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Short communication

Fecal markers of intestinal inflammation and intestinal permeability are elevated in Parkinson's disease

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ABSTRACT

Background/Objective: Intestinal inflammation and increased intestinal permeability (both possibly fueled by dysbiosis) have been suggested to be implicated in the multifactorial pathogenesis of Parkinson's disease (PD). The objective of the current study was to investigate whether fecal markers of inflammation and impaired intestinal barrier function corroborate this pathogenic aspect of PD.
Methods: In a case-control study, we quantitatively analyzed established fecal markers of intestinal inflammation (calprotectin and lactoferrin) and fecal markers of intestinal permeability (alpha-1-antitrypsin and zonulin) in PD patients (n = 34) and controls (n = 28, group-matched for age) by enzyme-linked immunosorbent assay. The study design controlled for potential confounding factors.
Results: Calprotectin, a fecal marker of intestinal inflammation, and two fecal markers of increased intestinal permeability (alpha-1-antitrypsin and zonulin) were significantly elevated in PD patients compared to age-matched controls. Lactoferrin, as a second fecal marker of intestinal inflammation, showed a non-significant trend towards elevated concentrations in PD patients. None of the four fecal markers correlated with disease severity, PD subtype, dopaminergic therapy, or presence of constipation.
Conclusions: Fecal markers reflecting intestinal inflammation and increased intestinal permeability have been primarily investigated in inflammatory bowel disease so far. Our data indicate that calprotectin, alpha-1-antitrypsin and zonulin could be useful non-invasive markers in PD as well. Even though these markers are not disease-specific, they corroborate the hypothesis of an intestinal inflammation as contributing factor in the pathogenesis of PD. Further investigations are needed to determine whether calprotectin, alpha-1-antitrypsin and zonulin can be used to define PD subgroups and to monitor the effect of interventions in PD.

1. Introduction

The etiology of Parkinson's disease (PD) includes genetic and environmental factors. Intestinal inflammation (possibly fueled by dysbiosis) has been suggested to be implicated as a contributing factor to PD pathogenesis [1,2]. Colonic inflammation has been shown in vivo in a biopsy study in PD patients [3]. Inflammation compromises the intestinal barrier and increases intestinal permeability. Increased intestinal permeability has been described in PD [4,5] [esupp ref 1] and has been associated with the preponderance of pro-inflammatory bacteria [5] [esupp ref 2]. Especially chronic inflammatory conditions in the intestine are thought to contribute to neurodegeneration [1]. Gut microbiota have been shown to promote neuroinflammation on a mouse model of PD [esupp ref 3].

Early involvement of the gastrointestinal tract in PD is also evidenced by clinical symptoms (constipation, disturbed gastric emptying) and histological findings in the enteric nervous system [esupp ref 4-5]. In addition, PD and inflammatory bowel disease, e.g. Crohn's disease, share a common genetic background [esupp ref 6-8]. In accordance with these genetic studies, a recent nation-wide cohort study from Taiwan reported an increased risk of PD among patients with Crohn's disease [esupp ref 9].

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Intestinal inflammation results in leukocyte migration to the mucous layer and subsequent leakage into the lumen. Calprotectin, a protein of the S100 family, is released by neutrophils upon activation. Lactoferrin, an iron-binding glycoprotein of the transferrin family, is secreted by activated neutrophils and mucosal epithelial cells as part of an inflammatory response. Calprotectin and lactoferrin are resistant to enzymatic degradation and are therefore sensitive fecal markers of intestinal inflammation. Fecal calprotectin even reflects subclinical inflammation [6].

Alpha-1-antitrypsin (A-1-AT), a protease inhibitor, reflects loss of proteins to the intestinal lumen, e.g. due to disruption of the mucosal barrier. Zonulin regulates intestinal permeability by modulating the function of tight junctions. Increased zonulin concentrations are linked to loss of integrity of the gastrointestinal barrier.

The objective of this study was to investigate these established fecal markers of intestinal inflammation (calprotectin, lactoferrin) and intestinal permeability (A-1-AT, zonulin) in an exploratory approach in PD patients compared to age-matched controls. Hitherto, these fecal markers have been mainly used for monitoring disease activity in inflammatory bowel disease (IBD).

1.1. Subjects and methods

The study was approved by the Ethics committee of the medical association of Saarland. All enrolled subjects provided informed consent to participate. Samples were pooled from subjects of a previous study [7] and subjects who participate in an ongoing study (clinicaltrials.gov, NCT02784145). Concerning samples from the NCT02784145 trial, only baseline samples were included. Additional information is provided online (Supplemental Material).

Subjects. Sixty-four subjects were enrolled between February 2014 and September 2016. All subjects were on an omnivorous diet. None of the subjects reported special dietary habits or dietary restrictions. Intake of antibiotic drugs, intake of probiotic or prebiotic products during the last 3 months as well as a history of acute or chronic gastrointestinal disorder and a history of gastrointestinal surgery (except appendectomy) were exclusion criteria. Mean age, sex distribution and presence of constipation among PD patients (n = 36) and controls (n = 28) are shown in Table 1. All PD patients were enrolled at the Department of Neurology, Saarland University. Diagnosis of PD was made according to the UK PD Society Brain Bank Clinical Diagnostic Criteria. All PD patients were on dopaminergic drugs. Levodopa equivalent daily dose (LEDD) was calculated for each enrolled PD patient as suggested by Tomlinson and colleagues [8]. Mean disease duration (defined by the time the first motor symptoms were experienced by the patient), modified Hoehn and Yahr stage and distribution of PD subtypes are shown in Table 1. We did not perform individual matching of cases and controls. Cases and controls were group-matched to have a comparable mean age (see Table 1). None of the control subjects reported pre-existing medical conditions. None of the control subjects was on permanent or intermittent use of drugs affecting gastrointestinal motility or on any other permanent medication.

Collection of fecal samples. Subjects were provided with sterile containers (MED AUXIL stool collector set, Suesse, Gudensberg, Germany) and instructed how to collect the fecal samples at home. Stool samples were sent to the Institute of Microoecology, Herborn, Germany, immediately frozen at −35 °C until analysis.

Quantitative analyses of fecal markers. The quantitative analyses were carried out the Institute of Microoecology, Herborn, Germany. All persons involved in the analyses were blinded to the clinical data and the diagnosis of the subjects. Fecal calprotectin and zonulin concentrations were measured by an enzyme-linked immunosorbent assay as described elsewhere [9]. Fecal lactoferrin concentrations were measured using the *IBD-SCAN*® test (TechLab®, Inc., Blacksburg, VA), fecal alpha-1-antitrypsin concentrations were measured using the *AAT* test (Maier Analytic, Sinsheim, Germany) following the providers' instructions.

Statistical analyses. Statistics was carried out using IBM SPSS statistics version 22.0 (SPSS Inc., Chicago, IL, USA). For comparison of binary variables the chi-squared test was used. For comparison of continuous variables the Mann-Whitney *U* test was used. In order to avoid a statistical bias caused by outliers in our cohort, we performed a non-parametric rank-sum test. Bonferroni correction was applied to correct for multiple comparisons when comparing the mean concentrations of the four fecal markers between patients and controls. For exploratory analyses concerning possible associations of demographic and clinical parameters with the four investigated fecal markers, uncorrected p values are reported. Correlation was investigated by calculating Spearman's rank correlation coefficient.

2. Results

Samples of all 64 enrolled subjects were eligible for analyses. Data on a history of appendectomy were available only for PD patients (n = 36). Detailed clinical data of the enrolled PD patients

Table 1
Demographic and clinical characteristics of enrolled subjects.

	PD patients	controls	p
number of subjects	36	28	
age (median, in years) [range]	65.53 [44–78]	64.25 [54–75]	0.534
sex (female/male)	14/22	17/11	0.083
constipation	8 of 36	2 of 28	0.099
disease duration (mean, in months) [range]	85.6 [12–228]	not applicable	
modified Hoehn and Yahr Scale	HY stage 2.0 n = 16 HY stage 2.5 n = 2 HY stage 3.0 n = 13 HY stage 4.0 n = 5	not applicable	
PD subtypes	akinetic-rigid: n = 15 mixed type: n = 16 tremor-dominant: n = 5	not applicable	
positive family history for neurodegenerative disorders	10 of 36	none	
smoker	02 of 26	07 of 28	
levodopa equivalent daily dose (median, in mg) [range]	555 [100–1380]	not applicable	
use of laxatives	02 of 36	none	

Supplemental information related to individual PD patients can be found online in Supplemental Table 1.