Gut microbiota across early stages of synucleinopathy: abridged secondary publication

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KEY MESSAGES

1. Gut dysbiosis is present in individuals with early and prodromal stages of synucleinopathy, including patients with rapid eye movement sleep behaviour disorder and their first-degree relatives who exhibit earlier prodromal features of the disorder.
2. Gut microbiome features might be a promising target for the early prevention and treatment of synucleinopathy.

Hong Kong Med J 2023;29(Suppl 7):S24-30
HMRF project number: 05162876

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Introduction

Synucleinopathies, such as Parkinson's disease (PD), are characterised by the abnormal aggregation of alpha-synuclein protein in the central nervous system. However, there is increasing evidence that alpha-synuclein pathology occurs in the enteric nervous system prior to central nervous system, which strongly supports the gut-to-brain propagation of synucleinopathy. In parallel, gut microbiota disturbance (gut dysbiosis), an emerging biomarker and treatment target for various complex diseases, has been consistently reported in patients with PD.[1] It is hypothesised that PD-associated gut dysbiosis, especially the depletion of short-chain fatty acid (SCFA)–producing bacteria and enrichment of putative pathobionts, is related to intestinal hyperpermeability, immune activation, and pathological alpha-synuclein aggregation.

Rapid eye movement sleep behaviour disorder (RBD) is the most specific prodromal marker of PD. Patients with RBD display an increased prevalence of constipation, along with increased immunostaining of phosphorylated alpha-synuclein in the enteric nervous system. Additionally, a recent case-control-family study reported that the first-degree relatives of patients with RBD (RBD-FDR) had increased constipation and a range of RBD features: from isolated RBD symptoms (indicative of prodromal RBD) to video polysomnography–diagnosed RBD. Therefore, RBD-FDR might comprise a group of susceptible individuals at a much earlier stage of synucleinopathy, compared with patients with RBD.[2]

We performed a large cross-sectional study across various early stages of disease to identify the associations of gut microbiota with the progression of synucleinopathy.

Methods

We recruited four groups of individuals representing different stages of PD: patients with PD but without dementia, patients with RBD, RBD-FDR, and healthy controls (Fig 1). Their sociodemographics, lifestyle, excessive daytime sleepiness, autonomic function, and RBD features were recorded with a comprehensive questionnaire, as were psychiatric disorders, motor dysfunction, orthostatic hypotension, olfactory function, and dementia. Bowel disorders, such as functional constipation, were diagnosed using the Rome IV diagnostic questionnaire.

Fresh stool samples were frozen at -80°C within 4 hours of collection. DNA extraction was performed using the DNeasy PowerSoil Pro DNA Kit (Qiagen). DNA libraries were constructed using primers spanning the targeted V3-V4 hypervariable regions of 16S ribosomal RNA genes.

Univariate analyses of categorical data were performed using the Chi-squared test or Fisher's exact test. For continuous data with a normal distribution, analysis of variance was performed

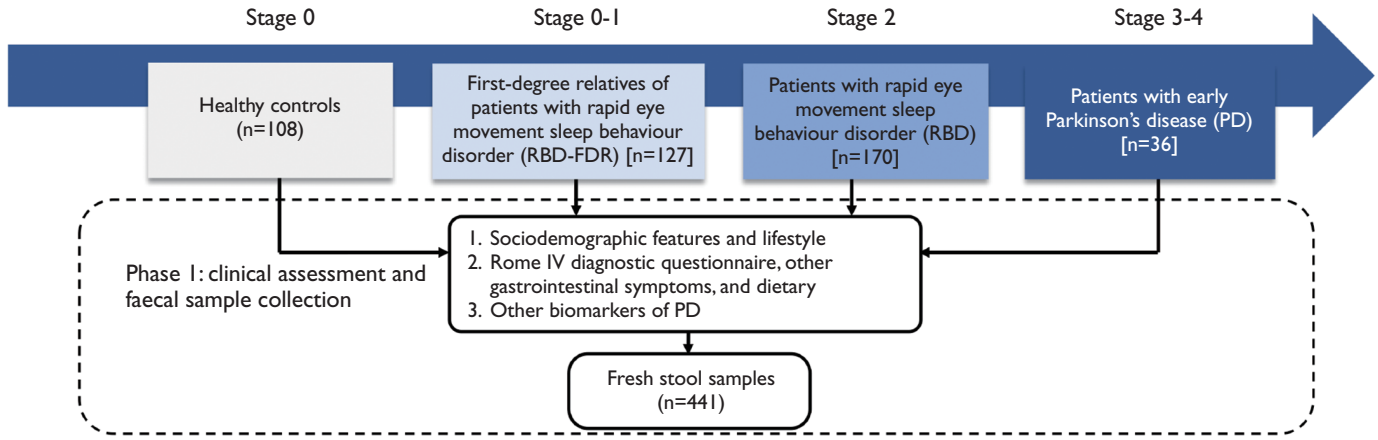


FIG 1. Flowchart of the study

followed by a post hoc test; otherwise, the Kruskal-Wallis H test was used. Considering potential associations between participants from the same family, a generalised estimating equation model was used to adjust for family clustering.[2] A two-sided P value of <0.05 was considered statistically significant. Compositional differences between each pair of groups were analysed using permutational multivariate analysis of variance (PERMANOVA) and then visualised using principal coordinates analysis. Differential taxa between groups were identified using the Kruskal-Wallis test with post hoc analysis. Correlations of significantly different faecal microbiota and host factors were assessed using microbiome multivariable associations with linear model. For multiple comparisons, P values were adjusted using the Benjamini-Hochberg false discovery rate, and a false positive rate of <5% (q<0.05) was considered statistical significance.

Results

With additional funding from the Faculty of Medicine, the Chinese University of Hong Kong, the proposed sample size (36 participants per arm) was increased to 108 controls, 127 RBD-FDR, 170 patients with RBD, and 36 patients with early PD.[3] Age and sex were comparable among controls (67.3 years, 63.9% men), patients with RBD (68.6 years, 73.5% men), and patients with early PD (67.8 years, 86.1% men), but the RBD-FDR group (58.0 years, 48.8% men) was younger and comprised more women, compared with controls (q<0.001). The prevalence of functional constipation showed an increasing trend across groups: from controls to RBD-FDR to patients with RBD to patients with early PD (8.3% vs 9.4% vs 45.3% vs 69.4%, P<0.001, Tables 1 and 2).

Faecal microbiome analysis showed that the early PD group displayed a distinct microbiota clustering pattern, compared with controls (R^2=0.035, q<0.001).[3] The RBD group was similar to the early PD group (R^2=0.0081, q=0.07) but differed from the control and RBD-FDR groups (all q<0.001, Fig 2). The RBD-FDR and control groups did not differ significantly. Differential taxa analysis revealed that SCFA-producing bacteria (eg, *Roseburia, Lachnospiraceae_ND3007_group*, *Lachnospira, [Eubacterium]_ventriosum_group*, *Butyricicoccus, Faecalibacterium, and Lachnospiraceae*), hydrogen sulphide-producing *Desulfovibrio*, mucin-degrading *Akkermansia*, *Collinsella*, *UBA1819*, and *Oscillospiraceae_UCG-002* and *-005*) were significantly (and similarly) altered in RBD and early PD groups, compared with controls (all q<0.05). Notably, the enrichment of pro-inflammatory *Collinsella* occurred in RBD-FDR, an early stage of synucleinopathy (adjusted (β=0.58, q=0.035).[3]

Correlations of differential microbe abundance with clinical biomarkers of synucleinopathy were explored. Genus *UBA1819* was associated with olfactory impairment (*r*=0.15, P=0.004), constipation/bowel movement frequency (*r*=0.32, P<0.001), and total likelihood ratio of prodromal PD (*r*=0.22, P<0.001). Among faecal samples, gut microbes enriched in early synucleinopathy (eg, *Desulfovibrio, Akkermansia, UBA1819, Family_XIII_AD3011_group*, and *Oscillospiraceae_UCG-005*) were positively correlated with cognitive decline and motor dysfunction, whereas microbes

TABLE 1. Sociodemographic, gastrointestinal, and clinical characteristics of participants (permission from: Huang B, Chau SWH, Liu Y, et al. Gut microbiome dysbiosis across early Parkinson's disease, REM sleep behavior disorder and their first-degree relatives. Nat Commun 2023;14:2501)

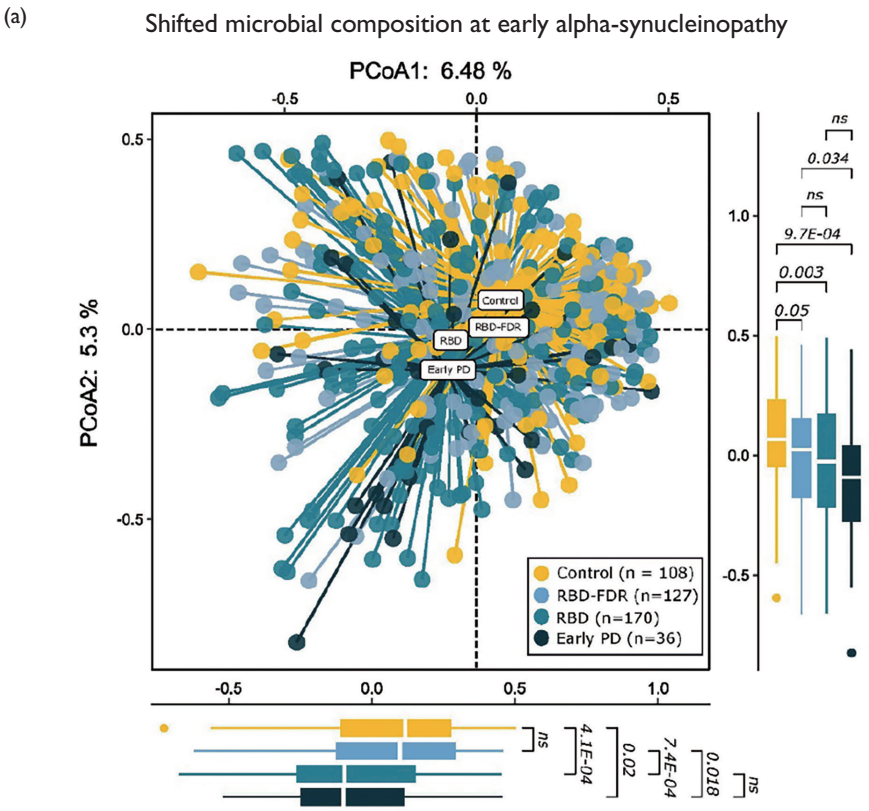
Characteristic	Healthy controls (n=108)*	First-degree relatives of patients with rapid eye movement sleep behaviour disorder (RBD-FDR) [n=127]*	Patients with rapid eye movement sleep behaviour disorder (RBD) [n=170]*	Patients with early Parkinson's disease (PD) [n=36]*	P value	Post hoc analysis
Age, y	67.3±7.0	58.0±9.5	68.6±7.6	67.8±5.6	<0.001	RBD-FDR<control=RBD=PD
Male sex	69 (63.9)	62 (48.8)	125 (73.5)	31 (86.1)	<0.001	RBD-FDR<control=RBD=PD
Body mass index, kg/m^2	24.8±3.7	24.4±3.1	24.5±3.7	24.5±2.7	0.94	-
Comorbidities						
Cardiovascular disease	16 (14.8)	7 (5.7)	33 (19.4)	5 (13.9)	0.19	-
Diabetes, type 2	15 (13.9)	19 (15.0)	28 (16.5)	5 (13.9)	0.30	-
Dyslipidaemia	36 (33.3)	39 (30.7)	54 (31.8)	13 (36.1)	0.51	-
Fatty liver	4 (3.7)	14 (11.0)	10 (5.9)	2 (5.6)	0.98	-
Depression, lifetime	15 (13.9)	28 (22.0)	54 (31.8)	10 (27.8)	<0.001	control=RBD-FDR<RBD=PD
Anxiety disorder, lifetime	5 (4.6)	14 (11.0)	34 (20.0)	5 (13.9)	0.001	control=RBD-FDR<RBD
Gout	2 (1.9)	5 (3.9)	14 (8.2)	2 (5.6)	0.31	-
Medications						
Proton pump inhibitors	8 (7.4)	8 (6.3)	21 (12.4)	2 (5.6)	0.51	-
Statins	26 (24.1)	18 (14.2)	58 (34.1)	5 (13.9)	0.12	-
Osmotic laxatives	1 (0.9)	2 (1.6)	9 (5.3)	11 (30.6)	<0.001	control=RBD-FDR=RBD<PD
Beta blockers	11 (10.2)	5 (3.9)	13 (7.6)	6 (16.7)	0.19	-
Antidepressants	5 (4.6)	6 (4.7)	43 (25.3)	5 (13.9)	<0.001	control=RBD-FDR<RBD
Benzodiazepines	2 (1.9)	4 (3.1)	99 (58.2)	21 (58.3)	<0.001	control=RBD-FDR<RBD=PD
Metformin	7 (6.5)	11 (8.7)	18 (10.6)	3 (8.3)	0.42	-
Urate-lowering drugs	2 (1.9)	1 (0.8)	6 (3.5)	0	0.78	-
Supplements						
Vitamin	13 (12.6)	16 (13.4)	25 (15.3)	6 (17.1)	0.58	-
Calcium	13 (12.6)	13 (10.9)	25 (15.3)	6 (17.1)	0.13	-
Probiotics, ≥1 time/week	17 (15.7)	13 (10.2)	24 (14.1)	7 (19.4)	0.11	-
Prebiotics, ≥1 time/week	2 (1.9)	6 (4.7)	13 (7.6)	2 (5.6)	0.12	-
Rapid eye movement sleep behaviour disorder questionnaire–Hong Kong	6.3±7.0	9.2±8.4	39.2±17.7	32.8±16.1	<0.001	control=RBD-FDR<RBD=PD
Factor 1	4.8±4.4	6.1±4.7	13.3±6.3	11.1±5.8	<0.001	control=RBD-FDR<RBD=PD
Factor 2	1.5±3.8	3.1±5.4	25.9±12.7	21.7±12.0	<0.001	control<RBD-FDR<RBD=PD
Rome IV diagnostic questionnaire for adult functional gastrointestinal disorders						
Functional constipation	9 (8.3)	12 (9.4)	77 (45.3)	25 (69.4)	<0.001	control=RBD-FDR<RBD<PD
Straining with defecation, ≥50% bowel movement	9 (8.8)	19 (15.8)	74 (45.4)	24 (68.6)	<0.001	control<RBD-FDR<RBD<PD
Incomplete evacuation, ≥50% bowel movement	14 (13.7)	15 (12.5)	51 (31.3)	13 (38.2)	0.001	control=RBD-FDR<RBD=PD
Anorectal obstruction/blockage, ≥40% bowel movement	14 (14.0)	10 (8.3)	52 (32.1)	13 (38.2)	<0.001	control=RBD-FDR<RBD=PD
Manual manoeuvres, ≥20% bowel movement	9 (8.8)	18 (15.0)	51 (31.3)	14 (41.2)	0.001	control=RBD-FDR<RBD=PD
Irritable bowel syndrome	2 (1.9)	6 (5.0)	6 (3.6)	1 (2.8)	0.60	-
Functional diarrhoea	7 (6.5)	7 (5.8)	7 (4.2)	0	0.44	-
Bowel movement frequency score	2.0±0.81	2.1±0.70	2.7±1.0	3.4±1.2	<0.001	control=RBD-FDR<RBD<PD
Reversed Bristol Stool Form Scale	2.9±1.0	3.1±1.1	3.8±1.4	4.4±1.1	<0.001	control=RBD-FDR<RBD<PD
Scales for Outcomes in Parkinson's Disease–Autonomic						
Swallowing/choking, 1st item ≥2	16 (15.1)	21 (16.5)	39 (23.9)	8 (22.9)	0.58	-
Sialorrhea, 2nd item ≥2	3 (2.8)	1 (0.8)	9 (5.5)	4 (11.8)	0.07	-
Early satiety, 4th item ≥2	7 (6.7)	11 (8.7)	11 (6.7)	3 (8.8)	0.89	-

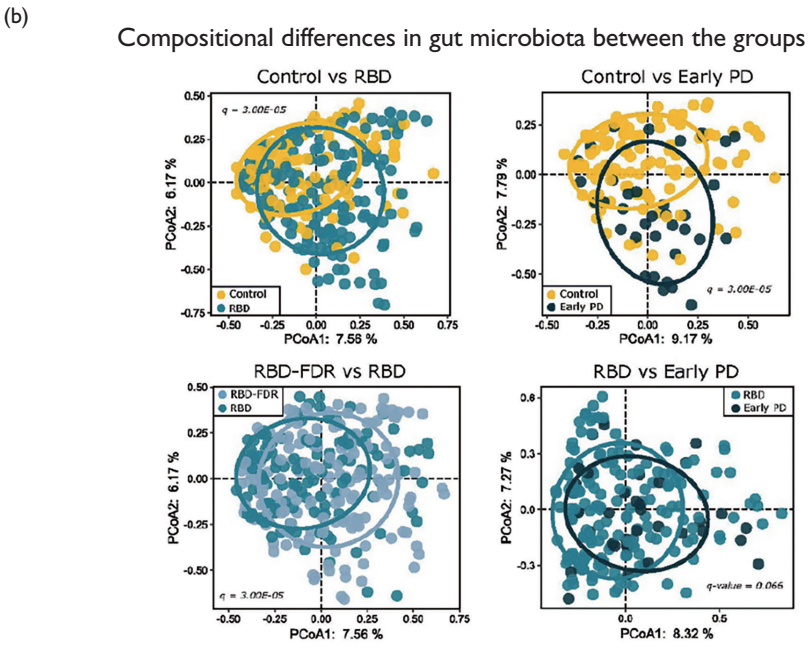
* Data are presented as mean ± standard deviation or No. (%) of participants

TABLE 2. Risk factors and prodromal markers of neurodegenerative diseases (permission from: Huang B, Chau SWH, Liu Y, et al. Gut microbiome dysbiosis across early Parkinson's disease, REM sleep behavior disorder and their first-degree relatives. Nat Commun 2023;14:2501)

Risk factor and prodromal marker	Healthy controls (n=108)*	First-degree relatives of patients with rapid eye movement sleep behaviour disorder (RBD-FDR) [n=127]*	Patients with rapid eye movement sleep behaviour disorder (RBD) [n=170]*	Patients with early Parkinson's disease (PD) [n=36]*	P value	Post hoc analysis
Male sex	69 (63.9)	62 (48.8)	125 (73.5)	31 (86.1)	<0.001	RBD-FDR<control=RBD=PD
Regular pesticide exposure	10 (8.5)	4 (3.3)	12 (7.1)	3 (8.3)	0.54	-
Consumption of caffeinated beverage ≤3 times/week	42 (35.3)	42 (34.1)	63 (37.3)	15 (42.9)	0.68	-
First-degree relative with Parkinson's disease	2 (1.9)	32 (25.2)	11 (6.5)	5 (13.9)	0.002	control=RBD<RBD-FDR, control<PD
Diabetes, type 2	15 (13.9)	19 (15.0)	28 (16.5)	5 (13.9)	0.30	-
Moderate to vigorous physical activity <1 hour/week	33 (33.7)	52 (42.6)	70 (42.4)	10 (27.8)	0.24	-
Smoking status						
Never	89 (84.0)	101 (80.2)	121 (72.5)	25 (69.4)	0.32	-
Former	11 (10.4)	15 (11.9)	32 (19.2)	10 (27.8)	0.03	control=RBD-FDR=RBD=PD
Current	6 (5.7)	10 (7.9)	14 (8.4)	1 (2.8)	0.36	-
Unified Parkinson's Disease Rating Scale part III score excluding action tremor	3.6±3.9	2.9±3.9	5.6±6.7	22.7±11.6	<0.001	control<RBD<PD
Olfactory impairment, Olfactory Identification test <3	21 (21.0)	20 (16.0)	108 (74.5)	29 (85.3)	<0.001	control=RBD-FDR<RBD=PD
Excessive daytime somnolence, Epworth sleepiness Score ≥14	19 (17.8)	23 (18.1)	32 (19.3)	5 (13.9)	0.80	-
Orthostatic hypotension	1 (0.9)	0	11 (6.5)	2 (5.6)	0.002	RBD-FDR<RBD
Erectile dysfunction, male only	14 (21.9)	6 (10.0)	45 (42.9)	13 (50.0)	0.007	control=RBD-FDR<RBD=PD
Urinary dysfunction	6 (5.7)	5 (3.9)	17 (10.3)	4 (11.4)	0.54	-
Depression, lifetime	15 (13.9)	28 (22.0)	54 (31.8)	10 (27.8)	<0.001	control=RBD-FDR<RBD=PD
Anxiety disorder, lifetime	5 (4.6)	14 (11.0)	34 (20.0)	5 (13.9)	0.001	control=RBD-FDR<RBD
Hong Kong version of Montreal Cognitive Assessment score	25.6±3.1	26.8±2.5	25.4±2.8	24.8±3.0	0.50	-
Farnsworth–Munsell 100 hue test, total error score	176.8±89.8	144.5±62.9	194.5±85.2	194.5±89.4	0.37	-
Total estimated likelihood ratio with log transformation	0.58±0.72	0.46±0.55	1.4±0.98	-	<0.001	control=RBD-FDR<RBD
Probable prodromal Parkinson's disease (>80%)	6 (5.6)	2 (1.6)	32 (18.8)	-	<0.001	control=RBD-FDR<RBD
Possible prodromal Parkinson's disease (30%-80%)	12 (11.1)	6 (4.7)	48 (28.2)	-	<0.001	control=RBD-FDR<RBD

* Data are presented as mean ± standard deviation or No. (%) of participants





Pairwise PERMANOVA tests

	Control		RBD		Early PD	
	R^2	*q-value*	R^2	*q-value*	R^2	*q-value*
Control	0.0075	0.060	0.017	**3.0E-05**	0.035	**3.0E-05**
RBD-FDR	/	/	0.016	**3.0E-05**	0.030	**3.0E-05**
RBD	/	/	/	/	0.008	0.066

FIG 2. (a) Faecal microbial composition across early stages of synucleinopathy, and (b) compositional differences in gut microbiota between groups (permission from: Huang B, Chau SWH, Liu Y, et al. Gut microbiome dysbiosis across early Parkinson's disease, REM sleep behavior disorder and their first-degree relatives. Nat Commun 2023;14:2501)
Abbreviations: RBD=rapid eye movement sleep behaviour disorder, RBD-FDR=first-degree relatives of patients with RBD, PD=Parkinson's disease, PERMANOVA=permutational multivariate analysis of variance

enriched in controls (eg, SCFA-producing bacteria *Faecalibacterium*, *Roseburia*, and *Lachnospiraceae_ND3007_group*) showed opposite correlations.

Discussion

Faecal microbiota communities in RBD-FDR, patients with RBD, and patients with early PD differed significantly from those communities in controls. The overall microbiota composition was similar in patients with RBD and patients with early PD, including depletion of SCFA-producing bacteria, and overabundance of *Collinsella*, *Desulfovibrio*, and *Oscillospiraceae UCG-005*. In RBD-FDR, comprising a younger population with an even earlier prodromal stage, there were emerging RBD/PD-like microbial changes, including an increase in pro-inflammatory *Collinsella* and a decrease in butyrate-producing *[Eubacterium]_ventriosum_group*.[3] Gut dysbiosis and enteric alpha-synuclein pathology were present at a much earlier stage, preceding the onset of RBD and PD. These findings highlight the critical role of the brain-gut-microbiota axis in the pathogenesis of synucleinopathy and provide a foundation for future research of specific gut microbes for the prevention of PD.

Gut dysbiosis occurred in the preclinical prodromal stages (RBD and RBD-FDR) of PD. Microbial community shifts were already present in patients with RBD, consistent with prior findings in patients with video polysomnography–diagnosed RBD and patients with possible RBD defined by a screening questionnaire.[4,5] PD-like gut dysbiosis—depletion of SCFA-producing bacteria—occurred prior to prodromal PD. Short-chain fatty acids, especially butyrate, are used as a source of energy by colonic epithelial cells; they modulate tight junctions between adjacent epithelial cells. The depletion of SCFA-producing bacteria may disrupt the integrity of the intestinal barrier, thereby contributing to intestinal hyperpermeability, activation of the enteric immune response, and subsequent aggregation of enteric alpha-synuclein. Furthermore, certain gut microbes (eg, *Collinsella* and *Desulfovibrio*) consistently increased in early stages of synucleinopathy. Most of these microbes were previously identified as RBD/PD-enriched bacteria. Among them, *Desulfovibrio* constitutes a group of hydrogen sulphide and lipopolysaccharide–producing bacteria. Lipopolysaccharide-treated mice developed intestinal hyperpermeability and greater accumulation of pathological alpha-synuclein. Notably, *Collinsella* is a hydrogen-reducing bacteria that can cross-feed with *Desulfovibrio*. *Collinsella* enrichment is associated with low-fibre diets and metabolic diseases; it may cause intestinal hyperpermeability by downregulating the expression of epithelial tight junctions.

Consistent with previous studies, constipation symptoms (ie, bowel movement frequency score) showed the strongest associations with gut microbiota features; 30% of the total effect of gut microbiota on prodromal PD passed through the mediator (bowel movement frequency score), indicating the potential direction of causality from gut dysbiosis to constipation to synucleinopathy.[3] This observation was supported by prior clinical trials in PD, whereby patients with PD who received pro-/prebiotics had significantly increased spontaneous bowel movement. Thus, drugs targeting constipation and specific microbes in early stages of disease might be important for future prevention and disease-modifying treatment of synucleinopathy.

Conclusions

Gut dysbiosis is likely present in early and prodromal stages of synucleinopathy. Gut microbiota might be a promising target for the early prevention and treatment of synucleinopathy.

Funding

This study was supported by the Health and Medical Research Fund, Health Bureau, Hong Kong SAR Government (#05162876) and Centre for Gut Microbiota Research, Faculty of Medicine, The Chinese University of Hong Kong. The full report is available from the Health and Medical Research Fund website (https://rfs2.healthbureau.gov.hk).

Disclosure

The results of this research have been previously published in:

1. Huang B, Chau SWH, Liu Y, et al. Gut microbiome dysbiosis across early Parkinson's disease, REM sleep behavior disorder and their first-degree relatives. Nat Commun 2023;14:2501.

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