Gastrointestinal infections are associated with an increased risk of Parkinson's disease

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We have read with interest the recent publication of Perez-Pardo and colleagues reporting the role of the TLR4 in the gut-brain axis in Parkinson's disease (PD).[1] These findings prompted us to investigate the role of common gastrointestinal infections (GIIs) in the pathogenesis of PD. In this prospective cohort study, we assessed the risk of PD in patients who previously suffered from GIIs compared to the control group not exposed to GIIs (table 1). At study entry (January 1, 2005), the analysis sample from health claims data of the largest German health insurer consisted of 228,485 individuals aged 50 and older, which were followed for a mean time of 8.6 years (median = 11y; IQR = 7.6y). PD and GIIs were defined by ICD-10 codes as described in the supplementary material. Overall, 6,195 individuals (2.7%) developed PD and 50,492 individuals (22.1%) were affected by any GII during the observation period between 2005 and 2015. The most frequent GIIs were those that caused gastroenteritis and colitis of unspecified origin (IGCUs, 39,093 individuals, 17.1%), followed by viral intestinal infections (VIIs, 9,328 individuals, 4.1%), and bacterial intestinal infections (BIIs, 9,298 individuals, 4.1%). The cumulative incidence of PD was significantly higher among individuals with GIIs ($p < 0.001$, supplementary figure S1). Multivariable analyses (table 2) using Cox regression to compute hazard ratios (HR) revealed an increased risk of PD in patients with GIIs when compared to the control group (HR = 1.42; 95% CI = 1.33–1.52). Subgroup analyses (table 2) revealed positive associations of GIIs for men (HR = 1.48; 95% CI = 1.34–1.63), women (HR = 1.38; 95% CI = 1.27–1.50), individuals aged 70 or older (HR = 1.25; 95% CI = 1.04–1.49), and individuals with (HR = 1.40; 95% CI = 1.23–1.59) or without COPD (HR = 1.43; 95% CI = 1.33–1.54). To solidify our results we performed sensitivity analyses and found no remarkable changes compared to our primary analysis

(supplementary table S1). In a secondary analysis, where we considered GIIs separately (supplementary table S2), BIIs (HR = 1.30; 95% CI = 1.12–1.50), VIIs (HR = 1.31; 95% CI = 1.14–1.50), and IGCUs (HR = 1.34; 95% CI = 1.24–1.44) were each associated with an increased risk of PD.

Our findings suggest that GIIs are associated with an increased risk of PD. In sporadic PD, Lewy pathology defined by aggregated alpha-synuclein is first observed in the olfactory bulb and the enteric plexuses, from where it propagates via the vagus nerve to the dorsal motor nucleus in the CNS.[2] This prion-like ability of pathological alpha-synuclein to retrogradely spread from the periphery to the CNS is supported by a growing body of experimental work in rodents.[3, 4, 5] In the light of these findings, our results point to the missing link of what may cause alpha-synuclein pathology in the enteric nervous system (ENS): bacterial and viral pathogens, which breach the mucosal lining of the GI tract during GIIs, may trigger aggregation of alpha-synuclein in enteric neurons and initiate its retrograde transport to the CNS. Several species of gut bacteria express amyloid proteins, which could potentially cross-seed aggregation of alpha-synuclein.[6] In line with this, oral challenge of rats with a wild-type *E. coli* strain expressing the extracellular amyloid curli led to deposition of pathological alpha-synuclein in their ENS and subsequently CNS.[7] Another study in patients showed that expression of alpha-synuclein in enteric neurites of the GI tract was elevated in response to BIIs and VIIs.[8] Also, biopsy samples from intestinal allograft subjects after a norovirus infection showed elevated alpha-synuclein expression in enteric neurons that persisted months after the

virus was no longer detected.[8] Overall, our findings are consistent with the concept that in some patients PD may start in the GI tract.

Table 1. Characteristics of the study population by exposition to GIIs, No (%)

Characteristics	**Not exposed to GIIs; N = 177,993 (77.9)**	**Exposed to GIIs; N = 50,492 (22.1)**
Age (SD)[a]	67.5 (10.7)	68.6 (12)
Men	77,355 (43.5)	19,184 (38)
Women	100,638 (56.6)	31,308 (62)
Diabetes mellitus	72,574 (40.8)	24,629 (48.8)
Cerebrovascular diseases	64,749 (36.4)	24,176 (47.9)
Hypertension	147,078 (82.6)	45,612 (90.3)
Ischemic heart diseases	78,948 (44.4)	28,347 (56.1)
Hypercholesterolemia	67,242 (37.8)	22,590 (44.7)
Chronic obstructive pulmonary disease	40,208 (22.6)	15,159 (30)
Smoking related cancers	19,839 (11.2)	6,831 (13.5)
Intracranial injury	7,835 (4.4)	3,422 (6.8)
N = 228,485		
a Mean age in years at January 1, 2005		
GIIs, gastrointestinal infections; SD, standard deviation		

Table 2. Incidence rates[a] and hazard ratios of PD for the total sample and subgroups

Types of Analysis	Not exposed to GIIs			Exposed to GIIs			Cox regression (ref.: not exposed to GIIs)			
	Events	Person years	IR	Events	Person years	IR	Cr. HR	95% CI	Adj. HR	95% CI
Overall[b]	5,020	1,704,049	2.95	1,175	250,573	4.69	1.42	1.33–1.52	1.42	1.33–1.52
Men[c]	2,327	724,388	3.21	493	93,896	5.25	1.48	1.34–1.63	1.48	1.34–1.63
Women[c]	2,693	979,661	2.75	682	156,677	4.35	1.38	1.27–1.50	1.38	1.27–1.50
Age < 70[d]	1,062	862,501	1.23	162	114,127	1.42	1.17	0.99–1.38	1.17	0.99–1.38
Age ≥ 70[d]	3,958	841,548	4.70	1,013	136,446	7.42	1.25	1.04–1.49	1.25	1.04–1.49
Without COPD[e]	4,051	1,438,104	2.82	858	191,598	4.48	1.65	1.53–1.78	1.43	1.33–1.54
With COPD[e]	969	265,945	3.64	317	58,975	5.38	1.51	1.33–1.72	1.40	1.23–1.59
Without SRC[f]	4,650	1,613,378	2.88	1,065	230,044	4.63	1.66	1.55–1.78	1.40	1.35–1.55
With SRC[f]	370	90,671	4.08	110	20,529	5.36	1.93	1.59–2.33	1.20	0.96–1.48
N = 228,485; PD cases = 6,195										
a per 1,000 person years										
b HR were adjusted for gender, age, diabetes mellitus, cerebrovascular diseases, hypertension, ischemic heart diseases, hypercholesterolemia, chronic obstructive pulmonary disease, and intracranial injury										
c HR were adjusted for age, diabetes mellitus, cerebrovascular diseases, hypertension, ischemic heart diseases, hypercholesterolemia, chronic obstructive pulmonary disease, and intracranial injury										
d HR were adjusted for gender, diabetes mellitus, cerebrovascular diseases, hypertension, ischemic heart diseases, hypercholesterolemia, chronic obstructive pulmonary disease, and intracranial injury										
e HR were adjusted for gender, age, diabetes mellitus, cerebrovascular diseases, hypertension, ischemic heart diseases, hypercholesterolemia, and intracranial injury										
f HR were adjusted for gender, age, diabetes mellitus, cerebrovascular diseases, hypertension, ischemic heart diseases, hypercholesterolemia, and intracranial injury										
PD, Parkinson's disease; GII, gastrointestinal infections; SRC, smoking related cancers; IR, incidence rate; Cr. HR, crude hazard ratio; Adj. HR, adjusted hazard ratio; CI, confidence interval										

FOOTNOTES

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SUPPLEMENTARY MATERIAL

Data source

We used longitudinal routine claims data from the years 2004–2015 collected by the largest German statutory health insurance company, the *Allgemeine Ortskrankenkasse* (AOK). In Germany, about 70 million people are insured through statutory programs, one third of whom are members of the AOK. The AOK covers more than 50% of the population of higher ages.[1] The data holder provided us with an age-stratified random sample of 250,000 individuals aged 50 years and older, which was about 2% of all individuals insured in the AOK. This sample was drawn in the first quarter of 2004 and enabled us to follow individuals through the end of 2015. The AOK sample is almost representative of the overall population in Germany in terms of gender but not of age, because individuals from the sample are somewhat older than average. The data contain types of diagnoses by the German modification of the International Classification of Diseases, Tenth Revision (ICD-10-GM), all treatments in the inpatient and outpatient sector which are relevant for billing, drug prescriptions filled in the outpatient sector according to the Anatomical Therapeutic Chemical Classification System, benefits from the long-term-care-insurance, and date of death.[2] This information is reported quarterly and covers every insured individual, regardless of actual utilization. Data access was legally approved by the Scientific Institute of the AOK. This study is based on anonymized administrative claims data which never involved patients directly, therefore individual patients cannot be identified and the analyses presented do not affect those patients whose anonymized records were used.

Analysis sample and outcome measure

The analysis sample consists of individuals aged 50 years and older who had not received any diagnosis of PD in the year 2004. PD was identified based on ICD-10-GM code G20. We developed internal validation strategies to rule out false positive diagnoses. First, we included only those diagnoses internally marked as "verified" in the outpatient sector and as "discharge diagnosis" or "secondary diagnosis" in the inpatient sector. Second, only valid PD diagnoses were considered; a diagnosis was considered to be valid, if a patient had also received a confirmative PD diagnosis (ICD-10 code for PD) during the observation period. If this was the case, the individual was identified as a PD patient starting at the time the first diagnosis was made. Third, PD cases in which the last diagnosis in our longitudinal data was atypical parkinsonism (G21, G22) or essential tremor (G25.0) were not considered valid.[2] After excluding prevalent PD cases we arrived at an analysis sample of 228,485 individuals, whom we followed from January 1, 2005 until one of the following occurred: a valid PD diagnosis, death, exit from the AOK insurance, or December 31, 2015 (end of the study period).

Exposure and comorbidities

In the primary analysis, we considered the impact of GIIs according to ICD-10-GM codes A00–A09 (intestinal infectious diseases). Diagnoses of GIIs had to be marked as "verified" in the outpatient sector and as "discharge diagnosis" or "secondary diagnosis" in the inpatient sector. We controlled for gender and age in five-year age groups (from 50 to 95+), and for common morbidities and risk factors for PD in old age: diabetes mellitus (E10–E14), cerebrovascular diseases (G45, G46, H34.0, I60, I69), hypertension (I10–

I15), ischemic heart diseases (I20–I25), hypercholesterolemia (E78.0), chronic obstructive pulmonary disease (COPD, J44), and intracranial injury (S06). In the bias analysis we controlled for smoking related cancers (SRC, C00–C14, C18–C20, C25, C30–C34, C64–C67) instead of COPD. Exposure and comorbidity variables were defined as time-varying dummy variables, receiving a value of one from the first time a specific diagnosis was made until the outcome or censoring. In the secondary analysis, we divided GIIs according to their origin: BIIs (A00-A05), VIIs (A08), and IGCUs (A09). We omitted amoebiasis (A06) and other protozoal intestinal diseases (A07) due the low number of cases.

Statistical analysis

We showed the cumulative risk of developing PD stratified by the presence of the diseases of interest.[3] Differences were assessed using a log-rank test. We used Cox proportional hazards models to examine whether the incidence of PD was associated with GIIs, adjusting for the covariates mentioned above. We examined the Schoenfeld residuals and found that the proportional hazards assumption was satisfied. Analysis time was defined by the number of months since January 1, 2005. For individuals transitioning to PD, the analysis time was set to the middle of the quarter. Deaths were assigned to the middle of the month of death. Furthermore, we performed subgroup analyses and calculated interaction effects for gender and age. To solidify our results we performed the following sensitivity analyses: (1) applying different disease-free periods prior to the first incident PD diagnosis (two, three, or four years), (2) PD identification based on one PD diagnosis (without the algorithm of repeated diagnoses), (3) exposure lagging by 4 and 5

years for each variable except for gender and age, (4) repeating the primary analysis including appendicitis (K35–K37) as a negative exposure control variable, (5) excluding individuals with prodromes of PD in 2004 (other sleep disorders, G47.8; constipation, K59.0; anosmia, R43.0) in the year 2004, and (6) conducting a competing risk model as proposed by Fine and Grey.[4] All analyses were conducted using the statistical software Stata 12.1 (StataCorp LLC).

Limitations

We did not have information on the exposure to GIIs prior to the age of 50, the severity of a GII, of individuals who did not seek medical attention, or of lifestyle (tobacco or coffee consumption) and occupational (working in the agricultural sector or heavy industry) risk factors known to be associated with PD and could not consider these factors in our analysis. However, we considered the effect of smoking by taking conditions into account which are strongly related to smoking. GIIs were still associated with PD for individuals with or without COPD or smoking related cancers (table 2).

Supplementary references

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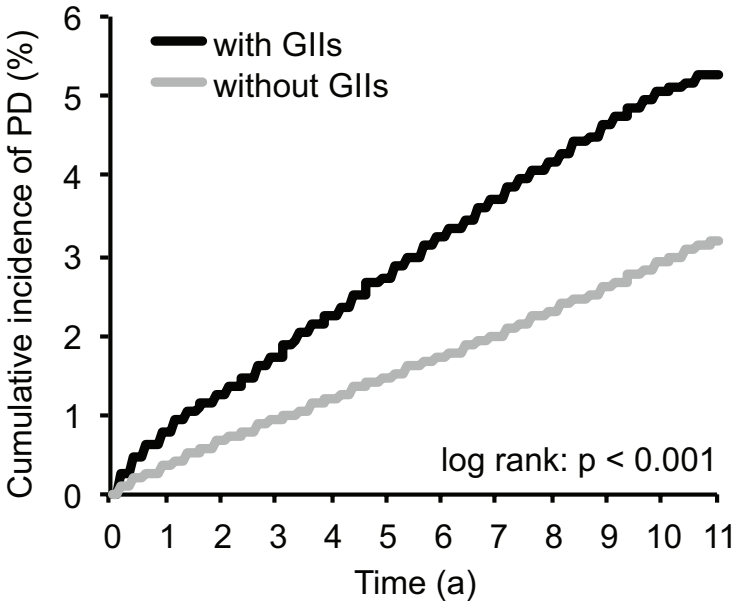
Supplementary table S1. Sensitivity analyses showing adjusted[a] hazard ratios for association between GIIs and risk of PD

Sensitivity Analysis	Exposure	HR	95% CI
Varying disease-free periods, in years			
2[b]	GIIs	1.40	1.31–1.50
3[c]	GIIs	1.43	1.33–1.53
4[d]	GIIs	1.42	1.32–1.52
PD incidence based on one PD diagnosis[e]	GIIs	1.41	1.33–1.50
Exposure lagging, in years			
4	GIIs	1.18	1.06–1.32
5	GIIs	1.18	1.04–1.34
Primary analysis including a negative exposure control	Appendicitis	1.18	0.93–1.50
Without patients with prodromes of PD in 2004[f]	GIIs	1.40	1.31–1.50
Competing risk analysis (taking the risk of death into account)[g]	GIIs	1.31	1.22–1.40
a Adjusted for gender, age, diabetes mellitus, cerebrovascular diseases, hypertension, ischemic heart diseases, hypercholesterolemia, chronic obstructive pulmonary disease, and intracranial injury			
b N = 217,980; PD cases = 5,370			
c N = 208,298; PD cases = 4,681			
d N = 197,786; PD cases = 4,105			
e N = 228,221; PD cases = 7,783			
f N = 221,682; PD cases = 5,878			
g N = 228,485; PD cases = 5,884; HR are given here as subdistribution HR			
GIIs, gastrointestinal infections; CI, confidence interval			

Supplementary table S2. Adjusted[a] hazard ratio for association between BII, VII, IGCU, and risk of PD

Risk Factor[b]	HR	95% CI
BIIs	1.30	1.12–1.50
VIIs	1.31	1.14–1.50
IGCUs	1.34	1.24–1.44
N = 228,485; PD cases = 6,195		
a Adjusted for gender, age, diabetes mellitus, cerebrovascular diseases, hypertension, ischemic heart diseases, hypercholesterolemia, chronic obstructive pulmonary disease, and intracranial injury		
b Reference group: not exposed to BIIs, VIIs, or IGCUs		
BIIs, bacterial intestinal infections; VIIs, viral intestinal infections; IGCU, infectious gastroenteritis and colitis of unspecified origin; HR, hazard ratio; CI, confidence interval		



Supplementary figure S1 Cumulative incidence of PD for exposed and non-exposed individuals

The cumulative incidence of PD was increased in patients with GIIs in comparison to the control group not exposed to GIIs.