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Research report

Gut microbiota in Parkinson disease in a northern German cohort

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ABSTRACT

Pathologic and epidemiologic studies suggest that Parkinson disease (PD) may in some cases start in the enteric nervous system and spread via the vagal nerve to the brainstem. Mounting evidence suggests that the gut microbiome plays an important role in the communication between gut and brain and that alteration of the gut microbiome is involved in the pathogenesis of numerous diseases, including Parkinson disease. The aim of this study was to determine whether Parkinson disease is associated with qualitative or quantitative changes in the gut microbiome.

We analyzed the gut microbiome in 29 PD cases and 29 age-matched controls by next-generation-sequencing of the 16S rRNA gene and compared diversity indices and bacterial abundances between cases and controls. Alpha diversity measures and the abundance of major phyla did not differ between cases and controls. Beta diversity analyses and analysis on the bacterial family level revealed significant differences between cases and controls for four bacterial families. In keeping with recently published studies, Lactobacillaceae were more abundant in cases. Barnesiellaceae and Enterococcacea were also more abundant in cases in this study but not in other studies. Larger studies, accounting for drug effects and further functional investigations of the gut microbiome are necessary to delineate the role of the gut microbiome in the pathogenesis of PD.

1. Introduction

Parkinson disease (PD) is the second most common neurodegenerative disease with a steady increase in prevalence with age. A prevalence peak with approximately 1% affected individuals is reported around the age of 70 (Pringsheim et al., 2014). The cardinal motor symptoms are bradykinesia, rigidity, resting tremor and postural instability (Kalia and Lang, 2015). Non-motor symptoms such as constipation, hyposmia, orthostatic hypotension and urogenital dysfunction are an important part of PD (Chaudhuri et al., 2011). Constipation is consistently associated with an increased risk of PD in epidemiologic studies and often appears years prior to motor symptoms. Based on autopsy studies, Braak and colleagues proposed that PD starts in the enteric nervous system and/or the olfactory bulb (Dickson et al., 2009; Hawkes et al., 2010). The gut microbiome influences the enteric nervous system, and via the vagal nerve the central nervous system (Ulusoy et al., 2013; Cryan and Dinan, 2012). Complete vagotomy reduces the risk to develop PD (Svensson et al., 2015). This study explores whether PD patients (n = 29) have gut microbiota differences compared to control subjects (n = 29), which might support a role of the gut microbiota in the PD pathophysiology.

2. Results

2.1. Demographics and Clinical data

Table A.1 contains detailed demographic and clinical data. The age distribution in cases (mean: 69.2 yrs., standard deviation (sd): 6.5 yrs) and controls (mean: 69.4 yrs., sd: 6.7 yrs) was nearly identical. The sex ratio differed between cases (23 male, 6 female) and controls (13 male, 16 female) (p_{Chi2} = 0.014). Constipation was more frequent in patients (20/29) than controls (4/29) (p_{Chi2} = 0.014), nicotine use was more frequent in controls (8/29) than patients (0/29) (p_{Chi2} = 0.008) and caffeine consumption was more frequent in controls (23/29) than in patients (8/29)

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(p_{Chi2} = 0.0002). Alcohol consumption (yes/no) and eating fermented milk products did not differ between patients and controls. The mean age of onset in the case group was 57.9 yrs. with an sd of 7.1 yrs., the mean UPDRS part III score was 21 and 6 PD cases were treated with deep brain stimulation (DBS).

2.2. Intestinal microbiota

We detected no significant differences between cases and controls in the abundances of major phyla and also no significant differences in alpha diversity (species richness of a group) measures (Simpson, Shannon, Chao1, Phylogenetic distance; Mann-Whitney *U* test, $p > 0.05$). Alpha diversity indices were also not dependent on sex and age (ANOVA, $p > 0.05$ for each factor). We assessed potential community-level differences between samples using beta diversity analyses, which consider the actual bacterial community composition of each group. The unweighted UniFrac dissimilarity significantly differs between cases and controls (*adonis*: unweighted UniFrac $R^2 = 0.0241$, $p = 0.0491$), but not between sexes ($R^2 = 0.01621$, $p = 0.553$). Next, we analyzed bacterial family-level abundances to identify the bacterial families responsible for the differences in the beta diversity. The ten most abundant bacterial groups accounted for ~80% of the total bacterial count (Fig. A.1). Using a 1000-fold Monte-Carlo simulation Wilcoxon test the abundance of four bacterial families differed at first sight significantly between cases and controls. However, quasi-Poisson regression analysis with bacterial counts as dependent variable and manual backwards selection of case/control status, age, sex, constipation, nicotine use, caffeine consumption, alcohol consumption, use of fermented milk products, cardiovascular disease and sequencing batch as independent variables for these families showed that sex and not disease status was the significant determinant of Lachnospiraceae count ($p = 0.0001$). Case/control status was a significant determinant ($p < 0.05$) of bacterial abundance for Barnesiellaceae and Enterococcaceae and a suggestive determinant ($p < 0.1$) for Lactobacillaceae (Tables 1, 2 and Fig. 1). Since a previous large study explicitly mentioned that COMT-inhibitors had an influence on bacterial counts (Hill-Burns et al., 2017), we performed an analysis without the four patients taking COMT-inhibitors, which did not substantially change the results (Table 1).

2.3. Comparison to previous studies

Four previous studies that are in principle comparable addressed differences in the microbiota between PD patients and controls (Hill-Burns et al., 2017; Hasegawa et al., 2015; Keshavarzian et al., 2015; Scheperjans et al., 2015). The studies are not directly comparable due to differences in wet-lab techniques as well as in statistical analysis strategies. Therefore, we limit the comparison to bacterial families showing significant differences between cases and controls in at least one study (Table 1). Bacteria of the Lactobacillaceae group were identified to be more abundant in cases than controls in four out of five studies, including this one (Hill-Burns et al., 2017; Hasegawa et al., 2015; Scheperjans et al., 2015). Verrucomicrobiaceae were more abundant in cases than controls in two studies but not in this one (Hill-Burns et al., 2017; Keshavarzian et al., 2015). The distribution of bacterial families identified in previous studies in our study population is shown in Fig. A.2. We did not include another recently published study in the comparison because the bacterial nomenclature used is not compatible with our study (Unger et al., 2016).

2.4. ROC analysis

To explore the potential usefulness of microbiota analysis as a biomarker for PD we performed a ROC analysis. The area under the curve (AUC) for disease status with Lactobacillaceae abundance as predictor was 0.68 (95% CI: 0.58–0.79) (Fig. A.3). The same analysis with Lactobacillaceae, Barnesiellaceae and Enterococcaceae abundance as predictors resulted in an AUC of 0.83 (95% CI: 0.73–0.94; Fig. A.4), indicating that it might be possible to derive a PD biomarker from intestinal microbiota analysis.

3. Discussion

We found a significant increase of three bacterial families in PD patients compared to age matched controls (Table 1). A significant increase of Lactobacillaceae in PD patients is in accordance with three previous studies, while the fourth study provides no information on Lactobacillaceae (Hill-Burns et al., 2017; Keshavarzian et al., 2015; Scheperjans et al., 2015). In our study, PD patients did not report probiotic use or other dietary habits explaining the difference in Lactobacillaceae abundance. We also found higher abundances of Enterococcaceae and Barnesiellaceae in the case group compared to the control group. ROC analysis showed that microbiota might have a predictive value for PD. However, it should be kept in mind that the AUC in the discovery stage is in nearly all cases overestimated, stressing the need for replication in independent samples. The main weakness of our study is the low number of participants and the imbalanced gender distribu-

Table 1
Comparison of p-values of bacteria associated with PD in this study or in previous studies. Significant p-values (≤0.05) in bold print, in brackets: without patients taking COMT-inhibitors. Due to different sequencing approaches and statistical methods, the p-values are not directly comparable. +bacterial count higher in cases, −bacterial count higher in controls, =bacterial count in patients and controls nearly identical.

Bacterial family	This Study	Hill-Burns et al. (2017)	Scheperjans et al. (2015)	Keshavarzian et al. (2015)	Hasegawa et al. (2015)
Statistical method	Permut.	K-W, ANCOM	Permutat.	FDR-BH	MWU
Lactobacillaceae	**0.002+ (0.014)**	**0.0000002+**	**0.004+**	–	**0.003+** (Lactobacillus)
Barnesiellaceae	**0.007+ (0.011)**	–	–	–	–
Enterococcaceae	**0.005+ (0.004)**	–	–	–	0.082+ (Enterococcus)
Prevotellaceae	0.936−	**–**	**0.001**−	0.88=	0.28 (Prevotella)
Verrucomicrobiaceae	0.444+	**0.0004+**	**0.014+**	**0.05+**	–
Bradyrhizobiaceae	0.581−	**–**	**0.021+**	**–**	–
Ruminococcaceae	0.309−	–	**0.029+**	0.31+	–
Bacteroidaceae	0.364+	–	**–**	**0.05+**	–
Bifidobacteriaceae	0.142+	**0.000002+**	–	0.2	0.47
Tissierellaceae	0.699+	**0.00000008+**	–	–	–
Christensenellaceae	0.320=	**0.000000004+**	–	–	–
Pasteurellaceae	–	**0.00003**−	–	–	–