Mild cognitive impairment is not associated with gut microbiota alterations in Parkinson's disease

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Brief Communication

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Brief Communication

Mild cognitive impairment is not associated with gut microbiota alterations in Parkinson's disease

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Abstract

Gut microbiome differences between people with Parkinson's disease (PD) and control subjects without parkinsonism are widely reported, but potential alterations related to PD with mild cognitive impairment (MCI) have yet to be comprehensively explored. We compared gut microbial features of PD with MCI (n=58) to cognitively unimpaired PD (n=60) and control subjects (n=90) without MCI. Our results did not support a specific microbiome signature related to MCI in PD.

Mild cognitive impairment (MCI) is a non-motor symptom of Parkinson's disease (PD) that represents a risk factor for developing dementia, and can significantly impact quality of life.[1] While gut microbial community differences between people with PD and individuals without parkinsonism are well established[2–7], only a single publication has investigated the gut microbiome in PD with MCI, suggesting significant differences in several taxa when contrasting PD with MCI to PD with unimpaired cognition or to control subjects.[8] To investigate whether these results could be replicated in a larger, geographically distinct cohort, we performed similar comparisons using data from the Luxembourg Parkinson's Study[4].

Our dataset comprised 58 people with PD and MCI (PD-MCI), 60 people with PD without cognitive impairment (PD-NC), and 90 control subjects without cognitive impairment (Ctrl). While there were differences in demographic and clinical variables between the Ctrl and PD groups, including that controls were younger and had lower frequency of constipation, the PD-MCI and PD-NC groups had similar profiles (Table 1).

We did not observe any difference between the PD-MCI, PD-NC, and Ctrl groups in microbial community richness and evenness (alpha diversity) when tested without confounders (Fig 1A-B, Supplementary Table 1A). In a linear regression model for the inverse Simpson index, including the three groups and potential confounding variables, both PD groups tended to have lower diversity than controls ($0.1 > p > 0.05$; Supplementary Table 1B). In a within-PD model with confounders, there was no difference between PD with or without MCI (Supplementary Table 1C).

In comparisons of community composition (beta diversity), there was a difference between the three groups when tested with or without confounding variables ($p < 0.001$ for both) (Fig 1C, Supplementary Tables 2A-B). Pairwise tests between controls and each of the PD groups also showed a significant group effect, but a within-PD test indicated no difference in relation to MCI status (Supplementary Tables 2C-E). In tests of sample dispersions between the groups, the difference was significant between PD-MCI and Ctrl ($p < 0.05$), close to significant between PD-NC and Ctrl ($0.1 > p > 0.05$) and not significant between PD-MCI and PD-NC (Fig 1D; Supplementary Tables 2F-G).

We performed differential abundance comparisons with three tools: DESeq2[9] and ANCOM-BC2[10], commonly used methods with different statistical backgrounds, and DA.lic from the DAtest[11] package, selected based on its performance compared to other tests (Supplementary Fig 1A). Comparing controls to the PD groups resulted in many significant taxonomic clades when comparing either PD-MCI or PD-NC to Ctrl (Fig 1E, Supplementary Fig 1B, Supplementary File 2). Taxa which were significant with more than one test included, among others, decreased abundances of the family *Lachnospiraceae*, *Clostridiaceae* and *Butyricicoccaceae* in PD, and increases in *Enterobacteriaceae* and the genera *Hungatella* and DTU089 (family *Ruminococcaceae*). DESeq2 indicated increases in many additional taxa, such as the genera *Escherichia/Shigella* and *Methanobrevibacter*. However, when comparing PD-MCI to PD-NC, two out of three tests detected no significant taxa (Fig 1E), and all three taxa highlighted by DESeq2 seemed likely to result from outlier values, with the possible exception of an Amplicon Sequence Variant (ASV) classified as *Akkermansia muciniphila* (Supplementary Fig 1C).

Many of the taxa detected as differentially abundant between the PD and Ctrl groups were in line with previous publications, including the increased abundances of *Enterobacteriaceae*[7], *Hungatella*[5,6] and *Methanobrevibacter*[6], and decreased abundances of *Lachnospiraceae*[5–7] and *Butyricicoccaceae*[6,7] in PD. The differences in beta diversity between control and PD subjects were also in line with the literature.[2–4,6,7] As for comparisons related to PD with MCI, the previous publication on the topic reported a significant difference in beta diversity between PD-NC and PD-MCI, higher abundances of two families and four genera in PD-MCI compared to either PD-NC or Ctrl, and decreases in two genera when contrasting PD-MCI and PD-NC.[8] In our study, there was no difference in beta diversity between PD with and without MCI. When comparing specific taxa, only one of three tests indicated any differences between PD with and without MCI, and none of those taxa overlapped with the previous publication[8]. The most compelling taxon detected in the present study was an *A. muciniphila* ASV, which was almost entirely absent in PD-MCI. *A. muciniphila* is typically increased in in PD[3–7], and more research regarding the significance of this taxon in PD and its subtypes is warranted.

To conclude, our comparisons reproduced previously detected differences between PD and control subjects but did not lend support to microbial community patterns specific to PD with MCI.

Methods

Subject recruitment, faecal sample collection and processing as well as amplification and sequencing of the 16S rRNA gene (regions V3–V4) have been described previously[4]. The Luxembourg Parkinson's Study[12] was conducted according to the Declaration of Helsinki, with approval from the National Ethics Board (CNER Ref: 201407/13) and Data Protection Committee (CNPD Ref: 446/2017). All participants signed written informed consent.

The present analyses were limited to subjects with age > 64 years due to overrepresentation of younger individuals in the Ctrl group. Participants were included if they matched the UKPDSBB clinical diagnostic criteria[13] for typical PD; subjects with atypical or not yet specified parkinsonism were excluded. Control subjects genetically related to participants with PD were also excluded. MCI was defined as Montreal Cognitive Assessment (MoCA) score[14] < 26.

Sequence data was processed with dadasnake[15]. Statistical comparisons and visualisations were performed in R, using the packages vegan[16], DAtest[11], DESeq2[9], and ANCOM-BC2[10] for statistics. Differential abundance tests were corrected for age, sex, BMI, constipation, and years of education. Detailed information is provided in the Supplementary Methods (Supplementary File 1).

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Tables and figures

Table 1. Clinical characteristics of study subjects.

Fig 1. A. Boxplot for richness (Chao1). **B.** Boxplot for richness and evenness (inverse Simpson). **C.** Community composition visualized as NMDS ordination of Bray-Curtis dissimilarity; ellipses indicate 95% confidence intervals. **D.** Boxplot for groupwise distances to centroid from the ordination, with significances for pairwise comparisons from Tukey HSD test. **E.** Numbers of differentially abundant taxa (multiple comparison corrected $p < 0.05$). In boxplots, box hinges represent the 1st and 3rd quartiles, whiskers range from hinge to the highest and lowest values that are within 1.5*IQR of the hinge, and outlines represent data distributions.

Data Availability

Patient data used in the preparation of this manuscript were obtained from the National Centre of Excellence in Research on Parkinson's Disease (NCER-PD). NCER-PD datasets are not publicly available, as they are linked to the Luxembourg Parkinson's Study and its internal regulations. The NCER-PD Consortium is willing to share its available data. Its access policy was devised based on the study ethics documents, including the informed consent form, as approved by the national ethics committee. Requests to access datasets should be directed to the Data and Sample Access Committee via email: request.ncer-pd@uni.lu

Code Availability

The R code for this study is available at https://gitlab.lcsb.uni.lu/ESB/ncer-mci-microbiome.

Author Contributions

Conceptualization: VTEA, RK, PM, PW; Data curation: VTEA, ZL, LP, and NCER-PD; Formal analysis: VTEA, PM, AHB, MK; Writing –original draft: VTEA, Writing –review & editing: all authors; Funding acquisition: PM, AKL, RK, PW; Project administration: VTEA, PM, AKL, RK, PW; Supervision: PM, AKL, RK, PW. All authors read and approved the final manuscript.

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The sequence data analyses for this study were carried out using the HPC facilities of the University of Luxembourg.

Competing Interests

AKL has served on advisory boards and as speaker for Roche. The other authors declare no financial or non-financial competing interests.

Supplementary information

Supplementary File 1

Supplementary Methods

Supplementary Table 1. Alpha diversity results. **A.** Single-variable comparisons (binary categorical variables: Wilcoxon rank sum test; categorical variables with more than two categories: Kruskal-Wallis test; continuous numeric variables: Pearson correlations). **B.** Linear model for inverse Simpson diversity, main grouping variable and confounders. **C.** Linear model for inverse Simpson diversity without Ctrl subjects.

Supplementary Table 2. Beta diversity results. Tables B-E show marginal effects. **A.** PERMANOVA for individual variables (model: distance matrix ~ variable). **B.** PERMANOVA with multiple variables (model: distance matrix ~ Group + Sex + Age + BMI + Constipation + Education). **C.** PERMANOVA with multiple variables, Ctrl vs PD-NC (model: distance matrix ~ Group + Sex + BMI + Constipation). **D.** PERMANOVA with multiple variables, Ctrl vs PD-MCI

(model: distance matrix ~ Group + Sex + BMI + Constipation). **E.** PERMANOVA with multiple variables, PD-NC vs PD-MCI (model: distance matrix ~ Group + Sex + BMI + Constipation + LEDD + Disease duration). **F.** ANOVA for group dispersions. **G.** Pairwise comparisons for differences between group dispersions with Tukey HSD.

Supplementary Fig 1. A. Results of differential abundance test comparisons with DAtest; for test abbreviations and descriptions, consult package documentation. In the "Score" panel, lines indicate 90% confidence limits. **B.** Heatmap summarizing taxa that were differentially abundant ($q < 0.05$) in at least 2 out of 6 possible result lists (2 contrasts [PD-MCI vs Ctrl, PD-NC vs Ctrl] and 3 tests [ANCOM-BC2, DESeq2, DA.lic from DAtest]). **C.** Boxplots of taxa that were differentially abundant between PD patients with and without MCI according to the DESeq2 test (multiple comparison corrected *p*-value (*q*-value) < 0.05). In both figures, · : $0.1 > q > 0.05$; * : $q < 0.05$; ** : $q < 0.01$; *** : $q < 0.001$.

Supplementary File 2

Full results for the differential abundance comparisons. **A.** Results from comparisons of differential abundance tests on PD-only data with testDA. **B.** Results from DAtest: DA.lic. **C.** Results from DESeq2. **D.** Results from ANCOM-BC2.

Table 1

Clinical characteristics of study subjects.

Characteristic	Ctrl, n = 90[1]	PD-NC, n = 60[1]	PD-MCI, n = 58[1]	p-value[2]	Ctrl vs. PD-MCI[3]	Ctrl vs. PD-NC[3]	PD-NC vs. PD-MCI[3]
Sex				0.315			
Female	39 (43%)	20 (33%)	19 (33%)				
Male	51 (57%)	40 (67%)	39 (67%)				
Constipation	6 (6.7%)	25 (42%)	28 (48%)	<0.001			
Age (years)	68.9 (66.1, 72.5)	71.3 (69.2, 74.9)	73.1 (68.8, 77.9)	0.002	0.001	0.078	0.162
MoCA	28 (27, 29)	28 (27, 29)	23 (22, 25)	<0.001	<0.001	0.777	<0.001
BMI (kg/m^2)	26.8 (24.1, 29.3)	27.6 (24.1, 30.3)	27.7 (25.3, 31.3)	0.055	0.060	0.276	0.427
Years of education	14 (11, 17)	14 (12, 17)	12 (10, 15)	0.061	0.092	0.714	0.092
PD duration since diagnosis (years)		5 (3, 9)	4 (2, 8)				0.400

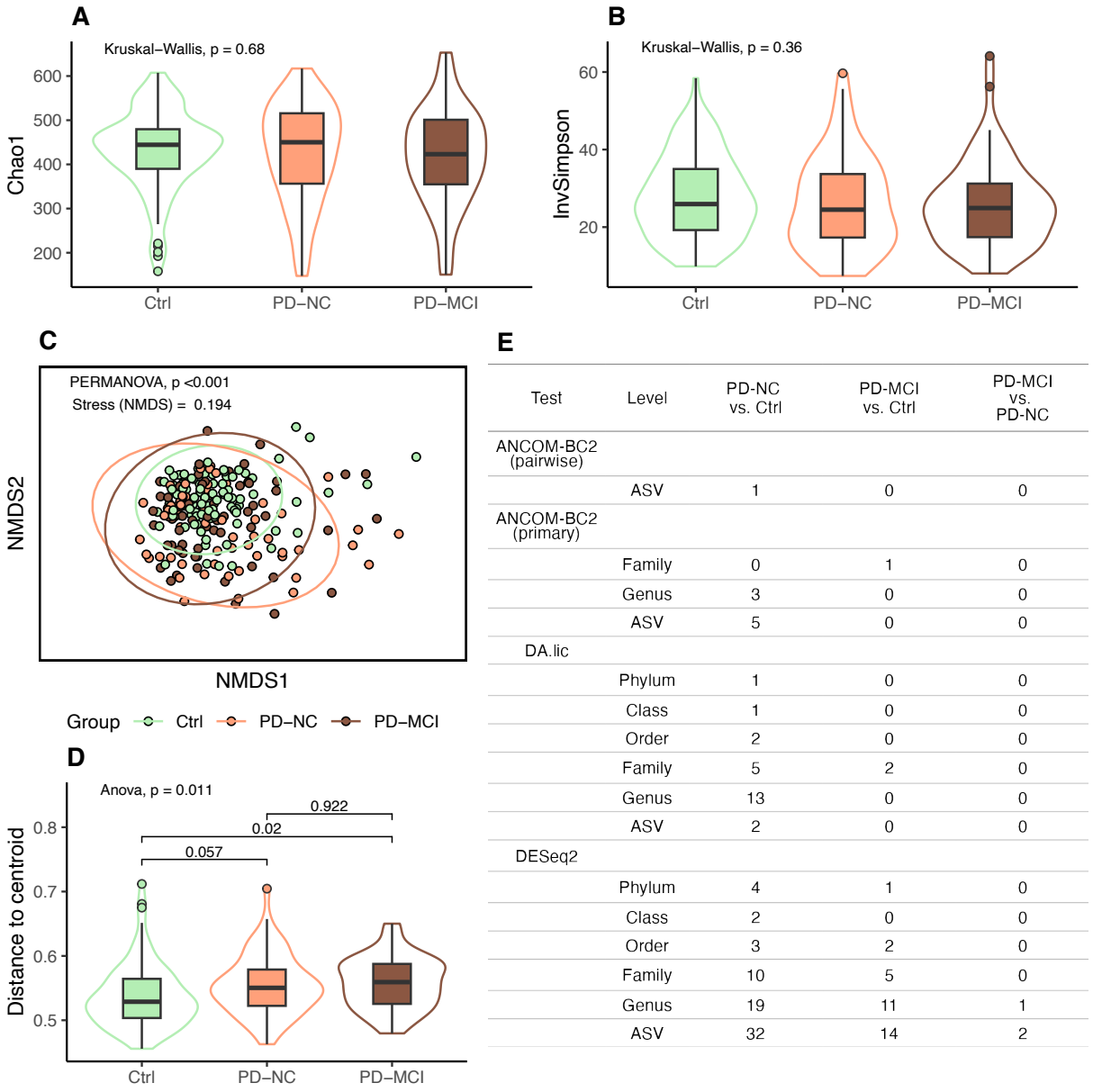
[1] Categorical variables: n (%); continuous variables: median (IQR)

[2] Categorical variables: Pearson's Chi-squared test; continuous variables: one-way ANOVA

[3] Pairwise t-test

Ctrl: control subjects; PD-NC: people with Parkinson's disease without cognitive impairment; PD-MCI: people with Parkinson's disease and mild cognitive impairment; MoCA: Montreal Cognitive Assessment score; BMI: Body Mass Index.

Figure 1



E

Test	Level	PD-NC vs. Ctrl	PD-MCI vs. Ctrl	PD-MCI vs. PD-NC
ANCOM-BC2 (pairwise)				
	ASV	1	0	0
ANCOM-BC2 (primary)				
	Family	0	1	0
	Genus	3	0	0
	ASV	5	0	0
DA.lic				
	Phylum	1	0	0
	Class	1	0	0
	Order	2	0	0
	Family	5	2	0
	Genus	13	0	0
	ASV	2	0	0
DESeq2				
	Phylum	4	1	0
	Class	2	0	0
	Order	3	2	0
	Family	10	5	0
	Genus	19	11	1
	ASV	32	14	2

Fig 1. A. Boxplot for richness (Chao1). **B.** Boxplot for richness and evenness (inverse Simpson). **C.** Community composition visualized as NMDS ordination of Bray-Curtis dissimilarity; ellipses indicate 95% confidence intervals. **D.** Boxplot for groupwise distances to centroid from the ordination, with significances for pairwise comparisons from Tukey HSD test. **E.** Numbers of differentially abundant taxa (multiple comparison corrected $p < 0.05$). In boxplots, box hinges represent the 1st and 3rd quartiles, whiskers range from hinge to the highest and lowest values that are within 1.5*IQR of the hinge, and outlines represent data distributions.

Supplementary Files

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- mcipdsupplementaryfile1.pdf
- mcipdsupplementaryfile2.xlsx