Supplementary Material

A Critical Analysis of Intestinal Enteric Neuron Loss and Constipation in Parkinson's Disease

Supplementary Table 1. Studies comparing intestinal enteric neuron quantities between PD and control groups

Reference	Region of intestine examined Tissue processing (myenteric and/or submucosal?)	• Subjects assessed - How intestinal tissue was obtained	Method of assessing and comparing intestinal enteric neuron quantities	Submucosal neuron loss in the intestinal region in PD?	Myenteric neuron loss in the intestinal region in PD?
A) Singaram et al., 1995 [1]	Colon (ascending) Cross-sections (myenteric, submucosal)	• **PD**: 11 severely constipated PD patients - 9/11: Samples of ascending colon were taken during colectomy (for treatment of intractable constipation). - 2/11: Samples of ascending colon were taken during autopsy. • **CC**: 5 constipated control subjects - Samples of ascending colon were taken during colectomy (for treatment of intractable constipation). • **control**: 17 non-constipated control subjects - Samples of ascending colon were taken during colectomy (for adenocarcinoma removal).	• **PGP 9.5** used as enteric neuron marker. • **VIP** used as VIPergic neuron marker. • **TH** used as catecholaminergic (dopaminergic and noradrenergic) neuron marker. • **DA** used as dopaminergic neuron marker. *NOTE:* Immunostaining for DA is not a validated method of identifying dopaminergic neurons *in situ* [2, 3]. For each subject: 5cm segments of ascending colon were fixed in formaldehyde before being cryosectioned at 16µm thickness. At least 500 PGP 9.5-positive neurons were counted per five sections, and at least 20 sections from the colon segment were assessed for VIP-positive neurons, TH-positive neurons and DA-positive neurons. For the submucosal plexus and myenteric plexus, separately, calculated the number of VIP-positive neurons, TH-positive neurons and DA-positive neurons per 100 PGP 9.5-positive neurons (i.e., in each plexus, VIPergic, catecholaminergic and dopaminergic neurons were quantified as a percentage of total enteric neurons). In each group (**PD**, **CC** and **control**): For the submucosal plexus and myenteric plexus, separately, calculated the mean number (with **SEM**) of VIP-positive neurons, TH-positive neurons and DA-positive neurons per 100 PGP 9.5-positive neurons (i.e., in each plexus, the mean (with SEM) percentage of total enteric neurons that were VIPergic, catecholaminergic or dopaminergic was quantified). *Statistical analysis*: Unpaired t-test (parametric; 1 variable, 2 categories, between-subjects [4]).	**N/A** No significant difference in the mean percentage of VIP-positive neurons, TH-positive neurons nor DA-positive neurons in the submucosal plexus between the three groups — This does not rule out submucosal neuron loss in PD.	**N/A** No significant difference in the mean percentage of VIP-positive neurons nor TH-positive neurons in the myenteric plexus between the three groups — This does not rule out myenteric neuron loss in PD. Significant reduction in the mean percentage of DA-positive neurons in the myenteric plexus of PD patients relative to controls (constipated and non-constipated) – Immunostaining for DA: • Is not a validated method of identifying dopaminergic neurons *in situ* [2, 3]. • Result conflicts with TH immunostaining result, and is therefore thought to be artifactual [2, 3].
B) Lebouvier et al., 2008 [5] *NOTE:* This pilot study utilized very small sample sizes, impacting on the validity of its findings [6]**.**	Colon (ascending) Wholemounts (submucosal)	• **PD**: 5 subjectively constipated PD patients - 4 biopsies were taken from the ascending colon during colonoscopy. • **CC**: 3 constipated control subjects - 4 biopsies were taken from the ascending colon during colonoscopy (for assessment of a chronic intractable constipation). • **control**: 5 non-constipated control subjects - 4 biopsies were taken from the ascending colon during colonoscopy (for colorectal cancer screening).	• **HuC/D** used as enteric neuron marker. For each subject: After biopsies underwent microdissection to obtain submucosa samples, the number of neurons per ganglion was averaged to produce 'neurons per ganglion' (the number of ganglia per biopsy was also averaged to produce 'ganglia per biopsy'). In each group (**PD**, **CC** and **control**): Calculated the mean number (with **SD**) of: - Neurons per ganglion - Ganglia per biopsy *Statistical analysis*: Not specified.	No — No significant difference in mean neurons per ganglion in the submucosal plexus of PD patients relative to controls (both constipated and non-constipated, separately). Also: No significant difference in mean ganglia per biopsy in PD patients relative to controls (both constipated and non-constipated, separately).	**N/A** Myenteric plexus not assessed.
C) Lebouvier et al., 2010 [6]	Colon (ascending, descending) Wholemounts (submucosal)	• **PD**: 29 PD patients - 4 biopsies were taken from the ascending colon and descending colon, respectively, during colonoscopy. • **control**: 10 control subjects	• **NF** used as enteric neuron marker. For each subject: Neurons were counted in all available ganglia of one submucosa sample from the ascending colon and one submucosa sample from the descending colon (submucosa samples derived from biopsy	Yes — Significant decrease (14%) in mean neurons per ganglion in the submucosal plexus of PD patients relative to controls.	**N/A** Myenteric plexus not assessed.

		- 4 biopsies were taken from the ascending colon and descending colon, respectively, during colonoscopy (for colorectal cancer screening).	tissue). The mean number of neurons per ganglion in the ascending colon submucosa sample and the mean number of neurons per ganglion in the descending colon submucosa sample were then averaged to produce 'neurons per ganglion'. In each group (**PD** and **control**): Calculated the mean number (with **SD**) of neurons per ganglion. *Statistical analysis*: Unpaired t-test (parametric; 1 variable, 2 categories, between-subjects [4]).		
D) Annerino et al., 2012 [2]	Duodenum, Colon (transverse) Cross-sections (myenteric)	• **PD**: 13 PD patients - 6µm cross-sections of duodenum and transverse colon tissue. • **control**: 12 control subjects - 6µm cross-sections of duodenum and transverse colon tissue (from individuals with no known neurological disease). *NOTE:* All cross-sections were formalin-fixed, paraffin-embedded and obtained from the Arizona Parkinson's Disease Consortium and the Banner Sun Health Research Institute Brain and Body Donation Program in Sun City, AZ.	• **HuC/D** used as enteric neuron marker. For each subject: For determination of myenteric neuron density in the duodenum and transverse colon, separately: 1. Examined 10 cross-sections (taken at 60µm intervals). 2. Counted all neurons (nucleated and non-nucleated) within the myenteric ganglion in each cross-section, then added up the neuron counts in each of the 10 cross-sections to produce the 'neuron count'. 3. Measured myenteric ganglion area (in mm^2) in each cross-section, then added up the ganglion area measurements in each of the 10 cross-sections to produce 'ganglion area' (in mm^2). 4. Divided the 'neuron count' by the 'ganglion area' to produce the 'myenteric neuron density' (neurons/mm^2). In each group (**PD** and **control**): Calculated the mean (with **SEM**) myenteric neuron density (neurons/mm^2) in the duodenum and transverse colon, separately. *Statistical analysis*: Mixed-factor repeated measures **ANOVA** (parametric; 1 variable, >2 categories, within-subjects [4]) with post hoc Bonferroni tests.	**N/A** Submucosal plexus not assessed.	No — No significant difference in mean myenteric neuron density (neurons/mm^2) in the duodenum nor transverse colon between PD patients and controls.
E) Corbillé et al., 2014 [3]	Colon (region/s not specified) Wholemounts (submucosal)	• **PD**: 35^ PD patients - 4 biopsies were taken from the colon during colonoscopy. • **control**: 10 control subjects - 4 biopsies were taken from the colon during colonoscopy (for colorectal cancer screening).	• **NF** used as enteric neuron marker. • **TH** used as catecholaminergic neuron marker. For each subject: Submucosa samples were obtained from biopsies⁺ and neurons positive for TH and/or NF were counted in all available ganglia of each submucosa sample. The percentage of TH-positive neurons (as a proportion of NF-positive neurons) was then calculated. In each group (**PD** and **control**): Calculated the mean (with **SEM**) percentage of TH-positive neurons (as a proportion of NF-positive neurons). *Statistical analysis*: Mann-Whitney U test (non-parametric; 1 variable, 2 categories, between-subjects [4]). *NOTE:* The mean area of submucosa samples was not significantly different between PD patients and controls.	**N/A** No significant difference in the mean percentage of TH-positive neurons in the submucosal plexus of PD patients relative to controls — This does not rule out submucosal neuron loss in PD.	**N/A** Myenteric plexus not assessed.
F) Barrenschee et al., 2017 [7]	Upper dorsal rectal wall Cross-sections (submucosal)	• **PD**: 12 PD patients - 2 biopsies were taken from the upper dorsal rectal wall during colonoscopy. • **control**: 11# control subjects - 2 biopsies were taken from the upper dorsal rectal wall during colonoscopy (for colorectal cancer screening or GI symptom assessment).	• **PGP 9.5** used as enteric neuron marker. For each subject: Biopsies (containing the mucosal and submucosal layer) were fixed in paraformaldehyde (4% in PBS) and embedded in paraffin, before being sectioned at 6µm thickness. Every 7th section was screened (via PGP 9.5 immunostaining) for submucosal neurons and ganglia, then the number and area of submucosal neurons + the area of submucosal ganglia were recorded. In each group (**PD** and **control**): Calculated the mean (with **SD**) of: - Neuronal area (in μm^2) - Ganglionic area (in μm^2) - Neuronal number per ganglionic area (neurons/μm^2) — i.e., submucosal neuron density *Statistical analysis*: Mann-Whitney U test (non-parametric; 1 variable, 2 categories, between-subjects [4]).	No — No significant difference in mean submucosal neuron density (neurons/μm^2) between PD patients and controls. Also: No significant difference in mean neuronal area, nor mean ganglionic area, in PD patients relative to controls.	**N/A** Myenteric plexus not assessed.

G) Desmet et al., 2017 [8]	Second part of the duodenum Wholemounts (submucosal)	• **PD**: 15 PD patients • **control**: 15~ control subjects - 8 biopsies were taken from the second part of the duodenum during endoscopy in each PD patient and control subject. *NOTE:* PD patients and control subjects were recruited in pairs — each pair consisted of a PD patient and their healthy partner.	• **NF** and **HuC/D** used as enteric neuron markers. For each subject: From 3 biopsies, the submucosal plexus was isolated and the number of neurons per ganglion was averaged to produce 'neurons per ganglion' (the number of ganglia per biopsy was also averaged to produce 'ganglia per biopsy', and the total number of neurons per biopsy was averaged to produce 'neurons per biopsy'). In each group (**PD** and **control**): Calculated the mean number (with **SD**) of: - Neurons per ganglion - Ganglia per biopsy - Neurons per biopsy *Statistical analysis*: Wilcoxon matched-pairs signed rank test (non-parametric; 1 variable, 2 categories, within-subjects [4]).	No — No significant difference in mean neurons per ganglion in the submucosal plexus of PD patients relative to controls. Also: No significant difference in mean neurons per biopsy, nor mean ganglia per biopsy, in PD patients relative to controls.	**N/A** Myenteric plexus not assessed.
H) Giancola et al., 2017 [9]	Colon (descending) Wholemounts (submucosal)	• **PD**: 29***** PD patients with Rome III criteria-defined constipation - 4 biopsies were taken from the descending colon during colonoscopy. • **CC**: 10 control subjects with Rome III criteria-defined constipation - 4 biopsies were taken from the descending colon during colonoscopy. • **control**: 20 control subjects without Rome III criteria-defined constipation - 4 biopsies were taken from the descending colon during colonoscopy (for polyp screening).	• **HuC/D** used as enteric neuron marker. For each subject: 3/4 biopsies underwent microdissection to obtain submucosal wholemounts. At least 20-30 neurons from 5-6 ganglia per submucosal wholemount were counted, and the number of neurons per ganglion was averaged to produce 'neurons per ganglion'. In each group (**PD**, **CC** and **control**): Calculated the mean number (with **SD**) of neurons per ganglion. *Statistical analysis*: One-way **ANOVA** (parametric; 1 variable, >2 categories, between-subjects [4]).	No — No significant difference in mean neurons per ganglion in the submucosal plexus between the three groups.	**N/A** Myenteric plexus not assessed.
I) Ohlsson and Englund, 2019 [10]	Jejunum, Colon (region/s not specified) Cross-sections (myenteric, submucosal)	• **PD**: 20 PD patients - Full-thickness samples of jejunum and colon were taken during autopsy. • **control**: 10 control subjects - Full-thickness samples of jejunum and colon were taken during autopsy (from non-PD, intestinally asymptomatic individuals).	• **H&E** staining used to identify the presence of atrophic/pycnotic (degenerating) neurons in the submucosal plexus and myenteric plexus, separately. For each subject: Full-thickness samples were fixed in formaldehyde, dehydrated and embedded in paraffin, before being sectioned at 5µm thickness.	**N/A** Degenerating neurons identified in the submucosal plexus of 10/15 PD patients, no degenerating neurons identified in controls. Unclear if the degenerating neurons identified were in the jejunum, colon or both. No quantification of enteric neuron loss.	**N/A** Degenerating neurons identified in the myenteric plexus of 13/15 PD patients, no degenerating neurons identified in controls. Unclear if the degenerating neurons identified were in the jejunum, colon or both. No quantification of enteric neuron loss.

PD, PD patient group; CC, Constipated control group; control, Healthy and/or non-constipated control group; PGP 9.5, Protein gene product 9.5; VIP, Vasoactive intestinal peptide; TH, Tyrosine hydroxylase; DA, Dopamine; HuC/D, HuC/HuD; NF, Neurofilament; SEM, Standard error of the mean; SD, Standard deviation; ANOVA, Analysis of variance; H&E, Hematoxylin and eosin; N/A, Not answered. ^Mean percentage of TH-positive neurons in the PD group was derived from only 29/35 PD patients who underwent the colonoscopy required for biopsy collection and subsequent submucosal plexus assessment [3]—the reason for this is unclear. ⁺The number of biopsies per subject used to derive submucosa samples is unclear (but one or more biopsies were instead used for western blot analysis) [3]. #Mean neuronal area, mean ganglionic area and mean neuronal number per ganglionic area (mean submucosal neuron density) in the control group were each derived from 10/11 control subjects who underwent the colonoscopy required for biopsy collection and subsequent submucosal plexus assessment—this is likely because no ganglia could be found in one control biopsy [7]. ~Mean of neurons per ganglion, mean of neurons per biopsy and mean of ganglia per biopsy in the control group were each derived from 14/15 control subjects who underwent the endoscopy required for biopsy collection and subsequent submucosal plexus assessment [8]—the reason for this is unclear. *Only 22/29 PD patients consented to the colonoscopy necessary for biopsy collection [9]. Furthermore, mean of neurons per ganglion in the PD group was derived from only 15/22 PD patients who underwent the colonoscopy required for biopsy collection [9]—the remaining 7/22 PD patients had biopsies collected for RNA extraction rather than neuronal quantification [11].

Supplementary Table 2. PD patient demographics in studies that sought to assess intestinal enteric neuron loss/death

Reference	PD patient age (mean/range) PD patient sex (Male: Female)	PD diagnosis (diagnostic criteria) Proportion of patients with definite/clinically established PD (%)	Age at PD onset (mean/range) Proportion of patients with early-onset PD (%)	PD duration (mean/range) Proportion of patients with early PD (%)	PD severity (mean/range) Proportion of patients with severe PD (%)
Singaram et al., 1995 [1]	Age Mean: 64 (**SD** 7.3) Range: **N/A** Sex **N/A**	PD "well-documented" in 11/11 (No diagnostic criteria specified)	**N/A**	PD "longstanding" in 11/11, > 20 years in 8/11 (No mean or range provided) **Early PD** 0% (0/11)	PD "severe" in 11/11 (No mean or range provided) **Severe PD** 100% (11/11)
Lebouvier et al., 2008 [5]	Age Mean: 63 (**SD** 7) Range: **N/A** Sex 3:2	**N/A**	**N/A**	> 5 years in 5/5 (No mean or range provided) **Early PD** 0% (0/5)	**N/A**
Lebouvier et al., 2010 [6] *(continued on next page)* Lebouvier et al., 2010 [6] *(continued from last page)*	Age Mean: 62.8 (**SD** 7.4) Range: 44 - 72 Sex 17:12 *(59%:41%)*	The United Kingdom Parkinson's Disease Society Brain Bank clinical diagnostic criteria [12] Definite PD[V] % unclear	Mean: 52[A] Approximated[@] range: 36 - 70 Approximated[@] **Early-onset PD** 28% (8/29)	≤ 6 years in 9/29 (group 1) 7 - 12 years in 10/29 (group 2) ≥ 13 years in 10/29 (group 3) Mean: 10 years[A] Range: 1 - 24 years **Early PD** 17% (5/29)	Assessed via the **UPDRS-III** [13] score**.** Specifically: • Dopa-responsiveness**~** = % of **UPDRS-III** [13] score improvement from **OFF-state** following levodopa administration. Mean: 66%[A] Range: 29% - 100% **Severe PD** (as potentially indicated by dopa-responsiveness**~** ≤ 30% [14]) 5% (1[T]/19) • **UPDRS-III** axial score = Sum of responses to **UPDRS-III** [13] items (during **ON-state**): - 18 (Dysarthria/slurred speech) - 19 (Hypomimia/facial masking) - 22 (Rigidity) - 27 (Difficulty arising from chair) - 28 (Stooped posture) - 29 (Difficulty walking due to/or festination/gait disturbance) - 30 (Postural instability) Mean: 6[A] Range: 1 - 21 **Severe PD** (as potentially indicated by **UPDRS-III** [13] axial score ≥ 21) 3% (1[T]/29)
Annerino et al., 2012 [2]	Age Mean: 76[A] Range: 63 - 87 Sex 9:4	**N/A**	Mean: 63[A] Range: 47 - 78 **Early-onset PD** 15% (2/13)	Mean: 14 years[A] Range: 4 - 22 years **Early PD** 8% (1/13)	Assessed via **Modified HY** [15] stage (state **N/A**): *Mode:* 3[A] Range: 2.5 - 4 **Severe PD** (**Modified HY** stage ≥ 4 [15]) 25% (3/12[K])
Corbillé et al., 2014 [3]	Age Mean: 62.8 (**SEM** 5)***** Range: 40 - 80***^** Sex 55%:45%***** *NOTE*: This sex ratio is only possible if derived from 29[#] (rather than 35) PD patients (i.e., 16:13).	The United Kingdom Parkinson's Disease Society Brain Bank clinical diagnostic criteria [12] Definite PD[V] % unclear	**N/A**[#]	**N/A**[#]	**N/A**[#]

Barrenschee et al., 2017 [7]	Age Mean: 65 Range: 43 - 77 Sex **N/A**	The United Kingdom Parkinson's Disease Society Brain Bank clinical diagnostic criteria [12] Definite PD[V] % unclear	**N/A** *NOTE:* Considering PD patient age range, the proportion of patients with **early-onset PD** is at least 8% (1/12)	**N/A**	Assessed via the **MDS-UPDRS (III)** [16] score (during **ON-state**): Mean: 26.5 (**SD** 16.1) Range: 0 - 59: **Severe PD** (**MDS-UPDRS (III)** score ≥ 59 [17]) 8% (1/12)
Desmet et al., 2017 [8]	Age Mean: 58.9 (**SD** 9.2) Range: 45 - 71 Sex 11:4	Gelb Diagnostic Criteria for Parkinson's Disease [18] *NOTE*: According to the Gelb criteria, a diagnosis of 'definite PD' can only be made post-mortem [18]. Living patients must have either possible or probable PD[O], but the proportions of these diagnoses are unclear.	Mean: 51.1 (**SD** 9.4) Range: 36 - 69 **Early-onset PD** 53% (8/15) *NOTE:* No mutations in the PARK2 gene were discovered upon analysis of 5/6 patients with PD onset ≤ age 45.	Mean: 7.8 (**SD** 3.9) years Range: 2 - 17 years **Early PD** 20% (3/15)	Assessed via the **UPDRS-III**/**MDS-UPDRS (III)**[?] score (during **OFF-state**): Mean: 23.3 (**SD** 10.0) Range: 12 - 46 **Severe PD** (**MDS-UPDRS (III)** score ≥ 59 [17]) 0% (0/12) OR **Severe PD** (as potentially indicated by **UPDRS-III** [13] score **≥ 45**) 7% (1/15) Additionally assessed **HY**/**Modified HY**[?] stage (during **OFF-state**): *Median:* 2 *Interquartile range (IQR):* 2 - 3 **Severe PD** (**HY**/**Modified HY** stage ≥ 4 [15, 19]) 7% (1/15)
Giancola et al., 2017 [9]	Age Mean: 71[A] Range: 48 - 85 Sex 20:9 *Among the 15 PD patients from whom mean neurons per ganglion was derived [11]:* *Age* *Mean: 74* *Range: 49 - 85* *Sex* *11:4*	The United Kingdom Parkinson's Disease Society Brain Bank clinical diagnostic criteria [12] Definite PD[V] % unclear	Approximated[@] mean: 66 Approximated[@] range: 43 - 82 Approximated[@] **Early-onset PD** 7% (2/27[D]) *Among the 13/15 PD patients with known disease duration (missing PD duration for 2/15) from whom mean neurons per ganglion was derived [11]:* *Approximated[@]* ***Early-onset PD*** *8% (1/13)*	Mean: 5 years[A] Range: 1 - 10 years **Early PD** 52% (14/27[D]) *Among the 13/15 PD patients with known disease duration (missing PD duration for 2/15) from whom mean neurons per ganglion was derived [11]:* ***Early PD*** *54% (7/13)* *Among the 22/23 PD patients with known disease duration (missing PD duration for 1/23) who underwent CTT evaluation and ARM:* ***Early PD*** *55% (12/22) [11]*	Assessed via the **UPDRS-III** [13] score (state **N/A**): Mean: N/A Range: 2 - 27 **Severe PD** (as potentially indicated by **UPDRS-III** [13] score **≥ 45**) 0% (0/28[U]) Additionally assessed **Modified HY** [15] stage (state **N/A**): Mean: N/A Range: 1 - 3 **Severe PD** (**Modified HY** stage ≥ 4 [15]) 0% (0/28[U])
Ohlsson and Englund, 2019[P] [10]	Age Median: 77 Range: 73 - 84 Sex 11:9	The Movement Disorder Society Clinical Diagnostic Criteria for Parkinson's Disease [14] Clinically established PD[C] 100% (20/20)	**N/A**	**N/A**	**N/A**

N/A, Not available; SD, Standard deviation; SEM, Standard error of the mean; early-onset PD, PD onset before age 50 [20-23]; early PD, PD duration under 5 years [24, 25]; UPDRS-III, Unified Parkinson's Disease Rating Scale — Part III: Motor Examination (assessment of PD motor symptom severity [13]); MDS-UPDRS (III), MDS-sponsored Revision of the Unified Parkinson's Disease Rating Scale — Part III: Motor Examination [16]; OFF-state, PD patient functional state without levodopa treatment (symptomatic relief from PD motor symptoms [26]); ON-state, PD patient functional state with levodopa treatment; HY, Original Hoehn and Yahr scale (assessment of disability and impairment severity in PD patients [15,

19]); Modified HY, Modified Hoehn and Yahr scale [15]. [@]Based on the age minus disease duration (in years) of each PD patient in the relevant study [6, 9]. [*]Presumably derived from all 35 PD patients, but mean percentage of TH-positive neurons in the PD group in this study was derived from only 29/35 PD patients who underwent the colonoscopy required for biopsy collection and subsequent submucosal plexus assessment. [^]This study is from the same research group behind Lebouvier et al. [6] — Lebouvier et al. [6] stated in their methods that the PD patient age range was 40-75, but it was 44-72 (according to the individual PD patient data provided in Table 1 [6]). Therefore, the PD patient age range stated in the methods of Corbillé et al. [3] may be inaccurate (individual PD patient data not provided). [#]The 29/35 patients from whom mean percentage of TH-positive neurons in the PD group was derived may be the same 29 PD patients assessed by Lebouvier et al. [6], but this is unable to be clarified by available data. [~]Dopa-responsiveness data derived from PD patients in group 2 and 3 only. [?]Version utilized in this study is unclear. [V]Fulfilment of ≥ 3/8 supportive criteria are required for a diagnosis of definite PD [12] — 2 of these criteria ('levodopa response for 5 years or more' and 'clinical course of 10 years or more') cannot be met by patients with **early PD,** and it is also challenging for **early PD** patients to meet the dramatic levodopa response and dyskinesia criteria [25]. [A]Estimated based on individual PD patient results and/or demographics published in the relevant study [2, 6, 9]. [T]PD patient 28 [6]. [K]PD patients with known **Modified HY** stage (12/13) [2]. [O]Disease duration ≥ 3 years and dramatic levodopa/dopamine agonist response is required for diagnosis — both of these criteria are challenging for **early PD** patients to meet [25]. [D]PD patients with known disease duration (27/29) [9]. [U]PD patients with known **UPDRS-III** score and known **Modified HY** stage (28/29 – all bar PD patient 21) [9]. [P]Assessed presence of degenerating enteric neurons in intestinal tissue from PD patients and control subjects [10] (i.e. investigated presence of intestinal enteric neuron death) but did not quantify/compare enteric neurons quantities in intestinal tissue between PD and control groups (i.e. did not investigate PD-related intestinal enteric neuron loss). [C]The diagnostic criteria for clinically established PD [14, 25], approximate to earlier definitions of definite PD [12] and probable PD [18], are very difficult for **early PD** patients to satisfy [12, 18, 25].

Supplementary Table 3. PD-related studies that included assessment of intestinal enteric neuron loss and constipation

Reference	Region of intestine examined Tissue processing (myenteric and/or submucosal?)	• Subjects assessed - How intestinal tissue was obtained	Method of assessing constipation presence and/or severity	Enteric neuron loss in the intestinal region in PD?	Correlation between intestinal enteric neuron loss and constipation presence and/or severity?
A) Lebouvier et al., 2008 [5] *NOTE:* This pilot study utilized very small sample sizes, impacting on the validity of its findings [6].	Colon (ascending) Wholemounts (submucosal)	• **PD**: 5 subjectively constipated PD patients - 4 biopsies were taken from the ascending colon during colonoscopy. • **CC**: 3 constipated control subjects - 4 biopsies were taken from the ascending colon during colonoscopy (for assessment of a chronic intractable constipation). • **control**: 5 non-constipated control subjects - 4 biopsies were taken from the ascending colon during colonoscopy (for colorectal cancer screening).	Constipation presence and severity (**PD**): PD patients "complaining of functional constipation" were recruited into this study — No formal diagnosis of constipation nor assessment of constipation severity was reported. Constipation presence and severity (**CC**): All constipated controls were undergoing assessment of a chronic intractable constipation (severe constipation).	*In the submucosal plexus:* No **—** No significant difference in mean neurons per ganglion in the submucosal plexus of PD patients relative to controls (both constipated and non-constipated, separately). Also: No significant difference in mean ganglia per biopsy in PD patients relative to controls (both constipated and non-constipated, separately). *In the myenteric plexus:* **N/A** — Myenteric plexus not assessed.	**N/A —** No correlations performed. *NOTE:* Mean of neurons per ganglion within the submucosal plexus of the ascending colon was not significantly different in PD patients relative to constipated and non-constipated controls, respectively (see previous column).
B) Lebouvier et al., 2010 [6]	Colon (ascending, descending) Wholemounts (submucosal)	• **PD**: 29 PD patients - 4 biopsies were taken from the ascending colon and descending colon, respectively, during colonoscopy. • **control**: 10 control subjects - 4 biopsies were taken from the ascending colon and descending colon, respectively, during colonoscopy (for colorectal cancer screening).	Constipation presence (**PD** and **control**): Constipation was diagnosed according to the Rome III Criteria for Functional Constipation**^**: Constipation severity (**PD** and **control**): A constipation severity score (0-6) was derived from the sum of positive responses to 6 constipation items (Questions 9-14) on the Rome III Diagnostic Questionnaire for Adult Functional Gastrointestinal Disorders.	*In the submucosal plexus:* Yes — Significant decrease (14%) in mean neurons per ganglion in the submucosal plexus of PD patients relative to controls. *In the myenteric plexus:* **N/A** — Myenteric plexus not assessed.	No — Mean of neurons per ganglion within the submucosal plexus of the ascending + descending colon was not correlated with constipation severity score. *Statistical analysis*: Spearman's correlation.
C) Giancola et al., 2017 [9]	Colon (descending) Wholemounts (submucosal)	• **PD**: 29***** PD patients with Rome III criteria-defined constipation - 4 biopsies were taken from the descending colon during colonoscopy. • **CC**: 10 control subjects with Rome III criteria-defined constipation - 4 biopsies were taken from the descending colon during colonoscopy. • **control**: 20 control subjects without Rome III criteria-defined constipation - 4 biopsies were taken from the descending colon during colonoscopy (for polyp screening).	Constipation presence (**PD** and **CC**): All PD patients and constipated controls were recruited into this study on the basis of being constipated according to the Rome III Criteria for Functional Constipation (see previous column). *NOTE:* Assessment of constipation severity was not described in the main article, but a correlation involving the severity of Rome III criteria-defined constipation in PD (on a scale of 0-6) was included in the supporting results.	*In the submucosal plexus:* No — No significant difference in mean neurons per ganglion in the submucosal plexus between the three groups. *In the myenteric plexus:* **N/A** — Myenteric plexus not assessed	**N/A —** No relevant correlation performed. *NOTE:* Mean of neurons per ganglion within the submucosal plexus of the descending colon was not significantly different between PD patients, constipated controls and non-constipated controls (see previous column).
D) Barrenschee et al., 2017 [7]	Upper dorsal rectal wall Cross-sections (submucosal)	• **PD**: 12 PD patients - 2 biopsies were taken from the upper dorsal rectal wall during colonoscopy. • **control**: 11**#** control subjects - 2 biopsies were taken from the upper dorsal rectal wall during colonoscopy (for colorectal cancer screening or GI symptom assessment).	Constipation severity (**PD** and **control**): A constipation severity score (0-20**~**) was derived from the Constipation Scoring System (CSS) questionnaire. *NOTE:* Assessment of constipation presence was not described**,** but 7/12 PD patients and 3/11 control subjects had a CSS score > 0. However, a CSS score > 15 defines 'constipation' according to the original CSS [27], and no PD patient nor control subject had a CSS score > 15. Studies involving PD patients have utilized a CSS score > 15 to define 'severe constipation' [28] or 'very severe constipation' [29], although no justification for these re-definitions were provided in the relevant studies. Therefore, although constipation severity was assessed, it is unclear whether any PD patient or control subject was 'constipated' according to their CSS score.	*In the submucosal plexus:* No **—** No significant difference in mean submucosal neuron density (neurons/μm^2) between PD patients and controls. Also: No significant difference in mean neuronal area, nor mean ganglionic area, in PD patients relative to controls. *In the myenteric plexus:* **N/A** — Myenteric plexus not assessed.	**N/A** — No relevant correlation performed. *NOTE:* Mean neuron density within the submucosal plexus of the rectum was not significantly different between PD patients and controls (see previous column). Mean constipation severity score was also not significantly different between PD patients and controls.

PD, PD patient group; CC, Constipated control group; control, Healthy and/or non-constipated control group; N/A, Not answered. ^Rome III criteria-defined constipation was present in 23/29 PD patients and 1/10 control subjects [6]. *Only 22/29 PD patients consented to the colonoscopy necessary for biopsy collection [9]. Furthermore, mean of neurons per ganglion in the PD group was derived from only 15/22 PD patients who underwent the colonoscopy required for biopsy collection [9] — the remaining 7/22 PD patients had biopsies collected for RNA extraction rather than neuronal quantification [11]. #Mean neuronal area, mean ganglionic area and mean neuronal number per ganglionic area (mean submucosal neuron density) in the control group were each derived from 10/11 control subjects who underwent the colonoscopy required for biopsy collection and subsequent submucosal plexus assessment — this is likely because no ganglia could be found in one control biopsy [7]. ~CSS scores can range between 0-30 [27] — it is unclear why the CSS score range was changed to 0-20 [7].

Supplementary Table 4. Correlations between PD patient demographics and intestinal enteric neuron quantities

Reference[+]	Correlation between intestinal enteric neuron quantity and PD patient age?	Correlation between intestinal enteric neuron quantity and age at PD onset?	Correlation between intestinal enteric neuron quantity and PD duration?	Correlation between intestinal enteric neuron quantity and PD severity?
Lebouvier et al., 2010 [6]	No — Submucosal neurons per ganglion in the ascending+descending colon was not correlated with age of PD patients. *Statistical analysis*: Spearman's correlation	**N/A** — Age at PD onset was not reported nor correlated with submucosal neurons per ganglion in the ascending+descending colon.	No — Submucosal neurons per ganglion in the ascending+descending colon was not correlated with duration of PD. *Statistical analysis*: Spearman's correlation	No — Submucosal neurons per ganglion in the ascending+descending colon was not correlated with severity of PD (neither **dopa-responsiveness** nor **UPDRS-III axial score** of PD patients). *Statistical analysis*: Spearman's correlation
Annerino et al., 2012 [2]	No — Myenteric neuron density* was not correlated with age of PD patients. *Statistical analysis*: Unspecified correlation test	No — Myenteric neuron density* was not correlated with age at PD onset. *Statistical analysis*: Unspecified correlation test	No — Myenteric neuron density* was not correlated with duration of PD. *Statistical analysis*: Unspecified correlation test	No — Myenteric neuron density* was not correlated with severity of PD (**Modified HY** [15] stage (state **N/A**)). *Statistical analysis*: Unspecified correlation test
Desmet et al., 2017 [8]	No — Submucosal neurons per ganglion in the duodenum was not correlated with age of PD patients. Also: Neither submucosal neurons per biopsy nor submucosal ganglia per biopsy were correlated with age of PD patients. *Statistical analysis*: Spearman's correlation	**N/A** — Correlation not performed.	No — Submucosal neurons per ganglion in the duodenum was not correlated with duration of PD. Also: Neither submucosal neurons per biopsy nor submucosal ganglia per biopsy were correlated with duration of PD. *Statistical analysis*: Spearman's correlation	No — Submucosal neurons per ganglion in the duodenum was not correlated with severity of PD (neither **UPDRS-III/MDS-UPDRS (III)**[?] score (during **OFF-state**) nor **HY/Modified HY**[?] stage (during **OFF-state**) of PD patients). Also: Neither submucosal neurons per biopsy nor submucosal ganglia per biopsy were correlated with severity of PD (neither **UPDRS-III/MDS-UPDRS (III)**[?] score (during **OFF-state**) nor **HY/Modified HY**[?] stage (during **OFF-state**) of PD patients). *Statistical analysis*: Spearman's correlation

N/A, Not answered; UPDRS-III, Unified Parkinson's Disease Rating Scale — Part III: Motor Examination (assessment of PD motor symptom severity [13]); MDS-UPDRS (III), MDS-sponsored Revision of the Unified Parkinson's Disease Rating Scale — Part III: Motor Examination [16]; OFF-state, PD patient functional state without levodopa treatment (symptomatic relief from PD motor symptoms [26]); ON-state, PD patient functional state with levodopa treatment; Dopa-responsiveness, % of UPDRS-III [13] score improvement from OFF-state following levodopa administration [6]; UPDRS-III axial score, Sum of responses to UPDRS-III [13] items 18, 19, 22, 27-30 (during ON-state) [6]; HY, Original Hoehn and Yahr scale (assessment of disability and impairment severity in PD patients [15, 19]); Modified HY, Modified Hoehn and Yahr scale [15]. [+]Study which sought to investigate intestinal enteric neuron loss in PD and also performed at least one correlation between PD patient demographic/s and intestinal enteric neuron quantities.*Myenteric neuron density was calculated for two intestinal regions (duodenum and transverse colon, separately) and the stomach — unclear if correlation was run between total myenteric neuron density across all examined areas (stomach + duodenum + transverse colon) and/or between myenteric neuron density in each examined area, separately. [?]Version utilized in this study is unclear.

Supplementary Table 5. Correlations between PD patient demographics and constipation severity score

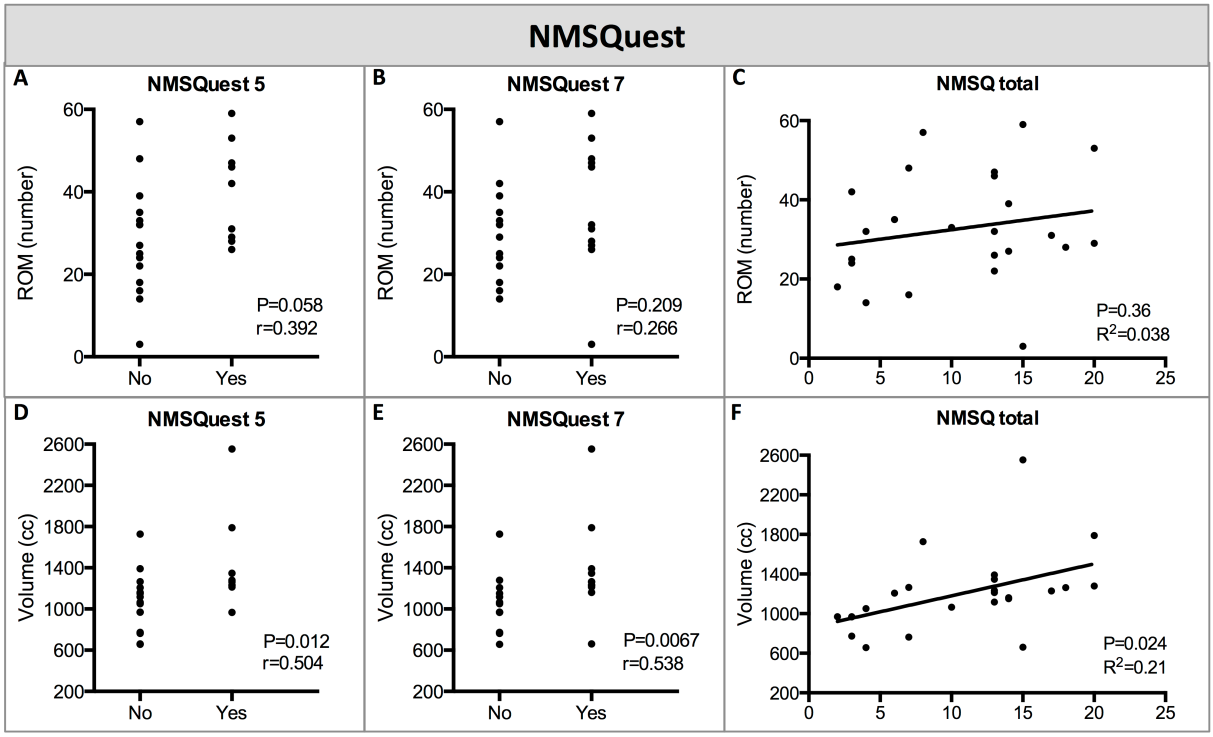
Reference[+]	Correlation between constipation severity score and PD patient age?	Correlation between constipation severity score and age at PD onset?	Correlation between constipation severity score and PD duration?	Correlation between constipation severity score and PD severity?
Giancola et al., 2017 [9]	**N/A** — Correlation not performed.	**N/A** — Age at PD onset was not reported nor correlated with constipation severity score.	**N/A** — Correlation not performed. *NOTE:* Number of residual intracolonic pellets/**ROM** (**CTT**) was positively correlated**^** with duration of PD (*Statistical analysis*: Spearman's correlation).	Yes — Constipation severity score***** was positively correlated**#** with severity of PD (**UPDRS-III** score). *Statistical analysis*: Spearman's correlation
Barrenschee et al., 2017 [7]	**N/A** — Correlation not performed.	**N/A** — Age at PD onset was not reported nor correlated with constipation severity score.	**N/A** — PD duration was not reported nor correlated with constipation severity score.	No — Constipation severity score**~** was not correlated with severity of PD (**UPDRS-III** score). *Statistical analysis*: Spearman's correlation

N/A, Not answered; ROM, Radiopaque markers; CTT, Colonic transit time (prolonged CTT typically indicative of slow transit constipation in PD patients [30-32]); UPDRS-III, Unified Parkinson's Disease Rating Scale — Part III: Motor Examination (assessment of PD motor symptom severity [13]).[+]Study which sought to investigate intestinal enteric neuron loss in PD and also performed at least one correlation between PD patient demographic/s and constipation severity score. **^**This correlation should be interpreted with caution (Supplementary Figure 2). *****Assessment of constipation severity was not described in the main article, but a constipation severity score appears to have been derived from the sum of positive responses to 6 constipation items on the Rome III Diagnostic Questionnaire for Adult Functional Gastrointestinal Disorders (Supplementary Figure 2). **#**This correlation appears to have involved less than half of the PD patients who had their UPDRS-III score recorded (Supplementary Figure 2). **~**It is unclear whether any PD patient was 'constipated' according to their CSS score (Supplementary Table 3).

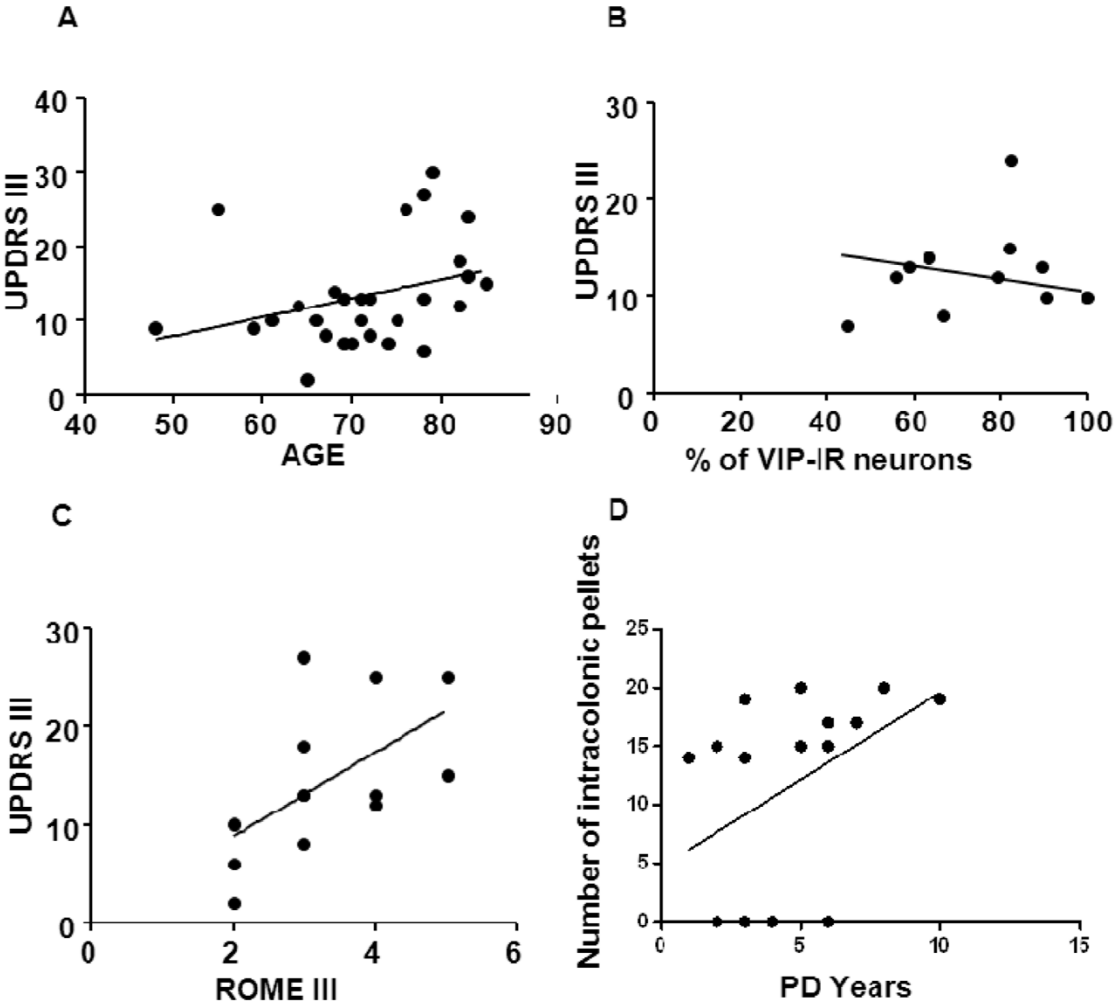
Supplementary Table 6. Outcomes were incompletely/inconsistently reported across the relevant PD-related studies

Reference[+]	Relevant descriptive statistics provided?	Relevant p-value(s) provided?	Individual participant data provided?	Relevant data approximate a normal distribution? Parametric or non-parametric tests performed?
Lebouvier et al., 2008 [5]	*Neurons per ganglion:* Yes[^] (Mean ± **SD**)	*Neurons per ganglion:* Yes[^] (From an unspecified statistical test)	*Neurons per ganglion:* No	**N/A[*]** Parametric tests seem to have been performed
Lebouvier et al., 2010 [6]	*Neurons per ganglion:* Yes[#] (Mean ± **SD**) *Constipation severity score [R]:* No	*Neurons per ganglion:* Yes (From an unpaired t-test) *Constipation severity score [R]:* No[~]	*Neurons per ganglion:* Reported mean number for each PD patient but not for individual control subjects *Constipation severity score [R]:* Reported for each PD patient but not for individual control subjects[~]	**N/A[*]** Parametric tests were performed
Annerino et al., 2012 [2]	*Neurons per ganglion area (neurons/mm^2):* Yes (Mean ± **SEM**)	*Neurons per ganglion area (neurons/mm^2):* No (Used a mixed-factor repeated measures **ANOVA**)	*Neurons per ganglion area (neurons/mm^2):* Yes	**N/A[*]** Parametric tests were performed
Desmet et al., 2017[P] [8]	*Neurons per ganglion:* No[@]	*Neurons per ganglion:* Yes (From a Wilcoxon matched-pairs signed rank test)	*Neurons per ganglion:* Yes[@]	**N/A[*]** Non-parametric tests were performed[&]
Giancola et al., 2017 [9]	*Neurons per ganglion:* Yes (Mean ± **SD**) *Constipation severity score [R]:* No[?] *Number of residual intracolonic pellets/**ROM** (**CTT**):* Yes (Mean ± **SD**)	*Neurons per ganglion:* Yes (From a one-way **ANOVA**) *Constipation severity score [R]:* No[?] *Number of residual intracolonic pellets/**ROM** (**CTT**):* Yes (Potentially from a one-way **ANOVA**)	*Neurons per ganglion:* No[!] *Constipation severity score [R]:* No[?] *Number of residual intracolonic pellets/**ROM** (**CTT**):* Reported for each PD patient tested but not for the constipated controls[!]	**N/A[*]** Parametric tests were performed
Barrenschee et al., 2017 [7]	*Neurons per ganglion area (neurons/µm^2):* Yes (Mean ± **SD**) *Constipation severity score (CSS score):* Yes (Mean ± **SD**)	*Neurons per ganglion area (neurons/µm^2):* No (Used a Mann-Whitney U test) *Constipation severity score (CSS score):* No (Used a Mann-Whitney U test)	*Neurons per ganglion area (neurons/µm^2):* No *Constipation severity score (CSS score):* No	**N/A[*]** Non-parametric tests were performed[&]

SD, Standard deviation; SEM, Standard error of the mean; N/A, Not answerable; ANOVA, Analysis of variance; ROM, Radiopaque markers; CTT, Colonic transit time. [+]Study which sought to quantitatively evaluate intestinal enteric neuron loss in PD. [^]The relevant sample sizes (n = 3-5 in each group) are too small for statistical testing to produce meaningful results. [*]Regardless of how the sample data appear to be distributed, sample size is too low to be confident that the sample distribution reflects the population data distribution. [#]For both the PD and control group, two disparate mean ± SD results were reported (specifically, the mean ± SD for neurons per ganglion in PD patients vs. control subjects reported in the results section (main text) were different from those reported in Table 2 [6]). [R]The sum of positive responses to 6 constipation items on the Rome III Diagnostic Questionnaire for Adult Functional Gastrointestinal Disorders [6] or appears to be [9] (Supplementary Figure 2). [~]It is unclear whether control subjects had their constipation severity scores determined [6]. [P]Implemented pairwise participant recruitment (allowed within-pairs comparisons between each PD patient and their healthy partner [8]) – this was not a feature of any other study[+]. [@]A supplementary Prism file containing individual participant data and results of the relevant Wilcoxon matched-pairs signed rank test was provided [8]. [&]When only p-values are provided, non-parametric test results cannot be included in a meta-analysis. Mean and SD information allows results of non-parametric tests to be approximately combined with other studies using parametric tests. [?]Assessment of constipation severity was not described in the main article, but a correlation involving the severity of Rome III criteria-defined constipation in PD (on a scale of 0-6) was included in the supporting results [9]. [!]These results can be obtained from the authors (Prof Roberto De Giorgio and Prof Fiorella Giancola) upon reasonable request.



Supplementary Figure 1. Correlations between PD non-motor symptoms (evaluated via the NMSQuest) and colonic volume/ROM (CTT) [33]. One PD patient who reported < 3 bowel movements per week or straining during defecation (Responded 'Yes' to NMSQuest Question 5, D) and sensation of incomplete bowel emptying following defecation (Responded 'Yes' to NMSQuest Question 7, E) had a colonic volume much greater than any other PD patient examined (colonic volume of 2600cc), which appears to be responsible for the strength of the correlation between NMSQuest Question 5 and colonic volume (D, r = 0.504) and between NMSQuest Question 7 and colonic volume (E, r = 0.538). Reprinted from Journal of Parkinson's Disease, 7, Knudsen et al., Objective Colonic Dysfunction is Far more Prevalent than Subjective Constipation in Parkinson's Disease: A Colon Transit and Volume Study, 359-367 © 2017 [33], with permission from IOS Press and Dr Karoline Knudsen. The publication is available at IOS Press through http://dx.doi.org/10.3233/JPD-161050.



Supplementary Figure 2. Significant Spearman's correlations involving PD patient demographics and/or results [9]. A) Age was positively correlated with PD motor symptom severity (UPDRS-III score). Data from 28/29 PD patients were included in this correlation — UPDRS-III score was missing for one patient [9]. B) Proportion of submucosal neurons that were VIP-positive in the descending colon was negatively correlated with UPDRS-III score. It appears that data from 11/15 PD patients who underwent biopsy collection for subsequent submucosal plexus assessment were included in this correlation.^* Furthermore, the regression line direction (downwards = negative correlation) does not appear to fit the data presented. C) Constipation severity score (appears to be derived from the sum of positive responses to 6 constipation items on the Rome III Diagnostic Questionnaire for Adult Functional Gastrointestinal Disorders, although constipation severity assessment was not described in the main article) was positively correlated with UPDRS-III score. It appears that data from only 12/28 PD patients who had their UPDRS-III score recorded were included in this correlation.^* D) Number of residual intracolonic pellets/ROM (CTT) was positively correlated with PD duration. It appears that data from 15/22 PD patients who underwent CTT evaluation and had their PD duration recorded were included in this correlation.^* Regardless, the steepness of the regression line (correlation strength) does not appear to fit the data presented. ^It may be the case that data from a greater number of PD patients were included in this correlation, but that data points overlap in the scatterplot. *PD patients who did not complete the entire design of this study [9] were excluded from correlation analyses [11]. Reproduced by permission from John Wiley and Sons: Neurogastroenterology & Motility, 29, Giancola et al., Downregulation of neuronal vasoactive intestinal polypeptide in Parkinson's disease and chronic constipation, e12995 © 2016 John Wiley & Sons Ltd [9].

REFERENCES

[1] Singaram C, Ashraf W, Gaumnitz EA, Torbey C, Sengupta A, Pfeiffer R, Quigley EM (1995) Dopaminergic defect of enteric nervous system in Parkinson's disease patients with chronic constipation. *Lancet* **346**, 861-864.

[2] Annerino DM, Arshad S, Taylor GM, Adler CH, Beach TG, Greene JG (2012) Parkinson's disease is not associated with gastrointestinal myenteric ganglion neuron loss. *Acta Neuropathol* **124**, 665-680.

[3] Corbillé A-G, Coron E, Neunlist M, Derkinderen P, Lebouvier T (2014) Appraisal of the dopaminergic and noradrenergic innervation of the submucosal plexus in PD. *J Parkinsons Dis* **4**, 571-576.

[4] Chng YS. Demystifying Statistical Analysis 7: Data Transformations and Non-Parametric Tests, https://towardsdatascience.com/demystifying-statistical-analysis-7-data-transformations-and-non-parametric-tests-7c02cc4c8168, Accessed September 27, 2021.

[5] Lebouvier T, Chaumette T, Damier P, Coron E, Touchefeu Y, Vrignaud S, Naveilhan P, Galmiche J-P, Brunley des Varannes S, Derkinderen P, Neunlist M (2008) Pathological lesions in colonic biopsies during Parkinson's disease. *Gut* **57**, 1741-1743.

[6] Lebouvier T, Neunlist M, Bruley des Varannes S, Coron E, Drouard A, N'Guyen J-M, Chaumette T, Tasselli M, Paillusson S, Flamand M, Galmiche J-P, Damier P, Derkinderen P (2010) Colonic biopsies to assess the neuropathology of Parkinson's disease and its relationship with symptoms. *PLoS One* **5**, e12728.

[7] Barrenschee M, Zorenkov D, Böttner M, Lange C, Cossais F, Scharf AB, Deuschl G, Schneider SA, Ellrichmann M, Fritscher-Ravens A, Wedel T (2017) Distinct pattern of enteric phospho-alpha-synuclein aggregates and gene expression profiles in patients with Parkinson's disease. *Acta Neuropathol Commun* **5**, 1.

[8] Desmet A-S, Cirillo C, Tack J, Vandenberghe W, Vanden Berghe P (2017) Live calcium and mitochondrial imaging in the enteric nervous system of Parkinson patients and controls. *eLife* **6**, e26850.

[9] Giancola F, Torresan F, Repossi R, Bianco F, Latorre R, Ioannou A, Guarino M, Volta U, Clavenzani P, Mazzoni M, Chiocchetti R, Bazzoli F, Travagli RA, Sternini C, De Giorgio R (2017) Downregulation of neuronal vasoactive intestinal polypeptide in Parkinson's disease and chronic constipation. *Neurogastroenterol Motil* **29**, e12995.

[10] Ohlsson B, Englund E (2019) Atrophic myenteric and submucosal neurons are observed in Parkinson's disease. *Parkinsons Dis* **2019**, 7935820.

[11] De Giorgio R, Giancola F (2021), personal communications.

[12] Hughes AJ, Daniel SE, Kilford L, Lees AJ (1992) Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. *J Neurol Neurosurg Psychiatry* **55**, 181-184.

[13] Fahn S, Elton R, Members of the UPDRS Development Committee (1987) The Unified Parkinson's Disease Rating Scale. In *Recent Developments in Parkinson's Disease, Vol 2.*, Fahn S, Marsden CD, Calne DB, Goldstein M, eds. Macmillan Healthcare Information, Florham Park, NJ, pp. 153-163, 293-304.

[14] Postuma RB, Berg D, Stern M, Poewe W, Olanow W, Oertel W, Obeso J, Marek K, Litvan I, Lang AE, Halliday G, Goetz CG, Gasser T, Dubois B, Chan P, Bloem BR, Adler CH, Deuschl G (2015) MDS clinical diagnostic criteria for Parkinson's disease. *Mov Disord* **30**, 1591-1599.

[15] Goetz CG, Poewe W, Rascol O, Sampaio C, Stebbins GT, Counsell C, Giladi N, Holloway RG, Moore CG, Wenning GK, Yahr MD, Seidl L (2004) Movement Disorder Society Task Force Report on the Hoehn and Yahr Staging Scale: status and recommendations. *Mov Disord* **19**, 1020-1028.

[16] Goetz CG, Tilley BC, Shaftman SR, Stebbins GT, Fahn S, Martinez-Martin P, Poewe W, Sampaio C, Stern MB, Dodel R, Dubois B, Holloway R, Jankovic J, Kulisevsky J, Lang AE, Lees A, Leurgans S, LeWitt PA, Nyenhuis D, Olanow CW, Rascol O, Schrag A, Teresi JA, van Hilten JJ, LaPelle N (2008) Movement Disorder Society-Sponsored Revision of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS): scale presentation and clinimetric testing results. *Mov Disord* **23**, 2129-2170.

[17] Martínez-Martín P, Rodríguez-Blázquez C, Alvarez M, Arakaki T, Arillo VC, Chaná P, Fernández W, Garretto N, Martínez-Castrillo JC, Rodríguez-Violante M, Serrano-Dueñas M, Ballesteros D, Rojo-Abuin JM, Chaudhuri KR, Merello M (2015) Parkinson's disease severity levels and MDS-Unified Parkinson's Disease Rating Scale. *Parkinsonism Relat Disord* **21**, 50-54.

[18] Gelb DJ, Oliver E, Gilman S (1999) Diagnostic criteria for Parkinson disease. *Arch Neurol* **56**, 33-39.

[19] Hoehn MM, Yahr MD (1967) Parkinsonism: onset, progression, and mortality. *Neurology* **17**, 427-442.

[20] Elkouzi A. Young Onset Parkinson's, https://www.parkinson.org/Understanding-Parkinsons/What-is-Parkinsons/Young-Onset-Parkinsons, Accessed October 6, 2021.

[21] John Hopkins Medicine. Young-Onset Parkinson's Disease, https://www.hopkinsmedicine.org/health/conditions-and-diseases/parkinsons-disease/youngonset-parkinsons-disease, Accessed October 6, 2021.

[22] The Michael J. Fox Foundation. Young-Onset Parkinson's Disease, https://www.michaeljfox.org/news/young-onset-parkinsons-disease, Accessed October 6, 2021.

[23] Parkinson's Australia. Young onset Parkinson's, https://www.parkinsons.org.au/young-onset-parkinson-s, Accessed October 6, 2021.

[24] Adler CH, Beach TG, Hentz JG, Shill HA, Caviness JN, Driver-Dunckley E, Sabbagh MN, Sue LI, Jacobson SA, Belden CM, Dugger BN (2014) Low clinical diagnostic accuracy of early vs advanced Parkinson disease: Clinicopathologic study. *Neurology* **83**, 406-412.

[25] Postuma RB, Poewe W, Litvan I, Lewis S, Lang AE, Halliday G, Goetz CG, Chan P, Slow E, Seppi K, Schaffer E, Rios-Romenets S, Mi T, Maetzler C, Li Y, Heim B, Bledsoe IO, Berg D (2018) Validation of the MDS Clinical Diagnostic Criteria for Parkinson's disease. *Mov Disord* **33**, 1601-1608.

[26] Mercuri NB, Bernardi G (2005) The 'magic' of L-dopa: why is it the gold standard Parkinson's disease therapy? *Trends Pharmacol Sci* **26**, 341-344.

[27] Agachan F, Chen T, Pfeifer J, Reissman P, Wexner SD (1996) A constipation scoring system to simplify evaluation and management of constipated patients. *Dis Colon Rectum* **39**, 681-685.

[28] Krogh K, Østergaard K, Sabroe S, Laurberg S (2008) Clinical aspects of bowel symptoms in Parkinson's disease. *Acta Neurol Scand* **117**, 60-64.

[29] Frazzitta G, Ferrazzoli D, Folini A, Palamara G, Maestri R (2019) Severe constipation in Parkinson's disease and in parkinsonisms: prevalence and affecting factors. *Front Neurol* **10**, 621.

[30] Pfeiffer RF (2018) Gastrointestinal dysfunction in Parkinson's disease. *Curr Treat Options Neurol* **20**, 54.

[31] Su A, Gandhy R, Barlow C, Triadafilopoulos G (2017) A practical review of gastrointestinal manifestations in Parkinson's disease. *Parkinsonism Relat Disord* **39**, 17-26.

[32] Skjærbæk C, Knudsen K, Horsager J, Borghammer P (2021) Gastrointestinal dysfunction in Parkinson's disease. *J Clin Med* **10**, 493.

[33] Knudsen K, Fedorova TD, Bekker AC, Iversen P, Østergaard K, Krogh K, Borghammer P (2017) Objective colonic dysfunction is far more prevalent than subjective constipation in Parkinson's disease: a colon transit and volume study. *J Parkinsons Dis* **7**, 359-367.