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Short chain fatty acids and gut microbiota differ between patients with Parkinson's disease and age-matched controls

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ABSTRACT

Background: Patients with Parkinson's disease (PD) frequently have gastrointestinal symptoms (e.g. constipation) and exhibit the PD-typical pathohistology in the enteric nervous system (ENS). Both, clinical symptoms and pathohistological changes in the ENS occur at early stages and can precede the motor manifestations of PD. Two recent studies reported an association between changes in gut microbiota composition and PD. We hypothesized that alterations in gut microbiota might be accompanied by altered concentrations of short chain fatty acids (SCFA), one main metabolic product of gut bacteria.

Methods: We quantitatively analyzed SCFA concentrations (using gas chromatography) and microbiota composition (using quantitative PCR) in fecal samples of 34 PD patients and 34 age-matched controls.

Results: Fecal SCFA concentrations were significantly reduced in PD patients compared to controls. The bacterial phylum *Bacteroidetes* and the bacterial family *Prevotellaceae* were reduced, *Enterobacteriaceae* were more abundant in fecal samples from PD patients compared to matched controls.

Conclusions: Our study confirms the recently reported association between PD and the abundance of certain gut microbiota and shows a reduction in fecal SCFA concentrations (one main metabolic product of certain gut bacteria). The reduction in SCFA might, theoretically, induce alterations in the ENS and contribute to gastrointestinal dysmotility in PD. Prospective longitudinal studies in subjects at risk for PD are required to further elucidate the causal role of gut microbiota and microbial products in the development of PD and PD-associated dysmotility.

1. Introduction

Parkinson's disease (PD) is a neurodegenerative disorder clinically characterized by motor and non-motor symptoms. The pathohistological hallmark of PD is the presence of aggregated proteins (Lewy bodies) in the nervous system. Lewy body pathology in PD is found in structures of the central nervous system (CNS) as well as in the peripheral autonomic [1] and enteric nervous system (ENS) [2–5]. PD patients frequently exhibit non-motor symptoms, including signs and symptoms of gastrointestinal dysmotility (e.g. delayed gastric emptying [6–8], constipation [9,10]). Lewy body pathology in the ENS might represent the pathohistological correlate for gastrointestinal symptoms in PD. Current hypotheses suggest that the ENS might be one of the first sites where Lewy body pathology appears in PD [2,11]. Interestingly, an animal model of PD shows that chronic intragastric administration of rotenone, a complex-1-inhibitor, results in a spatio-temporal distribution of alpha-synuclein immunoreactivity that is compatible with the above mentioned hypotheses [12,13]. A similar trigger might be responsible for the formation of Lewy bodies in humans.

Due to the immediate vicinity of the ENS with feces, gut microbiota and microbiota's metabolic products are among potential candidates that could initiate a process that eventually results in the formation of Lewy bodies in the ENS. Two recent studies

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showed an association between the abundance of certain gut microbiota and PD [14,15]: amongst others, bacteria that have the capacity to produce short chain fatty acids (SCFA) were reduced in PD. SCFA modulate the activity of the ENS and thereby increase gastrointestinal motility [16]. Hence, altered concentrations of SCFA might contribute to gastrointestinal dysmotility in PD.

We hypothesized that a shift in gut microbiota might be associated with a shift in gut microbiota's metabolic products, e.g. SCFA. We therefore analyzed microbiota and SCFA in fecal samples of patients with PD and age-matched healthy controls.

2. Subjects and methods

This study was approved by the Ethics committee of the medical association of Saarland. All enrolled subjects provided written informed consent.

2.1. Subjects

All subjects (patients as well as controls) were on an omnivorous diet and none of the subjects reported special dietary habits or dietary restrictions (e.g. due to food intolerance). None of the subjects reported intake of antibiotic drugs or intake of probiotic or prebiotic products during the last 3 months. None of the enrolled subjects had a history of acute or chronic gastrointestinal disorder. PD patients (24 male, 10 female) were diagnosed according to UK PD Society Brain Bank Clinical Diagnostic Criteria by a movement disorder specialist. All 34 PD patients were on dopaminergic drugs. Mean disease duration for PD (defined by the time the first motor symptoms were experienced by the patient) was 82 months (range: 12−228 months). Median Hoehn and Yahr stage was 2.5 (range: 1 to 4). All PD patients were interviewed for autonomic symptoms (including constipation and symptoms of gastrointestinal discomfort), pre-existing gastrointestinal disorders and previous surgical interventions on the gastrointestinal tract (individual data of all PD patients are provided in Supplemental Table 1). Seven of the 34 PD patients reported constipation. Mean age in the PD patient group was 67.7 ± 8.9 years. The fecal samples of PD patients were age-matched to samples of healthy controls (HC, n = 34, 18 male, 16 female) from the repository of the Institute of Microoecology in Herborn (mean age of HC 64.6 ± 6.6 years). None of the control subjects reported preexisting medical conditions. None of the control subjects was on chronic or intermittent use of drugs affecting gastrointestinal motility or on any other permanent medication. Two of the 34 control subjects reported constipation. No other gastrointestinal symptoms were reported by the control subjects. In order to identify age-related changes we also included a small group of younger healthy controls (n = 10, 5 male, 5 female, 33.3 ± 11.6 years). Additional information is provided online (Supplemental Table 1 and Supplemental Material).

2.2. Collection of fecal samples

All subjects were provided with sterile containers and instructed how to collect the fecal samples at home and how to send the samples to the Institute of Microoecology, Herborn. The stool samples were immediately frozen at - 35 °C and analyzed within a few days.

2.3. Sample preparation and DNA extraction

Microbial DNA was extracted using the QIAsymphony® DSP Virus/Pathogen Mini-Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. Automated isolation and pipetting of 96-well plates (Micro Amp Optical 96-Well Reaction Plate, Applied Biosystems, Darmstadt, Germany) were performed by the QIAsymphony® SP/AS instrument (QIAGEN, Hilden, Germany) using the QIAsymphony DSP Virus/Pathogen Mini-Kit.

2.4. Quantification of target bacteria by real-time quantitative PCR (qPCR)

Primers were selected to recognize either the whole bacterial phylum (Firmicutes, Bacteroidetes) or main representatives of a phylum (*Akkermansia muciniphila*, the genus *Bifidobacterium, Enterobacteriaceae, Methanobrevibacter smithii*). In addition, the genera *Lactobacillus* and *Lactococcus* were enumbered. PCR amplification and detection was performed using an ABI PRISM 7900HT sequence detection system (Applied Biosystems, Darmstadt, Germany) in optical-grade 96-well plates. A standard curve was produced using the appropriate reference organism to quantify the qPCR values into number of bacteria/g. The fluorescent products were detected in the last step of each cycle. A melting curve analysis was carried out following amplification to distinguish the targeted PCR product from the non-targeted PCR product. The melting curves were obtained by slow heating at temperatures from 55 to 95 °C at a rate of 0.2 °C/s, with continuous fluorescence collection. The data was analyzed using the ABI Prism software. The real-time PCRs were performed in triplicate.

2.5. Determination of SCFA concentrations

Fecal samples were lyophilized and analyzed using gas chromatography. The lyophilisate was dissolved in 100 µl 5 M HCOOH and 400 µl Aceton and centrifuged (5 min at 4000 × g). Concentrations of SCFA were determined in the supernatant using a GC-2010 Plus gas chromatograph (Shimadzu Deutschland GmbH, Duisburg, Germany) equipped with a flame ionization detection with a thin-film capillary column Stabilwax®-DA 30 m × 0,25 mm × 0,5 µm (Restek, Bad Homburg, Germany). The samples were spread out by split injection using the auto-sampler AOC-20s/I (Shimadzu Deutschland GmbH). GCsolution Chromatography Data System (Shimadzu Deutschland GmbH) was used for data processing. An external standard (Supelco™ WSFA-1 Mix, Supelco Sigma-Aldrich Co., Bellefonte PA) was used for quantification of SCFA.

2.6. Statistical analysis

Statistics was carried out using GraphPad Prism 6.0 and PASW statistics software version 22.0 (SPSS Inc., Chicago, IL, USA). Data are displayed as mean ± standard error of the mean. D'Agostino-Pearson omnibus normality test and Shapiro-Wilk normality test were applied to analyze Gaussian distribution. The Kruskal-Wallis test was applied to analyze global differences between the three groups. Mann-Whitney *U* test was used for post-hoc comparison. In order to control family-wise error rate caused by multiple comparison Bonferroni correction was carried out. Correlation was investigated using nonparametric Spearman correlation with a two-tailed confidence interval (95%). Binary logistic regression was performed to investigate the relationship between constipation and microbiota, SCFA concentrations respectively (all bacteria and SCFA were integrated in the binary logistic regression model).

3. Results

The percentage of bacterial phyla and bacterial groups in fecal samples of PD patients and age-matched controls were determined by quantitative PCR. Results are shown in Fig. 1: The bacterial phylum *Bacteroidetes* was significantly reduced in fecal samples of

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