Parkinsonism and Related Disorders 95 (2022) 11–17

Contents lists available at ScienceDirect

Parkinsonism and Related Disorders

journal homepage: www.elsevier.com/locate/parkreldis

Altered gut microbiota in Parkinson's disease patients with motor complications

Kai Takahashi [a], Hiroshi Nishiwaki [b], Mikako Ito [b], Kazuhiro Iwaoka [a], Kenta Takahashi [a], Yoshio Suzuki [a], Keita Taguchi [a], Kanako Yamahara [a], Yoshio Tsuboi [c], Kenichi Kashihara [d,e], Masaaki Hirayama [f], Kinji Ohno [b], Tetsuya Maeda [a,*]

[a] Division of Neurology and Gerontology, Department of Internal Medicine, School of Medicine, Iwate Medical University, Iwate, Japan
[b] Division of Neurogenetics, Center for Neurological Disease and Cancer, Nagoya University Graduate School of Medicine, Nagoya, Japan
[c] Department of Neurology, Fukuoka University, Fukuoka, Japan
[d] Department of Neurology, Okayama Kyokuto Hospital, Okayama, Japan
[e] Okayama Neurology Clinic, Okayama, Japan
[f] Department of Pathophysiological Laboratory Sciences, Nagoya University Graduate School of Medicine, Nagoya, Japan

ARTICLE INFO

Keywords:
Gut microbiota
Motor complications
Parkinson's disease
Dyskinesia
Wearing-off

ABSTRACT

Introduction: Parkinson's disease (PD) is associated with gut dysbiosis. However, whether gut dysbiosis can cause motor complications is unclear.
Methods: Subjects were enrolled from four independent movement disorder centers in Japan. We performed 16S ribosomal RNA gene sequence analysis of gut microbiota. Relative abundance of gut microbiota and relationships between them and clinical characteristics were statistically analyzed. Analysis of co-variance (ANCOVA) was used to assess altered gut microbiota associated with wearing-off or dyskinesia.
Results: We enrolled 223 patients with PD. Wearing-off was noted in 47.5% of patients and dyskinesia in 21.9%. We detected 98 genera of bacteria. Some changes in the gut microbiota were observed in patients with PD and motor complications. After Bonferroni correction, patients with wearing-off showed decreased relative abundance of *Lachnospiraceae Blautia* ($p < 0.0001$) and increased relative abundance of *Lactobacillaceae Lactobacillus* ($p < 0.0001$), but patients with dyskinesia no longer showed significant changes in the gut microbiota. Adjustment with two models of confounding factors followed by ANCOVA revealed that age ($p < 0.0001$), disease duration ($p = 0.01$), and wearing-off ($p = 0.0004$) were independent risks for the decreased relative abundance of *Lachnospiraceae Blautia*, and wearing-off ($p = 0.009$) was the only independent risk factor for the increased relative abundance of *Lachnospiraceae Lactobacillus*.
Conclusion: Relative abundance of *Lachnospiraceae Blautia* and *Lactobacillaceae Lactobacillus* was significantly decreased and increased, respectively, in the gut microbiota of PD patients with motor complications. This indicates that an altered gut microbiota is associated with the development of motor complications in patients with advanced PD.

1. Introduction

Recently, it has been suggested that the gut microbiota is involved in abnormal α-synuclein accumulation in the enteric plexus in patients with Parkinson's disease (PD) [1]. Analysis of autopsied brains of normal subjects and PD patients shows that Lewy bodies migrate from the dorsal nucleus of the vagus nerve to the nucleus accumbens and the substantia nigra [2]. In the gastrointestinal tract, colonic biopsies of PD patients almost universally show abnormal α-synuclein accumulation in the enteric plexus, in both Meissner and Auerbach plexuses [3]. Total vagus nerve resection for the treatment of duodenal ulcers led to a 50% reduction in the incidence of PD in Denmark [4] and Sweden [5]. Intraperitoneal administration of abnormal α-synuclein in PD mouse models [6] or administration via the gastric wall in normal mice [7]

* Corresponding author. Division of Neurology and Gerontology, Department of Internal Medicine, School of Medicine, Iwate Medical University, 1-1-1 Idaidori, Yahaba, Shiwa, Iwate, 028-3694, Japan.
E-mail address: maeda@iwate-med.ac.jp (T. Maeda).

https://doi.org/10.1016/j.parkreldis.2021.12.012
Received 1 October 2021; Received in revised form 15 December 2021; Accepted 19 December 2021
Available online 21 December 2021

causes abnormal α-synuclein accumulation in the central nervous system (CNS). These findings suggest that pathological changes in PD may potentially originate from the enteric plexus via the vagus nerve. Involvement of the gut microbiota in abnormal α-synuclein accumulation in the enteric plexus is also indicated by the following findings. PD is associated with increased intestinal permeability [8,9], and when normal mice are intraperitoneally administered with blood lipopolysaccharide, α-synuclein accumulates abnormally in the dorsal nucleus of the vagus nerve [10]. When α-synuclein-overexpressing mice is rendered sterile, motor deficits and constipation are mild, and transplantation of stool from PD patients into α-synuclein-overexpressing PD models causes severe motor deficits [11]. The pathogenesis of PD is thought to involve abnormal α-synuclein transmission to the CNS, and the gut microbiota plays an important role in α-synuclein accumulation and PD development. In addition to abnormal α-synuclein aggregates in the CNS after intraperitoneal [6] or gastric [7] injection of α-synuclein fibrils, it has been reported that vagotomy ameliorates oral rotenone-induced accumulation of α-synuclein fibrils in the dorsal vagal nucleus [12].

We previously conducted a multinational meta-analysis that revealed PD to be associated with gut dysbiosis [13]. In PD, the abundance of *Akkermansia*, an intestinal mucin layer-degrading bacteria, is increased while that of *Roseburia* and *Faecalibacterium*, short-chain fatty acid-producing bacteria, is decreased. However, whether gut microbiota are altered in patients with advanced PD and showing motor complications remains unclear.

As the disease progresses, PD patients receiving chronic dopamine replacement therapy experience motor complications known as wearing-off phenomenon and dyskinesia [14]. In clinical practice, the onset of motor complications is important, as it highlights an initial sign of progression to advanced stage PD. One of the hallmarks for the development of motor complications is the narrowing of the therapeutic range of dopaminergic drugs [15]. In the advanced stage of the disease, the rate of drug absorption from the small intestine becomes unstable because of poor gastrointestinal motility [16]. Gastrointestinal dysmotility and achlorhydria lead to abnormal growth of small intestinal bacteria [17], which is frequently seen in PD [18]. Patients with abnormal growth of small intestinal bacteria are more likely to have delayed-on or no-on episodes compared with patients without such abnormal growth [19]. Concomitantly, higher doses of dopaminergic drugs may induce a higher complication rate associated with abnormal small intestinal bacterial growth [18], which is related to duration of illness, Hoehn and Yahr (H&Y) severity classification, unified PD rating scale (UPDRS) part III severity, and severity of motor complications. These changes necessitate stabilization of plasma or central nervous system levels of dopaminergic drugs. In addition, gut microbiota may inhibit the absorption of L-dopa by decreasing the acidity of gastric juice. L-dopa dissolves under acidic conditions; therefore, administration of L-dopa in lemon water increases plasma levels of L-dopa and improves motor function [20]. In addition, inhibiting the growth of bacteria in the small intestine improves the decline in motor function without affecting the pharmacokinetics of L-dopa [18]. Therefore, the intestinal microbiota may be associated with the development of motor complications. In this study, we aimed to compare gut microbiota in PD patients with and without motor complications.

2. Methods

2.1. Study design and subjects

We conducted a multicenter cross-sectional study at four independent movement disorder centers in Japan. The study protocol was explained to PD patients and their healthy spouses living in the same household. The registration period was from September 2015 to February 2018. All PD patients were diagnosed according to the International Parkinson and Movement Disorder Society (MDS) PD criteria and were 20 years old or more. Confirmed information, including duration of illness and therapeutic history, was collected for all patients. Chronic systemic diseases, including diabetes mellitus, heart failure, cirrhosis, malignant neoplasms, hematopoietic system diseases, autoimmune diseases, and neurological diseases other than PD were excluded. Subjects who claimed to have taken antibiotics in the past month were also excluded.

This study received ethical approval from the ethics review committees of each of the four centers involved: the Nagoya University Graduate School of Medicine (approval number 2016-0151), Iwate Medical University (H28-123), Okayama Kyokuto Hospital (kyoIR-2016002), and Fukuoka University Graduate School of Medicine (2016M027).

2.2. Sample collection, DNA isolation and V3–V4 16S rRNA sequencing

The details of these procedures are described elsewhere [13]. Briefly, after obtaining written informed consent, clinical characteristics were recorded, including age, sex, smoking history, previous medical history, duration of illness, body mass index (BMI), and anti-parkinsonian medications. Stool appearance was also scored by the participants at their homes using the Bristol stool form scale. Neurological examinations were performed by neurologists, and the clinical severity of parkinsonism was assessed using H&Y staging and the MDS-UPDRS by movement disorder specialists certified by the MDS. Fecal samples were collected in special collection tubes and sent under refrigeration directly to Nagoya University. DNA was extracted using the QIAmp PowerFecal DNA Kit (QIAGEN, Hilden, Germany) and the bacterial composition was analyzed by next-generation sequencing. The V3–V4 hypervariable region of the bacterial 16S rRNA gene was amplified using primer 341F, 5′-CCTACGGGNGGCWGCAG-3′ and primer 805R, 5′-GACTACHVGGGTATCTAATCC-3′. Paired-end sequencing of 300-nucleotide fragments was performed using the MiSeq reagent kit V3 on a MiSeq System (Illumina). Taxonomic analysis with QIIME2 was also previously described in detail [13]. Briefly, amplicon sequence variants were generated using DADA2, and the SILVA taxonomy database release 132 (60) was used for taxonomic identification. We excluded gut bacteria with a relative abundance of less than 0.001% in each of the 223 PD patients, because they were only found in a limited number of patients. FASTQ files of our dataset are available at the DNA Data Bank of Japan under the accession numbers DRA009229 and DRA009322.

2.3. Statistical analysis

We used Wilcoxon's rank-sum test to analyze the clinical characteristics and gut microbiota of patients with and without motor complications. We analyzed the relationship between gut microbiota and sex using Wilcoxon's rank-sum test and Bonferroni correction, and the relationship between gut microbiota and age, duration of illness, BMI, daily L-dopa dose, constipation, and antacid drugs use using generalized linear model analysis. Analysis of co-variance (ANCOVA) was performed to detect independent co-variants and exclude the effects of confounding factors, which included age, sex, duration of illness, daily L-dopa dose, catechol-o-methyl transferase (COMT) inhibitor use, wearing-off, and dyskinesia. We have previously shown that COMT inhibitors affect the overall composition of the gut microbiota of PD patients [13]. Therefore, we designed two analysis models with and without both COMT inhibitor and daily L-dopa dose as confounding factors. Model 1 was constructed with both these factors excluded and model 2 was with all the confounders included. A p-value less than 0.05 was considered statistically significant.

3. Results

3.1. Clinical background of PD patients

A total of 223 patients with PD were enrolled in this study. Their

clinical characteristics are shown in Table 1. Power analysis showed this to be a sufficient number of patients.

The general mean age was 68.2 ± 8.5 (41–85) years, BMI was 21.7 ± 3.0 (15.2–30.8) kg/m^2, and duration of illness was 7.5 ± 6.1 (0.2–42) years. Clinical severity was 2.4 ± 0.8 (stage 1: 30 patients, stage 2: 99 patients, stage 3: 73 patients, stage 4: 16 patients, and stage 5: 5 patients) according to H&Y staging and 50.1 ± 23.1 (0–153) according to the MDS-UPDRS. Mean MDS-UPDRS part IV score was 2.7 ± 3.2 (0–13). PD patients were also categorized by the presence of wearing-off and dyskinesia and their clinical characteristics are shown in Table 1. Wearing-off was noted in 106 patients (47.5%) and dyskinesia in 49 patients (21.9%). Between with and without wearing-off, there were significant differences in age ($p = 0.0148$), BMI ($p = 0.0291$), and duration of illness ($p < 0.0001$). Similarly, between with and without dyskinesia, there were significant differences in age ($p = 0.0224$), BMI ($p = 0.0350$), and duration of illness ($p < 0.0001$).

3.2. Relationship between gut microbiota and motor complications

Ninety-eight genera of bacteria satisfied the detection criteria of this study. Differences in the relative abundance of these species were observed in PD patients with motor complications. In PD patients with wearing-off, there was increased relative abundance of *Lachnospiraceae NK4A136* ($p = 0.0285$), *Lactobacillaceae Lactobacillus* ($p < 0.0001$), *Bifidobacteriaceae Bifidobacterium* ($p = 0.0326$), *Desulfovibrionaceae Bilophila* ($p = 0.0238$), *Ruminococcaceae Oscillibacter* ($p = 0.0460$), and *Lachnospiraceae Tyzzerella* ($p = 0.0100$), and decreased relative abundance of *Lachnospiraceae Blautia* ($p < 0.0001$), *Lachnospiraceae Fusicatenibacter* ($p = 0.0071$), *Lachnospiraceae Anaerostipes* ($p = 0.0440$), and *Lachnospiraceae Eligens group* ($p = 0.0390$). In patients with PD and dyskinesia, increased relative abundance of *Prevotellaceae Alloprevotella* ($p = 0.0294$) and *Lachnospiraceae Pediococcus* ($p = 0.0130$), and decreased relative abundance of *Lachnospiraceae Blautia* ($p = 0.0408$) and *Eggerthellaceae Eggerthella* ($p = 0.0226$) were observed. Significant decrease in *Lachnospiraceae Blautia* and increase in *Lactobacillaceae Lactobacillus* were associated with wearing-off and dyskinesia, respectively.

The relative abundance of *Lachnospiraceae Blautia* and *Lactobacillaceae Lactobacillus* after adjustment for confounding factors using Bonferroni correction are shown in Fig. 1. In patients with wearing-off, *Lachnospiraceae Blautia* showed a significant decrease (A) ($p < 0.0001$) and *Lactobacillaceae Lactobacillus* showed a significant increase (C) ($p < 0.0001$). Patients with dyskinesia demonstrated no changes in the relative abundance of *Lachnospiraceae Blautia* (B) and *Lactobacillaceae Lactobacillus* (D).

3.3. Relationship between motor complications and Lachnospiraceae Blautia or Lactobacillaceae Lactobacillus

Generalized linear model analysis showed significant clinical features associated with decreased relative abundance of *Lachnospiraceae Blautia* and increased relative abundance of *Lactobacillaceae Lactobacillus*. As shown in Fig. 2, a decrease of *Lachnospiraceae Blautia* was significantly correlated with age (A) ($r^2 = 0.03$, $p = 0.0057$), duration of illness (B) ($r^2 = 0.07$, $p < 0.0001$), COMT inhibitor use (C) ($r^2 = 0.03$, $p = 0.005$), and daily L-dopa dose (D) ($r^2 = 0.07$, $p < 0.0001$). There was no significant correlation with BMI. Increased relative abundance of *Lactobacillaceae Lactobacillus* was significantly correlated with duration of illness (E) ($r^2 = 0.03$, $p = 0.007$), BMI (F) ($r^2 = 0.02$, $p = 0.019$), COMT inhibitor use (G) ($r^2 = 0.18$, $p < 0.0001$), and daily L-dopa dose (H) ($r^2 = 0.18$, $p < 0.0001$). There was no significant correlation with age.

3.4. Factors associated with decreased Lachnospiraceae Blautia and increased Lactobacillaceae Lactobacillus relative abundance

The decreased relative abundance of *Lachnospiraceae Blautia* and increased relative abundance of *Lactobacillaceae Lactobacillus* were assessed by two adjustment models for different confounding factors with ANCOVA analysis (Table 2A and B, respectively). A relative decrease of *Lachnospiraceae Blautia* abundance was significantly associated with age ($p < 0.0001$, 95% confidence intervals (CI) [−0.001, −0.0003]), duration of illness ($p = 0.01$, 95% CI [−0.001, −0.00021]), and wearing-off ($p = 0.0004$, 95% CI [−0.027, −0.007]) in model 1, and with age ($p = 0.002$, 95% CI [−0.001, 0.0002]) and COMT inhibitor use ($p < 0.0001$, 95% CI [−0.03, 0.012]) in model 2. A relative increase of *Lachnospiraceae Lactobacillus* abundance was significantly associated with wearing-off ($p = 0.009$, 95% CI [0.006, 0.045]) in model 1, and with sex ($p = 0.02$, 95% CI [0.002, 0.03]) and daily L-dopa dose ($p < 0.0001$, 95% CI [0.00007, 0.0001]) in model 2. This analysis, using two models of confounding factors, estimated that age and COMT inhibitor use were strong confounding factors associated with relatively decreased abundance of *Lachnospiraceae Blautia* and that daily L-dopa dose was a strong confounding factor associated with relatively increased abundance of *Lactobacillaceae Lactobacillus*. This analysis showed that age, duration of illness, and wearing-off were independent factors associated with decreased relative abundance of *Lachnospiraceae Blautia* and wearing-off was an independent factor associated with

Table 1
Clinical background of patients with or without motor complications.

	Total	wearing off		dyskinesia	
		(−)	(+)	(−)	(+)
number, F:M, total	128:95, 223	60:57, 117	68:38, 106	94:80, 174	34:15, 49
age, mean ± SD, range (years)	68.2 ± 8.5, 41-85	69.1 ± 9.2, 41-84	67.2 ± 7.7, 47-85	68.7 ± 8.6, 41-84	66.6 ± 8.2, 49-85
BMI, mean ± SD, range (kg/m^2)	21.7 ± 3.0, 15.2–30.8	22.0 ± 2.8, 16.4–30.8	21.3 ± 3.2, 15.2–29.7	21.9 ± 3.0, 15.5–30.8	20.9 ± 2.9, 15.2–28.2
duration of illness, mean ± SD, range (years)	7.5 ± 6.1, 0.2–42	5.1 ± 5.2, 0.2–28	10.1 ± 6.07, 1-42	6.6 ± 6.0, 0.2–42	10.7 ± 5.6, 1-28
L-dopa dosage, mean ± SD, range (mg/day)	354.4 ± 238.5, 0-2000	299.1 ± 238, 0-1450	415.6 ± 224.8, 0-2000	337.3 ± 259.3, 0-2000	415.3 ± 126.7, 100-700
H&Y stage, mean ± SD	2.4 ± 0.8, 1-5	2.3 ± 0.9, 1-5	2.5 ± 0.8, 1-4	2.3 ± 0.9, 1-5	2.5 ± 0.8, 1-4
stage 1 (number)	30	19	11	26	4
stage 2 (number)	99	58	41	79	20
stage 3 (number)	73	31	42	53	20
stage 4 (number)	16	4	12	11	5
stage 5 (number)	5	5	0	5	0
MDS-UPDRS, mean ± SD, range	50.1 ± 23.1, 0-153	44.6 ± 23.0, 0-153	55.9 ± 21.8, 11-115	48.8 ± 23.6, 0-153	54.6 ± 21.0, 11-115
part I, mean ± SD, range	8.8 ± 5.0, 0-21	8.0 ± 5.1, 0-21	9.6 ± 4.9, 0-20	8.5 ± 5.2, 0-21	9.6 ± 4.4, 2-20
part II, mean ± SD, range	11.8 ± 8.1, 0-48	10.2 ± 8.0, 0-48	13.6 ± 7.8, 1-36	11.2 ± 8.1, 0-48	14.3 ± 7.7, 1-36
part III, mean ± SD, range	26.8 ± 13.4, 0-84	26.2 ± 13.5	27.4 ± 13.3, 4-61	27.7 ± 13.7, 0-84	23.8 ± 12.2, 4-58
part IV, mean ± SD, range	2.7 ± 3.2, 0-13	0.1 ± 0.7, 0-5	5.3 ± 2.6, 1-13	1.4 ± 2.1, 0-8	7.0 ± 2.5, 3-13

F, female; M, male; SD, standard deviation; BMI, body mass index; H&Y, Hoehn and Yahr; MDS-UPDRS, the International Parkinson and Movement Disorder Society version unified Parkinson's disease rating scale.

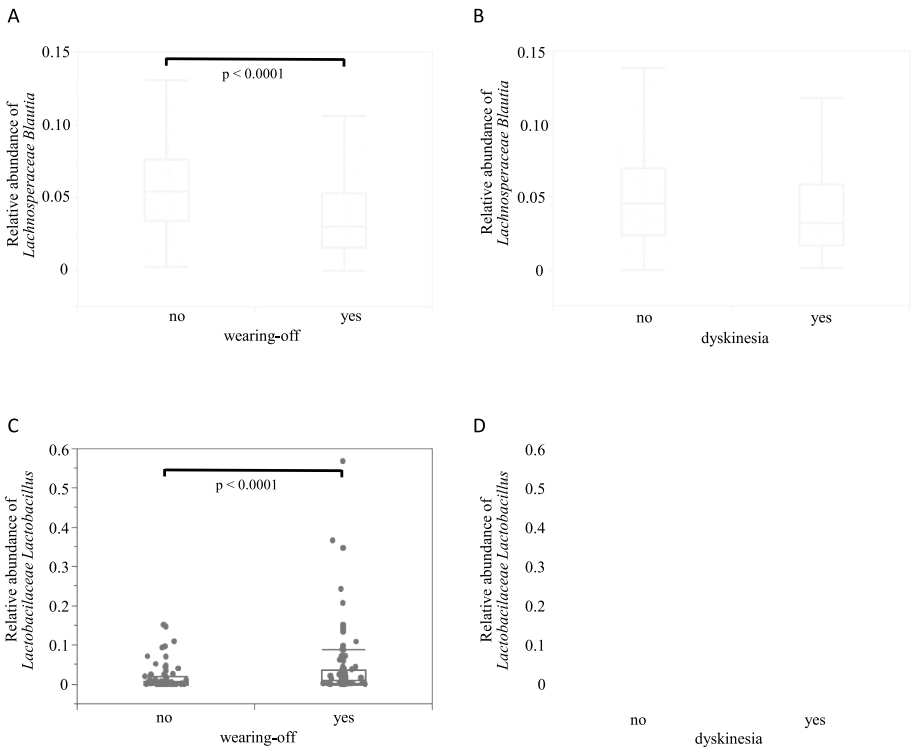

Fig. 1. Relationships between *Lachnospiraceae Blautia* or *Lactobacillaceae Lactobacillus* and motor complications
In patients with wearing-off, the relative abundance of *Lachnospiraceae Blautia* (A) and *Lactobacillaceae Lactobacillus* (C) significantly decreased and increased, respectively. Patients with dyskinesia (B and D) showed no change in relative abundance.

increased relative abundance of *Lactobacillaceae Lactobacillus*.

4. Discussion

We observed that the relative abundance of *Lachnospiraceae Blautia* was decreased and that of *Lactobacillaceae Lactobacillus* was increased in PD patients with motor complications. These findings disappeared after adjustments for clinical characteristics, including oral medications. Previous reports from ourselves and others [9,13,21,22] have shown that COMT inhibitors, which was entacapone in this study, have a strong influence on the overall composition of gut microbiota. Therefore, we used two models of confounders: one excluded the strong COMT inhibitor confounder and associated L-dopa, and the other included all confounders, because PD patients with motor complications, especially those with wearing-off, are ordinarily treated with relatively high doses of L-dopa combined with an adjunctive COMT inhibitor. Based on this analysis, we suggest that the altered gut microbiota can modify the clinical course of wearing-off development in advanced stage PD patients, and, as such, is a candidate pathogenic mechanism for motor complications. Gut dysbiosis has already been associated with the development of PD. Motor complications associated with dopamine replacement therapy manifest during the long-term clinical course of the disease and dyskinesia commonly occurs following wearing-off. Because wearing-off is a well-recognized correlated factor of dyskinesia, a statistical relationship between a relative change in *Lachnospiraceae Blautia* or *Lactobacillaceae Lactobacillus* abundance and dyskinesia may not be noticeable in multivariate analysis. In addition, the small sample size of patients with dyskinesia may also explain why we did not observe a significant relationship. We cannot exclude the possibility that the sample of patients with motor complications was too small to confirm whether or not dyskinesia is associated with an altered gut microbiota. It is extremely important to clarify whether the relative changes in *Lachnospiraceae Blautia* and *Lactobacillaceae Lactobacillus* have any impact on the development of motor complications. However, we cannot conclude whether or not our results are a primary cause of motor complications. Despite this, our study does indicate relationships between them that warrant further investigation.

In addition to wearing-off, age was also noted to be significantly associated with a decrease in the relative abundance of *Lachnospiraceae Blautia*, which is already known to decrease with aging [23]. Increased *Lactobacillaceae Lactobacillus* abundance was associated with sex and daily L-dopa dose. Female sex is a well-known risk factor for motor complications; therefore, an increase in *Lactobacillaceae Lactobacillus* is a possible risk factor of developing motor complications [24]. PD patients with wearing-off need relatively higher doses of L-dopa per day, which is a risk factor for developing motor complications. This evidence together with our results indicate that aging, female sex, and higher daily doses of L-dopa, together with an altered gut microbiota, are risk factors of motor complications. We also detected gut dysbiosis that was not associated with *Lachnospiraceae Blautia* or *Lactobacillaceae Lactobacillus*. There was a different microbiota distribution between patients with wearing-off and those with dyskinesia. We previously reported increased abundance of genera *Akkermansia* and *Catabacter* and families *Akkermansiaceae* and decreased abundance of genera *Roseburia* and *Faecalibacterium* and *Lachnospiraceae ND3007 group* in PD [9,13]. After adjusting for confounding factors, including COMT inhibitor use, the present results are consistent with previous reports. However, differences in gut dysbiosis between motor complications remain to be clarified.

Lachnospiraceae Blautia is a gram-positive, anaerobic, enteric bacteria that inhibits inflammatory reactions [25]. Its abundance is decreased in patients with liver cirrhosis, cancers, bowel diseases, and diabetes mellitus. *Lachnospiraceae Blautia* is relatively more abundant in Japanese people than in people of other countries [26]. PD is more prevalent in patients with inflammatory bowel disease [27] or diabetes mellitus [28]; therefore, *Lachnospiraceae Blautia* may modify the disease. This may also explain why the frequency of motor complications in Japan is lower than that in other countries [29]. The reason for the high prevalence of *Lachnospiraceae Blautia* in Japanese people is unclear, but a

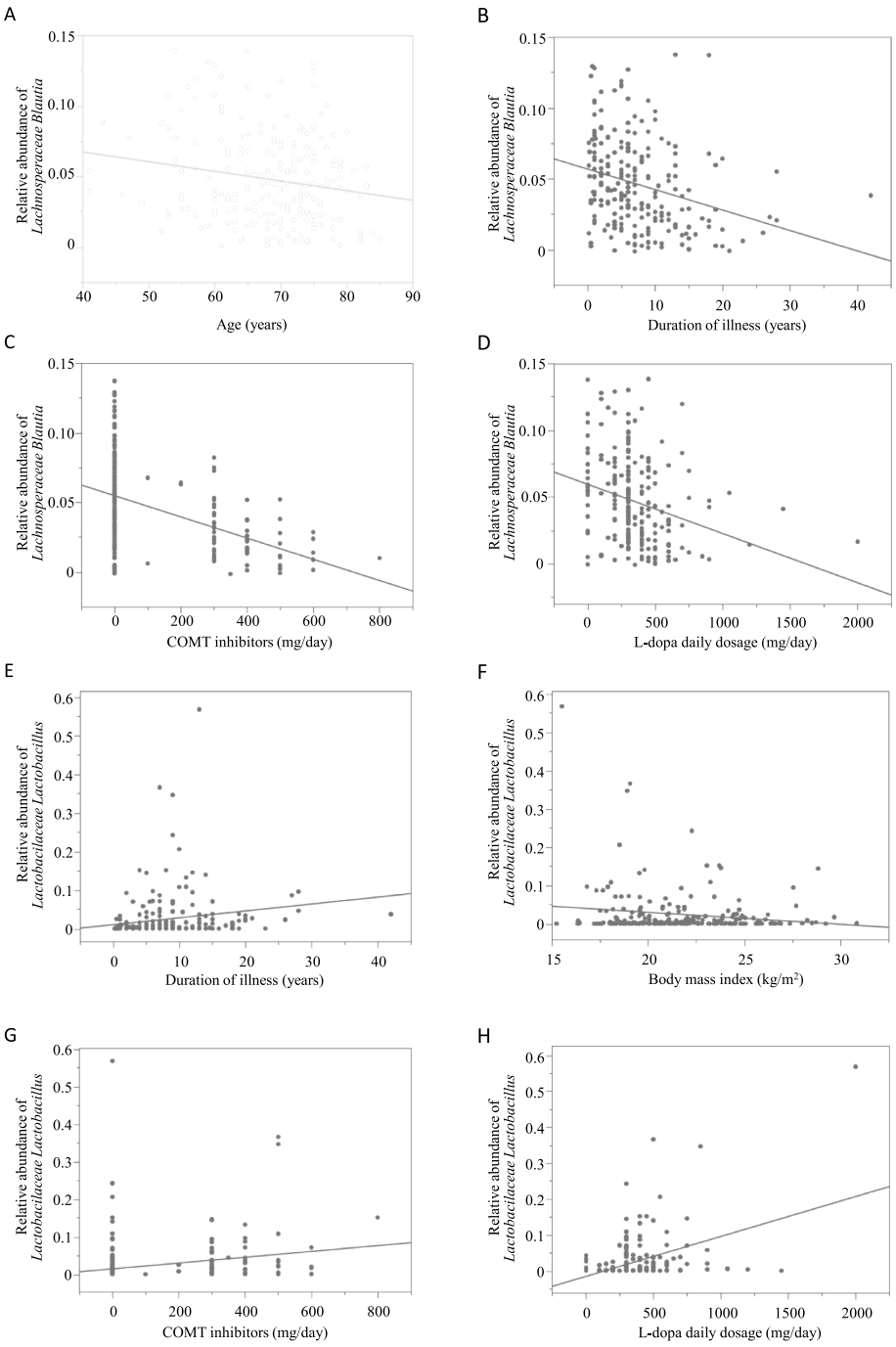

Fig. 2. Relationships between *Lachnospiraceae Blautia* or *Lactobacillaceae Lactobacillus* and clinical characteristics.
COMT, catechol-o-methyl transferase; BMI, body mass index.
Decreased abundance of *Lachnospiraceae Blautia* significantly correlated with age (A), duration of illness (B), daily L-dopa dose (C), and COMT inhibitor use (D). Increased abundance of *Lactobacillaceae Lactobacillus* was significantly correlated with duration of disease (E), BMI (F), daily L-dopa dose (G), and COMT inhibitor use (H).

plausible hypothesis is that the koji used in Japanese fermented foods, such as soy sauce, miso, traditional pickles, and Japanese sake, can contribute to an increased abundance of *Lachnospiraceae Blautia* [30]. Our study did not examine the dietary habits of individual patients. Changes in dietary habits are easy to introduce for PD patients; therefore, such a minor change may have a substantial disease-modifying effect in the long-term.

Lactobacillaceae Lactobacillus, a lactic acid-producing bacteria, has long been used in the production of cheese and other dairy products. Probiotics containing *Lactobacillaceae Lactobacillus* have some beneficial effects in PD via inhibition of inflammatory cytokines, antioxidant effects, and improving bioavailability of L-dopa [31]. In addition, supplemental probiotic *Lactobacillaceae Lactobacillus* reduced clinical scores of motor symptoms in a preliminary open-label small study [32]. Our study showed an increase in *Lactobacillaceae Lactobacillus* abundance in PD patients treated with a COMT inhibitor. The mechanism for this is still unclear.

This study has some limitations. First, this study is a cross-sectional observational study; therefore, the findings cannot clearly assert a causal relationship. Second, the number of PD patients enrolled may be insufficient, especially patients with PD and dyskinesia. In addition, all PD patients were Japanese. Third, daily L-dopa doses are relatively low in Japanese patients with motor complications compared with doses in other countries, which could influence the frequency or severity of motor complications. Fourthly, we did not collect dietary data. Future studies require larger enrollment and prospective tracking of the onset of motor complications.

Table 2
Results of multivariate analysis of *Lachnospiraceae Blautia* and *Lactobacilaceae Lactobacillus*.

A: *Lachnospiraceae Blautia*				
	Model 1		Mode 2	
	95% CI	p value	95% CI	p value
Age	−0.001 to −0.0003	<0.0001	−0.001 to 0.000	0.002
BMI	−0.001 to 0.001	0.917	−0.001 to 0.0009	0.564
COMT inhibitor	–		−0.031 to −0.012	<0.0001
Sex	−0.002 to 0.01	0.186	−0.006 to 0.009	0.667
Duration of illness	−0.001 to −0.00021	0.010	−0.001 to 0.000	0.176
L-dopa daily dosage	–		−0.0000295 to 0.00000579	0.186
Wearing off	−0.027 to −0.007	0.0004	−0.017 to 0.001	0.112
Dyskinesia	−0.008 to 0.014	0.589	−0.007 to 0.013	0.600
B: *Lactobacilaceae Lactobacillus*				
	Model 1		Model 2	
	95% CI	p value	95% CI	p value
Age	−0.0002 to 0.001	0.169	−0.119 to 0.062	0.534
BMI	−0.004 to 0.0004	0.106	−0.00082 to 0.00094	0.393
COMT inhibitor	–		−0.015 to −0.021	0.749
Sex	−0.009 to 0.023	0.418	0.002 to 0.034	0.020
Duration of illness	−0.0004 to 0.002	0.161	−0.001 to 0.001	0.893
L-dopa daily dosage	–		−0.000073 to 0.00014	<0.0001
Wearing off	0.006 to 0.045	0.009	−0.003 to 0.035	0.108
Dyskinesia	−0.033 to 0.010	0.298	−0.032 to 0.008	0.246

CI, confidence interval; BMI, body mass index; COMT, catechol-o-methyl transferase.

5. Conclusions

PD patients with motor complications showed significant changes to their gut microbiome, exemplified by a decrease of *Lachnospiraceae Blautia* and an increase of *Lactobacillaceae Lactobacillus*. Wearing-off was an independent associating factor common to both altered bacteria when involvement of a COMT inhibitor was excluded. This is the first evidence of gut dysbiosis as a possible mechanism for motor complications associated with PD. We suggest that gut dysbiosis is not only associated with the onset of PD but also with the development of motor complications.

Funding

This study was supported by Grants-in-Aid from the Japan Society for the Promotion of Science (JP17K07094 and JP19K16516), the Ministry of Health, Labour and Welfare of Japan (H29-Nanchi-Ippan-030), the Japan Agency for Medical Research and Development (JJP19gm1010002, JP19ek0109230, JP19ek0109281, and JP19bm0804005), the National Center of Neurology and Psychiatry (29-4), and the Hori Sciences and Arts Foundation.

Author contributions

Study conception: K.T., H.N., M.H., K.O., and T.M. Study organization: K.T., H.N., M.I., M.H., K.O., and T.M. Study conduct: K.T., H.K., M. I., K.I., K.Y., K.T., Y.S., K.T, Y.T., K.K., M.H., K.O., and T.M. Data and statistical analysis: K.T., H.N., M.H., K.O. and T.M. Manuscript preparation: K.T. and T.M. All authors reviewed and approved the final draft of the manuscript.

Declaration of competing interest

None.

Acknowledgements

We thank Kanako Abe at the Division of Neurology and Gerontology, Department of Internal Medicine, School of Medicine, Iwate Medical University, for general study support and Tomomi Yamada at the Nagoya University Graduate School of Medicine for sample delivery management. We thank Jeremy Allen, PhD, from Edanz (https://jp.edanz.com/ac) for editing a draft of this manuscript.

References

[1] A. Mulak, B. Bonaz, Brain-gut-microbiota axis in Parkinson's disease, World J. Gastroenterol. 21 (2015) 10609–10620.
[2] H. Braak, K. Del Tredici, H. Bratzke, J. Hamm-Clement, D. Sandmann-Keil, U. Rüb, Staging of the intracerebral inclusion body pathology associated with idiopathic Parkinson's disease (preclinical and clinical stages), J. Neurol. 249 (suppl. 3) (2002) III/1–5.
[3] M.G. Cersosimo, Gastrointestinal biopsies for the diagnosis of alpha-synuclein pathology in Parkinson's disease, Gastroenterol. Res. Pract. 2015 (2015) 476041.
[4] E. Svensson, E. Horváth-Puhó, R.W. Thomsen, J.C. Djurhuus, L. Pedersen, P. Borghammer, H.T. Sørensen, Vagotomy and subsequent risk of Parkinson's disease, Ann. Neurol. 78 (2015) 522–529.
[5] B. Liu, F. Fang, N.L. Pedersen, A. Tillander, J.F. Ludvigsson, A. Ekbom, P. Svenningsson, H. Chen, K. Wirdefeldt, Vagotomy and Parkinson disease: a Swedish register-based matched-cohort study, Neurology 88 (2017) 1996–2002.
[6] S. Breid, M.E. Bernis, J.T. Babila, M.C. Garza, H. Wille, G. Tamgüney, Neuroinvasion of alpha-synuclein prionoids after intraperitoneal and intraglossal inoculation, J. Virol. 90 (2016) 9182–9193.
[7] N. Uemura, H. Yagi, M.T. Uemura, Y. Hatanaka, H. Yamakado, R. Takahashi, Inoculation of alpha-synuclein preformed fibrils into the mouse gastrointestinal tract induces Lewy body-like aggregates in the brainstem via the vagus nerve, Mol. Neurodegener. 13 (2018) 21.
[8] S. Sharma, A. Awasthi, S. Singh, Altered gut microbiota and intestinal permeability in Parkinson's disease: pathological highlight to management, Neurosci. Lett. 712 (2019) 134516.
[9] H. Nishiwaki, T. Hamaguchi, M. Ito, T. Ishida, T. Maeda, K. Kashihara, Y. Tsuboi, J. Ueyama, T. Shimamura, H. Mori, K. Kurokawa, M. Katsuno, M. Hirayama, K. Ohno, Short-chain fatty acid-producing gut microbiota is decreased in Parkinson's disease but not in rapid-eye-movement sleep behavior disorder, mSystems 5 (2020) e00797-20.
[10] L.P. Kelly, P.M. Carvey, A. Keshavarzian, K.M. Shannon, M. Shaikh, R.A. Bakay, J. H. Kordower, Progression of intestinal permeability changes and alpha-synuclein expression in a mouse model of Parkinson's disease, Mov. Disord. 29 (2014) 999–1009.
[11] T.R. Sampson, J.W. Debelius, T. Thron, S. Janssen, G.G. Shastri, Z.E. Ilhan, C. Challis, C.E. Schretter, S. Rocha, V. Gradinaru, M.F. Chesselet, A. Keshavarzian, K.M. Shannon, R. Krajmalnik-Brown, P. Wittung-Stafshede, R. Knight, S. K. Mazmanian, Gut microbiota regulate motor deficits and neuroinflammation in a model of Parkinson's disease, Cell 167 (2016) 1469–1480.
[12] F. Pan-Montojo, M. Schwarz, C. Winkler, M. Arnhold, G.A. O'Sullivan, A. Pal, J. Said, G. Marsico, J.M. Verbavatz, M. Rodrigo-Angulo, G. Gille, R.H.W. Funk, H. Reichmann, Environmental toxins trigger PD-like progression via increased alpha-synuclein release from enteric neurons in mice, Sci. Rep. 2 (2012) 898.
[13] H. Nishiwaki, M. Ito, T. Ishida, T. Hamaguchi, T. Maeda, K. Kashihara, Y. Tsuboi, J. Ueyama, T. Shimamura, H. Mori, K. Kurokawa, M. Katsuno, M. Hirayama, K. Ohno, Meta-analysis of gut dysbiosis in Parkinson's disease, Mov. Disord. 35 (2020) 1626–1635.
[14] T.N. Tran, T.N.N. Vo, K. Frei, D.D. Truong, Levodopa-induced dyskinesia: clinical features, incidence, and risk factors, J. Neural. Transm. 125 (2018) 1109–1117.
[15] C.W. Olanow, J.A. Obeso, F. Stocchi, Drug insight: continuous dopaminergic stimulation in the treatment of Parkinson's disease, Nat. Clin. Pract. Neurol. 2 (2006) 382–392.
[16] R. Djaldetti, J. Baron, I. Ziv, E. Melamed, Gastric emptying in Parkinson's disease: patients with and without response fluctuations, Neurology 46 (1996) 1051–1054.
[17] M. Grover, M. Kanazawa, O.S. Palsson, D.K. Chitkara, L.M. Gangarosa, D. A. Drossman, W.E. Whitehead, Small intestinal bacterial overgrowth in irritable bowel syndrome: association with colon motility, bowel symptoms, and psychological distress, Neuro Gastroenterol. Motil. 20 (2008) 998–1008.
[18] M. Gabrielli, P. Bonazzi, E. Scarpellini, E. Bendia, E.C. Lauritano, A. Fasano, M. G. Ceravolo, M. Capecci, A. Rita Bentivoglio, L. Provinciali, P.A. Tonali, A. Gasbarrini, Prevalence of small intestinal bacterial overgrowth in Parkinson's disease, Mov. Disord. 26 (2011) 889–892.
[19] A. Fasano, F. Bove, M. Gabrielli, M. Petracca, M.A. Zocco, E. Ragazzoni, F. Barbaro, C. Piano, S. Fortuna, A. Tortora, R. Di Giacopo, M. Campanale, G. Gigante, E.

C. Lauritano, P. Navarra, S. Marconi, A. Gasbarrini, A.R. Bentivoglio, The role of small intestinal bacterial overgrowth in Parkinson's disease, Mov. Disord. 28 (2013) 1241–1249.
[20] I. Yazawa, Y. Terao, I. Sai, K. Hashimoto, M. Sakuta, Gastric acid secretion and absorption of levodopa in patients with Parkinson's disease the effect of supplement therapy to gastric acid, Rinsho Shinkeigaku 34 (1994) 264–266.
[21] F. Scheperjans, V. Aho, P.A. Pereira, K. Koskinen, L. Paulin, E. Pekkonen, E. Haapaniemi, S. Kaakkola, J. Eerola-Rautio, M. Pohja, E. Kinnunen, K. Murros, P. Auvinen, Gut microbiota are related to Parkinson's disease and clinical phenotype, Mov. Disord. 30 (2015) 350–358.
[22] E.M. Hill-Burns, J.W. Debelius, J.T. Morton, W.T. Wissemann, M.R. Lewis, Z. D. Wallen, S.D. Peddada, S.A. Factor, E. Molho, C.P. Zabetian, R. Knight, H. Payami, Parkinson's disease and Parkinson's disease medications have distinct signatures of the gut microbiome, Mov. Disord. 32 (2017) 739–749.
[23] T. Kurakawa, K. Ogata, K. Matsuda, H. Tsuji, H. Kubota, T. Takada, Y. Kado, T. Asahara, T. Takahashi, K. Nomoto, Diversity of intestinal Clostridium coccoides group in the Japanese population, as demonstrated by reverse transcription-quantitative PCR, PLoS One 10 (2015), e0126226.
[24] C.W. Olanow, K. Kieburtz, O. Rascol, W. Poewe, A.H. Schapira, M. Emre, H. Nissinen, M. Leinonen, F. Stocchi, Stalevo reduction in dyskinesia evaluation in Parkinson's disease (STRIDE-PD) investigators, factors predictive of the development of levodopa-induced dyskinesia and wearing-off in Parkinson's disease, Mov. Disord. 28 (2013) 1064–1071.
[25] E. Tuovinen, J. Keto, J. Nikkilä, J. Mättö, K. Lähteenmäki, Cytokine response of human mononuclear cells induced by intestinal Clostridium species, Anaerobe 19 (2013) 70–76.
[26] S. Nishijima, W. Suda, K. Oshima, S.W. Kim, Y. Hirose, H. Morita, M. Hattori, The gut microbiome of healthy Japanese and its microbial and functional uniqueness, DNA Res. 23 (2016) 125–133.
[27] T. Brudek, Inflammatory bowel disease and Parkinson's disease, J. Parkinsons Dis. 9 (Suppl 2) (2019) S331–S344.
[28] G. Pagano, S. Polychronis, H. Wilson, B. Giordano, N. Ferrara, F. Niccolini, M. Politis, Diabetes mellitus and Parkinson disease, Neurology 90 (2018) e1654–e1662.
[29] A. Yoritaka, Y. Shimo, M. Takanashi, J. Fukae, T. Hatano, T. Nakahara, N. Miyamato, T. Urabe, H. Mori, N. Hattori, Motor and non-motor symptoms of 1453 patients with Parkinson's disease: prevalence and risks, Park. Relat. Disord. 19 (2013) 725–731.
[30] H. Hamajima, H. Matsunaga, A. Fujikawa, T. Sato, S. Mitsutake, T. Yanagita, K. Nagao, J. Nakayama, H. Kitagaki, Japanese Traditional Dietary Fungus Koji *Aspergillus oryzae* Functions as a Prebiotic for *Blautia coccoides* through Glycosylceramide: Japanese Dietary Fungus Koji Is a New Prebiotic, vol. 5, Springer Plus, 2016, p. 1321.
[31] Y. Magistrelli, A. Amoruso, L. Mogna, T. Graziano, R. Cantello, M. Pane, C. Comi, Probiotics may have beneficial effects in Parkinson's disease: in vitro evidence, Front. Immunol. 10 (2019) 969.
[32] C.S. Lu, H.C. Chang, Y.H. Weng, C.C. Chen, Y.S. Kuo, Y.C. Tsai, The add-on effect of *Lactobacillus plantarum PS128* in patients with Parkinson's disease: a pilot study, Front. Nutr. 8 (2021) 650053.