Safety and efficacy of faecal microbiota transplantation in patients with mild to moderate Parkinson's disease (GUT-PARFECT): a double-blind, placebo-controlled, randomised, phase 2 trial

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Summary

Background Dysregulation of the gut microbiome has been implicated in Parkinson's disease (PD). This study aimed to evaluate the clinical effects and safety of a single faecal microbiota transplantation (FMT) in patients with early-stage PD.

Methods The GUT-PARFECT trial, a single-centre randomised, double-blind, placebo-controlled trial was conducted at Ghent University Hospital between December 01, 2020 and December 12, 2022. Participants (aged 50–65 years, Hoehn and Yahr stage 2) were randomly assigned to receive nasojejunal FMT with either healthy donor stool or their own stool. Computer-generated randomisation was done in a 1:1 ratio through permutated-block scheduling. Treatment allocation was concealed for participants and investigators. The primary outcome measure at 12 months was the change in the Movement Disorders Society-Unified Parkinson's Disease Rating Scale (MDS-UPDRS) motor score obtained during off-medication evaluations. Intention-to-treat analysis was performed using a mixed model for repeated measures analysis. This completed trial is registered on ClinicalTrials.gov (NCT03808389).

Findings Between December 2020 and December 2021, FMT procedures were conducted on 46 patients with PD: 22 in the healthy donor group and 24 in the placebo group. Clinical evaluations were performed at baseline, 3, 6, and 12 months post-FMT. Full data analysis was possible for 21 participants in the healthy donor group and 22 in the placebo group. After 12 months, the MDS-UPDRS motor score significantly improved by a mean of 5.8 points (95% CI −11.4 to −0.2) in the healthy donor group and by 2.7 points (−8.3 to 2.9) in the placebo group (p = 0.0235). Adverse events were limited to temporary abdominal discomfort.

Interpretation Our findings suggested a single FMT induced mild, but long-lasting beneficial effects on motor symptoms in patients with early-stage PD. These findings highlight the potential of modulating the gut microbiome as a therapeutic approach and warrant a further exploration of FMT in larger cohorts of patients with PD in various disease stages.

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Research in context

Evidence before this study

A comprehensive search was conducted on PubMed from database inception till September 1st, 2023, using the search terms "Parkinson" and "gut microbiota" or "gut microbiome", without any language or date restrictions. The existing literature supports an early involvement of the gastrointestinal system in the aetiology and progression of Parkinson's disease (PD) for a sub-group of patients, supporting the gut-first versus brain-first hypothesis. During the prodromal phase of the disease, evidence suggests the presence of alpha-synuclein in the enteric nervous system, subclinical gut inflammation, compromised intestinal barrier integrity, and gastrointestinal symptoms like constipation. Several recent meta-analyses comparing the gut microbiota of patients with PD to healthy controls have shown differential abundances of taxa associated with reduced mucosal barrier and increased intestinal inflammation. An additional search was performed on PubMed using the terms "Parkinson" and "microbiota transplantation" which yielded 82 reports predominantly focusing on faecal microbiota transplantation (FMT) as a potential treatment for PD. Four open-label studies with small number of patients have demonstrated beneficial effects on symptoms, mainly constipation. Although technically not a FMT study, a recently conducted pilot study with a randomized and placebo-controlled design (n = 11), using an orally lyophilised donor stool product or matching placebo, was well tolerated and reported reduced constipation, however objective UPDRS motor improvements were transient and not statistically different from placebo. In these studies, adverse events associated with FMT were mild and restricted to transient gastro-intestinal discomfort and diarrhoea.

Added value of this study

We present the results of a one-year, randomized, double-blind, placebo-controlled study of nasojejunal FMT in patients with early-stage PD. This study is the first of its kind with larger sample sizes compared to previously reported trials. The healthy donor FMT group demonstrated significantly greater improvements in motor symptom severity compared to the control group, with the effect becoming more pronounced starting from the 6 to 12 months interval. Objective measurements of gastrointestinal transit indicated improvements in the healthy donor group, starting from the 3 to 6 months interval. No severe adverse events were reported in either group, further supporting the safety profile of FMT.

Implications of all the available evidence

The findings of this study, combined with earlier smaller and open studies, highlight the potential of FMT as a treatment option for patients with PD. However, larger multicentre trials with extended follow-up periods are necessary to validate these results. Furthermore, the correlation between beneficial outcomes, alterations in microbiota composition and inflammatory markers needs to be further investigated and validated from a pathophysiological perspective.

Introduction

Parkinson's disease (PD) is a rapidly growing neurological disorder characterized by progressive degeneration of dopaminergic neurons in the substantia nigra, resulting in bradykinesia, rigidity, resting tremor, and postural instability.[1] Additionally, patients experience non-motor symptoms that significantly impact their quality of life and often precede the motor symptoms.[2] Prodromal gastrointestinal dysfunction is highly prevalent with approximately 80% of *de novo* untreated patients exhibiting prolonged colon transit time as a marker for constipation.[3] Several other findings emphasise the important role of the gut in PD. Patients with newly diagnosed, untreated PD exhibit evidence of increased intestinal inflammation and disturbed permeability of the intestinal epithelial barrier.[4,5] The pathological hallmark of PD, alpha-synuclein aggregates, has been observed in the gastrointestinal system during the prodromal phase of the disease.[6] This aggregated alpha-synuclein can reach the brain via the vagal nerve, which has been shown directly in animal models[7] and indirectly by an apparent reduced risk of developing PD after vagotomy.[8] These findings led to the dual-hit Braak hypothesis which states that alpha-synuclein aggregation is triggered by microbiota at the level of the gut and/or the olfactory nerves.[9] Recently, this hypothesis has been expanded to a body-first or brain-first onset of PD.[10] In the body-first phenotype, pathology is believed to start in the gut and emerging evidence suggests that the gut microbiome may play this pivotal role in PD pathogenesis and progression.[11] Meta-analyses comparing the gut microbiota of patients with PD and healthy controls have revealed differentially abundant taxa associated with reduced mucosal barrier and increased intestinal inflammation.[12] In preclinical studies, modulating the gut microbiota had neuroprotective effects.[13,14]

Faecal microbiota transplantation (FMT) represents the most effective method for achieving comprehensive and long-lasting changes in gut microbiota composition. FMT has been safely and successfully used for *Clostridioides difficile* infections, for which it is an approved indication.[15] However, evidence supporting the use of FMT in patients with PD is limited to case reports and open-label studies involving a small number of patients.[16–20] These studies have reported subjective and objective improvement in motor and non-motor symptoms, particularly constipation. Nevertheless, variations in inclusion criteria, FMT procedures and administration routes, clinical assessment, and follow-up periods, as well as the absence of placebo controls, have hindered the interpretation of results and the estimation of potential placebo effects.

Here, we present the results of the first randomised, double-blind, and placebo-controlled trial designed to evaluate the safety and efficacy of a single FMT procedure via nasojejunal administration of healthy donor stool (active treatment group) compared to own stool (placebo group) in patients with mild to moderate PD. This study aimed to provide robust evidence regarding the therapeutic potential of FMT in PD and address the limitations of previous investigations.

Methods

Study design

A single-centre randomised, double-blind and placebo-controlled phase 2 trial was performed at Ghent University Hospital (GUT-PARFECT trial) between December 01, 2020 and December 12, 2022. Treatment groups included a healthy donor FMT and a placebo FMT (own stool). The study included a baseline visit, a colonoscopy before and 3 months after FMT, the FMT procedure itself, and study visits at 3, 6, and 12 months after FMT. All clinical assessments were done at Ghent University Hospital by the same clinical investigator to avoid inter-investigator variability. The double-blind treatment period started at randomisation and lasted until the final study visit of the last participant. Changes in pharmacotherapy during the trial period were left at the discretion of the treating neurologist. The study did not use an independent data safety monitoring board, in view of the experience with FMT gained in various indications. The full trial protocol is available in the appendix.

Ethics

The trial protocol was approved by the ethical committee of Ghent University Hospital. Prospective written informed consent forms were obtained from every candidate prior to the start of the study. The study was performed in accordance with the ethical principles of the Declaration of Helsinki, Good Clinical Practice (GCP) and applicable regulatory requirements.

Participants

The inclusion criteria were a clinical PD diagnosis according to the Movement Disorder Society criteria, age limit of 65 years old, age of motor symptoms onset older than 50 years old, and Hoehn & Yahr stage II or III in an off-medication state. We excluded patients with a first degree relative or more than one relative with PD, patients with a diagnosis of dementia or Mini-Mental State Examination Score <25, patients with a diagnosis of depression or psychosis (DSM-V criteria), patients with gastrointestinal dysfunction unrelated to PD (primary disease or surgery leading to structural abnormalities of the intestines), patients with an immune disorder or under clinical immunosuppression. In addition, drug abuse, malignancy, or any severe comorbidity that might interfere with the study course were considered exclusion criteria. The FMT procedure was only performed if there was no use of probiotics or antibiotics in the three months prior to the FMT, and no gastrointestinal or respiratory tract infection in the two months prior to the FMT. Eligibility was established through in-person assessments following pre-screening over the phone or via e-mail. After inclusion via written informed consent, a formal baseline study visit was organised to minimize the time between baseline visit and date of FMT, and to allow for assessment in off-medication state.

Randomisation and masking

Eligible patients were randomly assigned to receive nasojejunal FMT with either healthy donor stool (active treatment group) or their own stool (placebo group). Computer-generated randomisation was done by an independent person involved in FMT preparation in a 1:1 ratio through a permutated-block schedule with a block size of 4. Treatment allocation was masked for participants, personnel involved in FMT administration and clinical investigators. Consequently, all patients had to deliver a stool sample to prepare a potential placebo FMT solution.

Procedures

Healthy donors were recruited via the Ghent Stool Bank following a strict inclusion protocol according to national (Superior Health Council of Belgium nr. 9202) and international guidelines (European FMT working group).[21] The selection process involves a meticulous review of the donor's clinical and personal information (collected through a questionnaire) as well as serology and stool testing. Faecal donations were collected over a period of 1 month and were released from quarantine after serological testing 3 months after the last faecal donation. This procedure helps to ensure safety, cost-efficiency, and availability. For each FMT preparation, 50 g of faecal product was used. The faecal product was diluted with sterile saline and subsequently homogenized anaerobically and filtered using a stomacher

(BagMixer, Interscience). Glycerol (10%) was added as a cryoprotectant to the filtered product resulting in a total volume of 200 ml. The faecal suspension was stored at –80 °C. Maximum 4 h before the FMT, the faecal suspension was thawed for 30 min in a water bath at 37 °C. Importantly, only healthy donor stools that were collected before the COVID-19 pandemic were used to avoid any potential confounder related to COVID-19. FMT solutions of 17 different healthy donors were used in the study. Due to the double-blind set-up of the study, each participant delivered a fresh stool sample to the lab 10–14 days preceding the FMT, and potential placebo FMT solutions were prepared from each participant. Participants collected this sample at home using a sampling kit containing a plastic collection box, cooler blocks, a sealable container, and an AnaeroGen™ Compact pouch to create an anaerobic environment in the sealable container. Participants delivered the stool sample to the Laboratory of Medical Microbiology of Ghent University Hospital within 2 h. The stool samples were aliquoted and the correct dilution for the faecal transplant solution was based on faecal weight as described above.

Seven days before the FMT, patients underwent a colonoscopy to screen for contra-indications. This colonoscopy was preceded by a bowel preparation according to the standard procedures of our centre, including low-fiber diet three days beforehand and solely clear liquid intake the day beforehand. Polyethylene glycol (Plenvu®, Norgine) preparations were taken the evening and the morning preceding the colonoscopy, according to the manufacturer's instructions. For FMT, candidates underwent this bowel preparation again. The FMT solution was thawed at 37 °C prior to the FMT procedure. The transplantation itself was performed through nasojejunal administration. The correct placement of the tube was confirmed through the Cortrak Enteral Access System,[15] followed by release of the FMT solution (200 ml). Afterwards, candidates lied still for 1 h before leaving the hospital again.

The study visits at baseline, 3, 6, and 12 months post-FMT were always performed in the morning in a fasting state. The off-medication state was defined as a period of withdrawal of levodopa for at least 8 h (i.e., overnight) or 36 h in the case of long-acting drugs such as ropinirole, pramipexole, rasagiline, and safinamide. Dopamine agonist doses were reduced to 50% of the initial dose three days before the study visit, and no dose was taken on the day before the study visit. In addition, we also included an overnight withdrawal of amantadine, and anticholinergic agents when prescribed for a resting tremor.

Prior to the study visit, the participants received questionnaires and instructions to achieve an off-medication state based on their individual medication list. The questionnaires included the Parkinson's Disease Questionnaire, Non-motor Symptoms Scale, Wexner Constipation Scale, Bristol Stool Chart, Geriatric Depression Scale, Parkinson Anxiety Scale, Lille Apathy Rating Scale, Parkinson's Disease Sleep Scale, and Parkinson's Fatigue Scale.

The MDS-UPDRS (all 4 parts) and the Montreal Cognitive Assessment was performed at the beginning of the study visit. During the study visit, the other filled-in questionnaires were briefly checked by the study investigator to minimize omissions. At the baseline visit, a 16-item Sniffin' Sticks identification test was performed (Burghart, Wedel, Germany), a result <11 points was defined as hyposmia. In addition, the REM Sleep Behavior Disorder Screening Questionnaire (RBDSQ) was administered and considered positive with a cut-off of >5 points. Furthermore, we performed an orthostatic hypotension test by measuring blood pressure in prone position, as well as upright 1 and 3 min later. A drop in systolic blood pressure of 20 mmHg or diastolic blood pressure drop of 10 mmHg was defined as orthostatic hypotension. Finally, to determine a more objective estimation of the colon transit time, we performed a radiopaque pellets test at baseline, 3, 6, and 12 months post-FMT. Ten radiopaque markers (3.5 mm x 3.5 mm; SAPA6210; Sapi Med, Allesandria, Italy) had to be ingested every morning starting from the sixth day before the study visit until the last day before the study visit. The study visit ended with an abdominal x-ray to determine the amount and location of radiopaque markers. Colon transit time was calculated using the following equation (total number of pellets on day 7 + 5)/10.[22] Safety assessments were performed at every study visit, as well as by telephone and/or e-mail one week after FMT, and included general questions concerning the presence of fever, gastrointestinal changes, or other self-reported symptoms.

Outcomes

The primary endpoint was the change of the motor section score of MDS-UPDRS, measured in an off-medication state (as defined above), from baseline to 12 months post-FMT for the healthy donor group compared to the placebo group. Other prespecified secondary endpoints at 12 months post-FMT were the levodopa-equivalent daily dose (LEDD), the radiopaque pellets test to determine colon transit time, and the scores of the MDS-UPDRS total score and scores of the other subdivisions (1, 2 and 4), Parkinson's Disease Questionnaire, Non-motor Symptoms Scale, Wexner Constipation Scale, Geriatric Depression Scale, Parkinson Anxiety Scale, Lille Apathy Rating Scale, Parkinson's Disease Sleep Scale, Parkinson's Fatigue Scale, and Montreal Cognitive Assessment.

Statistical analysis

Our study was powered to show an effect of FMT on MDS-UPDRS part 3 (motor score) in one year that was large enough to suggest a disease modifying effect of

gut microbiota alteration in PD. The Prospective Parkinson's Progression Markers Initiative (PPMI) cohort showed a mean MDS-UPDRS motor score progression over 12 months of 6.35 (SD 6.6) for patients with early PD.[23] A total sample size of 46 patients was calculated to provide 90% power to detect a MDS-UPDRS motor score difference of 6.35 (SD 6.6) between the two treatment groups with a two-sides significance level of 0.05.

A linear mixed model for repeated measurements (MMRM) was fitted to all the data combined, using the method of residual maximum likelihood, as implemented in Genstat version 22 (VSN International, Hemel Hempstead, UK). This procedure has the advantage of reducing the influence of missing data on the analysis because of LMM's benefit to model correlations between data. Briefly, the linear mixed model (random terms underlined) of the form y = μ + gender + treatment + time + treatment.time + covariates + patient + patient.time was fitted to the repeated measurements. Two kinds of analyses were performed: 1) either having the baseline measurements (T0) as covariate (MDS-UPDRS motor score was slightly higher at baseline compared to the placebo group, therefore we decided to correct for this in the model), or 2) having T0 as first level of the time factor. The constant μ represents an overall mean across all observations. The factor gender represents the effect of male and female averaged across all time points. The factor treatment represents the effect of either healthy donor FMT or placebo FMT averaged across all time points. The factor time indicates the effect at each time point, averaged across healthy donor FMT and placebo FMT. The interaction term treatment. time represents the differences between the two treatment levels as a function of time. We decided to include several covariates in the model to correct for disease duration, as well as factors that could indicate either a gut-first or brain-first PD phenotype. Covariates other than T0 included in the model are age, BMI, LEDD, duration of illness, amount of radiopaque pellets, Sniffin' Sticks test score, and constipation according to Rome IV criteria. The term patient time represents the residual error term with dependent errors because the repeated measurements are taken in the same individual, causing possible correlations among observations. Several covariance models were fitted to the data to account for the correlation present in the data. The power (city-block metric) correlation model was selected as best fitted model based on the Akaike's information criterion coefficient. Additional options selected to get a best fitting model included a common correlation between any pair of measurement points, done through the random term patient in the model, and allowance of unequal variances across time (heteroscedasticity). The significance of the fixed terms in the model and significance of changes in difference between healthy donor FMT and placebo FMT effects across time windows, were assessed using an approximate *F*-test as implemented in Genstat version 22. All data reported, including tables and figures, are results from this MMRM adjusted for covariates.

For safety data, the incidence of adverse events were summarized. This completed trial is registered on ClinicalTrials.gov (NCT03808389).

Role of the funding source

The sponsors had no role in study design, data collection, data analysis, data interpretation or in manuscript writing. All authors had full access to all the data in the study and the senior authors had final responsibility for the decision to submit for publication.

Results

Due to COVID-19-related restrictions, recruitment and treatment allocation were delayed from October 2019 to December 2020. During this period, a total of 289 patients were assessed for eligibility, with 47 patients ultimately enrolled (Fig. 1). The FMT procedures took place between December 2020 and December 2021, with one patient discontinuing the procedure upon request. Among the enrolled patients, 46 were randomly assigned to receive either healthy donor FMT (n = 22) or placebo FMT with their own stool (n = 24). Of these patients, 43 completed all study visits, with 21 in the healthy donor and 22 in the placebo FMT group. There was a limited amount of missing data, mainly caused by three participants that did not complete all study visits (as depicted in Fig. 1).

Baseline demographic variables were well-balanced between the treatment groups, although the healthy donor group had a higher baseline MDS-UPDRS motor score. There were no notable differences between the groups in terms of LEDD, Hoehn and Yahr stage, and duration since PD diagnosis, which are all markers of disease progression (Table 1). Both groups had a male/female ratio reflecting the usual pattern in PD (roughly 2/3 ratio) and were quite representative of the PD population in this stage of the disorder. Comorbidities and prescribed non-dopaminergic medication is listed for every participant in Supplementary Table S1.

At the 12-month mark, the primary outcome measure, namely the MDS-UPDRS motor scores in an off-medication state, showed improvement in the healthy donor FMT group with a decrease of 5.8 points (95% CI −11.4 to −0.2) compared to 2.7 points (−8.3 to 2.9) in the placebo group (Table 2; Fig. 2). The change in MDS-UPDRS motor score from baseline to 12 months post-FMT was significantly different between treatment groups (p = 0.0235; Table 2; Fig. 2), with the most important between-group deviation in the 6-to-12-months interval. The placebo FMT group experienced an increase in the number of radiopaque pellets by 6.9

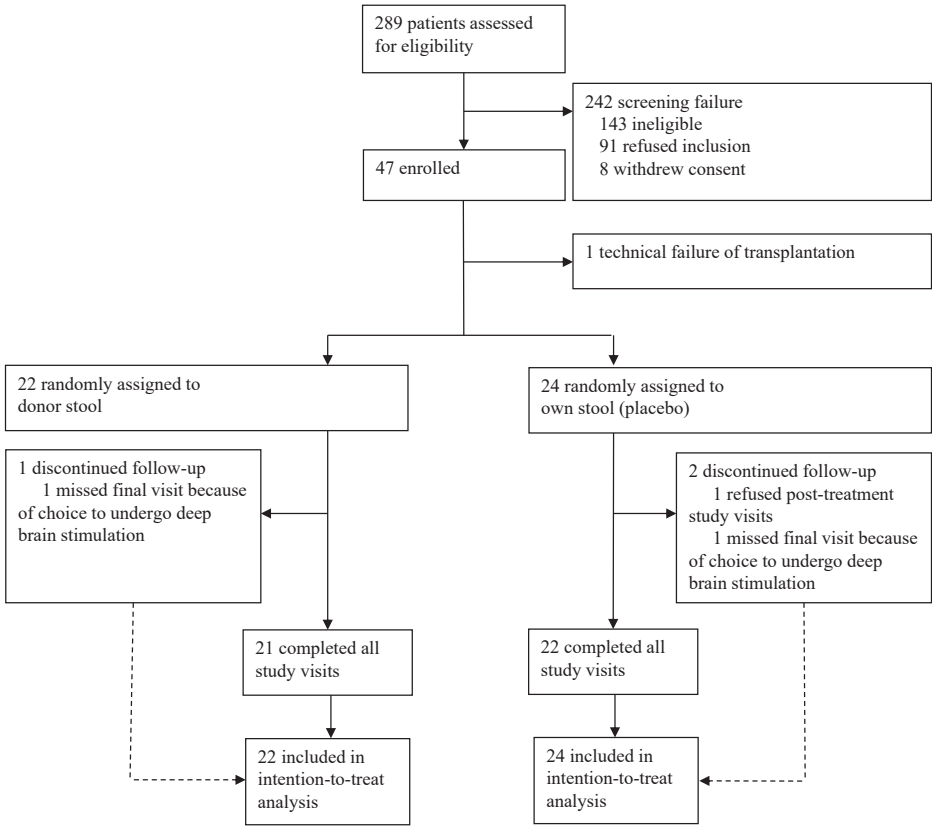

Fig. 1: **Trial profile.**

	Donor FMT (n = 22)	Placebo FMT (n = 24)
Sex		
Men	15 (68.2%)	14 (58.3%)
Women	7 (31.8%)	10 (41.7%)
Age (years)	61 (1.1)	60.5 (0.7)
Duration since Parkinson's disease diagnosis (years)	4.2 (0.7)	4.4 (0.7)
MDS-UPDRS part 3 off medication	40.3 (2.7)	37.1 (2.5)
Hoehn and Yahr stage 2 off medication	22 (100%)	24 (100%)
Levodopa-equivalent daily dose (mg)	383 (53)	431 (51)
Body mass index (kg/m^3)	24.6 (0.8)	24.3 (0.8)
MoCA score	27.6 (0.3)	28.2 (0.3)
Constipation		
Constipation (ROME-IV criteria)	14 (63.3%)	15 (62.5%)
Number of radiopaque pellets on day 7	20.6 (2.0)	18.6 (2.1)
Bristol stool chart score	3.4 (1.1)	3.0 (0.9)
16-item sniffing sticks identification test	7.9 (0.6)	7.1 (0.5)
RBDSQ >5 points	6 (27.3%)	5 (20.8%)
Orthostatic hypotension	9 (40.9%)	7 (29.2%)

Data are n (%) or mean (SEM). MDS-UPDRS, Movement Disorders Society Unified Parkinson's Disease Rating Scale. MoCA, Montreal Cognitive Assessment. RBDSQ, REM Sleep Behavior Disorder Screening Questionnaire.

Table 1: **Patient characteristics at baseline.**

	Baseline	3 months	6 months	12 months	Change (0–12 months)	p value
MDS-UPDRS part 3 (off-medication)						
Donor FMT	40.3 (2.7)	38.3 (2.7)	38.8 (2.6)	34.6 (3.0)	−5.8 (2.0)	**0.0235**
Placebo FMT	37.1 (2.5)	32.6 (2.6)	31.5 (2.5)	34.5 (2.9)	−2.7 (1.9)	
MDS-UPDRS part 1						
Donor FMT	11.0 (1.3)	11.1 (1.4)	10.6 (1.4)	11.0 (1.3)	0.1 (0.9)	0.7875
Placebo FMT	10.6 (1.3)	9.9 (1.4)	8.4 (1.4)	9.3 (1.2)	−1.3 (0.9)	
MDS-UPDRS part 2						
Donor FMT	10.7 (1.3)	11.2 (1.3)	12.0 (1.4)	12.1 (1.3)	1.4 (0.8)	0.7059
Placebo FMT	8.0 (1.2)	8.3 (1.3)	8.8 (1.3)	8.5 (1.3)	0.6 (0.8)	
MDS-UPDRS part 4						
Donor FMT	2.2 (0.6)	2.3 (0.5)	2.1 (0.6)	2.4 (0.6)	0.2 (0.4)	0.6308
Placebo FMT	2.6 (0.5)	2.5 (0.5)	2.8 (0.6)	2.5 (0.6)	−0.1 (0.4)	
MDS-UPDRS total						
Donor FMT	63.9 (4.2)	62.7 (4.3)	62.7 (4.4)	60.1 (4.6)	−3.7 (2.8)	0.2884
Placebo FMT	58.2 (4.0)	53.3 (4.1)	51.2 (4.2)	54.9 (4.5)	−3.3 (2.8)	

Data are mean (SEM). MDS-UPDRS, Movement Disorders Society Unified Parkinson's Disease Rating Scale. Significant differences ($p < 0.05$) are indicated in bold.

Table 2: **MDS-UPDRS scores between baseline and 12 months.**

pellets (2.0–11.8) corresponding to an increased colon transit time, whereas the active treatment group had a small decrease of 1.2 pellets (–6.1 to 3.7) ($p = 0.0252$; Table 3; Fig. 3). Additionally, the healthy donor FMT group demonstrated worse performance on the Parkinson's Fatigue Scale ($p = 0.0418$; Table 3). There were no significant differences between the treatment groups in other scores of the MDS-UPDRS (part 1, part 2, part 4, and part 1–4 total score; Table 2), the LEDD, the Non-Motor Symptoms Scale for Parkinson's Disease, the Parkinson's Disease Quality of Life Questionnaire, Wexner Constipation Scale, Geriatric Depression Scale, Parkinson Anxiety Scale, Lille Apathy Rating Scale, Parkinson's Disease Sleep Scale, and Montreal Cognitive Assessment (Table 3).

No severe adverse events associated with treatment were observed during the study. Mild transient gastrointestinal adverse events, such as abdominal cramps and nausea, were reported in the first week after treatment in 13 (59%) patients in the healthy donor FMT group and 6 (25%) patients in the placebo FMT group. Non-treatment-related hospital admissions occurred in

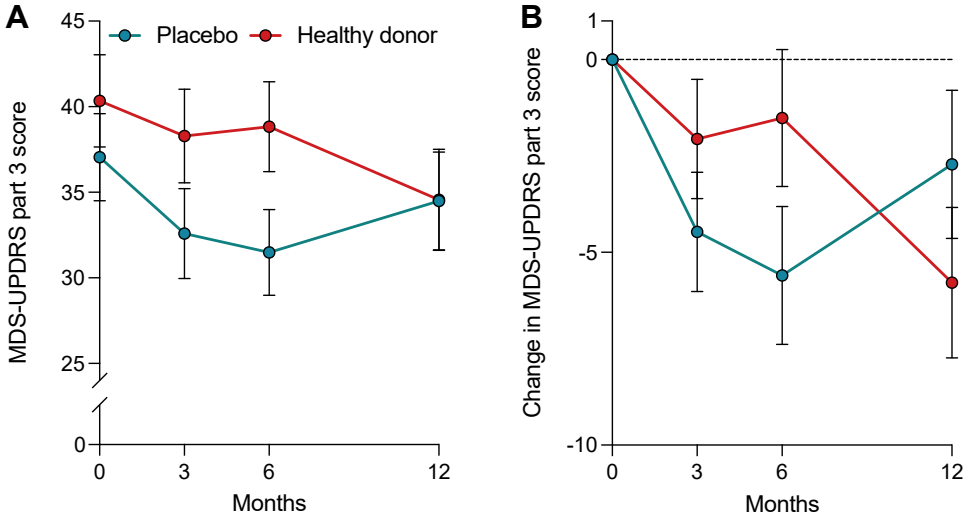

Fig. 2: **MDS-UPDRS part 3 motor scores (A) and changes in MDS-UPDRS part 3 motor scores (B), by study visit. Data are means for the off-medication state. Error bars represent standard error of the mean**. MDS-UPDRS, Movement Disorders Society Unified Parkinson's Disease Rating Scale.

	Baseline	3 months	6 months	12 months	p value
Radiopaque pellets test (number of radiopaque pellets on day 7)					
Donor FMT	20.6 (2.0)	22.1 (2.1)	19.2 (2.1)	19.4 (2.1)	**0.0252**
Placebo FMT	18.6 (2.1)	19.4 (2.1)	23.7 (2.1)	25.5 (2.2)	
Levodopa-equivalent daily dose (mg)					
Donor FMT	383.0 (53.3)	398.8 (57.2)	399.5 (57.0)	429.1 (59.0)	0.8350
Placebo FMT	431.1 (50.8)	429.7 (54.7)	442.1 (54.5)	455.9 (56.5)	
Non-Motor Symptoms Scale for Parkinson's Disease (NMSS)					
Donor FMT	45.8 (9.1)	50.1 (8.9)	48.2 (8.6)	54.7 (8.3)	0.1443
Placebo FMT	44.4 (8.6)	31.6 (8.5)	35.9 (8.3)	33.4 (8.0)	
Parkinson's Disease Quality of Life Questionnaire (PDQ-39)					
Donor FMT	34.4 (4.7)	32.8 (4.5)	35.4 (4.8)	36.5 (4.6)	0.5439
Placebo FMT	26.2 (4.4)	24.3 (4.3)	26.8 (4.6)	25.0 (4.5)	
Wexner Constipation Scale					
Donor FMT	6.7 (1.3)	6.8 (1.0)	6.7 (1.0)	6.7 (1.1)	0.7696
Placebo FMT	5.8 (1.2)	5.5 (1.0)	4.7 (1.0)	5.1 (1.0)	
Geriatric Depression Scale (GDS)					
Donor FMT	17.5 (0.7)	17.2 (0.6)	17.4 (0.6)	16.8 (0.6)	0.1674
Placebo FMT	17.3 (0.6)	17.3 (0.6)	17.7 (0.5)	18.3 (0.6)	
Parkinson Anxiety Scale (PAS)					
Donor FMT	8.7 (1.8)	9.7 (1.7)	8.1 (1.7)	9.6 (1.7)	0.1519
Placebo FMT	8.8 (1.7)	8.0 (1.6)	8.2 (1.6)	8.2 (1.6)	
Lille Apathy Rating Scale (LARS)					
Donor FMT	−19.4 (1.5)	−19.8 (1.6)	−19.0 (1.5)	−19.3 (1.4)	0.4253
Placebo FMT	−20.7 (1.4)	−21.2 (1.5)	−21.9 (1.5)	−22.4 (1.3)	
Parkinson's Disease Sleep Scale (PDSS)					
Donor FMT	101.1 (5.7)	104.5 (5.5)	101.3 (5.4)	102.7 (5.3)	0.7290
Placebo FMT	109.7 (5.4)	109.7 (5.3)	108.8 (5.3)	110.5 (5.1)	
Parkinson's Fatigue Scale (PFS)					
Donor FMT	37.3 (3.2)	39.6 (2.9)	40.0 (3.1)	43.4 (3.2)	**0.0418**
Placebo FMT	34.7 (3.0)	35.7 (2.8)	34.7 (3.0)	33.4 (3.2)	
Montreal Cognitive Assessment (MoCA)					
Donor FMT	27.6 (0.3)	–	28.4 (0.3)	28.2 (0.2)	0.4828
Placebo FMT	28.2 (0.3)	–	28.7 (0.3)	28.9 (0.2)	

Data are mean (SEM). MDS-UPDRS, Movement Disorders Society Unified Parkinson's Disease Rating Scale. Significant differences ($p < 0.05$) are indicated in bold.

Table 3: **Secondary outcomes between baseline and 12 months.**

three healthy donor FMT patients, including recurring paroxysmal atrial fibrillation, lower back pain, and an accidental fall resulting in a humerus fracture. One placebo FMT patient had a hospital admission for elective knee surgery.

Discussion

This study represents the first randomised, double-blind, placebo-controlled clinical trial demonstrating the improvement of motor symptoms in patients with mild to moderate PD through FMT via nasojejunal administration.

In GUT-PARFECT, a significant improvement of 5.8 points (95% CI −11.4 to −0.2) on the MDS-UPDRS motor score in an off-medication state was observed twelve months following FMT with healthy donor stool compared to 2.7 points (−8.3 to 2.9) after placebo FMT with autologous stool. Of note, this difference is considered clinically meaningful for patients with PD, as a within-group change of 3.25 indicates relevant improvement.[24]

Non-motor symptoms were assessed through questionnaires, except for the more objective testing of constipation through the radiopaque pellets test. Treatment group differences in the radiopaque pellets test were noticeable as early as 3–6 months after FMT, while the greatest improvement in motor symptoms (MDS-UPDRS part 3 in off-medication state) was observed in the 6-to-12-months interval, suggesting a primary beneficial effect of FMT at the gastrointestinal level, before neurological effects become apparent. No significant differences were observed between the treatment groups for LEDD and MDS-UPDRS parts 1, 2, 4,

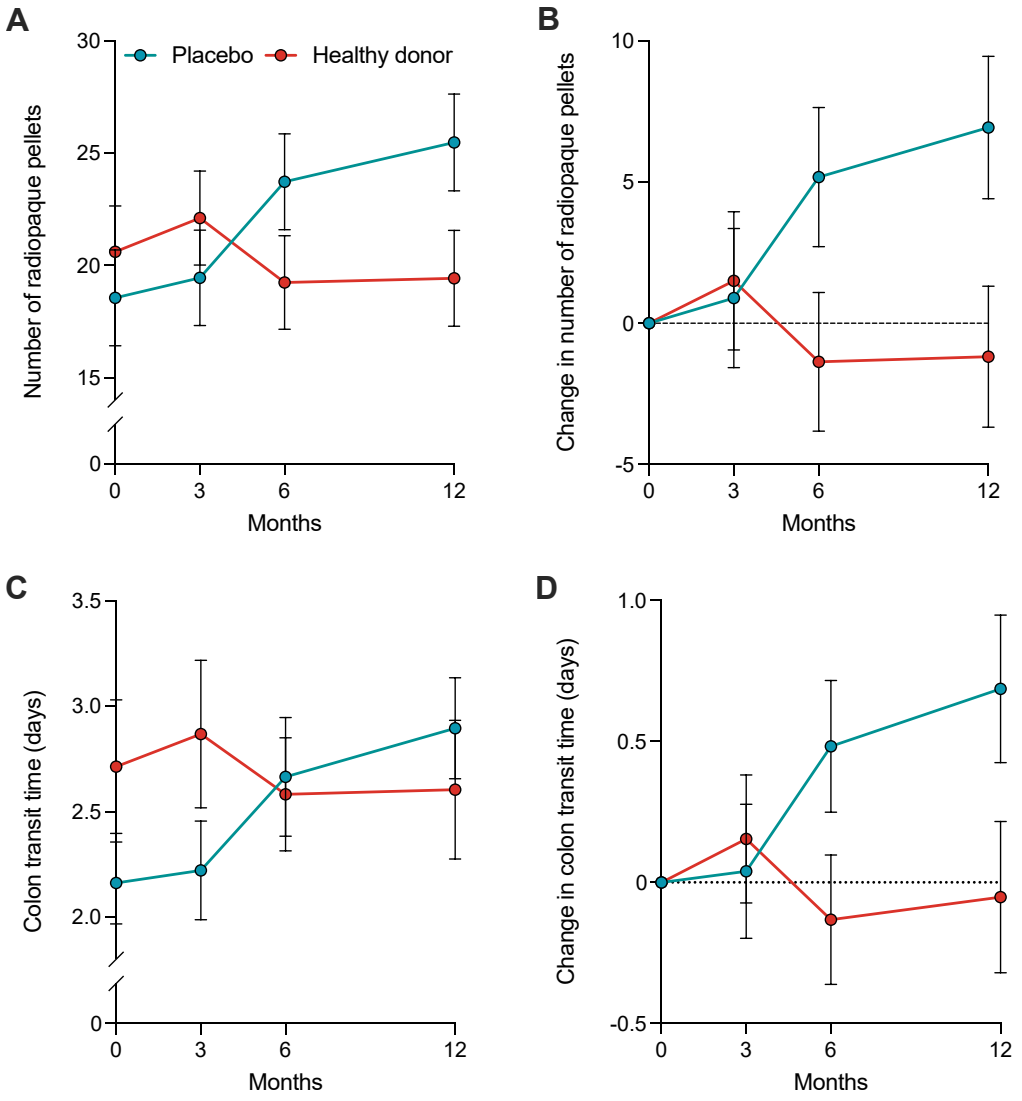

Fig. 3: **Number of radiopaque pellets on day 7 (A) and changes in number of radiopaque pellets (B), by study visit. Higher number of radiopaque pellets correlates to slower colon transit as a marker for constipation (C, D). Data are means. Error bars represent standard error of the mean.**

and total score, as well as for the other questionnaires, except for the Parkinson's Fatigue Scale where patients in the healthy donor group performed slightly worse. The positive effect that we report on constipation has also been reported in clinical trials investigating FMT for idiopathic slow transit constipation, as well as in the open-label studies of FMT in PD[16–19] and in probiotic trials in PD.[25] The results of the radiopaque pellets test indicate a slower progression of constipation in the treated group compared to the placebo group, with the latter exhibiting an increase in colon transit time of more than 12 h. The observed difference, although statistically significant, might still be too minimal after one year to result in a noticeable clinical improvement from the patients' perspective. This could explain why there is no significant difference in the patient-reported scores on the Wexner Constipation Scale.

FMT was well-tolerated and safe, consistent with its established use as a treatment for recurrent *C. difficile* infection. Mild gastrointestinal symptoms were more frequently observed in the healthy donor FMT group, but these were transient and resolved within one week after the intervention. We observed no severe adverse events associated to FMT. A systematic review examining potential adverse events associated with FMT over the last 20 years revealed that serious adverse events, such as infections and deaths, were observed in 1.4% of FMT procedures.[26] However, it is important to note that

all these reported severe adverse events were only observed in patients with mucosal barrier injury. Concerning long-term safety data (>1 year post-FMT), FMT does not appear to be linked to specific safety issues or adverse events.[26]

An important unresolved issue of this study is the large and relatively long-lasting response in the placebo FMT group. Indeed, up to 6 months after FMT, the response in the placebo group was not significantly different from that in the group receiving healthy donor FMT. Only after 12 months a significant difference in the evolution of MDS-UPRDS motor score could be demonstrated. While we can only speculate, it's plausible to hypothesize that the clinical differences observed might be aligned with the effects on gut motility, as evidenced by the radiopaque pellet test results at the 6-month evaluation. Another critical aspect to consider is the recent findings indicating significant alterations in the gut microbiome following autologous FMT.[27] These findings could play a crucial role in future evaluations of placebo-controlled FMT studies, highlighting the potential impact of microbiome changes on clinical outcomes. Finally, it remains to be recognized that studies of this kind dramatically raise expectations among participants, potentially leading to a considerable placebo response.

The study's strengths include its randomised placebo-controlled design and single-centre setting with one designated clinical investigator, which prevented both inter-site and inter-investigator variability in data collection, potentially facilitating the detection of significant effects, and possibly also limiting the drop-out rate. Previous literature has quantified the intra-rater and inter-rater variability of the (MDS-)UPDRS, with inter-rater variability showing good but not excellent Intraclass Correlation Coefficient (ICC) scores between 0.65 and 0.91. Intra-rater variability shows excellent reliability with ICCs between 0.90 and 0.91.[28] Changes in medication were left to the treating neurologist's discretion, ensuring the FMT intervention did not interfere with the routine clinical follow-up. During the trial there were no significant differences between treatment groups for medication changes as exemplified by the LEDD. The FMT solutions utilized 17 different healthy donors due to the absence of defined criteria for selecting suitable healthy donors for PD, while this is somewhat clearer for other indications such as inflammatory bowel diseases.[29] The inclusion of a diverse range of healthy donors aimed to avoid ambiguous results due to selecting an 'inadequate donor'. In GUTPARFECT, we chose to perform FMT through nasojejunal administration. While both nasojejunal and colonic FMT routes are equally effective for treating *C. difficile* infections, different considerations are warranted when trying to impact gut-brain communication.[21] Indeed, nasojejunal administration might be preferred for PD due to the significant role for the vagal nerve, as supported by potential protective effects of vagotomy.[8] The colon is only innervated by the vagal nerve for two-thirds of its length, making colonic FMT administration less suitable.[30] Furthermore, colonic FMT is less likely to alter gut microbiota composition in the small intestine compared to nasojejunal FMT. A disadvantage of nasojejunal administration is that it is technically challenging which might lead to failure or intolerability of the procedure, as was the case in one of our study candidates.

Our study also has several limitations. Based on the brain-first versus body-first hypothesis, certain PD phenotypes may be more likely to benefit from FMT. In light of this, inclusion criteria could have been adapted to increase likelihood of including patients with the body-first hypothesis. This group of patients would be expected to have a higher prevalence of prodromal autonomic symptoms such as constipation and orthostatic hypotension, as well as REM-sleep behaviour disorder. Pre-screening could also entail objective transit time testing (such as the radiopaque pellets tests) and MIBG scintigraphies.[10] The fact that we only chose to perform a single FMT could also be considered a limitation of the study. The debate continues regarding whether multiple consecutive FMTs produce superior results in altering the gut microbiome compared to a single FMT. At present, there is insufficient evidence to support the higher cost and burden of multiple FMTs over a single FMT.[21] In addition, only a limited number of studies have investigated the gut microbiome one year after FMT, however these studies still indicate similarity to the healthy donor's composition, thus indicating a repeat FMT was not necessary within a year.[31] Finally, our sample size was small, nonetheless still sufficient to show a significant difference in the primary outcome. One specific remark at this point is that the number of included patients was slightly below that calculated in the power analysis (43 versus 46). A simulation study based on the current data indicated a power of 74%, which is a strong indicator of a significant effect. In contrast with motor symptoms, the analysis non-motor symptoms might be more affected by this small sample size.

As this study represents the first randomised, double-blind, placebo-controlled trial of FMT in PD, it is crucial to independently reproduce these results. Ideally, a multicentre study with a larger sample size and the same primary outcome, *e.g.* MDS-UPDRS motor score, should be initiated. The pronounced difference in MDS-UPDRS motor score between treatment groups starting from the 6 to 12 months timepoint and not earlier suggests a potential disease-modifying effect rather than solely symptomatic improvement. This finding underscores the necessity for long-term follow-up for PD interventions to evaluate potential beneficial effects. The need for a one-year follow-up to observe the full treatment difference between placebo and treatment groups

in Parkinson's disease was explicitly shown in a recent meta-analysis.[32] However, clinical trials with a longer duration also entail increased patient burden and higher costs. The same holds true for the inclusion of neuroimaging outcome parameters such as DaT-SPECT or F-DOPA PET, which could yield additional measures of longitudinal evolution, although the correlation between imaging parameters and clinical findings is not always straightforward.

If the benefits of FMT are confirmed, further investigations are warranted to elucidate the precise mechanisms through which the microbiota exert their effects. A crucial next step is the sequential analysis of the microbiome before and after the FMT, which will allow an evaluation of the extent and duration of alterations and eventually of the correlations with clinical outcomes. As mentioned above this analysis will also explore the effects of autologous FMT as a potential explanation of the placebo response in this study. Another future aim is the development of less invasive microbiome-altering therapies such as increasing the abundance of beneficial microbiota by supplementing live organisms directly (i.e., probiotics), dietary interventions that promote their growth indirectly (i.e., prebiotics) or by administering specifically identified molecules excreted by such beneficial microbes (i.e., postbiotics).

In summary, FMT using healthy donor stool demonstrated a favourable benefit–risk profile, resulting in an improvement of objective measures of constipation (colon transit time) and later improvement in motor symptoms when compared to patients transplanted with their own stool (placebo). This trial provides evidence for the potential of gut microbiota-targeted treatments in PD. FMT as an intervention offers the advantages of being considered safe based on experience in other indications, and being cost-effective, which facilitate its rapid implementation in clinical practice if potential beneficial effects are confirmed.

Contributors

AB, CV, HH, BV, JR, DD, MV, LDC, LDV, DL, RV and PS contributed to study design. AB recruited patients and conducted all study visits. AB and CV have directly accessed and verified the underlying data reported in the manuscript. AB, CV, MV and PS were involved in statistical analysis and data interpretation. HH and BV were responsible for healthy donor selection and preparation of transplant solutions. DD was responsible for FMT procedures. DT was responsible for colonoscopies. PS, DL and RV were responsible for study oversight. PS was the principal investigator and oversaw study design. AB wrote the first draft of the article, which all authors critically revised and commented on. All authors had full access to all the data in the study and the senior authors had final responsibility for the decision to submit for publication.

Data sharing statement

De-identified participant data will be made available by the corresponding author to colleagues who propose a reasonable scientific request.

Declaration of interests

JR has received grants from Beneo, Cargill, Colruyt group, Danone, DSM, J&J, MRM/Prodigest, Nestle, Pfizer, and Takeda; and has received consulting and/or speaking fees from Aphea, Biofortis, DSM, Ferring, GSK, Janssen Pharmaceuticals, Metagenics, MSD, MRM/Prodigest, Nutricia, Sanofi, Takeda, Tsumura.

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All other authors declare no competing interests.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.eclinm.2024.102563.

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