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Complete List of Authors:	Pinel Ríos, Javier; Hospital Vithas Xanit Internacional, Neuroscience unit Madrid Navarro, Carlos; University Hospitals of Granada, Institute of Bio-health Research ibs, Department of Neurology Pérez Navarro, María; University Hospitals of Granada, Institute of Bio-health Research ibs, Department of Neurology Cabello Tapia, María; University Hospitals of Granada, Department of Digestive Diseases Piña Vera, María ; Andalusian Health Service Pharmacy and Benefit Support Department Campos Arillo, Víctor; Hospital Vithas Xanit Internacional, Neuroscience unit Gómez García, María; University Hospitals of Granada, Department of Digestive Diseases Mínguez Castellanos, Adolfo; University Hospitals of Granada, Institute of Bio-health Research ibs, Department of Neurology Escamilla Sevilla, Francisco; University Hospitals of Granada, Institute of Bio-health Research ibs, Department of Neurology
Keywords:	5-ASA, Parkinson’s disease, Inflammatory bowel disease < GASTROENTEROLOGY, alpha-synuclein, microbiota, prevalence

ASSOCIATION OF PARKINSON'S DISEASE AND TREATMENT WITH AMINOSALICYLATES IN INFLAMMATORY BOWEL DISEASE: A CROSS-SECTIONAL STUDY

Javier Pinel-Ríos[1], MD; Carlos Javier Madrid-Navarro[2], MD; María José Pérez-Navarro[2], MD; María José Cabello-Tapia[3], MD; María José Piña-Vera[4]; Víctor Campos-Arillo[1], MD, PhD; María Rosario Gómez-García[3], MD; Adolfo Mínguez-Castellanos[2], MD, PhD; Francisco Escamilla-Sevilla[2], MD, PhD.

1: Department of Neurology, Vithas Xanit International Hospital, Benalmadena, Malaga, Spain.
2: Department of Neurology, University Hospitals of Granada, Institute of Bio-health Research ibs. Granada, Spain.
3: Department of Digestive Diseases, University Hospitals of Granada, Granada, Spain.
4: Andalusian Health Service Pharmacy and Benefit Support Department. Spain.

Correspondence:
Dr. Javier Pinel-Ríos, MD
Unidad de Neurociencias, Av. de los Argonautas, s/n, 29631 Benalmadena, Malaga, Spain. Email: javierpinelr@gmail.com. Tel: +34652885523. Fax: +34952367197
Dr. Francisco Escamilla-Sevilla, MD PhD.
Email: fescamilla@hotmail.com

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Abstract

Objectives: To analyze the association between aminosalicylate-treated inflammatory bowel disease (IBD) and Parkinson´s disease (PD) at population level.

Design: Cross-sectional study.

Setting: The study was performed based on electronic drug prescription and dispensation records of the Andalusian Public Health System.

Participants: All individuals aged ≥ 50 yrs with at least one drug dispensation during December 2014 were identified from the records.

Primary and secondary outcome measures: Three groups were formed: "Possible PD" group, including all who received an anti-Parkinson agent; "Possible IBD" group, those treated with mesalazine and/or derivatives (5-aminosalicylic acid [ASA]); and "Possible PD and IBD", including those receiving both anti-Parkinson agent and 5-ASA. Prevalence of "Possible PD" was determined among those with "Possible IBD" and among those without this condition. The age- and sex-adjusted OR was calculated.

Results: We recorded 2,020,868 individuals (68±11 yrs., 56 % female), 19,966 were included in "Possible PD" group (75±9 yrs., 53 % female) and 7,485 in "Possible IBD" group (64±10 yrs., 47 % female); only 56 were included in both groups (76±8 yrs., 32 % female). The prevalence of "Possible PD" was 0.7% among those with "Possible IBD" and 1 % among those without this condition (OR=0.75; 95 % CI=0.57-0.98; p=0.036). The age- and sex-adjusted OR was 0.28 in individuals aged ≤65 years (95 % CI=0.10-0.74; p=0.01) and 1.17 in older individuals (95 % CI=0.89-1.54; p=0.257).

Conclusions: Within the limitations of this study, the results suggest a protective role for IBD and/or 5-ASA against PD development, especially among under 65-year-olds. Further studies are warranted to explore this association given its scientific and therapeutic implications.

Article Summary: Strengths and limitations of this study

- This study provides evidence for a better understanding on the association of inflammatory bowel disease and Parkinson's disease.
- Parkinson's disease and inflammatory bowel disease share some etiopathogenic features at the enteric level.
- This cross-sectional study explore associations between PD and IBD treated with mesalazine (5-aminosalicylic acid) or its derivative sulfasalazine.
- Matching by age and sex was performed.
- This study has limitations related to the utilization of a drug dispensation record, subjects therefore not be considered representative of the general population, and there is also a potential classification error related to the selection of proxy variables.

Introduction

Parkinson's disease (PD) is a multisystemic process with early involvement of the peripheral autonomic nervous system and subsequent propagation to the central nervous system [1]. After reports of deposits of alpha-synuclein aggregates that form neurites or Lewy bodies in gastric submucosal and myenteric plexi [1], various clinical and experimental studies described the enteric nervous system as fundamental in the etiopathogenesis of PD [1-5]. Braak's "dual-hit" hypothesis has been endorsed by evidence of the centripetal trans-synaptic and axonal migration of alpha-synuclein aggregates from neurons of the digestive system to brainstem structures such as the dorsal nucleus of the vagus nerve (Braak stage 2) [1-3]. It has been reported that certain components of the microbiota of PD patients can produce rupture of the intestinal barrier and bacterial translocation, which may be responsible for an intestinal proinflammatory status and subsequent alpha-synuclein aggregation and propagation [6,7].

Some features of inflammatory bowel disease (IBD) are similar to those of PD. Thus, their etiopathogenesis involves an anomalous immune response to specific microbiota that would trigger intestinal inflammatory activity in predisposed individuals unable to inhibit this inflammation and without the immunological tolerance characteristic of a healthy intestine [8]. The few studies on the relationship between these diseases have not considered IBD treatment and have published controversial results, with observations either of no association [9] or of an increased risk of PD only among patients with Crohn's disease [10], attributable to a genetic relationship between these diseases at different *loci,* as revealed in a genome-wide association study [11].

With this background, the objective of this study was to explore associations between PD and IBD treated with mesalazine (5-aminosalicylic acid) or its derivative sulfasalazine, hereafter 5-ASA.

Material and methods

A cross-sectional study was performed based on electronic drug prescription and dispensation records (*Receta XXI*) of the Andalusian Public Health System, which include 98 % of all prescriptions in the region. Andalusia is located in southern Spain and is the most populated autonomous community in the country, with 8,402,000 inhabitants. The study was approved by the Research Ethics Committee of Granada (CEI-GRANADA).

Subjects

All individuals aged ≥ 50 yrs with at least one drug dispensation during December 2014 were identified from the records. Three groups were formed: "Possible PD" group, including all who received an anti-Parkinson agent, such as levodopa in different formulations and/or dopaminergic agonist, excluding those only receiving a single low-dose dopaminergic agonist (ropinirole ≤ 1 mg; rotigotine ≤ 2 mg; pramipexole ≤ 0.26 mg; pergolide ≤1 mg; cabergoline ≤ 1 mg) and/or monoamine oxidase B inhibitor, including those exclusively treated with selegiline or rasagiline; "Possible IBD" group, including all receiving 5-ASA in its different formulations (mesalazine/mesalamine or 5 aminosalicylic acid and sulfasalazine); and "Possible PD and IBD", including those receiving both anti-Parkinson agent and 5-ASA.

Study variables

Membership of the "Possible PD" group was the dependent variable, and membership of the "Possible IBD" group, sex, and age were the predictive variables.

Data gathering

Data were obtained from the computerized databases of the Andalusian Health Service Pharmacy and Benefit Support Department. Anonymity was preserved by masking clinical record numbers.

Data analysis

In a descriptive analysis, central tendency and dispersion measurements were calculated for quantitative variables and absolute and relative frequencies for qualitative variables, plotting the corresponding graphs. After applying the Kolmogorov-Smirnov test to check data distribution normality, between-group comparisons were evaluated with the Mann-Whitney U for the quantitative variable (age) and with the chi-square test for categorical variables. After controlling for age and sex, the strength of between-group associations was established by constructing a multiple logistic regression model to estimate the odds ratio (OR) and 95 % confidence interval. All tests were two-tailed, and $p<0.05$ was considered significant. R and SPSS 21 statistical packages were used for the statistical analysis.

Results

During December 2014, 2,020,868 individuals aged ≥ 50 yrs were dispensed with at least one drug; their mean age was 67.9 ± 11.8 yrs., 56 % were female (mean of 68.6 ± 11.5 yrs) and 44 % were male (mean of 67.2 ± 10.7 yrs.). The study included 27,451 patients (mean age of 72.3 ± 10.9 yrs.; 51 % female) with at least one dispensation of anti-Parkinson agent ("Possible PD") and/or 5-ASA ("Possible IBD") after excluding 24 patients with inadequate records (e.g., missing data on age and/or sex) and 6 with unlikely PD (low-dose dopaminergic agonist [0.5 mg cabergoline in 1 case and 0.5 mg ropinirole in 5]); the mean age was slightly higher for the females than for the males (73.1 ± 10.9 *vs.* 71.4 ± 10.8 yrs.; p=0.0001)

A total of 19,966 individuals were considered as "Possible PD" (mean age of 75.4 ± 9.3 yrs.; 53 % females) and 7,485 as "Possible IBD" (64.15 ± 10.4 yrs.; 47 % females). Individuals with "Possible PD" were older (p=0.0001) and more frequently female (p=0.0001) in comparison to those with "Possible IBD" (figure 1). A group of 56 individuals (32 females) were considered both "Possible PD" and "Possible IBD; their mean age was 75.7 ± 8.1 yrs (range, 52-94 yrs), and they showed no differences in age (p=0.956) or sex (p=0.505) distribution with respect to the "Possible PD" group.

The prevalence of "Possible PD" was 0.7 % in the "Possible IBD" group and 1 % in those without this condition. The OR for an individual with "Possible PD" being in the "Possible IBD" group was 0.75 (95 % CI=0.57-0.98; p=0.036), rising to 0.94 (95 % CI=0.72-1.23; p=0.657) after controlling for age and sex; stratification by age yielded an OR of 0.28 (95 % CI=0.10-0.74; p=0.01) for individuals aged ≤65 years and 1.17 (95 % CI =0.89-1.54; p=0.257) for those aged > 65 years, after controlling for sex (Table 1).

Discussion

The results of this cross-sectional study suggest that the presence of IBD and/or treatment with 5-ASA may play a protective role against PD development, especially among under-65-year-olds. The pathogenesis of the two diseases may share certain features, including the role of enteric glial cells [12-14] and inflammation [15], genetic factors (PD and Crohn's disease) [11], the protective effect of tobacco (PD and ulcerous colitis) [16], the presence of proinflammatory flora, and altered permeability with rupture of the intestinal barrier [6,7]. In fact, the inflammatory condition and/or treatment with 5-ASA might have been expected to act as a risk rather than protective factor, unless they have some impact on the pathogenesis of this synucleinopathy.

Our results are similar to those of the recent case-control study of PD risk based on Medicare data; this group found an inverse association between IBD and PD, including both Crohn's disease and ulcerous colitis. Similarly, there was an inverse association between PD and IBD-associated conditions, as well as IBD associated surgical procedures and immunosuppressant use, even among beneficiaries without IBD. The authors suggest that IBD is associated with a lower risk of developing PD in the adjusted model for a population aged> 65 years (OR = 0.86; 95% CI=0.81-0.92) [17]. Conversely, a retrospective cohort study based on healthcare records found an increased risk of PD among IBD patients, especially those with Crohn's disease, given that this association was not observed in the subgroup with ulcerous colitis (OR = 0.94; 95 % CI=0.49-1.84) [10]. Although these cases were not specified in the present study, they can largely be considered to have ulcerous colitis, given reservations in clinical guidelines about the administration of 5-ASA in Crohn's disease [18]. In the aforementioned cohort study, there was a relative reduction in PD risk among patients

with longer follow-up times, similar to observations in individuals with truncal vagotomy [19,20] and in under 65year-olds, as in the present study.

Recently two studies have been published describing a risk association between IBD and PD. In the Danish nationwide population cohort study, covering the period 1977-2014, found a significantly increased risk of PD when comparing patients with IBD with non-IBD (HR=1.22; 95% CI=1.09-1.35), especially among patients with ulcerative colitis (HR=1.35; 95% CI=1.20-1.52). However, in those patients diagnosed with IBD under 40 years of age and with prolonged follow-up > 20 years, this increase was not statistically significant [21], suggesting, as we have previously referred, that IBD treatment may lessen the risk of PD. In a retrospective cohort study, based on the Market-Scan Commercial and Medicare databases, a higher incidence of PD was observed among patients with IBD than among individuals without IBD; the authors found a statistically significant 28% increase in the incidence of PD among patients with IBD compared with the unaffected matched controls. Conversely, they observed a markedly lower PD incidence rate (0.08 per 1000 patient-years) among patients with IBD who were exposed to anti-TNF therapy compared with patients without exposure (0.76 per 1000 patient-years), which means a 78% reduction in the PD incidence rate [22], a risk reduction in the same range as that found in our patients treated with 5-ASA. Despite the contradictory associations between both processes, two studies find a reduction in the risk of PD related to the use of immunomodulators [17,22], although they do not describe the degree of exposure to 5-ASA. It is presumable that those with a more active disease and therefore candidates for immunomodulators therapies will have had a greater exposure to 5-ASA, but it is also possible that those treated exclusively with 5-ASA have a more benign inflammatory disease.

5-ASA drugs are the first-line treatment in ulcerous colitis [18,23]. Their action mechanism is not fully elucidated but is considered to include: a) anti-inflammatory activity or inhibition of the local synthesis of prostaglandins and leukotrienes [24]; b) reduction in nitric oxide and IL-6 production and an antiapoptotic effect, diminishing phosphorylation by mitogen-activated protein kinases (MAPKs), e.g., p38 and JNK (c-Jun N-terminal kinases) [25]; and c) action on the microbiota, producing qualitative and quantitative changes in its composition [26].

In PD, alpha-synuclein aggregates are known to be present in the intestine some years before the onset of motor symptoms [4,5], especially in certain structures such as the vermiform appendix [27]. Local action mechanisms of 5-ASA described above may affect progression of the synucleinopathy, given that they may act on etiopathogenetic mechanisms that would activate microglia, associated with certain microbiota and bacterial membrane lipopolysaccharides or endogenous factors (e.g., JNK and p38) [28,29,30].

In a recent murine model with alpha-synuclein overexpression, transplantation from PD patients of fecal matter with greater short-chain fatty-acid content was followed by microglial activation, an increase in inflammation mediators (TNF-α and IL-6) and in alpha-synuclein aggregation and propagation, a poorer execution of motor tests, and even constipation; these changes were reduced by treatment with minocycline [31], suggesting that other drug groups, such as 5-ASA, may have a similar effect.

This study has limitations related to the utilization of a drug dispensation record, subjects therefore not be considered representative of the general population. The resulting ORs may be overestimated, because the prevalence of both diseases was over-represented but in an unequal manner, being 5-fold higher for PD in the study population than in the general population but only slightly higher for IBD. Cross-

sectional drug dispensation studies are more likely to include patients with PD than with IBD due to their older age and more frequent polymedication and because some biological and immunosuppressive therapies received by IBD patients in the hospital are not included in the dispensary records. Furthermore, individuals treated with 5-ASA are a subgroup of patients with IBD and ulcerous colitis, mainly in those with active disease. We did not control for the possibility of a group of patients receiving combined maintenance therapy (5-ASA and other drugs). There is also a potential classification error related to the selection of *proxy* variables, given that the diseases under study were identified according to the medication received. Notably, dopaminergic drugs are prescribed, although much less frequently, for diseases other than PD (atypical parkinsonism, chronic adult hydrocephaly, restless legs syndrome, etc.); however, this is unlikely to have affected the validity of our results given the large sample size.

Conclusions

A lower risk of PD was observed in individuals with IBD aged ≤65 years and treated with 5-ASA, which may be, as hypothesis, related to a theoretical pharmacological effect on alpha-synuclein aggregation and propagation that could reduce or delay PD onset in at-risk populations. The advantages of 5-ASA medication include its relatively low cost and lack of absorption. Within the limitations of the methodology applied, further experimental research with animal models and new longitudinal studies are warranted, given the important scientific and therapeutic implications of these findings.

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Conflict of interest: The authors declare they have no conflict of interest.

Author Contributions: JP, FE and VC contributed conception and design of the study; MPN, MPV and CM organized the database; FE and AM performed the statistical analysis; JP wrote the first draft of the manuscript; FE, CM, MC and MG wrote sections of the manuscript. All authors contributed to manuscript revision, read and approved the submitted version.

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Figures

Figure 1. Population pyramid with age and sex distribution of the groups with possible inflammatory bowel disease (IBD) and possible Parkinson's disease (PD)

Tables

Table 1. Age and sex adjusted risk of possible Parkinson's disease in individuals with possible inflammatory bowel disease

Variable		**β_1**	**OR (95 % CI)**	**p**
"Possible PD"		-0.28	0.75 (0.57-0.98)	**0.0362**
Adjusted model	"Possible PD"	-0.06	0.94 (0.72-1.23)	0.657
	"Possible PD" ≤ 65 yrs.	-1.28	0.28 (0.10-0.74)	**0.0103**
	"Possible PD" > 65 yrs.	0.16	1.17 (0.89-1.54)	0.257

Figure 1

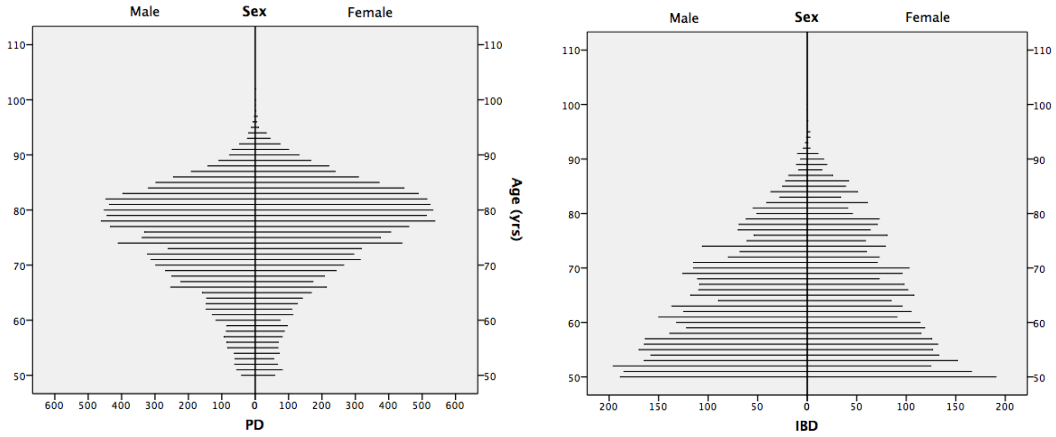

Figure 1. Population pyramid with age and sex distribution of the groups with possible inflammatory bowel disease (IBD) and possible Parkinson's disease (PD)

Reporting checklist for cross sectional study.

Based on the STROBE cross sectional guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

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		Reporting Item	Page Number
Title	#1a	Indicate the study's design with a commonly used term in the title or the abstract	1
Abstract	#1b	Provide in the abstract an informative and balanced summary of what was done and what was found	2
Background / rationale	#2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	#3	State specific objectives, including any prespecified hypotheses	5
Study design	#4	Present key elements of study design early in the paper	6,7
Setting	#5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6,7
Eligibility criteria	#6a	Give the eligibility criteria, and the sources and methods of selection of participants.	6,7

	#7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6,7
Data sources / measurement	#8	For each variable of interest give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group. Give information separately for for exposed and unexposed groups if applicable.	6,7
Bias	#9	Describe any efforts to address potential sources of bias	6,7
Study size	#10	Explain how the study size was arrived at	6,7
Quantitative variables	#11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why	6,7
Statistical methods	#12a	Describe all statistical methods, including those used to control for confounding	6,7
	#12b	Describe any methods used to examine subgroups and interactions	6,7
	#12c	Explain how missing data were addressed	6,7
	#12d	If applicable, describe analytical methods taking account of sampling strategy	6,7
	#12e	Describe any sensitivity analyses	6,7
Participants	#13a	Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed. Give information separately for for exposed and unexposed groups if applicable.	8
	#13b	Give reasons for non-participation at each stage	8
	#13c	Consider use of a flow diagram	8
Descriptive data	#14a	Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders. Give information separately for exposed and unexposed groups if applicable.	8

	#14b	Indicate number of participants with missing data for each variable of interest	8
Outcome data	#15	Report numbers of outcome events or summary measures. Give information separately for exposed and unexposed groups if applicable.	8
Main results	#16a	Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	8
	#16b	Report category boundaries when continuous variables were categorized	8
	#16c	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	8
Other analyses	#17	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	8
Key results	#18	Summarise key results with reference to study objectives	9
Limitations	#19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.	11,12
Interpretation	#20	Give a cautious overall interpretation considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.	9-11
Generalisability	#21	Discuss the generalisability (external validity) of the study results	12
Funding	#22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	13

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Complete List of Authors:	Pinel Ríos, Javier; Hospital Vithas Xanit Internacional, Neuroscience unit Madrid Navarro, Carlos; University Hospitals of Granada, Institute of Bio-health Research ibs, Department of Neurology Pérez Navarro, María; University Hospitals of Granada, Institute of Bio-health Research ibs, Department of Neurology Cabello Tapia, María; University Hospitals of Granada, Department of Digestive Diseases Piña Vera, María ; Andalusian Health Service Pharmacy and Benefit Support Department Campos Arillo, Víctor; Hospital Vithas Xanit Internacional, Neuroscience unit Gómez García, María; University Hospitals of Granada, Department of Digestive Diseases Mínguez Castellanos, Adolfo; University Hospitals of Granada, Institute of Bio-health Research ibs, Department of Neurology Escamilla Sevilla, Francisco; University Hospitals of Granada, Institute of Bio-health Research ibs, Department of Neurology
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Association of Parkinson's disease and treatment with aminosalicylates in inflammatory bowel disease: a cross-sectional study on drug dispensation records.

Javier Pinel-Ríos[1], MD; Carlos Javier Madrid-Navarro[2], MD; María José Pérez-Navarro[2], MD; María José Cabello-Tapia[3], MD; María José Piña-Vera[4]; Víctor Campos-Arillo[1], MD, PhD; María Rosario Gómez-García[3], MD; Adolfo Mínguez-Castellanos[2], MD, PhD; Francisco Escamilla-Sevilla[2], MD, PhD.

1: Department of Neurology, Vithas Xanit International Hospital, Benalmadena, Malaga, Spain.
2: Department of Neurology, University Hospitals of Granada, Institute of Bio-health Research ibs. Granada, Spain.
3: Department of Digestive Diseases, University Hospitals of Granada, Granada, Spain.
4: Andalusian Health Service Pharmacy and Benefit Support Department. Spain.

Correspondence:
Dr. Javier Pinel-Ríos, MD
Unidad de Neurociencias, Av. de los Argonautas, s/n, 29631 Benalmadena, Malaga, Spain. Email: javierpinelr@gmail.com. Tel: +34652885523. Fax: +34952367197
Dr. Francisco Escamilla-Sevilla, MD PhD.
Email: fescamilla@hotmail.com

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Language style: British English

Abstract

Objectives: To analyze the association between aminosalicylate-treated inflammatory bowel disease (IBD) and Parkinson´s disease (PD) at population level.

Design: Cross-sectional study.

Setting: The study was performed based on electronic drug prescription and dispensation records of the Andalusian Public Health System.

Participants: All individuals aged ≥ 50 yrs with at least one drug dispensation during December 2014 were identified from the records.

Primary and secondary outcome measures: Groups were formed: "Possible PD" group, including all who received an anti-Parkinson agent; "Possible IBD" group, those treated with mesalazine and/or derivatives (5-aminosalicylic acid [ASA]); and "Possible PD and IBD", including those receiving both anti-Parkinson agent and 5-ASA. Prevalence of "Possible PD" was determined among those with "Possible IBD" and among those without this condition. The age- and sex-adjusted OR was calculated.

Results: We recorded 2,020,868 individuals (68±11 yrs., 56 % female), 19,966 were included in "Possible PD" group (75±9 yrs., 53 % female) and 7,485 in "Possible IBD" group (64±10 yrs., 47 % female); only 56 were included in both groups (76±8 yrs., 32 % female). The prevalence of "Possible PD" was 0.7% among those with "Possible IBD" and 1 % among those without this condition (adjusted OR=0.94; 95 % CI=0.72-1.23; p=0.657). OR was 0.28 in individuals aged ≤65 years (95 % CI=0.10-0.74; p=0.01) and 1.17 in older individuals (95 % CI=0.89-1.54; p=0.257).

Conclusions: Within the limitations of this study, the results suggest a protective role for IBD and/or 5-ASA against PD development, especially among under 65-year-olds. Further studies are warranted to explore this association given its scientific and therapeutic implications.

Article Summary: Strengths and limitations of this study

- This study provides evidence for a better understanding on the association of inflammatory bowel disease and Parkinson's disease.
- Parkinson's disease and inflammatory bowel disease share some etiopathogenic features at the enteric level.
- This cross-sectional study explore associations between PD and IBD treated with mesalazine (5-aminosalicylic acid) or its derivative sulfasalazine.
- This study has limitations related to the utilization of a drug dispensation record, and there is also a potential classification error related to the selection of proxy variables.

Introduction

Parkinson's disease (PD) is a multisystemic process with early involvement of the peripheral autonomic nervous system and subsequent propagation to the central nervous system [1]. After reports of deposits of alpha-synuclein aggregates that form neurites or Lewy bodies in gastric submucosal and myenteric plexi [1], various clinical and experimental studies described the enteric nervous system as fundamental in the etiopathogenesis of PD [1-5]. Braak's "dual-hit" hypothesis has been endorsed by evidence of the centripetal trans-synaptic and axonal migration of alpha-synuclein aggregates from neurons of the digestive system to brainstem structures such as the dorsal nucleus of the vagus nerve (Braak stage 2) [1-3]. It has been reported that certain components of the microbiota of PD patients can produce rupture of the intestinal barrier and bacterial translocation, which may be responsible for an intestinal proinflammatory status and subsequent alpha-synuclein aggregation and propagation [6,7].

Some features of inflammatory bowel disease (IBD) are similar to those of PD. Thus, their etiopathogenesis involves an anomalous immune response to specific microbiota that would trigger intestinal inflammatory activity in predisposed individuals unable to inhibit this inflammation and without the immunological tolerance characteristic of a healthy intestine [8]. The few studies on the relationship between these diseases have published controversial results, with observations either of no association [9] or of an increased risk of PD only among patients with Crohn's disease [10], attributable to a genetic relationship between these diseases at different *loci,* as revealed in a genome-wide association study [11]. Specifically, mutations in the leucine-rich repeat kinase 2 (LRRK2) gene, recently associated with Crohn's disease, have revealed pleiotropy between this IBD and PD risk [12]. Instead, a meta-analysis of the recent literature

showed an increased risk of PD in the IBD population and the increased risk remained significant when analyzing Crohn's disease and ulcerous colitis subgroups [13].

With this background, the objective of this study was to explore associations between PD and IBD treated with mesalazine (5-aminosalicylic acid) or its derivative sulfasalazine, hereafter 5-ASA.

Material and methods

A cross-sectional study was performed based on electronic drug prescription and dispensation records (*Receta XXI*) of the Andalusian Public Health System, which include 98 % of all prescriptions in the region. Andalusia is located in southern Spain and is the most populated autonomous community in the country, with 8,402,000 inhabitants. The study was approved by the Research Ethics Committee of Granada (CEI-GRANADA).

Subjects

All individuals aged ≥ 50 yrs with at least one drug dispensation during December 2014 were identified from the records. Three groups were formed: "Possible PD" group, including all who received an anti-Parkinson agent, such as levodopa in different formulations and/or dopaminergic agonist, excluding those only receiving a single low-dose dopaminergic agonist (ropinirole ≤ 1 mg; rotigotine ≤ 2 mg; pramipexole ≤ 0.26 mg; pergolide ≤1 mg; cabergoline ≤ 1 mg) and/or monoamine oxidase B inhibitor, including those exclusively treated with selegiline or rasagiline; "Possible IBD" group, including all receiving 5-ASA in its different formulations (mesalazine/mesalamine or 5 aminosalicylic acid and sulfasalazine); and "Possible PD and IBD", including those receiving both anti-Parkinson agent and 5-ASA.

Study variables

Membership of the "Possible PD" group was the dependent variable, and membership of the "Possible IBD" group, sex, and age were the predictive variables.

Data gathering

Data were obtained from the computerized databases of the Andalusian Health Service Pharmacy and Benefit Support Department. Anonymity was preserved by masking clinical record numbers.

Data analysis

In a descriptive analysis, central tendency and dispersion measurements were calculated for quantitative variables and absolute and relative frequencies for qualitative variables, plotting the corresponding graphs. After applying the Kolmogorov-Smirnov test to check data distribution normality, between-group comparisons were evaluated with the Mann-Whitney U for the quantitative variable (age) and with the chi-square test for categorical variables. After controlling for age and sex, the strength of between-group associations was established by constructing a multiple logistic regression model to estimate the odds ratio (OR) and 95 % confidence interval. All tests were two-tailed, and $p<0.05$ was considered significant. R and SPSS 21 statistical packages were used for the statistical analysis.

Patient and Public Involvement

Patients were not involved.

Results

During December 2014, 2,020,868 individuals aged ≥ 50 yrs were dispensed with at least one drug; their mean age was 67.9 ± 11.8 yrs., 56 % were female (mean of 68.6 ± 11.5 yrs) and 44 % were male (mean of 67.2 ± 10.7 yrs.). The study included 27,451 patients (mean age of 72.3 ± 10.9 yrs.; 51 % female) with at least one dispensation of anti-Parkinson agent ("Possible PD") and/or 5-ASA ("Possible IBD") after excluding 24 patients with inadequate records (e.g., missing data on age and/or sex) and 6 with unlikely PD (low-dose dopaminergic agonist [0.5 mg cabergoline in 1 case and 0.5 mg ropinirole in 5]); the mean age was slightly higher for the females than for the males (73.1 ± 10.9 *vs.* 71.4 ± 10.8 yrs.; p=0.0001)

A total of 19,966 individuals were considered as "Possible PD" (mean age of 75.4 ± 9.3 yrs.; 53 % females) and 7,485 as "Possible IBD" (64.15 ± 10.4 yrs.; 47 % females). Individuals with "Possible PD" were older (p=0.0001) and more frequently female (p=0.0001) in comparison to those with "Possible IBD" (figure 1). A group of 56 individuals (32 females) were considered both "Possible PD" and "Possible IBD; their mean age was 75.7 ± 8.1 yrs (range, 52-94 yrs), and they showed no differences in age (p=0.956) or sex (p=0.505) distribution with respect to the "Possible PD" group.

The prevalence of "Possible PD" was 0.7 % in the "Possible IBD" group and 1 % in those without this condition (Table 1). "Possible IBD" showed a reduced risk for "Possible PD" with OR of 0.75 (95 % CI=0.57-0.98; p=0.036), rising to 0.94 (95 % CI=0.72-1.23; p=0.657) after controlling for age and sex; stratification by age yielded an OR of 0.28 (95 % CI=0.10-0.74; p=0.01) for individuals aged ≤65 years and 1.17 (95 % CI =0.89-1.54; p=0.257) for those aged > 65 years, after controlling for sex (Table 2).

Discussion

The results of this cross-sectional study suggest that the presence of IBD and/or treatment with 5-ASA may play a protective role against PD development, especially among under-65-year-olds. The pathogenesis of the two diseases may share certain features, including the role of enteric glial cells [14-16] and inflammation [17], genetic factors (PD and Crohn's disease) [11], the protective effect of tobacco (PD and ulcerous colitis) [18], the presence of proinflammatory flora, and altered permeability with rupture of the intestinal barrier [6,7]. In fact, the inflammatory condition and/or treatment with 5-ASA might have been expected to act as a risk rather than protective factor, unless they have some impact on the pathogenesis of this synucleinopathy.

Our results are similar to those of the recent case-control study of PD risk based on Medicare data; this group found an inverse association between IBD and PD, including both Crohn's disease and ulcerous colitis. Similarly, there was an inverse association between PD and IBD-associated conditions, as well as IBD associated surgical procedures and immunosuppressant use, even among beneficiaries without IBD. The authors suggest that IBD is associated with a lower risk of developing PD in the adjusted model for a population aged> 65 years (OR = 0.86; 95% CI=0.81-0.92) [19]. Conversely, a retrospective cohort study based on healthcare records found an increased risk of PD among IBD patients, especially those with Crohn's disease, given that this association was not observed in the subgroup with ulcerous colitis (OR = 0.94; 95 % CI=0.49-1.84) [10]. Although these cases were not specified in our study, they can largely be considered to have ulcerous colitis, given reservations in clinical guidelines about the administration of 5-ASA in Crohn's disease [20]. In the aforementioned cohort study, there was a relative reduction in PD risk among patients with longer

follow-up times, similar to observations in individuals with truncal vagotomy [21,22] and in under 65year-olds, as in the present study.

Recently three studies have been published describing a risk association between IBD and PD. In the Danish nationwide population cohort study, covering the period 1977-2014, found a significantly increased risk of PD when comparing patients with IBD with non-IBD (HR=1.22; 95% CI=1.09-1.35), especially among patients with ulcerative colitis (HR=1.35; 95% CI=1.20-1.52). However, in those patients diagnosed with IBD under 40 years of age and with prolonged follow-up > 20 years, this increase was not statistically significant [23], suggesting, as we have previously referred, that IBD treatment may lessen the risk of PD. In a similar way, a Nationwide Swedish Cohort Study, covering the period 2002-2014, found that IBD is associated with an increased risk of PD, however IBD patients who developed PD were much older at the end of follow-up (88% vs 35% at age ≥60 years; p= 0.001) and more likely to never have received thiopurine or anti-TNF compared with those without PD (88% vs 66%; p= 0.001), 80% of PD events occurred in patients with IBD onset ≥60 years and the relative risk of future PD in IBD was highest among patients diagnosed with IBD at age 60 years or older. Authors suggested a potential protective effect of selective IBD-directed therapies, thiopurines, or anti-TNF-α on PD, as IBD patients never exposed to thiopurines or anti-TNF were 60% more likely to develop PD (HR, 1.6;95% CI, 1.2–2.2) than their matched reference individuals [24], raising again the protective effect of the treatment of IBD on the development of PD. In a retrospective cohort study, based on the Market-Scan Commercial and Medicare databases, a higher incidence of PD was observed among patients with IBD than among individuals without IBD; the authors found a statistically significant 28% increase in the incidence of PD among patients with IBD compared with the unaffected matched controls. Conversely, they observed a

markedly lower PD incidence rate (0.08 per 1000 patient-years) among patients with IBD who were exposed to anti-TNF therapy compared with patients without exposure (0.76 per 1000 patient-years), which means a 78% reduction in the PD incidence rate [25], a risk reduction in the same range as that found in our patients treated with 5-ASA. Despite the contradictory associations between both processes, two studies find a reduction in the risk of PD related to the use of immunomodulators [19,25], although they do not describe the degree of exposure to 5-ASA. It is presumable that those with a more active disease and therefore candidates for immunomodulators therapies will have had a greater exposure to 5-ASA, but it is also possible that those treated exclusively with 5-ASA have a more benign inflammatory disease.

5-ASA drugs are the first-line treatment in ulcerous colitis [20,26]. Their action mechanism is not fully elucidated but is considered to include: a) anti-inflammatory activity or inhibition of the local synthesis of prostaglandins and leukotrienes [27]; b) reduction in nitric oxide and IL-6 production and an antiapoptotic effect, diminishing phosphorylation by mitogen-activated protein kinases (MAPKs), e.g., p38 and JNK (c-Jun N-terminal kinases) [28]; and c) action on the microbiota, producing qualitative and quantitative changes in its composition [29].

In PD, alpha-synuclein aggregates are known to be present in the intestine some years before the onset of motor symptoms [4,5], especially in certain structures such as the vermiform appendix [30]. Local action mechanisms of 5-ASA described above may affect progression of the synucleinopathy, given that they may act on etiopathogenetic mechanisms that would activate microglia, associated with certain microbiota and bacterial membrane lipopolysaccharides or endogenous factors (e.g., JNK and p38) [31-33].

In a recent murine model with alpha-synuclein overexpression, transplantation from PD patients of fecal matter with greater short-chain fatty-acid content was followed by microglial activation, an increase in inflammation mediators (TNF-α and IL-6) and in alpha-synuclein aggregation and propagation, a poorer execution of motor tests, and even constipation; these changes were reduced by treatment with minocycline [34], suggesting that other drug groups, such as 5-ASA, may have a similar effect.

This study has limitations related to the utilization of a drug dispensation record, subjects therefore not be considered representative of the general population. The resulting ORs may be overestimated, because the prevalence of both diseases was over-represented but in an unequal manner, being higher for PD in the study population than in the general population and only slightly higher for IBD. Cross-sectional drug dispensation studies are more likely to include patients with PD than with IBD due to their older age and more frequent polymedication and because some biological and immunosuppressive therapies received by IBD patients in the hospital are not included in the dispensary records. Furthermore, individuals treated with 5-ASA are a subgroup of patients with IBD and ulcerous colitis, mainly in those with active disease. We did not control for the possibility of a group of patients receiving combined maintenance therapy (5-ASA and other drugs). There is also a potential classification error related to the selection of *proxy* variables, given that the diseases under study were identified according to the medication received. Notably, dopaminergic drugs are prescribed, although much less frequently, for diseases other than PD (atypical parkinsonism, chronic adult hydrocephaly, restless legs syndrome, etc.); however, this is unlikely to have affected the validity of our results given the large sample size.

Conclusions

A lower risk of PD was observed in individuals with IBD aged ≤65 years and treated with 5-ASA, which may be, as hypothesis, related to a theoretical pharmacological effect on alpha-synuclein aggregation and propagation that could reduce or delay PD onset in at-risk populations. The advantages of 5-ASA medication include its relatively low cost and lack of absorption. Within the limitations of the methodology applied, further experimental research with animal models and new longitudinal studies are warranted, given the important scientific and therapeutic implications of these findings.

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Conflict of interest: The authors declare they have no conflict of interest.

Author Contributions: JP, FE and VC contributed conception and design of the study; MPN, MPV and CM organized the database; FE and AM performed the statistical analysis; JP wrote the first draft of the manuscript; FE, CM, MC and MG wrote sections of the manuscript. All authors contributed to manuscript revision, read and approved the submitted version.

Data sharing statement: No additional data are available.

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Figures

Figure 1. Population pyramid with age and sex distribution of the groups with possible inflammatory bowel disease (IBD) and possible Parkinson's disease (PD)

Tables

Table 1. 2x2 contingency table and prevalence of possible PD

	Without Possible PD	**With Possible PD**	**Prevalence Possible PD**
Without Possible IBD	1993473	19966	1%
With Possible IBD	7485	56	0.7%

Table 2. Age and sex adjusted risk of possible Parkinson's disease in individuals with possible inflammatory bowel disease

Variable		**β_1**	**OR (95 % CI)**	**p**
"Possible PD"		-0.28	0.75 (0.57-0.98)	**0.0362**
Adjusted model	"Possible PD"	-0.06	0.94 (0.72-1.23)	0.657
	"Possible PD" ≤ 65 yrs.	-1.28	0.28 (0.10-0.74)	**0.0103**
	"Possible PD" > 65 yrs.	0.16	1.17 (0.89-1.54)	0.257

Figure 1

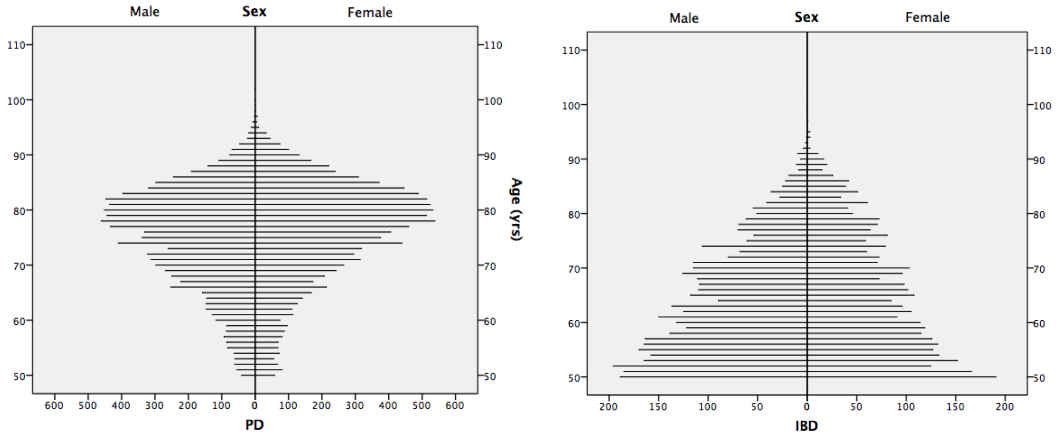

Figure 1. Population pyramid with age and sex distribution of the groups with possible inflammatory bowel disease (IBD) and possible Parkinson's disease (PD)

Reporting checklist for cross sectional study.

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Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

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		Reporting Item	Page Number
Title	#1a	Indicate the study's design with a commonly used term in the title or the abstract	1
Abstract	#1b	Provide in the abstract an informative and balanced summary of what was done and what was found	2
Background / rationale	#2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	#3	State specific objectives, including any prespecified hypotheses	5
Study design	#4	Present key elements of study design early in the paper	6,7
Setting	#5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6,7
Eligibility criteria	#6a	Give the eligibility criteria, and the sources and methods of selection of participants.	6,7

	#7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6,7
Data sources / measurement	#8	For each variable of interest give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group. Give information separately for for exposed and unexposed groups if applicable.	6,7
Bias	#9	Describe any efforts to address potential sources of bias	6,7
Study size	#10	Explain how the study size was arrived at	6,7
Quantitative variables	#11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why	6,7
Statistical methods	#12a	Describe all statistical methods, including those used to control for confounding	6,7
	#12b	Describe any methods used to examine subgroups and interactions	6,7
	#12c	Explain how missing data were addressed	6,7
	#12d	If applicable, describe analytical methods taking account of sampling strategy	6,7
	#12e	Describe any sensitivity analyses	6,7
Participants	#13a	Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed. Give information separately for for exposed and unexposed groups if applicable.	8
	#13b	Give reasons for non-participation at each stage	8
	#13c	Consider use of a flow diagram	8
Descriptive data	#14a	Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders. Give information separately for exposed and unexposed groups if applicable.	8

	#14b	Indicate number of participants with missing data for each variable of interest	8
Outcome data	#15	Report numbers of outcome events or summary measures. Give information separately for exposed and unexposed groups if applicable.	8
Main results	#16a	Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	8
	#16b	Report category boundaries when continuous variables were categorized	8
	#16c	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	8
Other analyses	#17	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	8
Key results	#18	Summarise key results with reference to study objectives	9
Limitations	#19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.	11,12
Interpretation	#20	Give a cautious overall interpretation considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.	9-11
Generalisability	#21	Discuss the generalisability (external validity) of the study results	12
Funding	#22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	13

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Association of Parkinson's disease and treatment with aminosalicylates in inflammatory bowel disease: a cross-sectional study in a Spain drug dispensation records.

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Association of Parkinson's disease and treatment with aminosalicylates in inflammatory bowel disease: a cross-sectional study in a Spain drug dispensation records.

Javier Pinel-Ríos[1], MD; Carlos Javier Madrid-Navarro[2], MD; María José Pérez-Navarro[2], MD; María José Cabello-Tapia[3], MD; María José Piña-Vera[4]; Víctor Campos-Arillo[1], MD, PhD; María Rosario Gómez-García[3], MD; Adolfo Mínguez-Castellanos[2], MD, PhD; Francisco Escamilla-Sevilla[2], MD, PhD.

1: Department of Neurology, Vithas Xanit International Hospital, Benalmadena, Malaga, Spain.
2: Department of Neurology, Virgen de las Nieves University Hospital, Institute of Bio-health Research ibs. Granada, Spain.
3: Department of Digestive Diseases, Virgen de las Nieves University Hospital, Granada, Spain.
4: Andalusian Health Service Pharmacy and Benefit Support Department. Spain.

Correspondence:
Dr. Javier Pinel-Ríos, MD
Unidad de Neurociencias, Av. de los Argonautas, s/n, 29631 Benalmadena, Malaga, Spain. Email: javierpinelr@gmail.com. Tel: +34652885523. Fax: +34952367197
Dr. Francisco Escamilla-Sevilla, MD PhD.
Email: fescamilla@hotmail.com

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Abstract

Objectives: To analyze the association between aminosalicylate-treated inflammatory bowel disease (IBD) and Parkinson´s disease (PD) at population level.

Design: Cross-sectional study.

Setting: The study was performed based on electronic drug prescription and dispensation records of the Andalusian Public Health System.

Participants: All individuals aged ≥ 50 yrs with at least one drug dispensation during December 2014 were identified from the records.

Primary and secondary outcome measures: Groups were formed: "Possible PD" group, including all who received an anti-Parkinson agent; "Possible IBD" group, those treated with mesalazine and/or derivatives (5-aminosalicylic acid [ASA]); and "Possible PD and IBD", including those receiving both anti-Parkinson agent and 5-ASA. Prevalence of "Possible PD" was determined among those with "Possible IBD" and among those without this condition. The age- and sex-adjusted OR was calculated.

Results: We recorded 2,020,868 individuals (68±11 yrs., 56 % female), 19,966 were included in "Possible PD" group (75±9 yrs., 53 % female) and 7,485 in "Possible IBD" group (64±10 yrs., 47 % female); only 56 were included in both groups (76±8 yrs., 32 % female). The prevalence of "Possible PD" was 0.7% among those with "Possible IBD" and 1 % among those without this condition (adjusted OR=0.94; 95 % CI=0.72-1.23; p=0.657). OR was 0.28 in individuals aged ≤65 years (95 % CI=0.10-0.74; p=0.01) and 1.17 in older individuals (95 % CI=0.89-1.54; p=0.257).

Conclusions: Within the limitations of this study, the results suggest a protective role for IBD and/or 5-ASA against PD development, especially among under 65-year-olds. Further studies are warranted to explore this association given its scientific and therapeutic implications.

Strengths and limitations of this study

- This is the first study that analyzes the association between aminosalicylate-treated inflammatory bowel disease and Parkinson's disease at population level.
- We conducted this study with a large population-based sample.
- The study was performed based on electronic drug prescription and dispensation records of the Andalusian Public Health System, Spain.
- This study has limitations related to the utilization of a drug dispensation record, and there is also a potential classification error related to the selection of proxy variables.

Introduction

Parkinson's disease (PD) is a multisystemic process with early involvement of the peripheral autonomic nervous system and subsequent propagation to the central nervous system [1]. After reports of deposits of alpha-synuclein aggregates that form neurites or Lewy bodies in gastric submucosal and myenteric plexi [1], various clinical and experimental studies described the enteric nervous system as fundamental in the etiopathogenesis of PD [1-5]. Braak's "dual-hit" hypothesis has been endorsed by evidence of the centripetal trans-synaptic and axonal migration of alpha-synuclein aggregates from neurons of the digestive system to brainstem structures such as the dorsal nucleus of the vagus nerve (Braak stage 2) [1-3]. It has been reported that certain components of the microbiota of PD patients can produce rupture of the intestinal barrier and bacterial translocation, which may be responsible for an intestinal proinflammatory status and subsequent alpha-synuclein aggregation and propagation [6,7].

Some features of inflammatory bowel disease (IBD) are similar to those of PD. Thus, their etiopathogenesis involves an anomalous immune response to specific microbiota that would trigger intestinal inflammatory activity in predisposed individuals unable to inhibit this inflammation and without the immunological tolerance characteristic of a healthy intestine [8]. The few studies on the relationship between these diseases have published controversial results, with observations either of no association [9] or of an increased risk of PD only among patients with Crohn's disease [10], attributable to a genetic relationship between these diseases at different *loci,* as revealed in a genome-wide association study [11]. Specifically, mutations in the leucine-rich repeat kinase 2 (LRRK2) gene, recently associated with Crohn's disease, have revealed pleiotropy between this IBD and PD risk [12]. Instead, a meta-analysis of the recent literature

showed an increased risk of PD in the IBD population and the increased risk remained significant when analyzing Crohn's disease and ulcerous colitis subgroups [13].

With this background, the objective of this study was to explore associations between PD and IBD treated with mesalazine (5-aminosalicylic acid) or its derivative sulfasalazine, hereafter 5-ASA, because they could have a role in reducing the risk of PD.

Material and methods

A cross-sectional study was performed based on electronic drug prescription and dispensation records (*Receta XXI*) of the Andalusian Public Health System, which include 98 % of all prescriptions in the region. Andalusia is located in southern Spain and is the most populated autonomous community in the country, with 8,402,000 inhabitants. The study was approved by the Research Ethics Committee of Granada (CEI-GRANADA).

Subjects

All individuals aged ≥ 50 yrs with at least one drug dispensation during December 2014 were identified from the records. Three groups were formed: "Possible PD" group, including all who received an anti-Parkinson agent, such as levodopa in different formulations and/or dopaminergic agonist, excluding those only receiving a single low-dose dopaminergic agonist (ropinirole ≤ 1 mg; rotigotine ≤ 2 mg; pramipexole ≤ 0.26 mg; pergolide ≤1 mg; cabergoline ≤ 1 mg) and/or monoamine oxidase B inhibitor, including those exclusively treated with selegiline or rasagiline; "Possible IBD" group, including all receiving 5-ASA in its different formulations (mesalazine/mesalamine or 5 aminosalicylic acid and sulfasalazine); and "Possible PD and IBD", including those receiving both anti-Parkinson agent and 5-ASA.

Study variables

Membership of the "Possible PD" group was the dependent variable, and membership of the "Possible IBD" group, sex, and age were the predictive variables.

Data gathering

Data were obtained from the computerized databases of the Andalusian Health Service Pharmacy and Benefit Support Department. Anonymity was preserved by masking clinical record numbers.

Data analysis

In a descriptive analysis, central tendency and dispersion measurements were calculated for quantitative variables and absolute and relative frequencies for qualitative variables, plotting the corresponding graphs. After applying the Kolmogorov-Smirnov test to check data distribution normality, between-group comparisons were evaluated with the Mann-Whitney U for the quantitative variable (age) and with the chi-square test for categorical variables. After controlling for age and sex, the strength of between-group associations was established by constructing a multiple logistic regression model to estimate the odds ratio (OR) and 95 % confidence interval. All tests were two-tailed, and $p<0.05$ was considered significant. R and SPSS 21 statistical packages were used for the statistical analysis.

Patient and Public Involvement

Patients were not involved.

Results

During December 2014, 2,020,868 individuals aged ≥ 50 yrs were dispensed with at least one drug; their mean age was 67.9 ± 11.8 yrs., 56 % were female (mean of 68.6 ± 11.5 yrs) and 44 % were male (mean of 67.2 ± 10.7 yrs.). The study included 27,451 patients (mean age of 72.3 ± 10.9 yrs.; 51 % female) with at least one dispensation of anti-Parkinson agent ("Possible PD") and/or 5-ASA ("Possible IBD") after excluding 24 patients with inadequate records (e.g., missing data on age and/or sex) and 6 with unlikely PD (low-dose dopaminergic agonist [0.5 mg cabergoline in 1 case and 0.5 mg ropinirole in 5]); the mean age was slightly higher for the females than for the males (73.1 ± 10.9 *vs.* 71.4 ± 10.8 yrs.; p=0.0001)

A total of 19,966 individuals were considered as "Possible PD" (mean age of 75.4 ± 9.3 yrs.; 53 % females) and 7,485 as "Possible IBD" (64.15 ± 10.4 yrs.; 47 % females). Individuals with "Possible PD" were older (p=0.0001) and more frequently female (p=0.0001) in comparison to those with "Possible IBD" (figure 1). A group of 56 individuals (32 females) were considered both "Possible PD" and "Possible IBD; their mean age was 75.7 ± 8.1 yrs (range, 52-94 yrs), and they showed no differences in age (p=0.956) or sex (p=0.505) distribution with respect to the "Possible PD" group.

The prevalence of "Possible PD" was 0.7 % in the "Possible IBD" group and 1 % in those without this condition (Table 1). "Possible IBD" showed a reduced risk for "Possible PD" with OR of 0.75 (95 % CI=0.57-0.98; p=0.036), rising to 0.94 (95 % CI=0.72-1.23; p=0.657) after controlling for age and sex; stratification by age yielded an OR of 0.28 (95 % CI=0.10-0.74; p=0.01) for individuals aged ≤65 years and 1.17 (95 % CI =0.89-1.54; p=0.257) for those aged > 65 years, after controlling for sex (Table 2).

Discussion

The results of this cross-sectional study suggest that the presence of IBD and/or treatment with 5-ASA may play a protective role against PD development, especially among under-65-year-olds. The pathogenesis of the two diseases may share certain features, including the role of enteric glial cells [14-16] and inflammation [17], genetic factors (PD and Crohn's disease) [11], the protective effect of tobacco (PD and ulcerous colitis) [18], the presence of proinflammatory flora, and altered permeability with rupture of the intestinal barrier [6,7]. In fact, the inflammatory condition and/or treatment with 5-ASA might have been expected to act as a risk rather than protective factor, unless they have some impact on the pathogenesis of this synucleinopathy.

Our results are similar to those of the recent case-control study of PD risk based on Medicare data; this group found an inverse association between IBD and PD, including both Crohn's disease and ulcerous colitis. Similarly, there was an inverse association between PD and IBD-associated conditions, as well as IBD associated surgical procedures and immunosuppressant use, even among beneficiaries without IBD. The authors suggest that IBD is associated with a lower risk of developing PD in the adjusted model for a population aged> 65 years (OR = 0.86; 95% CI=0.81-0.92) [19]. Conversely, a retrospective cohort study based on healthcare records found an increased risk of PD among IBD patients, especially those with Crohn's disease, given that this association was not observed in the subgroup with ulcerous colitis (OR = 0.94; 95 % CI=0.49-1.84) [10]. Although these cases were not specified in our study, they can largely be considered to have ulcerous colitis, given reservations in clinical guidelines about the administration of 5-ASA in Crohn's disease [20]. In the aforementioned cohort study, there was a relative reduction in PD risk among patients with longer

follow-up times, similar to observations in individuals with truncal vagotomy [21,22] and in under 65year-olds, as in the present study.

Recently three studies have been published describing a risk association between IBD and PD. In the Danish nationwide population cohort study, covering the period 1977-2014, found a significantly increased risk of PD when comparing patients with IBD with non-IBD (HR=1.22; 95% CI=1.09-1.35), especially among patients with ulcerative colitis (HR=1.35; 95% CI=1.20-1.52). However, in those patients diagnosed with IBD under 40 years of age and with prolonged follow-up > 20 years, this increase was not statistically significant [23], suggesting, as we have previously referred, that IBD treatment may lessen the risk of PD. In a similar way, a Nationwide Swedish Cohort Study, covering the period 2002-2014, found that IBD is associated with an increased risk of PD, however IBD patients who developed PD were much older at the end of follow-up (88% vs 35% at age ≥60 years; p= 0.001) and more likely to never have received thiopurine or anti-TNF compared with those without PD (88% vs 66%; p= 0.001), 80% of PD events occurred in patients with IBD onset ≥60 years and the relative risk of future PD in IBD was highest among patients diagnosed with IBD at age 60 years or older. Authors suggested a potential protective effect of selective IBD-directed therapies, thiopurines, or anti-TNF-α on PD, as IBD patients never exposed to thiopurines or anti-TNF were 60% more likely to develop PD (HR, 1.6;95% CI, 1.2–2.2) than their matched reference individuals [24], raising again the protective effect of the treatment of IBD on the development of PD. In a retrospective cohort study, based on the Market-Scan Commercial and Medicare databases, a higher incidence of PD was observed among patients with IBD than among individuals without IBD; the authors found a statistically significant 28% increase in the incidence of PD among patients with IBD compared with the unaffected matched controls. Conversely, they observed a

markedly lower PD incidence rate (0.08 per 1000 patient-years) among patients with IBD who were exposed to anti-TNF therapy compared with patients without exposure (0.76 per 1000 patient-years), which means a 78% reduction in the PD incidence rate [25], a risk reduction in the same range as that found in our patients treated with 5-ASA. Despite the contradictory associations between both processes, two studies find a reduction in the risk of PD related to the use of immunomodulators [19,25], although they do not describe the degree of exposure to 5-ASA. It is presumable that those with a more active disease and therefore candidates for immunomodulators therapies will have had a greater exposure to 5-ASA, but it is also possible that those treated exclusively with 5-ASA have a more benign inflammatory disease.

5-ASA drugs are the first-line treatment in ulcerous colitis [20,26]. Their action mechanism is not fully elucidated but is considered to include: a) anti-inflammatory activity or inhibition of the local synthesis of prostaglandins and leukotrienes [27]; b) reduction in nitric oxide and IL-6 production and an antiapoptotic effect, diminishing phosphorylation by mitogen-activated protein kinases (MAPKs), e.g., p38 and JNK (c-Jun N-terminal kinases) [28]; and c) action on the microbiota, producing qualitative and quantitative changes in its composition [29].

In PD, alpha-synuclein aggregates are known to be present in the intestine some years before the onset of motor symptoms [4,5], especially in certain structures such as the vermiform appendix [30]. Local action mechanisms of 5-ASA described above may affect progression of the synucleinopathy, given that they may act on etiopathogenetic mechanisms that would activate microglia, associated with certain microbiota and bacterial membrane lipopolysaccharides or endogenous factors (e.g., JNK and p38) [31-33].

In a recent murine model with alpha-synuclein overexpression, transplantation from PD patients of fecal matter with greater short-chain fatty-acid content was followed by microglial activation, an increase in inflammation mediators (TNF-α and IL-6) and in alpha-synuclein aggregation and propagation, a poorer execution of motor tests, and even constipation; these changes were reduced by treatment with minocycline [34], suggesting that other drug groups, such as 5-ASA, may have a similar effect.

This study has limitations related to the utilization of a drug dispensation record, subjects therefore not be considered representative of the general population. The resulting ORs may be overestimated, because the prevalence of both diseases was over-represented but in an unequal manner, being higher for PD in the study population than in the general population and only slightly higher for IBD. Cross-sectional drug dispensation studies are more likely to include patients with PD than with IBD due to their older age and more frequent polymedication and because some biological and immunosuppressive therapies received by IBD patients in the hospital are not included in the dispensary records. Furthermore, individuals treated with 5-ASA are a subgroup of patients with IBD and ulcerous colitis, mainly in those with active disease. We did not control for the possibility of a group of patients receiving combined maintenance therapy (5-ASA and other drugs). There is also a potential classification error related to the selection of *proxy* variables, given that the diseases under study were identified according to the medication received. Notably, dopaminergic drugs are prescribed, although much less frequently, for diseases other than PD (atypical parkinsonism, chronic adult hydrocephaly, restless legs syndrome, etc.); however, this is unlikely to have affected the validity of our results given the large sample size.

Conclusions

A lower risk of PD was observed in individuals with IBD aged ≤65 years and treated with 5-ASA, which may be, as hypothesis, related to a theoretical pharmacological effect on alpha-synuclein aggregation and propagation that could reduce or delay PD onset in at-risk populations. The advantages of 5-ASA medication include its relatively low cost and lack of absorption. Within the limitations of the methodology applied, further experimental research with animal models and new longitudinal studies are warranted, given the important scientific and therapeutic implications of these findings.

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Conflict of interest: The authors declare they have no conflict of interest.

Author Contributions: JP, FE and VC contributed conception and design of the study; MPN, MPV and CM organized the database; FE and AM performed the statistical analysis; JP wrote the first draft of the manuscript; FE, CM, MC and MG wrote sections of the manuscript. All authors contributed to manuscript revision, read and approved the submitted version.

Data sharing statement: No additional data are available.

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Figures

Figure 1. Population pyramid with age and sex distribution of the groups with possible inflammatory bowel disease (IBD) and possible Parkinson's disease (PD)

Tables

Table 1. 2x2 contingency table and prevalence of possible PD

	Without Possible PD	**With Possible PD**	**Prevalence Possible PD**
Without Possible IBD	1993473	19966	1%
With Possible IBD	7485	56	0.7%

Table 2. Age and sex adjusted risk of possible Parkinson's disease in individuals with possible inflammatory bowel disease

Variable		**β_1**	**OR (95 % CI)**	**p**
"Possible PD"		-0.28	0.75 (0.57-0.98)	**0.0362**
Adjusted model	"Possible PD"	-0.06	0.94 (0.72-1.23)	0.657
	"Possible PD" ≤ 65 yrs.	-1.28	0.28 (0.10-0.74)	**0.0103**
	"Possible PD" > 65 yrs.	0.16	1.17 (0.89-1.54)	0.257

Figure 1

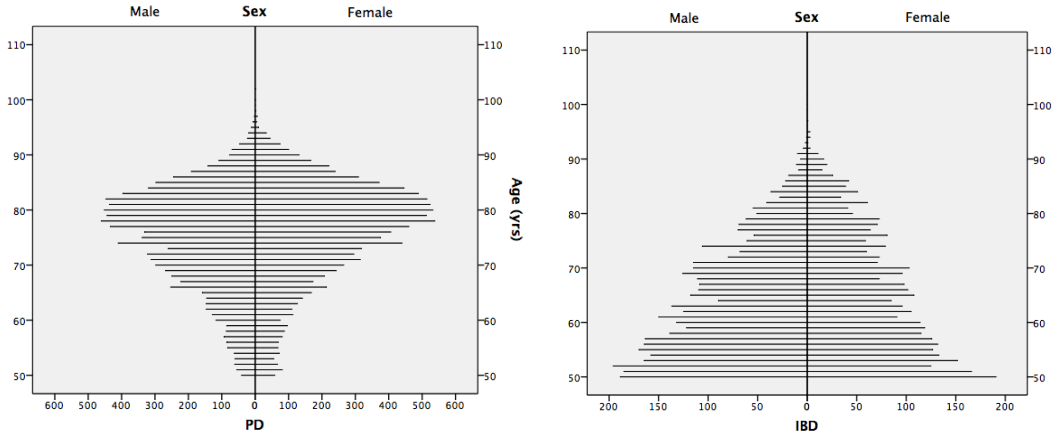

Figure 1. Population pyramid with age and sex distribution of the groups with possible inflammatory bowel disease (IBD) and possible Parkinson's disease (PD)

Reporting checklist for cross sectional study.

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		Reporting Item	Page Number
Title	#1a	Indicate the study's design with a commonly used term in the title or the abstract	1
Abstract	#1b	Provide in the abstract an informative and balanced summary of what was done and what was found	2
Background / rationale	#2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	#3	State specific objectives, including any prespecified hypotheses	5
Study design	#4	Present key elements of study design early in the paper	6,7
Setting	#5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6,7
Eligibility criteria	#6a	Give the eligibility criteria, and the sources and methods of selection of participants.	6,7

	#7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6,7
Data sources / measurement	#8	For each variable of interest give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group. Give information separately for for exposed and unexposed groups if applicable.	6,7
Bias	#9	Describe any efforts to address potential sources of bias	6,7
Study size	#10	Explain how the study size was arrived at	6,7
Quantitative variables	#11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why	6,7
Statistical methods	#12a	Describe all statistical methods, including those used to control for confounding	6,7
	#12b	Describe any methods used to examine subgroups and interactions	6,7
	#12c	Explain how missing data were addressed	6,7
	#12d	If applicable, describe analytical methods taking account of sampling strategy	6,7
	#12e	Describe any sensitivity analyses	6,7
Participants	#13a	Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed. Give information separately for for exposed and unexposed groups if applicable.	8
	#13b	Give reasons for non-participation at each stage	8
	#13c	Consider use of a flow diagram	8
Descriptive data	#14a	Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders. Give information separately for exposed and unexposed groups if applicable.	8

	#14b	Indicate number of participants with missing data for each variable of interest	8
Outcome data	#15	Report numbers of outcome events or summary measures. Give information separately for exposed and unexposed groups if applicable.	8
Main results	#16a	Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	8
	#16b	Report category boundaries when continuous variables were categorized	8
	#16c	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	8
Other analyses	#17	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	8
Key results	#18	Summarise key results with reference to study objectives	9
Limitations	#19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.	11,12
Interpretation	#20	Give a cautious overall interpretation considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.	9-11
Generalisability	#21	Discuss the generalisability (external validity) of the study results	12
Funding	#22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	13