	–	**2.52 [1.60–3.96]**	**<0.001**	**1.80 [1.08–3.00]**	**0.022**
Head injury	–	**0.49 [0.28–0.85]**	**0.012**	1.20 [0.73–1.95]	0.456
Diabetes	–	1.12 [0.61–2.06]	0.707	1.75 [0.97–3.18]	0.062
REM sleep behavior disorder	–	1.93 [0.90–4.13]	0.091	0.96 [0.33–2.76]	0.947
Family history					
Parkinson's disease	–	1.03 [0.62–1.72]	0.882	1.21 [0.71–2.07]	0.467
Dementia	–	0.79 [0.47–1.30]	0.361	0.63 [0.37–1.09]	0.105
Tremor	–	1.33 [0.75–2.35]	0.326	0.91 [0.46–1.82]	0.807
Initial presentation					
Tremor-predominant	–	0.95 [0.52–1.73]	0.871	**0.29 [0.16–0.54]**	**<0.001**
Akinetic/rigid predominant	–	**3.21 [1.86–5.51]**	**<0.001**	1.05 [0.63–1.75]	0.835

OR, odds ratio; 95% CI, 95% confidence interval. OR, 95% CI, and *p* values are in bold if $p < 0.05$.
[a]Modeled using a censored normal probability distribution for the dependent variable.
[b]Based on the weighted posterior probability.
[c]Group probability based on the posterior probabilities.
[d]Compared to membership in reference trajectory, all models are censored normal.

with a family history of dementia were more likely to be in Group 1 for UPDRS-III-total score.

When *study site* and *years of education* (for motor-score models) were added as covariates in these trajectory models, several important predictors of group membership in models without these covariates were retained (*cf.* Supplementary Tables S1–S5 to S6–S10, respectively). In both types of models, older age at motor-symptom onset was associated with membership in Group 3 for UPDRS-III bradykinesia-rigidity subscore, and in Groups 2 or 3 for H&Y and MMSE scores. A tremor-predominant initial presentation was less likely in Group 2 for UPDRS-III score and in Groups 2 or 3 for UPDRS-III bradykinesia-rigidity subscore and H&Y stage. Patients living with diabetes were more likely to be in Group 3 for UPDRS-III, UPDRS-III tremor subscore, and H&Y stage and in Group 2 for MMSE scores. Females were less likely to be in Group 3 for MMSE score.

Some associations gained significance when *study site* and *years of education* were included. Older age at motor-symptom onset was associated with membership in Group 2 or 3 for UPDRS-III score and in Group 2 for UPDRS-III bradykinesia-rigidity subscore. A tremor-predominant subtype at initial presentation was less likely to be in Group 3 for UPDRS-III score. Patients living with diabetes were more likely to be in Group 2 for

H&Y stage. Females were less likely to be in Group 3 for UPDRS-III and UPDRS-III bradykinesia-rigidity subscores. Fewer years of education were associated with membership in Group 3 for UPDRS-III score. Head injury was associated with Group 2 and 3 UPDRS-III tremor subscores.

Some associations lost significance when *study site* and *years of education* were included. Age at motor-symptom onset was not associated with membership in Group 2 for UPDRS-III tremor-subscore. Patients living with diabetes were not more likely to be in Group 3 for MMSE score. Head injury was not associated with Group 2 or 3 MMSE or UPDRS-III scores. Pesticide exposure was not associated with UPDRS-III Group 2 or 3. Family history of PD, dementia, or tremor were not associated with any trajectory group. An akinetic/rigid predominant subtype at initial presentation was not associated with Group 2 or 3 for UPDRS-III score or Group 3 for UPDRS-III bradykinesia-rigidity subscore.

Multivariable (UPDRS-III score, Hoehn & Yahr Stage, MMSE score) trajectories

The trajectories observed in the three groups identified when three variables (UPDRS-III total score, H&Y stage, MMSE score) were simultaneously modeled, generally followed the patterns identified in single-variable trajectories (Figure 2A). The trajectories modeled for Group 1 (50.6%), showed relatively benign progression in all three measures. Compared to Group 1, Group 3 (21.3%) showed a much faster rate of progression of motor dysfunction, disease stage, and cognitive decline. In contrast to Groups 1 and 3, Group 2 (28.1%) showed a faster rate of progression of both UPDRS-III and MMSE scores after year five. Group membership appeared stable with <5% likelihood of misclassification 5 years from motor symptom onset (Figures 2B,C).

Predictors of Group 2 or 3 membership relative to the more benign Group 1 (=reference group) are shown in Table 2. Individuals with the tremor-predominant subtype at initial presentation were less likely to be in Group 3, whereas individuals with the akinetic/rigid subtype at initial presentation were more likely to be in Group 2.

Females were less likely to be in Group 2 than in Group 1. Older AAO increased the likelihood for membership in Group 3, but decreased the likelihood for membership in Group 2. Individuals with fewer years of education were more likely to be in Group 2 or 3.

Individuals with self-reported pesticide exposure were more likely to be in Group 2 or 3; however, individuals with self-reported prior head injury were less likely to be in Group 2.

When *study site* was added as a covariate in this trajectory model, the trajectories remained very similar, and each trajectory group included a similar percentage of patients (*cf.* Table 2 to Supplementary Table S11; Figure 2 to Supplementary Figure S14). Three important characteristics – age at motor-symptom onset, years of education, and tremor-predominant disease subtype—remained significantly associated with group membership. Older age at motor-symptom onset was associated with both Groups 2 and 3, fewer years of education was associated with Group 3, and a tremor-predominant subtype at initial presentation was less likely in Group 3. However, sex, pesticide exposure, head injury, and akinetic/rigid predominant subtype were not associated with group membership.

Association of disease outcomes with single and multivariable trajectories

We used Kaplan–Meier survival analyses to evaluate whether survival free of each of 11 PD outcomes differed significantly across the three groups defined by each trajectory analysis. If a log-rank test identified differences in an outcome across the three groups, pairwise group differences were evaluated using Bonferroni correction for multiple tests. Figures 3, 4 and Supplementary Figures S3–S13 show the results of these analyses. Three patterns of outcome-free survival were common in both single and multivariable trajectory groups. In the first, the outcome was poorest in Group 3 (severe disease trajectory), less poor in Group 2 (intermediate trajectory) and least poor in Group 1 (most benign trajectory). In the second, the outcome was similar in Groups 2 and 3 but poorer than in Group 1. In the third, the outcome was poorest in Group 3, and similar but less poor in Groups 1 and 2. Table 3 summarizes the patterns observed for each outcome across the groups defined by the trajectory analyses.

While motor fluctuations and dyskinesias were complications of therapy that occurred in more than 12% of our patients (Supplementary Table S6), survival free of these outcomes varied relatively little across the groups identified by either single or multivariable trajectory analyses. Outcomes for motor fluctuations were poorer in Group 3 than Group 1 for H&Y stage (Supplementary Figure S3). In contrast, outcomes for dyskinesias were poorer for UPDRS-III-tremor-subscore in Group 1 than in Group 3 (Supplementary Figure S4).

All outcomes other than complications of levodopa therapy varied across the groups defined by the multivariable and the UPDRS-III-total-score trajectory models (Table 3, Figures 3, 4, Supplementary Figures S5–S13). However, they did not always vary across the groups defined by other single-variable trajectory models (e.g., H&Y-stage or MMSE-score GBTMs). Across the three groups defined in the multivariable trajectory model, the outcomes of persistent freezing (9.9% of patients, Supplementary Table S6), persistent falls (10.3%), and cognitive impairment (32.8%) was poorest in Group 3, less poor in Group 2, and least poor in Group 1 (Figure 3). That is, the severity of these outcomes paralleled the severity of disease course in the groups defined by the multivariable trajectory model. In contrast, the outcomes of dysphagia (17.6%) and psychosis (6.9%) in Groups 2 and 3 were similar but poorer than in Group 1 (Figure 3). This pattern was also seen for autonomic dysfunction outcomes: persistent orthostatism (10.3%), and persistent urinary incontinence (14.2%) also had similar outcomes in Groups 2 and 3 that were poorer than in Group 1 (Figure 4). The difference in outcomes for axial symptoms such as persistent freezing of gait and falls, and outcomes such as orthostatism and urinary incontinence may reflect response to treatment. While axial symptoms are treatment-resistant, other features are treatment-responsive at least for part of the disease course. A different pattern was seen for the outcomes of RBD (33.9%, analysis restricted to patients without RBD at the initial encounter) and ICD (3.1%). These outcomes were poorer in Group 2 than in Groups 1 and 3 (Supplementary Figures S10, S13).

Very similar patterns of disease outcomes across a set of trajectory groups were seen when trajectory groups were modeled together with the additional predictors *study site* and *years-of-education* (*cf.* Table 3 to Supplementary Table S13, Figures 3, 4,

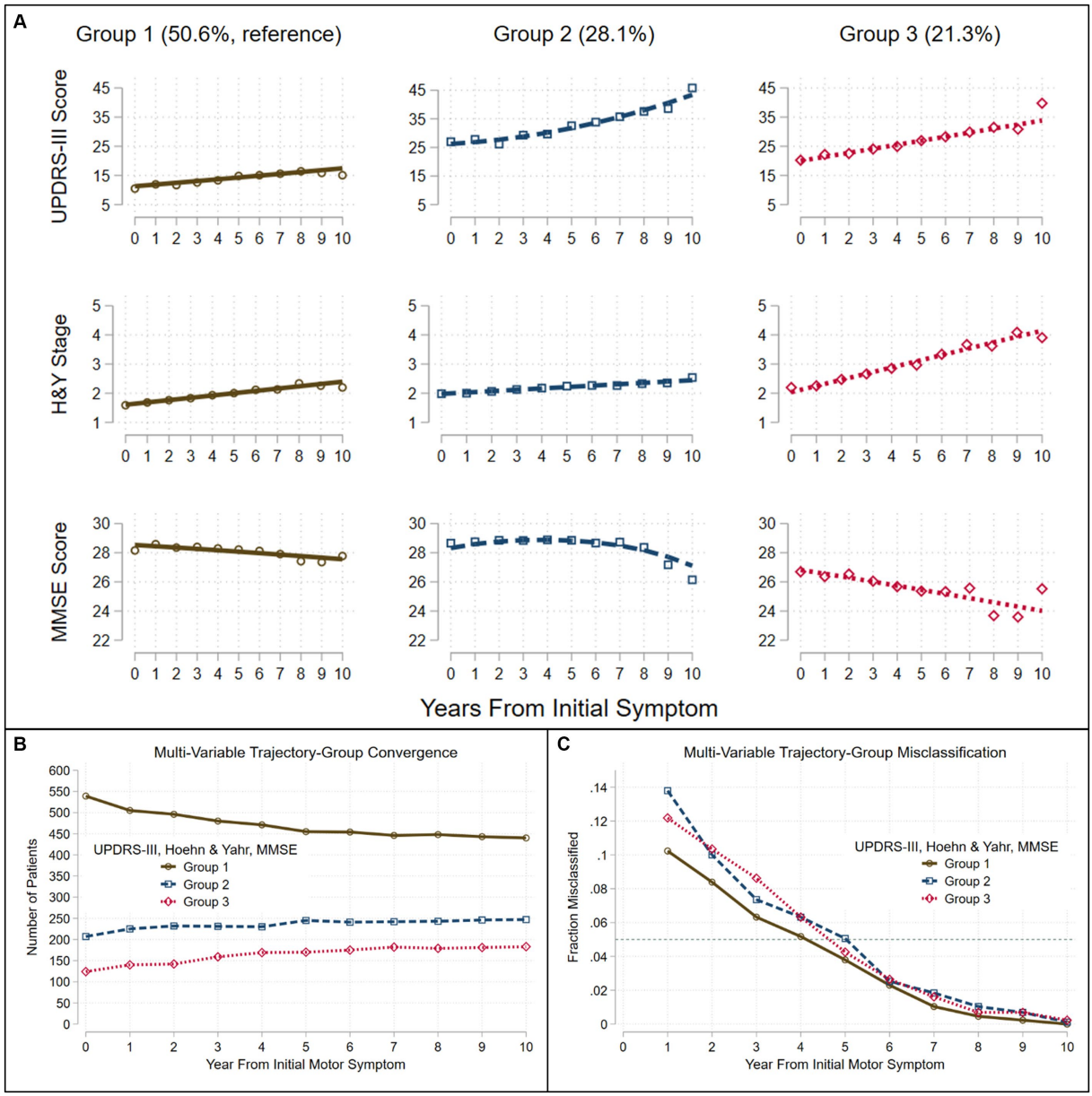

FIGURE 2
Trajectories seen in the LONG-PD cohort when three assessments are modeled jointly. Group-based trajectory modeling using UPDRS-III score, Hoehn and Yahr (H&Y) Stage and Mini-Mental Status Exam (MMSE) score identified three groups **(A)**. Assignment to group-membership trajectories converge **(B)** with <5% misclassification **(C)** (dashed teal line) by about 5 years after the onset of the initial motor symptom. Trajectories were modeled jointly with the predictors: sex, age at motor-symptom onset, education-years, pesticide exposure, head injury, diabetes, REM-behavior sleep disorder, family history (Parkinson's disease, dementia, or tremor), and initial presentation (tremor-predominant, akinetic/rigid predominant).

and Supplementary Figures S3–S13 to Supplementary Figures S15, S16, and S19–S29, respectively). When differences in these patterns were seen, they usually reflected whether a single intergroup difference gained or lost significance following a Bonferroni correction for multiple tests. It is notable that Group 3 remains distinct in that it is associated with the poorest outcomes regardless of whether *study site* and *years of education* were included as covariates.

The groups defined by the multivariable trajectory model better delineated disease outcomes than did the groups defined by the UPDRS-III-total-score trajectory model, even though outcomes in the groups defined by both models share similarities (Table 3, Figures 3, 4, Supplementary Figures S5–S13). More specifically, the benign disease group defined by the multivariable trajectory model showed better outcomes than the intermediate/severe disease groups for persistent falls, persistent urinary incontinence, dysphagia, and psychosis. These outcomes are similar in the benign and intermediate disease groups defined by the UPDRS-III-total-score trajectory model. Hence, outcomes that capture milestones in disease progression and have a significant effect on disease prognosis and quality of life, such as freezing, falls, autonomic dysfunction, cognitive impairment, and psychosis, were more consistently and accurately reflected in the multivariable trajectory analysis.

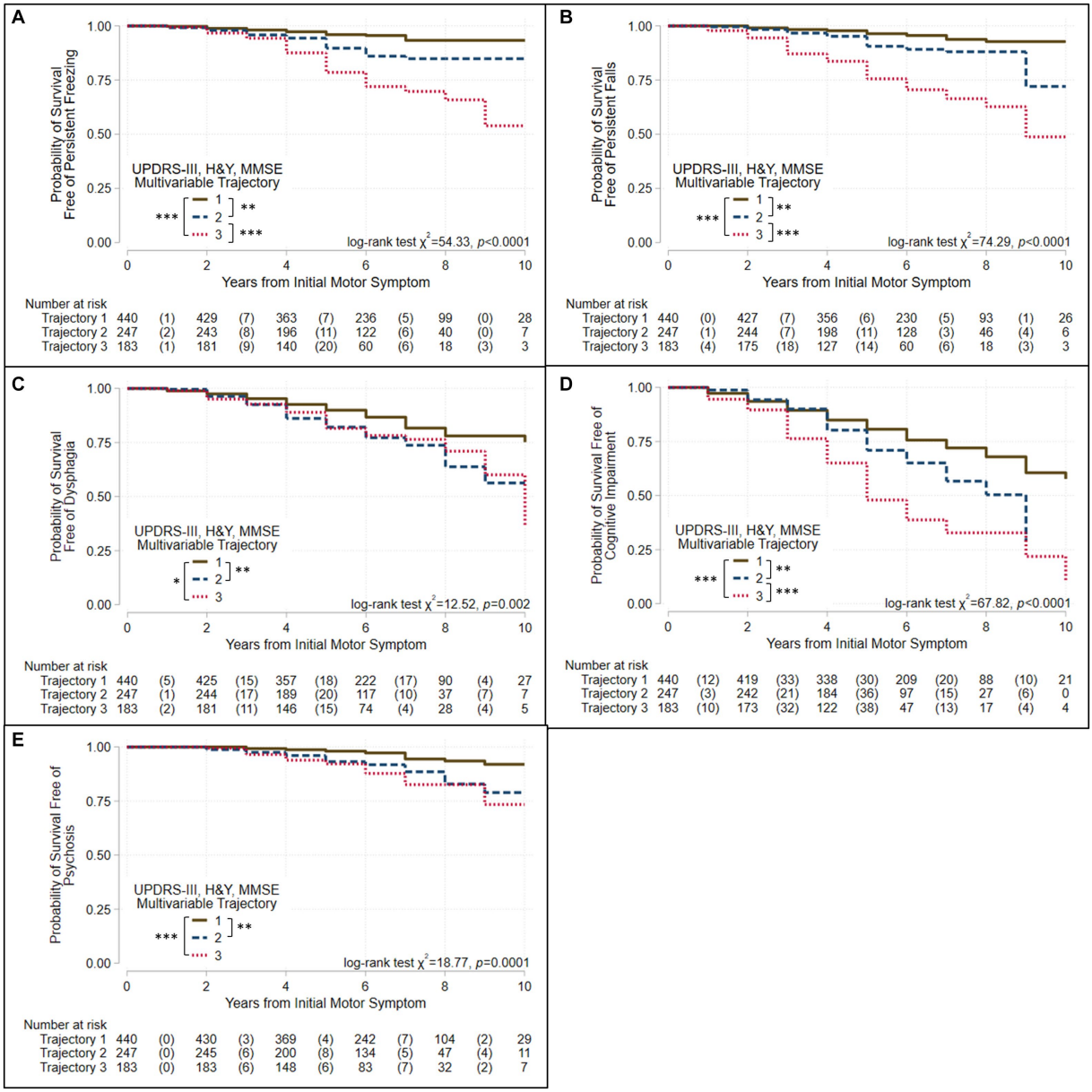

FIGURE 3
Survival free of clinically significant milestones in the groups identified when three assessments are modeled jointly. Kaplan–Meier analyses for survival free of **(A)** persistent freezing, **(B)** persistent falls, **(C)** dysphagia, **(D)** cognitive impairment, and **(E)** psychosis in trajectory-groups identified using group-based trajectory models simultaneously considering UPDRS-III total score, Hoehn & Yahr (H&Y) stage, and Mini-Mental Status Exam (MMSE) score. Trajectories were modeled jointly with the predictors: sex, age at motor-symptom onset, education-years, pesticide exposure, head injury, diabetes, REM-behavior sleep disorder, family history (Parkinson's disease, dementia, or tremor), and initial presentation (tremor-predominant, akinetic/rigid predominant). The at-risk table beneath each plot shows the number at-risk at each time point, with the number of failed (outcome reached) events listed in parentheses. Log-rank test results are shown. Asterisks identify pairs of trajectory-groups where outcomes differ in pairwise log-rank tests with a Bonferroni-corrected $p < 0.05$ (*), $p < 0.01$ (**), or $p < 0.001$ (***). The outcomes of persistent freezing, persistent falls, and cognitive impairment are poorest in Group 3, which show the most severe trajectories, and less poor in Group 2, which show intermediate trajectories, compared to Group 1, which shows trajectories that are more benign. The outcomes of dysphagia and psychosis are similar in Groups 2 and 3, but poorer than in Group 1.

Discussion

We present an analysis of disease trajectories and clinical outcomes in a longitudinal study of a large multiethnic, multisite PD cohort. We used GBTM with UPDRS part III, H&Y stage, and MMSE assessments, individually and in combination, to identify groups that follow one of three trajectories with differential severity and rates of progression. In general, patients show either a benign, slowly progressive disease course, an intermediate, intermediately progressive course, or a severe, more rapidly progressive course. Assignment to a group following a specific trajectory remained stable and misclassification was low 4 to 5 years after the initial motor symptom. This indicates that long-term follow-up of at least this duration is required to accurately determine disease course and prognosis. The groups defined by the multivariable trajectory model better delineate disease course and outcomes. Thus, this modeling approach provides a clinically useful framework to consistently

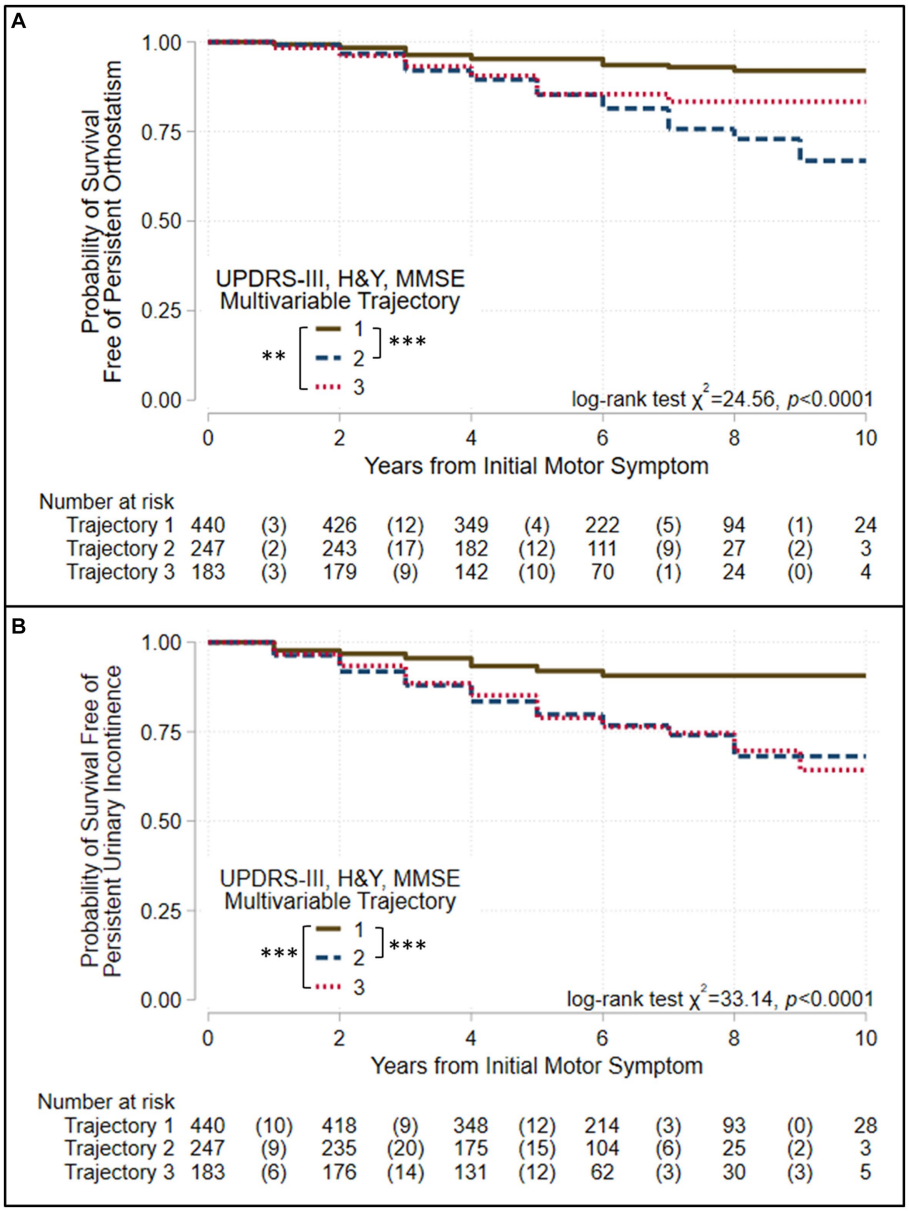

FIGURE 4
Survival free of autonomic symptoms in the groups identified when three assessments are modeled jointly. Kaplan–Meier analyses for survival free of **(A)** persistent orthostatism and **(B)** persistent urinary incontinence in trajectory-groups identified using group-based trajectory models simultaneously considering UPDRS-III total score, Hoehn & Yahr (H&Y) stage, and Mini-Mental Status Exam (MMSE) score. Trajectories were modeled jointly with the predictors: sex, age at motor-symptom onset, education-years, pesticide exposure, head injury, diabetes, REM-behavior sleep disorder, family history (Parkinson's disease, dementia, or tremor), and initial presentation (tremor-predominant, akinetic/rigid predominant). The at-risk table beneath each plot shows the number at-risk at each time point, with the number of failed (outcome reached) events listed in parentheses. Log-rank test results are shown. Asterisks identify pairs of trajectory-groups where outcomes differ in pairwise log-rank tests with a Bonferroni-corrected $p < 0.05$ (*), $p < 0.01$ (**), or $p < 0.001$ (***). All outcomes are similar in Groups 2 and 3, which show intermediate and severe trajectories, respectively, but poorer than Group 1, which shows trajectories that are more benign.

identify rates of disease progression and severity using a combination of objective measures rather than subjective assessment measures. These findings support the hypothesis that disease classification should not depend only on the presence of a single or a combination of symptoms, but rather include longitudinal trajectories that capture the rate of disease progression by taking advantage of standardized objective assessments that capture the full phenotypic spectrum of PD (Puschmann et al., 2015).

In the multivariable trajectory model, relative to Group 1 (more benign trajectories) membership, predictors of intermediate-group membership were akinetic/rigid subtype at initial presentation, fewer years of education, younger AAO, and pesticide exposure. Individuals were less likely to be in the intermediate group if they had reported a head injury or were female. Predictors of severe group membership were older AAO, fewer years of education, and pesticide exposure. Individuals were less likely to be in the severe group if they initially presented with a tremor-predominant subtype. Interestingly, a tremor-predominant initial presentation is not a good predictor of any UPDRS-III-tremor-subscore trajectory-group, but is a useful predictor of an

TABLE 3 Patterns of significant differences in the survival free of an outcome across trajectory groups.[a]

Outcome	Assessment used in group-based-trajectory model						Figures
	UPDRS-III score	UPDRS-III tremor subscore	UPDRS-III Bradykinesia-rigidity subscore	Hoehn & Yahr stage	MMSE score	UPDRS-III, Hoehn & Yahr, MMSE multivariable	
Motor fluctuations	ns	ns	ns	[3] < [1]	ns	ns	**S3**
Dyskinesia	ns	[1] < [3]	ns	ns	ns	ns	**S4**
Persistent freezing	[3] < [2] < [1]	ns	[2, 3] < [1]	[3] < [1,2]	[3] < [1]	[3] < [2] < [1]	3, **S5**
Persistent falls	[3] < [1,2]	ns	[2,3] < [1]	[3] < [2] < [1]	[2,3] < [1]	[3] < [2] < [1]	3, **S6**
Persistent orthostatism	[3] < [2] < [1]	ns	ns	ns	ns	[2,3] < [1]	4, **S7**
Persistent urinary incontinence	[3] < [1,2]	ns	ns	ns	[3] < [1]	[2,3] < [1]	4, **S8**
Dysphagia	[3] < [1,2]	ns	ns	[3] < [1,2]	ns	[2,3] < [1]	3, **S9**
REM sleep behavior disorder [b]	[2,3] < [1]	ns	[3] < [1]	ns	ns	[2] < [1,3]	**S10**
Cognitive impairment	[3] < [2] < [1]	ns	[3] < [1,2]	[3] < [2] < [1]	[3] < [2] < [1]	[3] < [2] < [1]	3, **S11**
Psychosis	[3] < [1,2]	ns	ns	[3] < [1]	[3] < [1]	[2,3] < [1]	3, **S12**
Impulse control disorder	[3] < [1,2]	nd	nd	[1] < [2]	ns	[2] < [1,3]	**S13**

UPDRS-III, Unified Parkinson's Disease Rating Scale part III (motor); MMSE, Mini-Mental Status Exam; ns, not significant, either a log-rank test failed to identify significant differences in survival free of the outcome across the three trajectories or pairwise differences were not significant following Bonferroni correction for multiple tests; nd, not done, none of the patients with available UPDRS-III tremor subscore and bradykinesia-rigidity subscore data developed impulse control disorder.

[a]If a log-rank test revealed a significant difference between an outcome across a set of three trajectory groups, pairwise log-rank tests were used to assess differences between pairs of trajectory groups. The table reports trajectory groups that show significant differences for an outcome after Bonferroni correction for multiple tests. A bracketed number refers to a trajectory group for an assessment. A trajectory group to the left of the "<" sign showed poorer survival free of the outcome than a trajectory group to the right. In some cases, no pairs or only one pair of trajectory groups showed differences that remained significant following Bonferroni correction. Compare the Kaplan–Meier survival probability plotted in the indicated figure.

[b]Patients with REM sleep behavior disorder at the initial visit were excluded from these analyses.

absence of membership in the intermediate and/or severe trajectory-groups for UPDRS-III, UPDRS-III bradykinesia-rigidity subscore, H&Y stage, and the multivariable trajectories. This suggests that the initial presence or absence of tremor is a less reliable indicator of later disease severity or progression.

In addition, specific outcomes that reflect clinically significant milestones of disease progression, such as persistent freezing of gait, persistent falls, orthostatism and development of complications of levodopa therapy, were differentially associated with trajectory groups. As a rule, these outcomes tended to occur earliest and most frequently in the group following the most severe trajectory. The development of some important clinical outcomes, such as freezing, falls, autonomic dysfunction, dysphagia, psychosis, and cognitive impairment, showed patterns that were most consistently found in the groups identified when GBTM jointly assessed the trajectories of UPDRS-III, H&Y, and MMSE scores. In one pattern, an outcome was poorest in Group 3 (severe trajectory), less poor in Group 2 (intermediate trajectory) and least poor in Group 1 (most benign trajectory). In a second, an outcome was poorest in Group 3, with Groups 2 and 1 showing less poor but similar outcomes. In a third, the outcome was poorest but similar in Groups 2 and 3, and less poor in Group 1. In general, treatment-resistant axial symptoms, such as persistent freezing and falls, occurred earlier and more frequently in the group following the severe trajectory, whereas features that may be treatment-responsive, at least for part of the disease course, occurred later and less frequently in the groups having more intermediate or benign trajectories.

Dysphagia is a significant late manifestation of bradykinesia and often co-occurs with hypokinetic dysarthria as the disease progresses. During the follow-up period, dysphagia was noted in 17.6% of patients, while dysarthria was noted in 3.3% of patients. Since we presented analyses of outcomes only if they appeared in >5% of patients, we did not present analyses of dysarthria in the results. The fact that dysarthria was recorded in <5% of patients may reflect lack of data entry for this symptom at some sites, or that was a later manifestation, not appearing in many patients during the assessment period at some sites. In addition, the severity of hypophonia was assessed by the UPDRS-III total and UPDRS-III bradykinesia-rigidity subscores, whereas the presence, but not the severity of dysphagia was assessed at successive annual evaluations. Indeed, in a Kaplan–Meier analysis to evaluate how dysarthria varied across trajectory groups (that were modeled with *study site*), dysarthria, like dysphagia, had a poorer outcome in UPDRS-III score Group 3 than either Groups 1 or 2, where it was similar (overall $p = 0.011$, Bonferroni corrected $p < 0.05$ for Group 3 vs. 1 and 3 vs. 2). While dysarthria did not vary across other sets of trajectory groups, only 1.2% (8/678) of the patients available for modeling UPDRS-III bradykinesia-rigidity subscore trajectories developed dysarthria during the follow-up period. A possible source of variability in the development of dysphagia and dysarthria are behavioral treatments. These include speech therapy or evaluation by video swallowing, which have been obtained in some patients, but were not included in the analysis as predictors of trajectory-group assignment.

Interestingly, the most benign tremor-subscore trajectory was associated with poorer outcomes for dyskinesias. This difference could

reflect differences in the underlying neurodegenerative process and/or treatment response.

In agreement with our previous study, the tremor-predominant subtype as ascertained at the initial visit was associated with the benign UPDRS-III, H&Y stage, and multivariable trajectories, whether or not study site was included in modeling the trajectory groups. The tremor-dominant subtype has been previously described as being distinct from the akinetic/rigid form of the disease (Lawton et al., 2018). Taken together, these findings suggest that the tremor-predominant disease subtype reflects a different underlying neurodegenerative process than the akinetic/rigid subtype.

Genetic factors clearly contribute to disease progression and the severity of specific disease characteristics when these are assessed relative to age and disease duration. For example, common genetic variation at *GBA1* and *APOE* affects the rate of cognitive decline (Szwedo et al., 2022). *GBA1* mutations are associated with earlier age of onset, greater disease severity and motor subtype (Malek et al., 2018), a disease subtype with weaker levodopa response and poorer prognosis (Zhou et al., 2023), and some *GBA1* mutations are associated with reduced survival and more rapid progression (Brockmann et al., 2014; Celia et al., 2016). Large genome-wide association studies (GWAS) have also provided insight into the genetic landscape influencing disease progression and severity. Liu et al. (2021), in a study of 3,821 patients, identified three novel loci associated with cognitive progression in PD, in addition to confirming associations with *GBA1* and *APOE*. Tan et al. (2022), in an analysis of 11 cohorts from Europe and the Americas, including the PPMI, Tracking Parkinson's, and Oxford Discovery cohorts (Lawton et al., 2022), identified six novel loci associated with PD motor progression or mortality, and found that the E326K *GBA1* variant was associated with increased mortality. Martínez Carrasco et al. (2023), in a GWAS meta-analysis, identified an association between axial motor progression and expression of *ACP6* that suggests mitochondrial lipid homeostasis plays a role in motor progression. Hence, the genetic architecture underlying motor or cognitive progression in PD appears to be somewhat separate from that for disease susceptibility. For these reasons, it will be important to elucidate the relationship between different disease subtypes and the underlying differential spatial dissemination of key pathological proteins (e.g., α-synuclein), genetic factors that influence disease course and outcomes, and environmental exposures. Longitudinal analyses of multiple cohorts are essential to address the role of genetic architecture in disease progression and outcomes. Phenotypic and genetic variability raises the significance of more accurate disease characterization and biological staging (Chahine et al., 2023; Höglinger et al., 2023).

Fewer years of education was associated with membership in the groups following the intermediate and more severe trajectories in the multivariable and H&Y-stage GBTM models. This supports prior findings suggesting a role for cognitive reserve in the development of motor and cognitive symptoms (Valenzuela et al., 2007; Hindle et al., 2016; Lee et al., 2019).

Impulse control disorders were associated with the most severe UPDRS-III trajectory, but also with the most benign H&Y stage and the intermediate multivariable trajectories. Within the follow-up period analyzed here, ICD was noted only in the LuxPark cohort, possibly reflecting different treatment practices.

Our results are in agreement with those of Bartl et al. (2022), who compared progression indicators in the DeNoPa and PPMI cohorts and found similar slopes of progression in both cohorts and that higher scores at baseline for ADLs, freezing, and rigidity were predictors of faster progression.

While individual cohorts, such as those in the DeNoPa, PPMI, and the TrackingPD studies, have identified individual characteristics that contribute to disease progression, it is important to incorporate these characteristics in a multifactorial mode of phenotypic assessment that may more accurately reflect determinants of disease progression.

Our results may be influenced by differences in sample size, treatment practices at the participating sites, the number of patients seen at each annual interval, the duration of follow-up, and/or minor variations in the instruments used for objective assessment of patients, e.g., the use of UPDRS-III vs. MDS-UPDRS-III and MMSE vs. MoCA vs. STMS, which were allowed in the study protocol.

When *study site* was included in the GTBMs, the trajectories shown by groups of patients, the numbers of patients assigned to each trajectory group, and the outcomes associated with different trajectory groups are very similar to those in GBTMs when *study site* was not included. This supports the important conclusion that the trajectory groups and the outcomes associated with them are relatively robust to site-specific effects. Also robust to site-specific effects are some predictors of trajectory-group membership, such as age at motor-symptom onset, years of education, and tremor-predominant subtype at initial presentation for the groups identified by GTBMs jointly considering UPDRS-III, H&Y and MMSE scores. Interestingly, the partial effects of other predictors, such as akinetic/rigid initial presentation, pesticide exposure, head injury in the aforementioned GBTM, are better captured by site. This would be expected for baseline attributes at sites that vary widely from DodoNA, which was used as the reference. For example, in the aforementioned GBTMs, *pesticide exposure* was a strong predictor of membership in Groups 2 and 3 in the model without *study site* (OR[CI]: 1.6[1.0–2.5], $p = 0.032$ and 4.2[2.5–7.2], $p < 0.001$, respectively), but failed to reach significance predicting membership in Group 3 in the model with *study site* (1.6[0.9–2.7], $p = 0.099$). Correspondingly, the incidence of pesticide exposure was considerably higher at the LuxPark site than the DodoNA site (67.0% vs. 18.6%, respectively). Therefore, it will be important to evaluate additional multiethnic cohorts at different sites to clarify how such predictors contribute to trajectory-group assignment.

While treatment effects were not directly assessed in our study, the development of complications of therapy was mostly similar across groups following different disease trajectories. This supports the hypothesis that treatment effects do not alter the underlying disease process. In addition, since two distinct objective measures that are more (UPDRS-III) or less (H&Y) sensitive to treatment effects have similar directions and rate of disease progression across trajectories, a strong treatment effect on group assignment seems unlikely.

Our study results are in overall agreement with analyses of two Canadian cohorts having a mean follow-up of 4.5 years (Fereshtehnejad et al., 2015) and the PPMI cohort having a mean follow-up of 2.7 years (Fereshtehnejad et al., 2017). These studies identified three disease subtypes using clustering on composite indicators: *mainly motor/slow progression*, *diffuse/malignant*, and *intermediate*. In the Canadian cohorts, patients with the diffuse/malignant phenotype were more likely to have mild cognitive impairment, orthostatic hypotension, and RBD at baseline, and at prospective follow-up showed a more rapid progression in cognition motor signs, motor symptoms and a global composite outcome. In the PPMI cohort, key classifiers were motor summary score, cognitive impairment, RBD and dysautonomia.

Interestingly, in the PPMI cohort, MRI based morphometry of a PD-specific brain network showed more atrophy in the diffuse malignant subtype, compared to the mild motor-predominant subtype and patients with the diffuse malignant subtype progressed with greater decline in cognition and in dopamine functional neuroimaging after an average of 2.7 years. These differences between the subtypes argue in favor of different underlying pathophysiology between the subtypes.

It is also interesting to note that in cohorts with autopsy-confirmed PD (De Pablo-Fernández et al., 2019), age at diagnosis was the only significant variable and that, staging of Lewy pathology and Alzheimer disease–related pathology did not differ between subtypes. This again suggests a contribution of different underlying pathophysiology between disease subtypes.

A review of subtyping studies by Mestre et al. (2021) reported significant methodologic shortcomings, questionable clinical applicability, and unknown biological relevance, and suggested that the clinical and biological signature of PD may be unique to the individual patient. The subtyping studies reported to date differ in their methods of analysis, duration of follow-up, and cohort composition. Despite these differences, similar classification patterns have begun to emerge based on clinical characteristics. Therefore, this review underscores the importance of using a common type of longitudinal analyses in evaluating disease progression. In agreement with this conclusion, based on our study's results, we argue that it is important to use multifactorial annual objective assessments in large multiethnic cohorts, apply a standard methodology in analyzing the cohorts, such as trajectory analysis, and clinical outcomes (De Roos et al., 2017), and perhaps most importantly, use a long duration of longitudinal follow-up. It is the long duration of follow-up that will allow stable patterns of clinically significant disease progression and severity to emerge.

In that vein, as reviewed by Berg et al. (2021), incorporating prodromal symptomatology and subtypes can inform the symptomatic phase of the disease. Since combinations of prodromal symptoms are also present in the symptomatic phase of the disease, it will be important to assess their effect on disease progression and severity in longitudinal cohorts, as we have begun to do. A combined analysis of the DeNoPa, PPMI and FOUND cohorts led to the development of the PREDIGT score, which was able to identify newly diagnosed PD patients before a motor examination (Li et al., 2022). Variables included in the model were hyposmia, constipation, caffeine intake, metal exposure, head injury, smoking, family history, depression, anxiety and RBD.

In summary, these analyses of a large multiethnic, multisite PD cohort identified three groups of patients that show different trajectories of disease progression based on objective longitudinal assessment, predictors of trajectory-group membership, and different patterns of outcome onset. This work demonstrates the importance of long-term annual follow-up (>5 years) with standardized clinical phenotypic assessment for accurately determining disease course and prognosis. It also supports the hypothesis that disease classification and prognosis are more reliable if longitudinal trajectories that capture the rate of disease progression in multiple phenotypic manifestations are considered. It is important to validate the findings of our study in other longitudinal cohorts using similar analytical methods and thereby determine the robustness of our findings. If indeed this type of analysis for predicting disease progression and outcomes is validated, it can inform clinical practice and the development of therapies that are disease-stage appropriate. In addition, accurate longitudinal phenotypic characterization is essential to inform genomic analyses that can elucidate the underlying neurodegenerative process, leading to targeted therapies that can improve disease outcomes.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by the Ethical Committee for Central Norway (NUST), the Ethics Committee at the University Hospital of Larissa (UT), the Institutional Review Board of Asan Medical Center (ASAN), the National Research Ethics Committee of the Luxembourg government (LuxPark), and the Institutional Review Board of NorthShore University HealthSystem (DodoNA). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent.

Author contributions

KM, JA, RK, SC, RF, DM, and BC contributed to study design and the analysis plan. BC wrote the first draft of the manuscript and performed the statistical analyses. KM, RK, LP, SC, JA, ED, AP, BS, DM, and NK collected clinical data. NA contributed to database management. BC, KM, RK, LP, SC, DM, and RF contributed to manuscript revision. All authors except JA (deceased) approved the manuscript.

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In memoriam

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnagi.2023.1240971/full#supplementary-material

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Glossary

AAO	Age at initial motor symptom onset
ADL	Activities of daily living
AIC	Aikake Information Criterion
ASAN	Asan Medical Center site
BIC	Bayesian information criterion
CI	Confidence interval
CSF	Cerebrospinal fluid
DBS	Deep-brain stimulation
DeNoPa	*De Novo* Parkinson cohort
DodoNA	NorthShore University HealthSystem site
Duration	Years from initial motor symptom onset
GBTM	Group-based trajectory model
GEoPD	Genetic epidemiology of Parkinson's disease consortium
GWAS	Genome-wide association study
H&Y	Hoehn and Yahr stage
HDL	High-density lipoprotein
ICD	Impulse Control Disorder
LuxPark	Luxembourg Institute of Health and Luxembourg Centre for Systems site
M	Mean
MD	Median
MDS-UPDRS	Movement Disorder Society – Unified Parkinson's Disease Rating Scale
MMSE	Mini-Mental Status Exam
MoCA	Montreal Cognitive Assessment
MRI	Magnetic resonance imaging
NUST	The Norwegian University of Science and Technology site
OR	Odds ratio
PD	Parkinson's disease
PIGD	Postural instability gait disorder subtype
PPMI	Primary progression markers initiative
R	Range
RBD	REM (rapid eye movement) sleep behavior disorder
STMS	Short Test of Mental Status
UPDRS-III	Unified Parkinson's disease rating scale part III (motor)
UT	University Hospital of Larissa site

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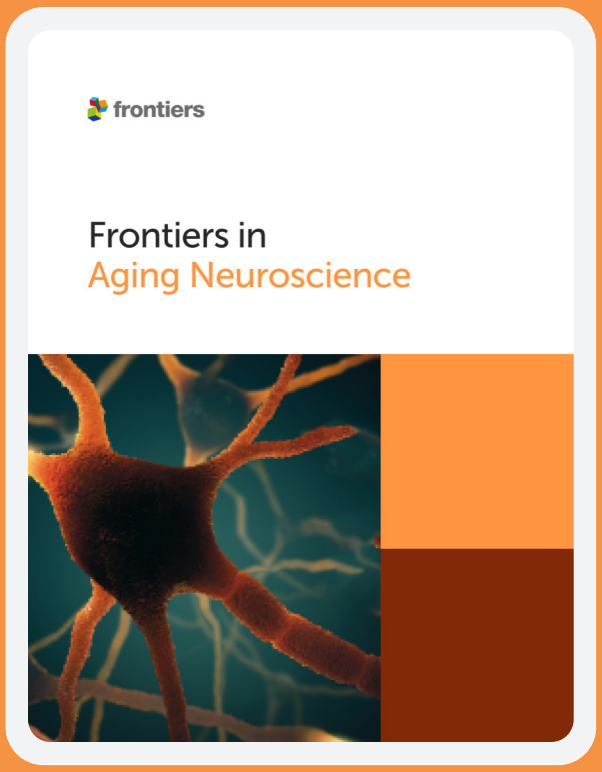

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