RESEARCH ARTICLE

Antibiotic Exposure and Risk of Parkinson's Disease in Finland: A Nationwide Case-Control Study

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ABSTRACT: Background: Gut microbiota alterations have been found in prodromal and established Parkinson's disease (PD). Antibiotic exposure can have long-term effects on the composition of human intestinal microbiota, but a potential connection between antibiotic exposure and risk of PD has not been studied previously.

Objective: To evaluate the impact of antibiotic exposure on the risk of PD in a nationwide, register-based, case-control study.

Methods: We identified all patients who were diagnosed with PD in Finland during the years 1998 to 2014. Information was obtained on individual purchases of orally administered antibiotics during the years 1993 to 2014. We assessed the association between prior antibiotic exposure and PD using conditional logistic regression.

Results: The study population consisted of 13,976 PD cases and 40,697 controls. The strongest connection with PD risk was found for oral exposure to macrolides and lincosamides (adjusted odds ratio up to 1.416; 95% confidence interval, 1.053–1.904). After correction for multiple comparisons, exposure to antianaerobics and tetracyclines 10 to 15 years before the index date, sulfonamides and trimethoprim 1 to 5 years before the index date, and antifungal medications 1 to 5 years before the index date were positively associated with PD risk. In post hoc analyses, further positive associations were found for broad-spectrum antibiotics.

Conclusions: Exposure to certain types of oral antibiotics seems to be associated with an elevated risk of PD with a delay that is consistent with the proposed duration of a prodromal period. The pattern of associations supports the hypothesis that effects on gut microbiota could link antibiotics to PD, but further studies are needed to confirm this. © 2019 International Parkinson and Movement Disorder Society

Key Words: antibiotic exposure; epidemiology; microbiota; Parkinson's disease; risk factors

The cause of Parkinson's disease (PD) remains unclear, although a variety of genetic and environmental factors appear to play a role in the pathogenesis. Known environmental risk factors include pesticide exposure and rural living, whereas smoking and coffee consumption are associated with a lower risk of PD.[1] Key factors in PD pathophysiology seem to include mitochondrial dysfunction, oxidative stress, protein aggregation, and neuroinflammation.[2]

It has been proposed that an exogenic factor such as a micro-organism or toxin could initiate PD pathology.[3] The pathologic process in PD might begin in the submucosal plexus of the enteric nervous system and spread retrogradely toward the central nervous system via preganglionic axons of the dorsal motor nucleus of the vagus nerve.[4] This hypothesis is supported by the observation that truncal vagotomy is associated with a lower risk of PD.[5] Also, several gastrointestinal disorders, such as constipation,[6] irritable bowel syndrome (IBS),[7] and inflammatory bowel disease[8] are associated with a higher risk of PD. Lewy-type pathology has been detected in the gastrointestinal tract up to 20 years prior to PD diagnosis,[9] and

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prominent denervation of the gut in prodromal and established PD has been demonstrated.[10]

The intestinal microbiota has an important role in protection against pathogens and in the maintenance of the structural integrity of the gut mucosal barrier, the production of nutrients and signaling molecules, and the regulation of microglia.[11] Antibiotic exposure can have long-term effects on the diversity and composition of human intestinal microbiota.[12] Although some antiinflammatory and neuroprotective properties unrelated to antimicrobial effects have been suggested for certain antibiotics,[13] antibiotic exposure has been recently linked to a number of disorders, including colorectal cancer,[14] Crohn's disease,[15] and certain psychiatric disorders.[16]

In PD, several studies have described alterations of gut microbiota composition, and changes in fecal microbiota abundance have been found to be associated with gastrointestinal and motor symptoms.[17,18] However, the cause of these changes is not known. We hypothesized that higher antibiotic exposure is associated with increased PD risk.

Methods

We conducted a case-control study using data from the Finnish Care Register for Health Care (HILMO), which contains nationwide linkable data on all discharges from inpatient care, day surgeries, and specialized outpatient care.[19] Data on specialized outpatient care includes all secondary and tertiary care outpatient visits in the public healthcare system since 1998. All diagnostic data in the HILMO register is coded according to the 10th version of the International Classification of Diseases, which has been in use in Finland since 1996. The diagnosis codes used for this study are explained in Supporting Information eTable 1.

We identified all patients who were discharged from inpatient care or specialized outpatient care during the years 1998 to 2014 with a diagnostic code of PD (G20) from the HILMO register and acquired their discharge data (Fig. 1). For each PD patient, 3 control subjects of the same age (±1 year) and sex and who were living in the same municipality were sought from a database maintained by the Population Register Center. For controls, HILMO register data were also acquired for the year 2015 to exclude subjects who already suffered from early PD in 2014 but who were only diagnosed in 2015. We obtained data from the Finnish National Prescription Register (established in 1987) on whether the subjects had been entitled to a special reimbursement of medical expenses for treatment of PD and comparable movement disorders (special reimbursement code 110, 10th version of the International Classification of Diseases codes G20, G23, G24.1, G24.8, G90.3) between the years 1987 and 2014. To be entitled to special reimbursement for PD medication, the diagnosis and need for treatment must be evaluated by a neurologist or a doctor working in a neurology unit and be justified to the insurance agency in writing.

For nearly all cases in Finland, the PD diagnosis is set in the neurology department of public secondary or tertiary healthcare units. However, in some occasions the diagnosis of PD can be first set by a neurologist working in the private sector, which is not included in the HILMO register. Hence a significant delay may occur before the first visit to a public health care unit as a result of PD. On the other hand, PD patients may have mild clinical symptoms at the time of diagnosis not requiring medication immediately, and the special reimbursement might be issued several years afterward. Therefore, the index date was defined as the first time when the patient was discharged with a PD diagnosis or the date the patient was entitled to special reimbursement for medication for PD, whichever occurred earlier. Special reimbursement data between the years 1987 and 1997 were used only to exclude subjects who were probably diagnosed with PD before the establishment of the HILMO register.

The exclusion criteria are presented in the study flow chart (Fig. 1). All subjects with a diagnosis of other movement disorders (G21-G25, G90.3, and I67.3), schizophrenia (F20), and those who were entitled to a special reimbursement for indications other than PD (G23, G24.1, G24.8, and G90.3) were excluded (Supporting Information eTable 1). To take into account the uncertainty of a PD diagnosis at a single visit and possible reading errors, we considered the PD diagnosis to be reliable if the subject had special reimbursement and at least 2 discharges with a PD diagnosis at a neurology unit. For those subjects with no special reimbursement for PD medication, at least 3 discharges at a neurology unit with a PD diagnosis were demanded. Cohen's κ statistic was used assess the consistency between discharge with a PD diagnosis and eligibility for reimbursement for PD medication.[20] Cohen's κ (0.855) indicated a strong level of agreement between these 2 methods for identifying PD patients.

We obtained information on purchases of antibiotics for systemic use between the years 1993 and 2014 from the Finnish National Prescription Register. In Finland, all antibacterial agents for systemic use are available by prescription only and sold in licensed pharmacies, and therefore all purchases are covered by this register. It contains information on the type of medication prescribed according to the Anatomical Therapeutic Chemical classification system and the dispension dates of the prescription since 1993. To avoid protopathic

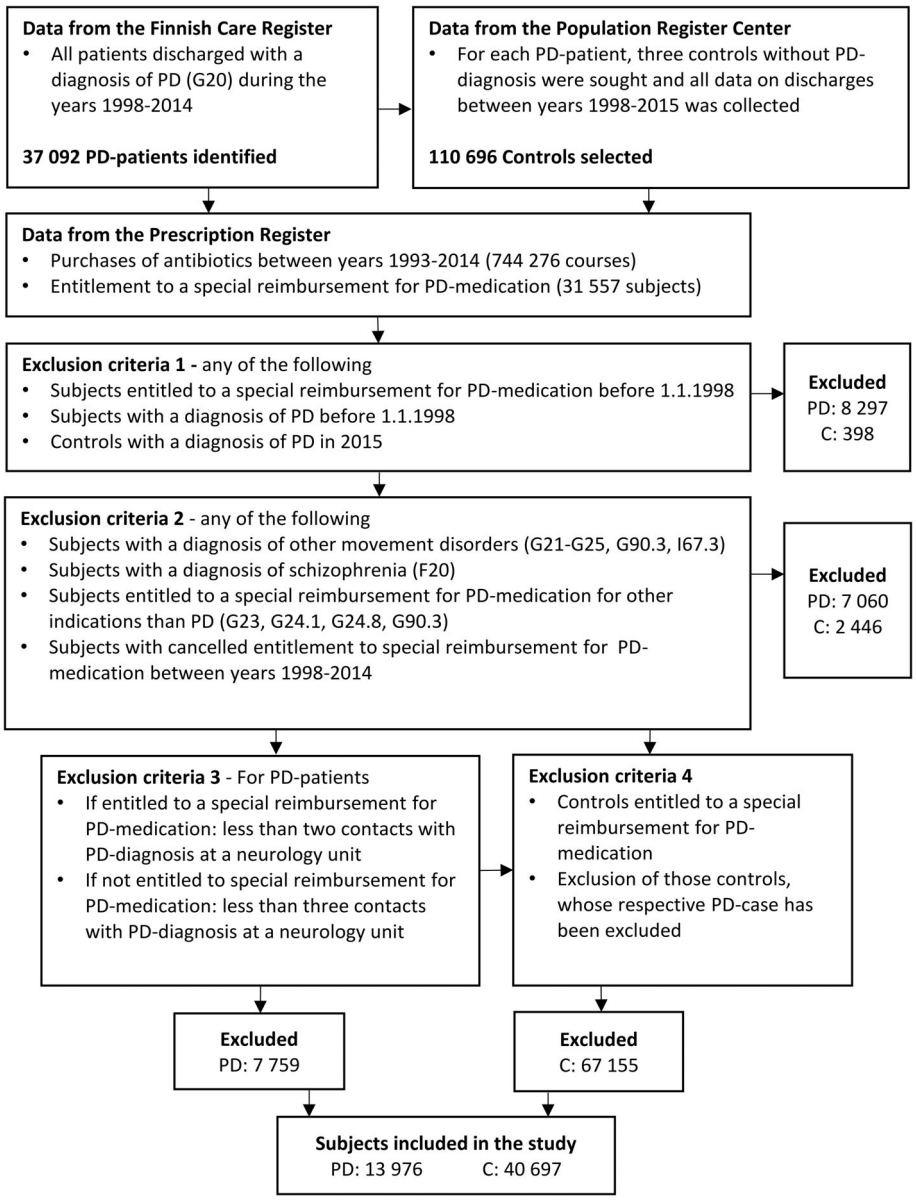

FIG. 1. The study flow chart. PD, Parkinson's disease patients; C, controls.

bias, only antibiotics purchased more than 1 year prior to the index date were assessed.[21] Because it has been proposed that the lag between initiation and motor manifestation could be several years,[6] we decided to assess antibiotic exposure in 3 separate time periods. For each subject, the number of purchased antibiotic courses 1 to 5 years prior to the index date was counted. For those subjects with a long enough observation period, antibiotic exposure 5 to 10 and 10 to 15 years before the index date was counted.

We assessed exposure to any type of systemic antibiotic (Anatomical Therapeutic Chemical classification system groups J01, A02BD, A07A, and P01AB). Besides overall exposure, exposure to antimycotics for systemic use (J02), antianaerobic and nonantianaerobic antibiotics,[22] and 7 antibiotic categories (tetracyclines, penicillins, other betalactam antibacterials, sulfonamides and trimethoprim, macrolides and lincosamides, fluoroquinolones, and other antimicrobials) were analyzed separately (Supporting Information eTable 2). The common usage of oral antibiotics in Finland is described in the supplementary material (Supporting Information eTable 3). In post hoc analyses, we classified the antibiotics based on the mechanism of action (nucleic acid inhibition, cell wall synthesis inhibition, or protein synthesis inhibition), antimicrobial spectrum (broad or narrow spectrum, according to the Danish Integrated Antimicrobial Monitoring and Research

Programme 2013)[23] and their effect on the targeted bacteria (bactericidal or bacteriostatic).[24]

To take into account a possible effect of infectious burden, the number of hospitalizations and all discharges as a result of bacterial and viral infections at least 1 year before index date were assessed. Furthermore, we analyzed the history of discharges as a result of bacterial pneumonia, urinary tract infection, skin infections, gastrointestinal infections, bacterial meningitis or encephalitis, sepsis, and other bacterial infections.

As other potential confounders, we assessed the history of chronic obstructive pulmonary disease (COPD) as a proxy for smoking and potential PD risk factors that could affect antibiotic exposure (IBS, ulcerative colitis, Crohn's disease, transient ischemic attack or stroke, and *Helicobacter pylori* eradication). Data from the Finnish National Prescription Register were used to identify subjects with a history of *H. pylori* eradication. We also calculated the Charlson Comorbidity Index,[25] which includes several comorbidities possibly affecting the risk of both PD and infection. All covariates were identified by International Classification of Diseases codes and measured prior to the index date (Supporting Information eTable 1). Antibiotic exposure was included in the adjusted model for antimycotic exposure because antibiotic treatment may predispose to fungal infections.

The statistical analyses were performed using IBM SPSS Statistics version 24.0.0.1 (IBM Corp., Armonk, NY). For baseline characteristics of the cohort, Fisher's 2-sided exact test was used for differences regarding the dichotomous categorical variables. For polytomous categorical variables, a chi-square test was used. The normality of the data was assessed graphically by evaluating Q-Q plots. An unpaired *t* test was used to analyze the group differences of baseline characteristics for normally distributed variables; otherwise the Mann-Whitney *U* test was used. *P* values below 0.05 were considered significant.

The odds ratio (OR) and 95% confidence intervals (CI) were estimated for the association between PD and number of antibiotic courses and bacterial infections, respectively, by conditional logistic regression. For this analysis, antibiotic exposure was classified into 0, 1, 2 to 4, and 5 or more purchases, if more than 5% of subjects had purchased more than 2 courses within a certain category. Otherwise, purchases were grouped as 0, 1, and 2 or more. All discharges as a result of bacterial infections were classified into 0, 1, and 2 or more discharges. The reference exposure group consisted of subjects without documented exposure to the specific antibiotic group. A multivariate logistic regression was used to adjust for potential confounders (Crohn's disease, ulcerative colitis, IBS, COPD, transient ischemic attack or stroke, Charlson Comorbidity Index, *H. pylori* eradication, and viral infections). Although not considered necessary in the current explorative setting,[26] the false discovery rate (FDR) was corrected using the Benjamini-Hochberg procedure[27] for potential associations between antibiotic exposure during certain time periods and the risk of PD.

We ran sensitivity analyses to see whether the definition of a PD case affected our results. First, we tested a more liberal approach with demanding only at least 1 discharge with a PD diagnosis from a neurology unit and the entitlement to special reimbursement for PD medication. In another sensitivity model, we used a stricter approach for determining a PD case, demanding at least 3 visits with a PD diagnosis at a neurology unit and the entitlement to special reimbursement for PD medication.

The study was conducted in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology recommendations.[28] Identifiers enabling direct identification of the research subject were replaced with research codes to maintain patient confidentiality. The study design was approved by the Data Protection Ombudsman from the Ministry of Social Affairs and Health and by all the register authorities who have provided data for the research. Ethical approval is not required for registry studies in Finland.

Results

After exclusions, the study population consisted of 13,976 PD cases and 40,697 matched controls, with a median follow-up of 14.40 (interquartile range 10.06–18.22) and 14.41 (interquartile range 10.08–18.24) years, respectively (Table 1). History of *H. pylori* eradication and diagnoses of IBS and transient ischemic attack or stroke were more common in PD cases, whereas the diagnosis of COPD was less common.

During the course of follow-up, 84.9% of PD patients and 83.6% of controls had purchased at least 1 antibacterial course ($P < 0.001$). Penicillins were purchased most frequently, constituting 22.8% of all purchases, followed by other betalactams (22.3%), tetracyclines (18.6%), macrolides and lincosamides (14.6%), sulfonamides and trimethoprim (10.0%), fluoroquinolones (7.3%), and other antibacterials (4.4%). The use of antimycotics was less prevalent: only 16.4% of PD cases and 14.7% of controls had purchased at least 1 antifungal course ($P < 0.001$). Most of the antifungals purchased were azoles (93.7%). In total, 342,559 antibiotic courses and 22,049 antifungal courses were bought during 770,439 person-years of follow-up.

On average, PD patients had purchased more antibiotic courses than controls (6.32 vs. 6.25; $P = 0.021$), whereas controls had more hospitalizations for bacterial infections (0.19 ± 0.73 vs. 0.16 ± 0.65; $P = 0.017$) and more discharges with a diagnosis of bacterial

TABLE 1. The baseline characteristics of PD cases and controls

Demographics	PD cases	Controls	*P*
Number of subjects	13,976	40,697	
Male, no. (%)	7876 (56.4)	22936 (56.4)	1.000
Age, mean ± SD, y	69.91 ± 9.74	69.87 ± 9.75	0.671
Special reimbursement for PD medication, no. (%)	13577 (97.1)	-	-
Number of discharges			
Outpatient visits, median [IQR]	7 [2–17]	5 [1–15]	<0.001
Hospitalizations, median [IQR]	2 [0–4]	1 [0–4]	<0.001
Follow-up time, median [IQR], y[a]	14.40 [10.06–18.22]	14.41 [10.08–18.24]	0.769
Comorbidities			
Charlson comorbidity index, mean ± SD	0.51 ± 0.87	0.53 ± 0.94	0.594
Irritable bowel syndrome, no. (%)	122 (0.9)	250 (0.6)	0.002
Crohn's disease, no. (%)	29 (0.2)	101 (0.2)	0.420
Ulcerative colitis, no. (%)	115 (0.8)	269 (0.7)	0.051
Helicobacter pylori eradication, no. (%)	772 (5.5)	2004 (4.9)	0.005
Chronic obstructive pulmonary disease, no. (%)	227 (1.6)	1091 (2.7)	<0.001
Transient ischemic attack or stroke, no. (%)	1203 (8.6)	3178 (7.8)	0.003
Antibiotic exposure and infections			
Antibiotic courses, mean ± SD	6.32 ± 8.97	6.25 ± 8.84	0.021
Antifungal courses, mean ± SD	0.44 ± 1.76	0.39 ± 1.64	<0.001
All bacterial infections, mean ± SD	0.32 ± 1.17	0.36 ± 1.37	0.168
Bacterial pneumonia, no. (%)	221 (1.6)	804 (2.0)	0.003
Urinary tract infection, no. (%)	474 (3.4)	1414 (3.5)	0.666
Skin infections, no. (%)	287 (2.1)	907 (2.2)	0.228
Gastrointestinal infections, no. (%)	701 (5.0)	1956 (4.8)	0.327
Sepsis, no. (%)	62 (0.4)	215 (0.5)	0.242
Bacterial meningitis or encephalitis, no. (%)	4 (0.03)	17 (0.04)	0.622
Other bacterial infections, no. (%)	232 (1.7)	683 (1.7)	0.906
All viral infections, mean ± SD	0.10 ± 0.51	0.11 ± 0.74	0.253
Hospitalizations for bacterial infection, mean ± SD	0.16 ± 0.65	0.19 ± 0.73	0.017
Hospitalizations for viral infections, mean ± SD	0.04 ± 0.24	0.04 ± 0.27	0.501

PD, Parkinson's disease; SD, standard deviation; IQR, interquartile range.
[a]Time between January 1, 1993 (the date when antibiotic purchase data is available) and the index date.

pneumonia (2.0% vs 1.6%; $P = 0.003$). However, there were no statistically significant differences in the number of all discharges because of bacterial or viral infections.

Antibiotic Exposure and PD

The association between the number of antibiotic courses for each antibiotic class and risk of PD was investigated with a conditional logistic regression (Table 2). After adjustment for potential confounders, most associations for antibacterials were found for exposure 10 to 15 years before the index date (Fig. 2). The connection was strongest for macrolides and lincosamides, showing a dose–response relationship between antibiotic exposure and PD with an adjusted OR reaching 1.416 (95% CI, 1.053–1.904) for subjects who had purchased at least 5 courses 10 to 15 years before the index date, although this finding did not remain statistically significant after FDR correction (Fig. 2). After FDR correction, exposure to antianaerobics and tetracyclines 10 to 15 years before the index date and to sulfonamides and trimethoprim 1 to 5 years before the index date remained significantly associated with PD risk.

Exposure to antifungal medications 1 to 10 years before the index date was also associated with a significantly higher OR for PD (Table 2 and Fig. 2). The association was strongest for subjects who had purchased at least 2 courses 1 to 5 years before the index date (adjusted OR 1.255; 95% CI, 1.110–1.420) and remained statistically significant after FDR correction.

Sensitivity analyses showed only modest changes in the association between antibiotic exposure and PD risk (Supporting Information eResults and eTables 4 and 5).

Because associations between antibiotic exposure and PD risk were clearly different between antibiotic classes and stronger for antianaerobics than for non-antianaerobics, we ran post hoc analyses to investigate whether the mechanism of action, bactericidicity, or broadness of the antimicrobial spectrum would be associated with the risk of PD. Although we did not find convincing association for the mechanism of action, bactericidity, or narrow-spectrum antibiotics, there were associations between broad-spectrum antibiotics and PD risk (Fig. 3, Supporting Information eTable 6).

TABLE 2. Antibiotic exposure, burden of bacterial infections, and risk of PD

Antibiotic group and number of courses	1–5 years before index date, n = 54,673			5–10 years before index date, n = 41,121			10–15 years before index date, n = 25,424		
	PD, n (%)	C (%), n (%)	Crude OR [95% CI]	PD, n (%)	C (%), n (%)	Crude OR [95% CI]	PD, n (%)	C (%), n (%)	Crude OR [95% CI]
Antibacterials									
0	5274 (37.7)	15,605 (38.3)	-	3643 (34.7)	10,857 (35.5)	-	2232 (34.4)	6794 (35.9)	-
1	3026 (21.7)	8663 (21.3)	1.033 [0.981–1.088]	2184 (20.8)	6302 (20.6)	1.030 [0.969–1.096]	1358 (20.9)	3895 (20.6)	1.060 [0.980–1.147]
2–4	3808 (27.2)	10947 (26.9)	1.030 [0.981–1.082]	3057 (29.1)	8768 (28.6)	1.040 [0.984–1.100]	1877 (28.9)	5419 (28.6)	1.055 [0.982–1.133]
5+	1868 (13.4)	5482 (13.5)	1.008 [0.947–1.073]	1619 (15.4)	4691 (15.3)	1.027 [0.959–1.101]	1024 (15.8)	2825 (14.9)	1.104 [1.012–1.205]
Antianaerobics[a]									
0	7875 (56.3)	23,144 (56.9)	-	5639 (53.7)	16,762 (54.7)	-	3548 (54.7)	10,577 (55.9)	-
1	3036 (21.7)	8770 (21.5)	1.020 [0.971–1.071]	2387 (22.7)	6817 (22.3)	1.040 [0.984–1.100]	1499 (23.1)	4235 (22.4)	1.145 [1.067–1.229]
2–4	2505 (17.9)	7149 (17.6)	1.029 [0.976–1.086]	1994 (19.0)	5736 (18.7)	1.035 [0.975–1.099]	1187 (18.3)	3397 (17.9)	1.137 [1.055–1.225]
5+	560 (4.0)	1634 (4.0)	1.008 [0.912–1.115]	483 (4.6)	1303 (4.3)	1.109 [0.994–1.237]	257 (4.0)	724 (3.8)	0.953 [0.827–1.097]
Nonantianaerobics									
0	7749 (55.4)	22,714 (55.8)	-	5374 (51.2)	15,747 (51.4)	-	3350 (51.6)	9713 (51.3)	-
1	2978 (21.3)	8606 (21.1)	1.019 [0.935–1.110]	2280 (21.7)	6650 (21.7)	1.004 [0.949–1.063]	1434 (22.1)	4127 (21.8)	0.998 [0.929–1.073]
2–4	2461 (17.6)	7032 (17.3)	1.035 [0.944–1.134]	2143 (20.4)	6180 (20.2)	1.017 [0.959–1.078]	1288 (19.8)	3849 (20.3)	1.050 [0.974–1.131]
5+	788 (5.6)	2354 (5.8)	1.046 [0.953–1.149]	706 (6.7)	2041 (6.7)	1.009 [0.920–1.106]	419 (6.5)	1244 (6.6)	1.035 [0.915–1.170]
Tetracyclines									
0	11,319 (81.0)	32,968 (81.0)	-	8052 (76.7)	23,616 (77.1)	-	4601 (70.9)	13,754 (72.6)	-
1	1798 (12.9)	5197 (12.8)	1.006 [0.949–1.066]	1596 (15.2)	4620 (15.1)	1.011 [0.950–1.077]	1208 (18.6)	3207 (9.3)	1.126 [1.045–1.213]
2–4	772 (5.5)	2242 (5.5)	1.005 [0.922–1.094]	754 (7.2)	2120 (6.9)	1.044 [0.956–1.140]	606 (9.3)	1759 (9.3)	1.026 [0.929–1.133]
5+	87 (0.6)	290 (0.7)	0.878 [0.690–1.117]	101 (1.0)	262 (0.9)	1.131 [0.897–1.424]	76 (1.2)	213 (1.1)	1.069 [0.821–1.392]
Penicillins									
0	9687 (69.3)	28,536 (70.1)	-	7264 (69.2)	21,518 (70.3)	-	4636 (71.4)	13,823 (73.0)	-
1	2557 (18.3)	7340 (18.0)	1.029 [0.978–1.083]	2009 (19.1)	5614 (18.3)	1.064 [1.005–1.128]	1222 (18.8)	3355 (17.7)	1.085 [1.008–1.168]
2–4	1497 (10.7)	4114 (10.1)	1.073 [1.006–1.146]	1056 (10.1)	3012 (9.8)	1.042 [0.966–1.124]	576 (8.9)	1567 (8.3)	1.094 [0.988–1.211]
5+	235 (1.7)	707 (1.7)	0.976 [0.839–1.136]	174 (1.7)	474 (1.5)	1.099 [0.920–1.312]	57 (0.9)	188 (1.0)	0.908 [0.673–1.226]
Other betalactams									
0	10,111 (72.3)	29,061 (71.4)	-	7269 (69.2)	21,028 (68.7)	-	4547 (70.1)	13,204 (69.7)	-
1	2463 (17.6)	7366 (18.1)	0.960 [0.912–1.010]	2012 (19.2)	5885 (19.2)	0.989 [0.933–1.047]	1209 (18.6)	3601 (19.0)	0.975 [0.906–1.050]
2–4	1262 (9.0)	3806 (9.4)	0.951 [0.889–1.018]	1097 (10.4)	3285 (10.7)	0.964 [0.895–1.038]	668 (10.3)	1924 (10.2)	1.009 [0.918–1.109]
5+	140 (1.0)	464 (1.1)	0.862 [0.712–1.044]	125 (1.2)	420 (1.4)	0.849 [0.694–1.038]	67 (1.0)	204 (1.1)	0.948 [0.717–1.253]
Sulfonamides & trimethoprim									
0	12,582 (90.0)	37,123 (31.2)	-	9500 (90.5)	27,858 (91.0)	-	5821 (89.7)	17,028 (89.9)	-
1	865 (6.2)	2139 (5.3)	1.200 [1.105–1.304]	638 (6.1)	1721 (5.6)	1.093 [0.994–1.202]	446 (6.9)	1292 (6.8)	1.005 [0.899–1.125]
2–4	326 (2.5)	952 (2.3)	1.111 [0.980–1.259]	269 (2.6)	740 (2.4)	1.064 [0.923–1.227]	178 (2.7)	478 (2.5)	1.088 [0.912–1.298]

(Continues)

TABLE 2. Continued

Antibiotic group and number of courses	1–5 years before index date, n = 54,673			5–10 years before index date, n = 41,121			10–15 years before index date, n = 25,424		
	PD, n (%)	C (%), n (%)	Crude OR [95% CI]	PD, n (%)	C (%), n (%)	Crude OR [95% CI]	PD, n (%)	C (%), n (%)	Crude OR [95% CI]
5+	173 (1.2)	483 (1.2)	1.050 [0.879–1.254]	96 (0.9)	299 (1.0)	0.925 [0.733–1.167]	46 (0.7)	138 (0.7)	0.965 [0.689–1.352]
Macrolides & lincosamides									
0	11,546 (82.6)	33,352 (82.0)	-	8030 (76.5)	23,415 (76.5)	-	4840 (74.6)	14,403 (76.1)	-
1	1709 (12.2)	5197 (12.8)	0.951 [0.897–1.009]	1608 (15.3)	4738 (15.5)	0.991 [0.931–1.055]	1052 (16.2)	2970 (15.7)	1.055 [0.975–1.141]
2–4	655 (4.7)	1902 (4.7)	0.994 [0.907–1.091]	774 (7.4)	2207 (7.2)	1.025 [0.940–1.117]	533 (8.2)	1415 (7.5)	1.129 [1.016–1.255]
5+	66 (0.5)	246 (0.6)	0.776 [0.590–1.020]	91 (0.9)	258 (0.8)	1.030 [0.809–1.312]	66 (1.0)	145 (0.8)	1.371 [1.022–1.838]
Fluoroquinolones									
0	12,399 (88.7)	36,371 (89.4)	-	9366 (89.2)	27,384 (89.4)	-	5944 (91.6)	17,423 (92.0)	-
1	1025 (7.3)	2804 (6.9)	1.073 [0.995–1.156]	746 (7.1)	2062 (6.7)	1.060 [0.971–1.156]	383 (5.9)	1042 (5.5)	1.077 [0.954–1.217]
2+	552 (3.9)	1522 (3.7)	1.065 [0.964–1.177]	391 (3.7)	1172 (3.8)	0.974 [0.866–1.096]	164 (2.5)	486 (2.5)	1.026 [0.856–1.230]
Other antibacterials									
0	13,181 (94.3)	38,406 (94.4)	-	9786 (93.2)	28,513 (93.1)	-	6005 (92.5)	17,501 (92.4)	-
1	613 (4.4)	1795 (4.4)	0.995 [0.905–1.093]	568 (5.4)	1663 (5.4)	0.997 [0.904–1.100]	373 (5.7)	1111 (5.9)	0.978 [0.866–1.105]
2+	182 (1.3)	496 (1.2)	1.066 [0.897–1.267]	149 (1.4)	442 (1.4)	0.981 [0.813–1.183]	113 (1.7)	321 (1.7)	1.026 [0.825–1.274]
Antifungals									
0	13,081 (93.6)	38,459 (94.5)	-	9619 (91.6)	28,397 (92.7)	-	5925 (91.3)	17,371 (91.7)	-
1	512 (3.7)	1347 (3.3)	1.119 [1.008–1.242]	485 (4.6)	1247 (4.1)	1.153 [1.035–1.284]	330 (5.1)	878 (4.6)	1.102 [0.967–1.256]
2+	383 (2.7)	891 (2.2)	1.267 [1.121–1.431]	399 (3.8)	974 (3.2)	1.209 [1.072–1.362]	236 (3.6)	684 (3.6)	1.015 [0.871–1.183]
Bacterial infections[b]									
0	9445 (89.9)	27,433 (89.6)	-	5995 (92.4)	17,366 (91.7)	-	1716 (93.8)	5039 (94.1)	-
1	506 (4.8)	1443 (4.7)	1.011 [0.910–1.122]	239 (3.7)	719 (3.8)	0.958 [0.824–1.114]	67 (3.7)	152 (2.8)	1.291 [0.959–1.736]
2+	552 (5.3)	1742 (5.7)	0.919 [0.832–1.014]	257 (4.0)	848 (4.8)	0.876 [0.759–1.011]	46 (2.5)	165 (3.1)	0.806 [0.578–1.124]

PD, Parkinson's disease; C, control; OR, odds ratio; CI, confidence interval.
[a]Penicillins, tetracyclines, clindamycin, moxifloxacin, metronidazole, cefoxitin, nitroimidazole derivatives, and vancomycin were defined as antianaerobic antibiotics. Other antibiotics were defined as nonantianaerobics.
[b]For bacterial infections, 1 to 5 years before index date n = 41,121, 5 to 10 years before index date n = 25,424, 10 to 15 years before index date n = 7185.

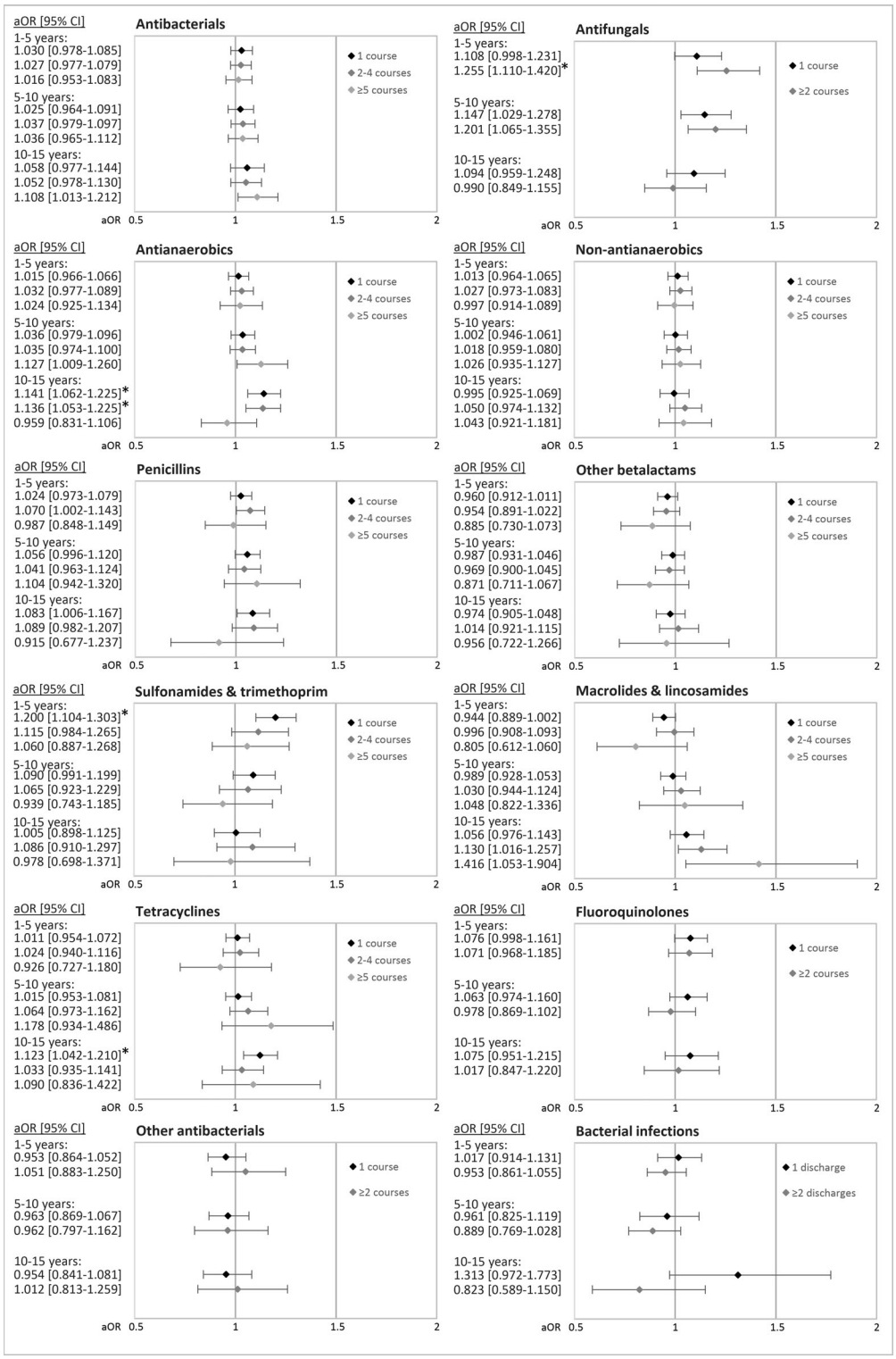

FIG. 2. Adjusted odds ratios for Parkinson's disease after exposure to antibiotics and bacterial infections. For antifungal exposure, antibiotic exposure was also added to the adjusted model. aOR, adjusted odds ratio (adjusted for Crohn's disease, ulcerative colitis, irritable bowel syndrome, chronic obstructive pulmonary disease, transient ischemic attack or stroke, Charlson Comorbidity Index, *Helicobacter pylori* eradication, and viral infections); CI, confidence interval. *Statistically significant after false discovery rate correction.

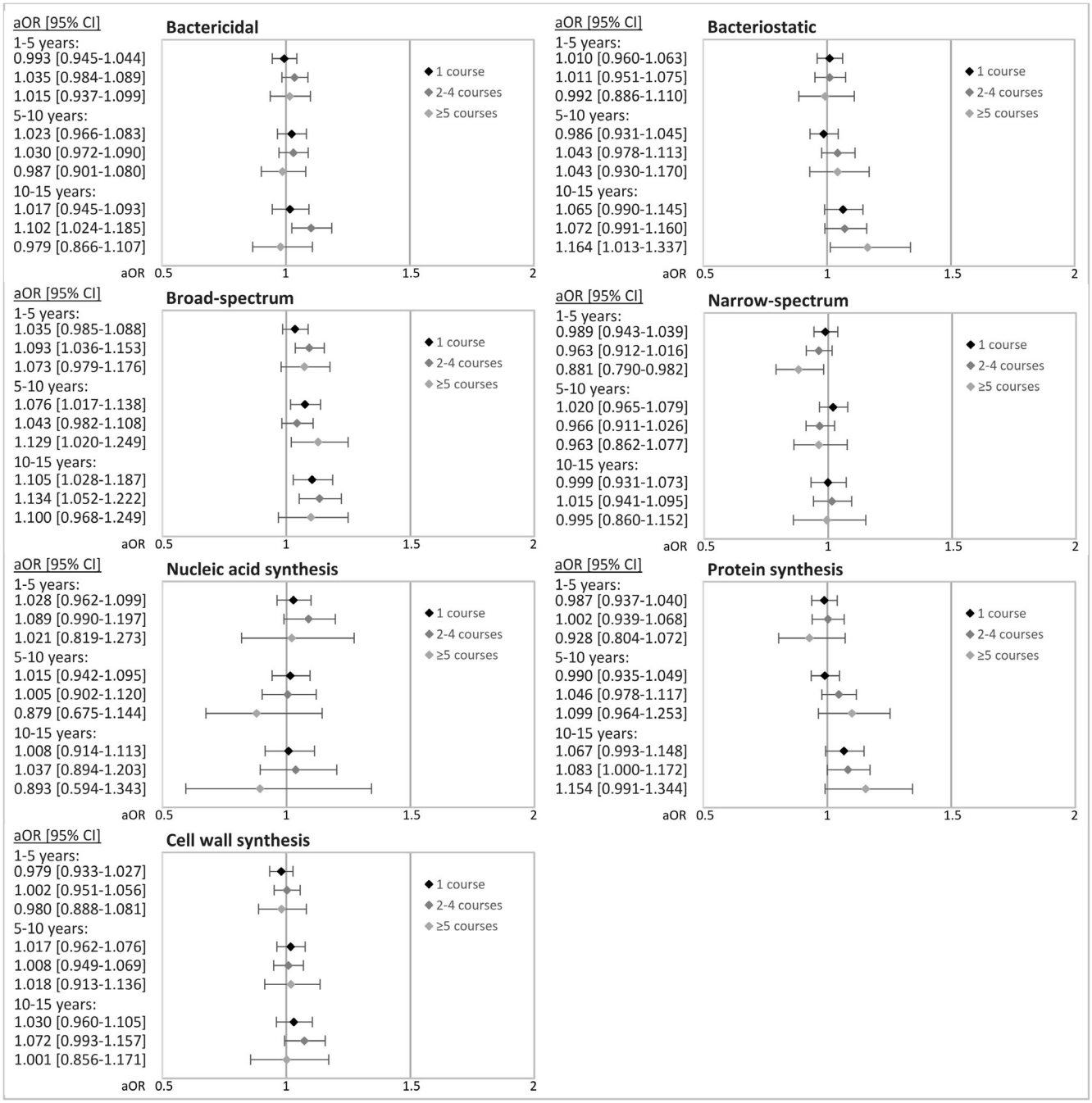

FIG. 3. Post hoc analysis: adjusted odds ratios for Parkinson's disease. For antifungal exposure, antibiotic exposure was also added to the adjusted model. aOR, adjusted odds ratio (adjusted for Crohn's disease, ulcerative colitis, irritable bowel syndrome, chronic obstructive pulmonary disease, transient ischemic attack or stroke, Charlson Comorbidity Index, *Helicobacter pylori* eradication, and viral infections); CI, confidence interval.

Discussion

To our knowledge, this is the first study specifically investigating the connection between exposure to antibiotics and PD. We found positive associations between oral exposure to certain antibiotics and PD risk that showed dependency on antianaerobic coverage, broadness of the antimicrobial spectrum, and period of exposure. After FDR correction, the exposure to antianaerobics and tetracyclines 10 to 15 years before the index date and to sulfonamides and trimethoprim 1 to 5 years before the index date remained significantly associated with PD risk.

In a study exploring association between rosacea and PD, sensitivity analysis suggested that tetracycline exposure regardless of rosacea appeared to be associated with a very modest 2% reduction of PD risk.[29] However, these results were not stratified by the duration

between antibiotic exposure and diagnosis of PD, making direct comparisons with our findings difficult.

Infectious burden has also been associated with PD[30] and potentially modifying the effect of antibiotic exposure. We did not have information on the clinical indications of prescriptions of antimicrobial agents. However, there were no statistically significant differences in the number of discharges as a result of viral or bacterial infections. Although controls had a higher prevalence of pneumonia (2.0% vs. 1.6%), which may be explained by their higher prevalence of COPD, their overall antibiotic exposure was less than in PD patients (Table 1). If antibiotics in general would have acted as proxy for infection, one would expect a stronger association between overall antibiotic exposure and PD. However, the associations found in this study were specific for certain antibiotic classes and their antimicrobial spectrum. Altogether this makes it unlikely that the observed associations are explained by a generally higher infectious burden in the PD group.

The delay of 10 to 15 years between the most strongly associated antibiotic exposures and PD diagnosis observed in this study is comparable to what has been proposed as the lag between peripheral initiation and motor manifestation of PD.[6] This would also explain the lack of association between antibiotic exposure 1 to 5 years before index date—if antibiotic exposure could induce or contribute to the pathogenesis of PD in the gastrointestinal tract, it would probably take several years before the clinical manifestation of PD. An exception is our observation that exposure to sulfonamides and trimethoprim 1 to 5 years before the index date, but not earlier, was associated with a higher risk of PD. This could be explained by protopathic bias because these antibiotics are mainly used against urinary tract infections that PD patients might be more susceptible to already in the prodromal phase.[31] On the contrary, it is unlikely that the associations between exposure to certain antibiotics 10 to 15 years before the index date and PD could be explained with protopathic bias. We adjusted for several risk factors or prodromal symptoms that could also be associated with antibiotic use, namely IBS and inflammatory bowel disease. For gastrointestinal disturbances such as small intestinal bacterial overgrowth or delayed gastric emptying that could increase the probability of antibiotic exposure, there is no solid evidence that they would be present already in the prodromal phase.[32] Truncal vagotomy has been associated with a reduced PD risk, but such procedures were extremely rare in Finland during our study period. Also, we did not expect any plausible association between vagotomy and antibiotic exposure.

Gut microbiome alterations have been described in prodromal and established PD.[18,33] Our study indicates that a repetitive exposure to certain types of antibiotics, rather than general or single exposure, is associated with PD risk. Exposure to antibiotics can have long-lasting impacts on the composition of the gut microbiota, which consists predominantly of anaerobic bacteria.[12] Broad-spectrum antibiotics and those that are effective against anaerobic bacteria have the strongest impact on gut microbiota.[34] Macrolides and lincosamides are mainly excreted into the bile resulting in high concentrations in feces.[35] They cause profound, long-lasting effects on the gut microbiota.[12] The exposure to such antibiotics also had the strongest associations with PD risk in our study, supporting the hypothesis that microbiota alterations could link antibiotic exposure and PD risk.

The alterations of microbiota composition caused by antibiotic exposure may be linked to impaired gut mucosal barrier function[36] and inflammation. Such changes of the local mucosal microenvironment can trigger the overexpression and aggregation of alpha synuclein in enteric neurons.[37] This could induce alpha synuclein–related PD pathology in the enteric nervous system with subsequent prion-like spread to the central nervous system.[38] Thus, even a transient peripheral trigger such as exposure to oral antibiotics could induce a self-propelling pathophysiological cascade that leads to PD motor symptoms and a diagnosis many years later. However, independent of the gut microbiota alterations, antibiotics can also have direct harmful effects on the gut epithelium,[39] and gut barrier dysfunction may expose the host also to other exogenic pathogens or toxins that may be linked to PD pathology.

The association between PD and antifungal exposure, with a shorter latency between exposure and PD diagnosis when compared with antibacterials, was surprising. This finding was adjusted for exposure to antibacterials and is thus not explained by fungal infections that are secondary to antibiotic use. The role of the mycobiome in human health and disease is much less studied than that of the bacterial component of the microbiota. Although it is likely that oral antifungal medications induce changes in the gut mycobiome,[40] azoles can also directly inhibit mitochondrial function, which is another potential link to PD.[41]

To have as accurate information as possible on when PD was actually diagnosed, we defined the onset of PD as the date of the first discharge with a PD diagnosis or the date when special reimbursement for PD medication was granted. In our data, the special reimbursement for PD medication was granted within 2 years of the first discharge with PD diagnosis in 90.5% of PD cases. The validity of the HILMO register has been evaluated in several studies, and the positive predictive value for common diagnoses varies between 75% and 99%.[19] Because data of the HILMO register have not been collected primarily for the purposes of specific research questions, we used strict inclusion and exclusion criteria for the study to reduce the probability of reading errors and misdiagnosis. We also used special reimbursement

data to exclude subjects who were entitled to reimbursement for PD medication for other indications than PD and those who were diagnosed with PD before the year 1998.

Sensitivity analyses showed that our findings were not susceptible to changes in the definition of a PD case. However, the recording of subsidiary diagnoses has been less accurate and therefore the data on comorbidities must be interpreted with caution. Smoking may predispose to respiratory infections giving rise to frequent antibiotic courses. Because information about smoking status was not available, we used COPD as a proxy. The higher prevalence of COPD in the control population is in agreement with the known link between smoking and reduced PD risk.[42] Still, differential smoking prevalence may not be completely accounted for. Thus, we cannot exclude that this may have weakened the observed association between antibiotic exposure and PD.

Data on the use of antibiotics were based on drug purchases, but the dosages and durations of antibiotic exposure and patient compliance could not be assessed. However, the observed dose–response relationship between certain antibiotic purchases and PD risk suggests that this measure reflects actual exposure. In Finland, antibiotics used in in-patient units are not included in the drug prescription register. Taking into account that in our study population, patients had purchased on average more than 6 antibiotic courses during the follow-up, in comparison with an average of less than 0.2 hospitalizations as a result of a bacterial infection, this limitation is unlikely to have caused significant bias. Finally, the classification of antibiotics based on the range of antimicrobial action and effect on the targeted bacteria is controversial.[43] However, we considered the post hoc analyses to be meaningful to investigate the possible mechanisms behind the connection between prior antibiotic exposure and PD in this observational study setting.

Finally, the possibility of false positive findings must be taken into consideration. Although not considered necessary in an exploratory setting, we report FDR-corrected results to adjust for multiple comparisons regarding our primary analyses.

In conclusion, our results suggest that prior exposure in particular to antianaerobic as well as broad-spectrum antibiotics is associated with subsequent PD with a delay of 10 to 15 years. A similar association was found for antifungal medication with a delay of 1 to 10 years. Although no conclusions regarding causality can be made, it is plausible that oral antibiotic exposure is one factor that makes the gastrointestinal tract more susceptible to PD pathology, increasing the risk of PD. The pattern of associations may point to a role of gut microbiota alterations in linking antibiotics and PD, but also other local and systemic pharmacological effects must be taken into consideration. Our findings demand confirmation in different cohorts. However, if confirmed in future studies, a connection between commonly prescribed oral antibiotics and neurodegeneration could have major implications for prescribing practices and public health.

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Supporting Data

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1. Research project: A. Conception, B. Organization, C. Execution; 2. Statistical Analysis: A. Design, B. Execution, C. Review and Critique; 3. Manuscript: A. Writing of the first draft, B. Review and Critique
T.H.M.: 1A, 1B, 1C, 2A, 2B, 2C, 3A, 3B
E.P.: 1A, 1B, 3B
F.S.: 1A, 1B, 1C, 3B

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