Effect of vagotomy on modulation of dopaminergic neurons in Parkinson's disease

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Effect of vagotomy on modulation of dopaminergic neurons in Parkinson's disease

Directed by Professor Phil Hyu Lee

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ABSTRACT

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Background: Growing evidence has suggested that Lewy body pathology would arise from the gastrointestinal system and spread to the brain via the vagal nerve, which is modulated by vagotomy. This study aimed to investigate whether vagotomy itself could have a protective effect on the survival of nigral dopaminergic neurons in a parkinsonian animal model. Additionally, we investigated whether gastrectomy and vagotomy prior to parkinsonian symptom onset could alleviate the neurodegenerative load in patients with Parkinson's disease.

Methods: To establish a parkinsonian animal model, male C57BL/6J mice at 5 weeks of age were intraperitoneally injected with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) for five days. A subset of mice underwent left cervical vagotomy prior to MPTP administration. Six weeks after MPTP administration, mice were

sacrificed and the substantia nigra (SN) was sectioned for immunohistochemical detection of dopaminergic neurons (tyrosine hydroxylase [TH]) and microglia (ionized calcium binding adaptor molecule-1 [Iba-1]). Western blotting analyses for TH and Iba-1 and inflammatory cytokine analyses using Enzyme-Linked Immunosorbent Assay were also performed.

Additionally, fifty-one patients with de novo Parkinson's disease who had undergone gastrectomy and vagotomy prior to motor symptom onset (gastrectomy group) were enrolled from three university hospitals and then, matched with 204 patients with Parkinson's disease without a gastrectomy history (non-gastrectomy group). We performed inter-group comparative analyses of the striatal dopamine transporter availability and level of cognitive performance. We assessed the longitudinal changes in the levodopa-equivalent dose over time in 29 patients with Parkinson's disease who had previously undergone gastrectomy/vagotomy (follow-up >2 years) and compared the estimated monthly levodopa-equivalent dose with that in patients without a previous history of gastrectomy/vagotomy using a linear mixed model.

Results: Decreases in the number of TH-positive neurons as well as immunoblotting band densities of TH in the SN were comparable between the parkinsonian animal models according to the vagotomy procedure. Meanwhile, Iba-1 expression in the SN was increased in the parkinsonian animal model without vagotomy, but was significantly attenuated in the parkinsonian animal model with vagotomy. Expression of interleukin-1β and interleukin-6 was increased in both parkinsonian animal models, while the parkinsonian animal model group with

vagotomy exhibited less markedly increased expression of these pro-inflammatory cytokines.

In clinical data, there was no significant difference in demographic characteristics between the Parkinson's disease groups. The gastrectomy group had less severely decreased dopamine transporter availability in the posterior putamen compared to the non-gastrectomy group (p = 0.006) after adjusting for age at onset, sex, and Parkinson's disease duration. The gastrectomy group had a slower longitudinal increase in dopaminergic medication doses than the non-gastrectomy group (p <0.001). However, the gastrectomy group showed poorer cognitive performance in the attention/working memory (p = 0.013), frontal/executive (p = 0.029), and memory function domains (p = 0.023) relative to the non-gastrectomy group.

Conclusions: Our results suggest that vagotomy has protective effects on nigrostriatal dopaminergic degeneration and disease progression in subsequent Parkinson's disease, but may be unfavorable for cognitive performance. The neuroprotective effect of vagotomy could be largely mediated by blocking the spread of α-synuclein from the gut to the brain, and the modulation of inflammatory response might also contribute to some extent.

Key words: dopaminergic neuron; gastrectomy; neuroprotection; vagotomy; parkinson's disease

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I. INTRODUCTION

Parkinson's disease is pathologically characterized by Lewy bodies (LB) in the substantia nigra (SN) and has been clinically regarded as having a complex clinical entity. Along with cardinal motor signs,[1] Parkinson's disease is frequently accompanied by nonmotor symptoms including hyposmia, rapid eye movement sleep behavior disorder (RBD), depression, and constipation, appearing several years before the onset of motor features.[2] A number of studies have reported that LB pathology was detected in the neural correlates of these nonmotor symptoms prior to the Parkinson's disease diagnosis,[3, 4] and Braak et al.[5] proposed the dual-hit hypothesis in their pathological series, suggesting that LB pathology would arise from the gastrointestinal and olfactory bulb systems and spread to the brain.

There is ample evidence to suggest that α-synuclein aggregates exhibit prion-like cell-to-cell transmission,[6, 7] and the spread of LB pathology from the gut to the brain occurs via the vagal nerve, which was blocked by vagotomy in animal studies.[8-11] Recently, two epidemiological studies have been reported regarding the protective effect of truncal vagotomy against developing Parkinson's disease.[12, 13] However, the lack of information on the clinical characteristics of patients with vagotomy prior to Parkinson's disease onset limits their clinical significance with some important questions: Is the dual-hit hypothesis clinically implicated in Parkinson's disease? Does vagotomy alleviate the pathological burden by preventing the spread of α-synuclein aggregates from the gut even after the onset of Parkinson's disease? Does vagotomy itself have a protective effect against the neurodegenerative processes underlying Parkinson's disease?

Furthermore, a recent study reported that vagal nerve stimulation had a protective effect on the survival of nigral dopaminergic neurons in a parkinsonian animal model.[14] This result suggests that the vagal nerve modulation (either vagal nerve stimulation or vagotomy, which may counteract each other or may not) would be one of promising disease-modifying strategies in Parkinson's disease. In this regard, further studies are needed to elucidate the effect of vagotomy on the modulation of dopaminergic neurons in Parkinson's disease and its possible underlying mechanisms.

In the present study, we hypothesized that vagotomy would favorably affect the loss of nigral dopaminergic neurons and clinical features in patients with Parkinson's disease, via the modulation of inflammatory and/or oxidative

stress processes as well as the blockade of the spread of α-synuclein from the gut to the brain. To clarify this issue, we first investigated the effect of vagotomy on the survival of nigral dopaminergic neurons in a parkinsonian animal model. Additionally, we explored the possible underlying mechanisms of the neuroprotective effect of vagotomy, including alterations of the glial activation state and inflammatory cytokines. Second, we investigated the neuroprotective effect of vagotomy in patients with Parkinson's disease who had previously undergone gastrectomy, considering that gastrectomy and vagotomy are usually performed together. We assessed the dopamine transporter (DAT) availability of the striatum, longitudinal disease progression, and level of cognitive performance in patients with Parkinson's disease depending on a previous history of gastrectomy.

II. MATERIALS AND METHODS

1. Experimental data

A. Animals

All procedures of this animal research were performed in accordance with the Laboratory Animal Welfare Act, the Guide for the Care and Use of Laboratory Animals and the Guidelines and Policies for Rodent experiment provided by the IACUC (Institutional Animal Care and Use Committee) in Yonsei University Health System. Male C57BL/6J mice (4 weeks old) were acclimated in a climate-controlled room with a constant 12-hour light/dark cycle (12-hour on, 12-hour off) for a week prior to the construction of

parkinsonian animal model (i.e., initiation of drug administration). At 5 weeks of age, the mice were randomly divided into four groups (A-D) as follows: Group A = Parkinsonian model group without vagotomy (n = 8); Group B = Parkinsonian model group with vagotomy (n = 8); Group C = Control group (n = 8); and Group D = Vagotomy group (n = 8).

B. Establishment of parkinsonian animal model

To establish the parkinsonian animal model, the mice in group A (parkinsonian model group without vagotomy) and group B (parkinsonian model group with vagotomy) received sub-acute injection of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP; Simga, St. Louis, MO, USA; 25 mg/kg), which was freshly dissolved in 20% dimethyl sulfoxide (DMSO) / 80% normal saline, intraperitoneally for 5 days. The mice in group C (control group) and group D (vagotomy group) did not receive sub-acute injection of MTPT.

C. Left cervical vagotomy procedure

Additionally, the mice in group B (parkinsonian model group with vagotomy) were subjected to left cervical vagotomy 2 days before MPTP administration. A unilateral vagotomy was chosen because bilateral vagotomy is lethal in mice. Briefly, mice were anesthetized and a small vertical skin incision on the neck was made to expose the left cervical vagus trunk. The left vagal nerve was identified in close contact with the carotid artery and sympathetic nerve using a microscope. Then, the left vagal nerve was isolated and teased

away from carotid artery using small curved forceps, and clearly resected. Finally, the skin was sutured and antibiotic ointment was applied to incision area (Figure 1). The mice in group D (vagotomy group) also underwent left cervical vagotomy at 5 weeks of age.

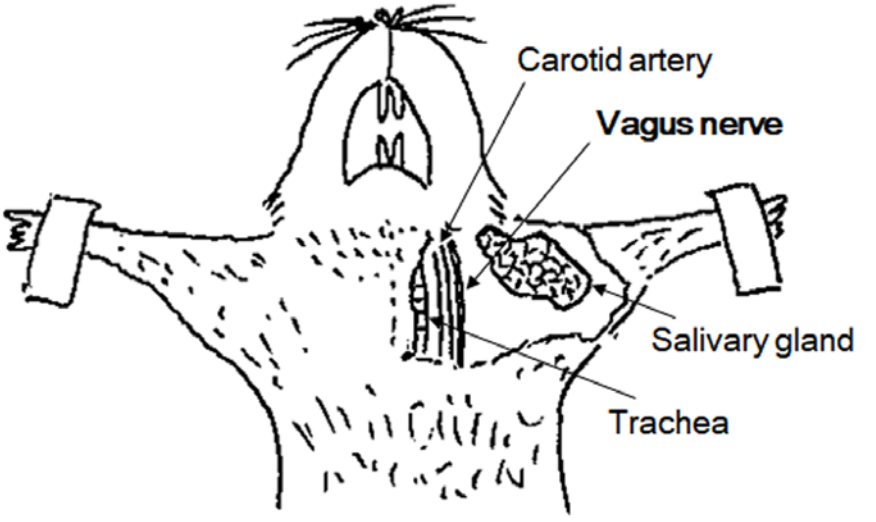

Figure 1. A schematic drawing presenting the left cervical vagotomy procedure. To expose the left cervical vagus trunk, the salivary gland and neck muscles need to be pulled aside or cut with straight scissors.

D. Experimental design

As described above, group A (parkinsonian model group without vagotomy) consisted of mice which received sub-acute injection of MPTP for 5 days at 5 weeks of age, while group B (parkinsonian model group with vagotomy) consisted of mice which were subjected to left cervical vagotomy 2 days before MPTP administration. Group C (control group) comprised mice which did not receive any intervention (i.e., either MPTP administration or left cervical vagotomy procedure), while group D (vagotomy group) underwent left cervical vagotomy at 5 weeks of age. Mice in this experimental study were sacrificed at 12 weeks of age. In other words, mice in the parkinsonian model groups (groups A and B) were injected with MPTP for 5 days at 5 weeks of age, and then were sacrificed after 6 weeks (Figure 2).

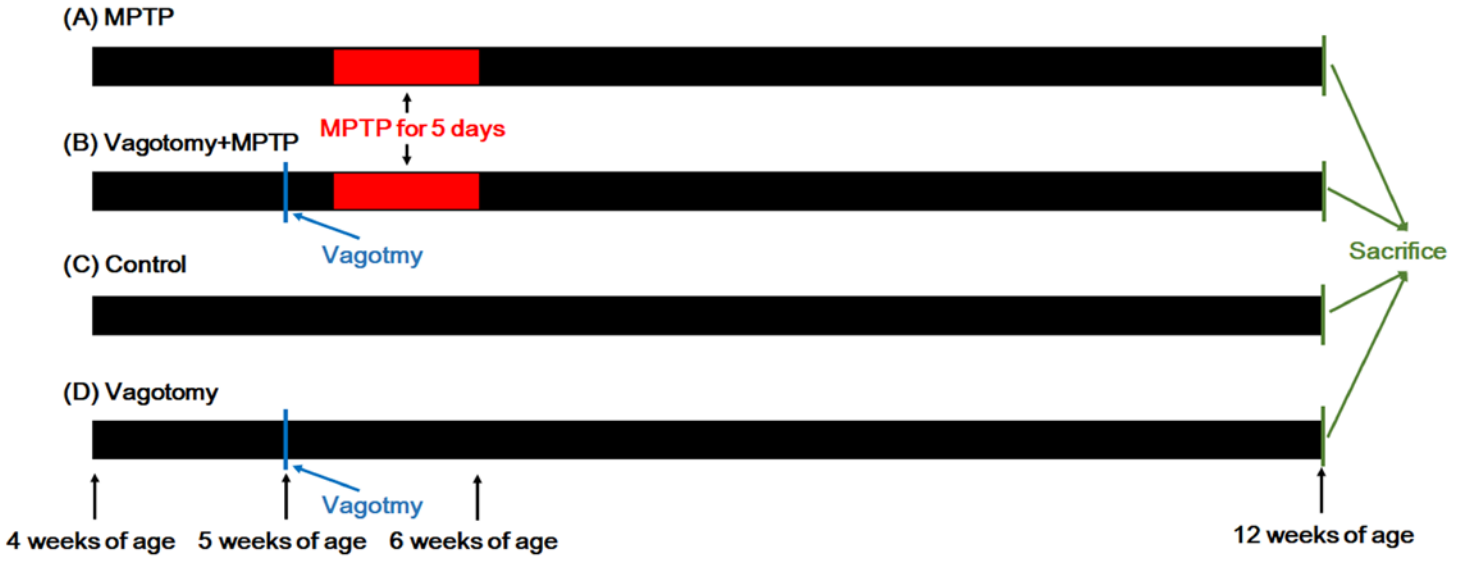

Figure 2. Experimental design. Male C57BL/6J mice at 5 weeks of age were randomly divided into four groups as follows: (A) Parkinsonian model group without vagotomy. (B) Parkinsonian model group with vagotomy. (C) Control group. (D) Vagotomy group.

E. Preparation of brain tissue

For immunohistochemistry, the mice were perfused with 4% paraformaldehyde. Brains were harvested from the skulls, post-fixed overnight in 4% paraformaldehyde, and stored in 30% sucrose solution for 1-2 days at 4 ℃ until they sank. Then, 25-μm-thick coronal sections were obtained using a cryostat. The sections were stored in tissue storage solution (30% glycerol, 30% ethylene glycol, 30% distilled water, 10% 0.2 M phosphate buffer [PB]) at 4 ℃ until required.

F. Immunohistochemistry

Brain tissue was immunostained with tyrosine hydroxylase (TH) and ionized calcium binding adaptor molecule-1 (Iba-1) in the SN, which identified dopaminergic neurons and activated microglia, respectively, to investigate the protective effect of vagotomy in MPTP parkinsonian animal model. First, brain tissues were frozen with optimal cutting temperature compound (OCT compound; Sakura Finetek) and 25-μm-thick coronal sections were obtained using a cryostat. Sections were immunostained using 3,3-diaminobenzidine (DAB) method. Brain sections were washed twice in phosphate-buffered saline (PBS) and incubated in 0.2% Triton X-100 (Sigma) for 15 minutes at room temperature. They were blocked with 0.5% bovine serum albumin (BSA; Sigma) for 30 minutes. After blocking, they were rinsed three times with 0.25% BSA and incubated overnight at 4 ℃ with specific primary antibodies. The antibodies were detected with 0.05% DAB (Vector Laboratories).

G. Cell counting of tyrosine hydroxylase-positive neurons

To determine the number of TH-positive cells in the granule cell layer, five sections of the midbrain per mouse were counted. Each experimental group consisted of four mice. All of the counting was performed under a microscope using a 10x objective in stacks of five optical sections.

H. Western blotting analysis

Brain tissues were dissolved in ice-cold radioimmunoprecipitation assay buffer (RIPA buffer; 50 mM Tris-HCl, pH 7.5, with 150 mM sodium chloride, 1% Triton X-100, 1% sodium deoxycholate, 0.1% sodium dodecyl sulfate [SDS], 2 mM Ethylenediaminetetraacetic acid [EDTA] sterile solution; Lugen Sci, Korea) plus protease inhibitor cocktail (Sigma). The lysates were centrifuged at 4 ℃ for 20 minutes (14,000 g) and supernatants were transferred to fresh tubes. Briefly, 30 μg of protein was separated by SDS-gel electrophoresis and transferred to hydrophobic polyvinylidene difluoride (PVDF) membranes (GE Healthcare, Little Chalfont, UK). The membranes were blocked in 5% skim milk in PBST (PBS with Tween 20). Membranes were probed with the following primary antibodies: rabbit anti-TH (Abcam), mouse anti-Iba-1 (Abcam), and mouse anti-Actin (Santacruz). As secondary antibodies, 1:5,000 dilutions of horseradish peroxidase-conjugated goat anti-rabbit antibody (Solarbio) and anti-mouse antibody (Solarbio) were used. Antigen-antibody complexes were visualized with Enhanced chemiluminescent (ECL) solution (GenDEPOT). For quantitative analyses, immunoblotting band densities were measured by image J.

I. Cytokine and Chemokine analysis

For cytokine and chemokine analysis, tissue lysate was analyzed for their cytokine content by Enzyme-Linked Immunosorbent Assay (ELISA): interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF-α). All were purchased from ELISA with the mouse enzyme immunoassay sets (R&D Systems, Inc., Minneapolis, MN, USA). Reactions were performed in duplicates. Analyses were performed according to the manufacturer's instructions. Data were analyzed via Legendplex V8.0 software (Biolegend) and specified as pg/mL.

J. Statistical analyses

The Kruskal-Wallis test was used to compare the number of TH-positive neurons and expression of TH, Iba-1, and pro-inflammatory cytokines between the groups. The statistical analyses were performed with SPSS software (version 23.0; IBM Corp., Armonk, NY, USA), and results with a two-tailed $p < 0.05$ were considered statistically significant.

2. Clinical data

A. Study design and participants

Patient data were collected from three tertiary referral hospitals (Severance Hospital, Ajou University Hospital, and Seoul St. Mary's Hospital). Among the patients who visited the Movement Disorders outpatient clinic from March 2006 and were diagnosed with Parkinson's disease according to the clinical

diagnostic criteria of the UK Parkinson's disease Society Brain Bank,[15] we reviewed the medical data of 51 patients with de novo Parkinson's disease with a history of gastrectomy due to gastric cancer a mean of 5.83 years prior to parkinsonian symptom onset (39 from Severance Hospital, seven from Ajou University Hospital, and five from Seoul St. Mary's Hospital). Of the 51 patients in the gastrectomy group, 14 underwent total gastrectomy and the other 37 underwent subtotal gastrectomy. Twenty-nine (56.9%) patients did not receive any additional treatment after gastrectomy, while 22 (8 in the total gastrectomy and 14 in the subtotal gastrectomy group) patients received adjuvant chemotherapy. Under general anesthesia, the patient was placed in supine position on the operative table. Omentectomy, vessel ligation, duodenal mobilization, and truncal vagotomy (either complete or partial) were performed during the surgical procedure. For total gastrectomy, two clamps were applied on the esophageal wall about 1 cm above the gastroesophageal junction to divide. Other two clamps were applied on the jejunal wall about 20 cm distance from the ligament of Treitz to resect the proximal jejunum, and then Roux-en-Y esophagojejunostomy was performed. For subtotal gastrectomy, the stomach was clamped in upper 1/4 portion to resect, and then gastroduodenostomy (Billroth-I) or gastrojejunostomy (Billroth-II) was performed. Among 51 patients in the gastrectomy group, a detailed description of gastrectomy procedure (i.e., operative note) was available in 32 patients (10 with total gastrectomy and 22 with subtotal gastrectomy). None of these 32 patients were reported to have undergone vagal nerve-preserving gastrectomy Eight (80.0%) patients in the total gastrectomy group and 11 (50.0%) patients in the subtotal

gastrectomy group were reported to have undergone complete truncal vagotomy. Three (13.6%) patients in the subtotal gastrectomy underwent incomplete truncal vagotomy. There was no description of whether or not vagotomy was performed in 10 patients (2 in the total gastrectomy group and 8 in the subtotal gastrectomy group) on the operative note. In addition, among 22 patients with subtotal gastrectomy, 10 (45.5%) underwent gastroduodenostomy (Billroth-I) and 12 (54.5%) underwent gastrojejunostomy (Billroth-II).

We also reviewed the database of the Yonsei Parkinson Center (483 consecutive patients with drug-naïve Parkinson's disease from April 2009 to August 2015; all patients from the database of the Yonsei Parkinson Center underwent an [^{18}F] N-(3-fluoropropyl)-2β-carbonethoxy-3β-(4-iodophenyl) nortropane (^{18}F-FP-CIT) positron emission tomography (PET) scan and were followed up for at least two years; 20 patients had a past history of gastrectomy and the other 463 patients did not) to perform comparative analyses between the Parkinson's disease groups according to the history of a previous gastrectomy (Figure 3). To reduce the effect of selection bias and potential confounding factors, estimated propensity scores were used to match the two Parkinson's disease groups with respect to the history of gastrectomy prior to Parkinson's disease onset. The propensity score was computed for each patient using a logistic regression model including the age at onset, sex, and Parkinson's disease duration as variables. Based on the propensity scores, the 51 patients with Parkinson's disease and a previous gastrectomy history (gastrectomy group) were matched to 204 of the 463 patients with Parkinson's disease without a history of gastrectomy (non-gastrectomy group). The propensity score

matching was performed with the R software package, version 3.4.0.

Parkinsonian motor symptoms were assessed using the Unified Parkinson's disease Rating Scale Part III (UPDRS- III), and olfactory function was measured by the cross-cultural smell identification test (CCSIT). The presence of RBD was evaluated using an RBD screening questionnaire with a cut-off score of 5/6.[16] Depression was evaluated using the Beck Depression Inventory (BDI). The Korean version of the Mini-Mental State Examination (K-MMSE) was used to assess general cognition, and the Parkinson's disease medication doses were calculated as levodopa-equivalent doses (LEDs).[17]

This study was approved by the Yonsei University Severance Hospital institutional review board. The need for informed consent was waived because of the retrospective nature of the study.

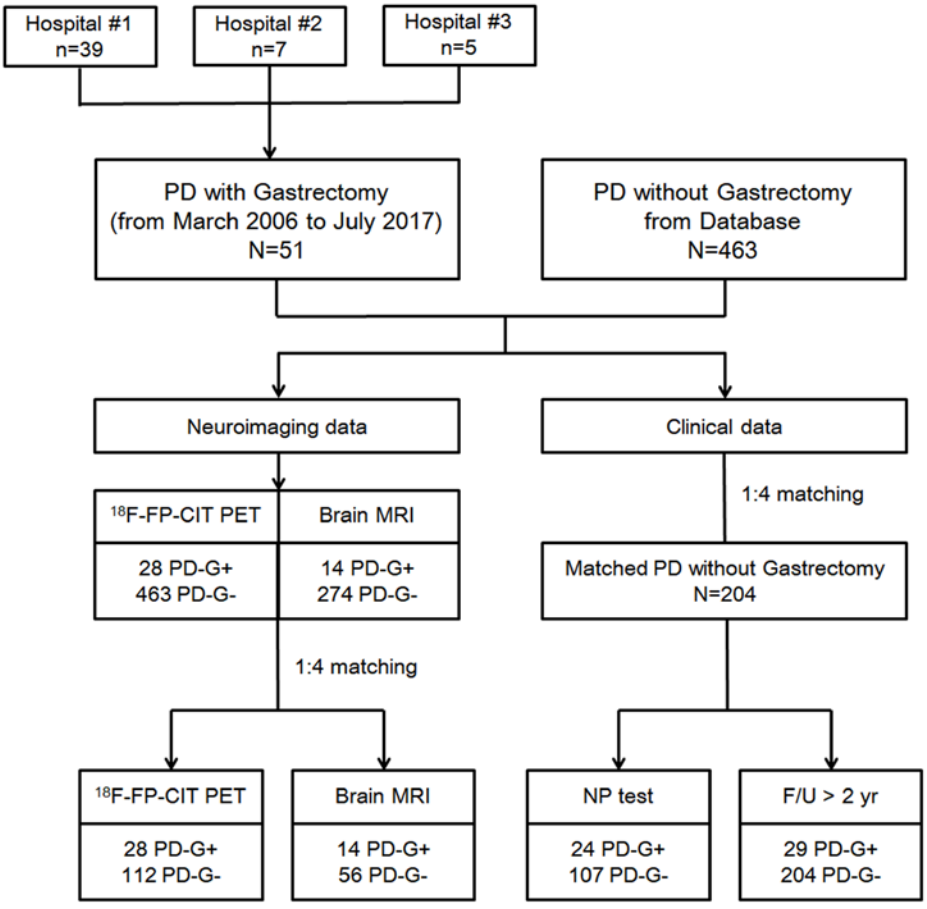

Figure 3. Flow diagram of participants enrollment. Abbreviation: PD, Parkinson's disease; PD-G+, PD with a previous history of gastrectomy; PD-G-, PD without a previous history of gastrectomy; ^{18}F-FP-CIT PET, [^{18}F] N-(3-fluoropropyl)-2β-carbonethoxy-3β-(4-iodophenyl) nortropane positron emission tomography; MRI, magnetic resonance imaging; NP test, neuropsychological test; F/U, follow-up.

B. Quantitative analysis of the ^{18}F-FP-CIT PET images

Twenty-eight out of 51 patients with Parkinson's disease in the gastrectomy group underwent an ^{18}F-FP-CIT PET scan. The propensity score matching was performed as described above to match these patients to 112 patients with Parkinson's disease without a gastrectomy history from the database of the Yonsei Parkinson Center (463 drug-naïve patients with Parkinson's disease who had no history of gastrectomy). We used the same methodology to obtain and analyze the ^{18}F-FP-CIT PET images as we employed in a previous study.[18]

The ^{18}F-FP-CIT PET scans were acquired using a Discovery STe PET-CT scanner (GE Healthcare, Milwaukee, WI, USA), which obtains images with three-dimensional resolution of 2.3-mm full width at half maximum. After the subjects fasted for at least 6 h, they were intravenously injected with 5mCi (185 MBq) of ^{18}F-FP-CIT. 90 min after the injection, PET images were acquired for 20 min in the three-dimensional mode at 12 kVp and 380 mA.

Image processing was performed using SPM8 (Wellcome Department of Imaging Neuroscience, Institute of Neurology, UCL, London, UK) with Matlab 2013a for Windows (Math Works, Natick, MA, USA). Quantitative analyses were based on volumes of interests (VOIs), which were defined based on a template in standard space. All reconstructed PET images were spatially normalized to the Montreal Neurology Institute (MNI) template space using a standard ^{18}F-FP-CIT PET template which was generated from ^{18}F-FP-CIT PET and T1 MR images of 13 normal controls. Eight VOIs of bilateral striatal sub-regions and one occipital VOI were drawn on a co-registered spatially

normalized single T1 MR and ^{18}F-FP-CIT PET template image on MRIcro version 1.37 (Chris Rorden, Columbia, SC, USA). The striatum was divided into the caudate, ventral striatum, anterior putamen, and posterior putamen. The outer boundaries of the striatal sub-regions were visually determined by the characteristic dense grey signal of the striatum. The boundary of between the anterior and posterior putamen was defined as the anterior commissure coronal plane. These VOIs were adjusted by a minor translation in our in-house editing software ANIQUE.[19] DAT availability was calculated by the non-displaceable binding potential, which was defined as (mean standardized uptake value of the striatal sub-regions VOI–mean standardized uptake value of the occipital VOI)/(mean standardized uptake of the occipital VOI).[20]

In addition, 70 healthy subjects without a neurological disease history (mean ± standard deviation age, 66.9 ± 7.8; female, 75.7%) were included as a control group as described in a previous study.[18] We also quantified the striatal DAT availability of the healthy controls to ascertain that the patients with Parkinson's disease had abnormally decreased DAT availability in the posterior putamen.

C. Longitudinal assessment of the changes in LED over time

Of the 51 patients in the gastrectomy group, 29 were treated with Parkinson's disease medications for at least two years. The doses of the Parkinson's disease medications were adjusted for effective symptom control by Y.J.H., K.J., S.Y.H., and L.P.H., according to the patients' response. A linear mixed model was then used to assess the changes in LED over time. Four fixed

effects were included in the model: three were between-subjects effects (Parkinson's disease subgroup according to a previous history of gastrectomy, age at Parkinson's disease onset, and baseline UPDRS-III scores), and one was within-subject effect (time). The subject factor was considered a random effect. The effects of the Parkinson's disease subgroup on the changes in LED over time were tested with a time × Parkinson's disease subgroup interaction term. We compared the rates of the longitudinal increases in LED between the gastrectomy group (n = 29) and the non-gastrectomy group (n = 463 before matching and n = 204 after matching).

D. Neuropsychological assessment

One hundred thirty-one patients with Parkinson's disease (24 out of 51 patients in the gastrectomy group and 107 out of 204 patients in the non-gastrectomy group) underwent a baseline detailed neuropsychological assessment (Seoul Neuropsychological Screening Battery [SNSB]).[21] The SNSB is a comprehensive neuropsychological test battery in the Korean language and covers five cognitive domains: attention and working memory (forward/backward digit span task and the Stroop test); frontal/executive function (contrasting program, go/no-go test, and the Controlled Oral Word Association Test [COWAT]); language and related functions (the Korean version of the Boston Naming Test [K-BNT], calculation, and praxis); verbal and visual memory (immediate recall/delayed recall/recognition test using the Seoul Verbal Learning Test [SVLT] for verbal memory; immediate recall/delayed recall/recognition test using the Rey Complex Figure Test

[RCFT] for visual memory); and visuospatial function (the RCFT copy and interlocking pentagon). The scores on each cognitive domain were classified as abnormal when they were below the 16th percentile (1SD) of the age-, sex-, and education-specific norms of 447 normal subjects. Then, we compared the levels of cognitive performance between the two groups using the composite scores calculated by dividing the sum of z-scores by the number of tests in each cognitive domain.

E. Cortical thickness analyses

Two hundred eighty-eight patients (14 with a previous history of gastrectomy and 274 without in the database of Yonsei Parkinson Center) underwent baseline brain MRI scans. MRI scans were acquired using a 3.0 T scanner (Achieva; Philips Medical Systems, Best, The Netherlands) with a 32-channel receiver array head coil. The high-resolution axial T1-weighted MRI data were obtained using a 3D T1-TFE sequence with the following parameters: 224 × 224 axial acquisition matrix; 256 × 256 reconstructed matrix with 170 slices; voxel size, 0.859 × 0.859 × 1 mm^3; field of view, 220 mm; echo time, 4.6 msec; repetition time, 9.8 msec; flip angle, 8°.

The propensity score matching was performed as described above to match 14 patients with Parkinson's disease and a history of prior gastrectomy to 56 patients with Parkinson's disease and no history of gastrectomy. We used the CIVET pipeline (http://mcin.ca/civet/) to measure cortical thickness. Each subject's T1-weighted image was corrected for intensity inhomogeneity and linearly registered to the MNI-152 template.[22, 23] The images were then tissue

classified[24] and the inner and the outer cortical surfaces were extracted, resulting in 81,924 polygons and 40,962 vertex points per hemisphere.[25, 26] The cortical thickness was calculated as the Euclidean distance between the linked vertices of the inner and outer surfaces.[27] The measured cortical thickness was smoothed using a surface-based diffusion smoothing kernel (30mm full width at half maximum).[28] Statistical analyses for the cortical thickness were performed using the SurfStat toolbox.[29] To identify regions of cortical thinning, we used general linear model adjusting for age, sex, years of education, disease duration, and intracranial volume as covariates. We mapped t-scores and statistical significance on the standard template. Multiple comparisons were corrected using the Random Field Theory (RFT).[30]

F. Statistical analyses

To compare the baseline demographic characteristics between the two groups, Student's *t*-tests and Pearson's χ^2 tests were used for continuous and categorical variables, respectively. In order to compare the DAT availability of each striatal sub-region, an analysis of covariance was used while adjusting for age, sex, and Parkinson's disease duration as covariates. A linear mixed model was used to compare the rate of longitudinal changes in LED between the groups. We compared the level of cognitive performance between the two groups using composite scores, calculated by dividing the sum of the z-scores by the number of tests in each cognitive domain. A Bonferroni-Holm correction was used for multiple comparisons after using the Student's t-test. The statistical analyses were performed with SPSS software (version 23.0; IBM Corp.,

Armonk, NY, USA), and results with a two-tailed p <0.05 were considered statistically significant.

III. RESULTS

1. Experimental data

A. Tyrosine hydroxylase

(A) Immunohistochemistry and cell counting

TH-positive neurons were counted in five sections per individual case in the midbrain stained with an antibody against TH. The number of TH-positive neurons in the SN was comparable between the control group (mean, 1618.5; standard error [SE], 60.0) and vagotomy group (mean, 1465.0; SE 108.0). MPTP resulted in a marked loss of TH-positive neurons in the SN 6 weeks after administration: 43.9% loss of TH-positive neurons in the parkinsonian model group without vagotomy (mean, 908.0; SE, 223.6) and 41.3% loss of TH-positive neurons in the parkinsonian model group with vagotomy (mean, 950.0; SE, 59.1). However, there was no significant difference in the number of TH-positive neurons between the two parkinsonian model groups according to the vagotomy procedure (Figures 4I, 4II).

(B) Western blotting analysis

MPTP-induced TH-positive neuronal loss was also assessed using western blotting analysis. TH expression was detected in homogenates from the SN of mice in the control group, while its expression was detected more faintly from

mice in the parkinsonian model group without vagotomy and the parkinsonian model group with vagotomy, which were sacrificed 6 weeks after MPTP administration. In particularly, immunoreactive band densities of TH in the SN of mice were significantly decreased in the two parkinsonian model groups compared to the control group. However, no difference in TH expression was seen between the parkinsonian model groups with and without vagotomy (Figure 4III).

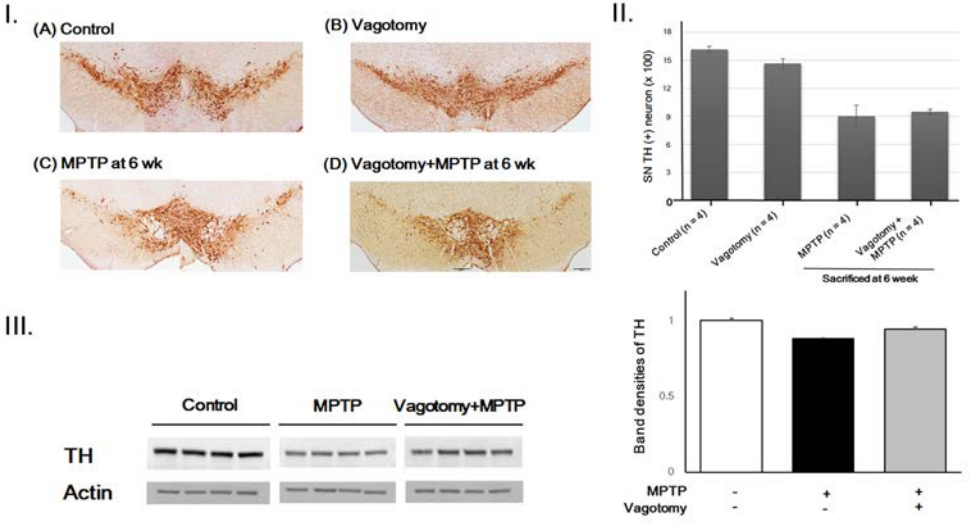

Figure 4. Tyrosine hydroxylase (TH). I. Immunohistochemistry. TH-positive neurons in the substantia nigra were stained from individual cases of four groups of mice. **II. Cell counting of TH-positive neurons.** The number of TH-positive neurons of each mouse was counted under a microscope using a 10x objective in stacks of five optical sections. **III. Western blotting analysis.** Immunoblotting band densities of TH in the substantia nigra of mice were decreased in the parkinsonian model group without vagotomy and the parkinsonian model group with vagotomy, compared to the control group. However, there was no difference in the immunoblotting band densities between the two parkinsonian model groups.

B. Ionized calcium binding adaptor molecule-1 (Iba-1)

(A) Microglial morphology

The morphology of microglial cells was visualized in the Iba-1 stained sections of SN of mice in the control group, the parkinsonian model group without vagotomy, and the parkinsonian model group with vagotomy, which were sacrificed at 12 weeks of age or 6 weeks after MPTP administration. In the parkinsonian model group without vagotomy, a substantial proportion of microglia displayed typical signs of microglial activation, i.e., an increased soma size and more irregular shape of cell body (an amoeboid morphology).[31-34] The prevalence of microglia which showed an amoeboid morphology rather than a ramified morphology in the parkinsonian model group with vagotomy was at a level between that of the control group and the parkinsonian model group without vagotomy (Figure 5I).

(B) Western blotting analysis

Iba-1 is a cytoplasmic protein whose expression is primarily restricted to microglia in the brain,[35] and an increase in Iba-1 expression would reflect microglial activation with alterations in cell numbers, size or function.[36] Immunoreactive band densities of Iba-1 in the SN of mice were significantly increased in the parkinsonian model group without vagotomy. However, the level of Iba-1 expression in homogenates from the SN of mice in the parkinsonian model group with vagotomy was significantly attenuated in the parkinsonian model group with vagotomy (p = 0.045), which was comparable with that in the control group (Figure 5II).

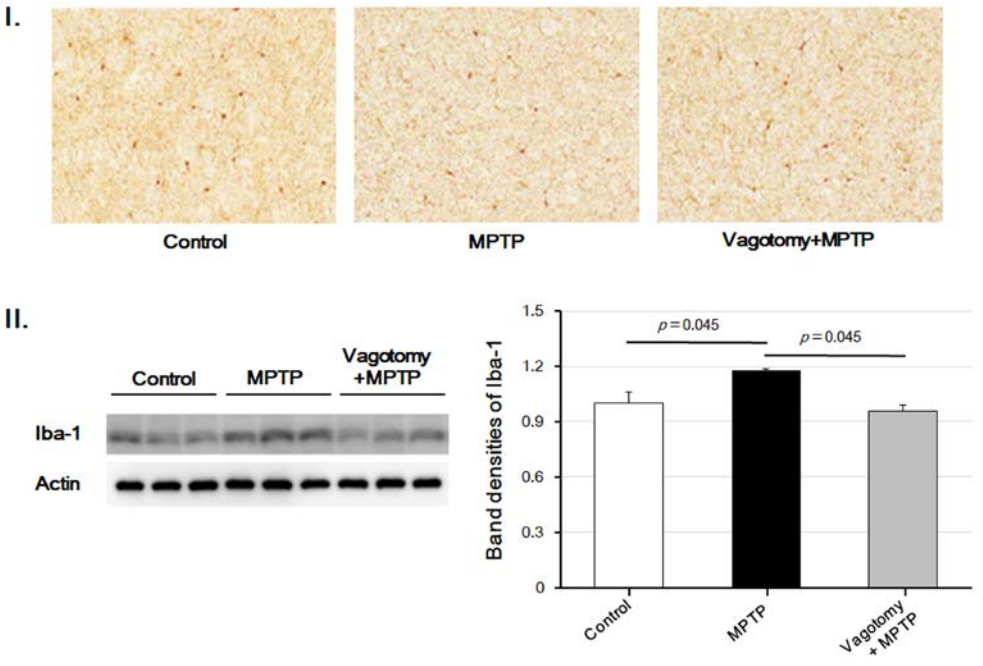

Figure 5. Ionized calcium binding adaptor molecule-1 (Iba-1). I. Immunohistochemistry. Morphology of microglia in the substantia as a marker for microglial activation was visualized using diaminobenzidine (DAB) method.. **II. Western blotting analysis.** Immunoblotting band densities of Iba-1 in the substantia nigra of mice were increased in the parkinsonian model group without vagotomy, but were significantly attenuated in the parkinsonian model group with vagotomy (p = 0.045).

C. Cytokine and Chemokine analysis

The level of IL-1β expression was increased in both parkinsonian model groups compared to the control group (mean, 138.05 pg/mL; SE, 16.69), while the parkinsonian model group with vagotomy (mean, 240.66 pg/mL; SE, 19.15) exhibited less markedly increased expression of IL-1β than the parkinsonian model group without vagotomy (mean, 671.93 pg/mL; SE, 50.25; $p = 0.006$). The level of IL-6 expression was also increased in both parkinsonian model groups compared to the control group (mean, 27.54 pg/mL; SE, 3.92), while the parkinsonian model group with vagotomy (mean, 102.98 pg/mL; SE, 12.20) exhibited less markedly increases in IL-6 expression than the parkinsonian model group without vagotomy (mean, 187.78 pg/mL; SE, 8.52; $p = 0.006$). Meanwhile, the level of TNF-α expression was comparable between the parkinsonian model groups with vagotomy (mean, 46.11 pg/mL; SE, 1.65) and without vagotomy (mean, 50.01 pg/mL; SE, 6.86; $p > 0.999$; Figure 6).

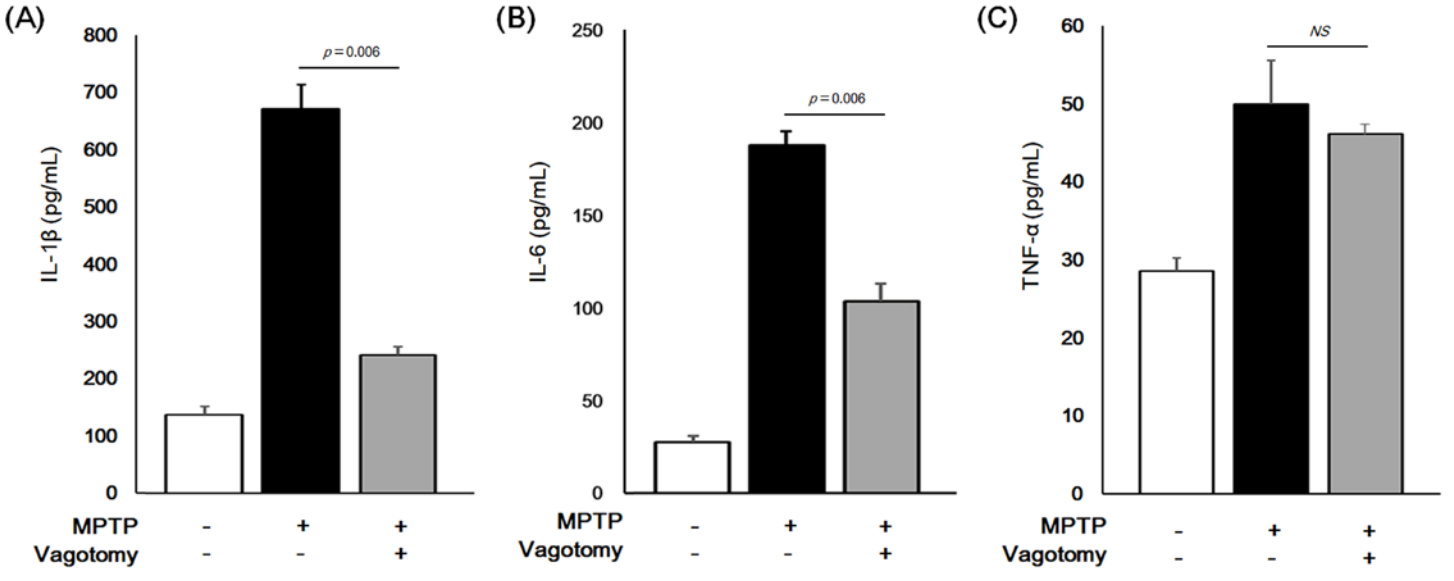

Figure 6. The level of expression of pro-inflammatory cytokines (ELISA). (A) Interleukin-1β (IL-1β). (B) Interleukin-6 (IL-6). (C) Tumor necrosis factor alpha (TNF-α).

2. Clinical data

A. Baseline clinical characteristics of patients with Parkinson's disease

The baseline demographic characteristics of the patients with Parkinson's disease are listed in Table 1. Of the 51 patients in the gastrectomy group, 14 underwent total gastrectomy and the other 37 underwent subtotal gastrectomy. There were no significant differences between the groups in the onset age of parkinsonian motor symptoms (71.22 ± 10.38 years in the gastrectomy group and 70.24 ± 8.98 years in the non-gastrectomy group; p = 0.502), sex (frequency of female sex, 31.4% in the gastrectomy group and 32.4% in the non-gastrectomy group; p = 0.893), Parkinson's disease duration (69.33 ± 9.95 months in the gastrectomy group and 68.69 ± 9.10 in the non-gastrectomy group; p = 0.660), or UPDRS-III scores (23.11 ± 12.34 in the gastrectomy group and 23.83 ± 9.46 in the non-gastrectomy group; p = 0.715). The CCSIT scores (6.03 ± 2.75 in the gastrectomy group and 6.12 ± 2.34 in the non-gastrectomy group; p = 0.830) and the frequency of RBD (31.4% in the gastrectomy group and 37.3% in the non-gastrectomy group; p = 0.434) did not differ between the two groups. The patients in the gastrectomy group (16.63 ± 9.06) had higher BDI scores than those in the non-gastrectomy group (11.89 ± 8.71; p = 0.009). There was no significant difference in demographic characteristics between the two gastrectomy groups (i.e., total gastrectomy group and subtotal gastrectomy group; Table 2).

Table 1. Baseline demographic characteristics of patients with Parkinson's disease

	PD-G+ (n = 51)	PD-G- (n = 204)	*p*-value
Age (years)	71.22 ± 10.38	70.24 ± 8.98	0.502
Female, No. (%)	16 (31.4%)	66 (32.4%)	0.893
Age at PD onset (years)	69.33 ± 9.95	68.69 ± 9.10	0.660
PD duration (months)	20.51 ± 26.21	18.55 ± 16.21	0.539
UPDRS-III	23.11 ± 12.34	23.83 ± 9.46	0.715
CCSIT	6.03 ± 2.75	6.12 ± 2.34	0.830
RBD	16 (31.4%)	76 (37.3%)	0.434
BDI	16.63 ± 9.06	11.89 ± 8.71	0.009
K-MMSE	25.12 ± 3.27	26.11 ± 3.48	0.095

The values are expressed as mean ± standard deviation or number (percentage). The propensity score was computed for each patient using a logistic regression model including the following variables: age at onset, sex, and PD duration. Abbreviations: PD, Parkinson's disease; PD-G+, PD with a previous history of gastrectomy; PD-G-, PD without a previous history of gastrectomy; UPDRS-III, Unified PD Rating Scale Part III; CCSIT, the cross-cultural smell identification test; RBD, rapid eye movement behavior disorder; BDI, Beck Depression Inventory; K-MMSE, the Korean version of the Mini-Mental State Examination.

Table 2. Comparison between the total gastrectomy and subtotal gastrectomy groups

	Total gastrectomy	Subtotal gastrectomy	*p*-value
Demographic characteristics	N = 14	N = 37	
Age (years)	71.29 ± 10.43	71.19 ± 10.51	0.977
Female, No. (%)	3 (21.4%)	13 (35.1%)	0.503
Age at PD onset (years)	69.00 ± 10.55	69.46 ± 9.86	0.885
PD duration (months)	23.00 ± 36.68	19.57 ± 21.56	0.681
UPDRS-III	24.50 ± 16.47	22.61 ± 10.72	0.716
CCSIT	5.14 ± 2.85	6.25 ± 2.73	0.348
RBD	5 (35.7%)	11 (29.7%)	0.742
BDI	13.40 ± 17.36	17.36 ± 9.14	0.387
Striatal DAT availability	N = 7	N = 21	
Whole striatum	2.093 (0.250)	1.918 (0.140)	0.556
Anterior caudate	2.122 (0.253)	1.956 (0.142)	0.580
Posterior caudate	1.378 (0.190)	1.153 (0.107)	0.323
Anterior putamen	2.617 (0.328)	2.322 (0.184)	0.450
Posterior putamen	1.984 (0.341)	1.664 (0.191)	0.431
Ventral striatum	1.929 (0.248)	2.070 (0.139)	0.631
Δ LED	N = 10	N = 19	
Estimated beta (SE)	3.7313 (0.6933)	8.1880 (0.7869)	< 0.001

The values are expressed as mean ± standard deviation or percentage (number) for the baseline demographic characteristics, and estimated mean (standard error) for the DAT availability. The estimate (β) is the change in LED per month (Δ LED), i.e., positive value indicates the dose-up of PD medications. Abbreviations: PD, Parkinson's disease; UPDRS-III, Unified PD Rating Scale

Part III; CCSIT, the cross-cultural smell identification test; RBD, rapid eye movement behavior disorder; BDI, Beck Depression Inventory; DAT, dopamine transporter; LED, levodopa-equivalent dose; SE, standard error.

B. Comparison of the ^{18}F-FP-CIT PET activities between two groups

Table 3 shows the baseline DAT availability in each striatal sub-region of the patients with Parkinson's disease. The striatal DAT availability of the patients with Parkinson's disease were markedly lower than that in the healthy controls from out database,[18] and all the patients with Parkinson's disease exhibited DAT availability that was lower than 2 SD below the normal mean of DAT availability of the healthy control in the posterior putamen. After adjusting for age at onset, sex, and Parkinson's disease duration, the estimated mean DAT availability of the posterior putamen in the gastrectomy group (1.74) was less severely decreased than that in the non-gastrectomy group (1.42; p = 0.006). The DAT availability in the anterior caudate (2.00 in the gastrectomy group and 1.92 in the non-gastrectomy group; p = 0.515), posterior caudate (1.21 in the gastrectomy group and 1.29 in the non-gastrectomy group; p = 0.398), anterior putamen (2.39 in the gastrectomy group and 2.21 in the non-gastrectomy group; p = 0.176), and ventral striatum (2.03 in the gastrectomy group and 2.01 in the non-gastrectomy group; p = 0.865) did not differ between the two groups. Additionally, the DAT availability in the whole striatum (1.96 in the gastrectomy group vs. 1.83 in the non-gastrectomy group; p = 0.210) did not differ between the two groups.

Table 3. The comparisons of the striatal dopamine transporter availability between the Parkinson's disease groups

	PD-G+ (n = 28)	PD-G- (n = 112)	*p*-value
Whole striatum	1.959 (0.094)	1.827 (0.047)	0.210
Anterior caudate	1.995 (0.106)	1.918 (0.053)	0.515
Posterior caudate	1.207 (0.086)	1.289 (0.043)	0.398
Anterior putamen	2.393 (0.120)	2.210 (0.060)	0.176
Posterior putamen	1.741 (0.103)	1.416 (0.052)	0.006
Ventral striatum	2.033 (0.108)	2.012 (0.054)	0.865

The values are expressed as estimated mean (standard error) for DAT availability. The propensity score was computed for each patient using a logistic regression model including the following variables: age at onset, sex, and Parkinson's disease duration. Abbreviations: DAT, dopamine transporter; PD-G+, Parkinson's disease with a previous history of gastrectomy; PD-G-, Parkinson's disease without a previous history of gastrectomy.

C. Comparison of longitudinal changes in LED between the groups

Twenty-nine patients with Parkinson's disease and a prior gastrectomy were treated with Parkinson's disease medications for at least two years. The LED changes were estimated with a linear mixed model that included the following covariates: Parkinson's disease subgroup according to a previous history of gastrectomy, age at Parkinson's disease onset, baseline UPDRS-III, time, and Parkinson's disease subgroup × time interaction. The patients in the gastrectomy group exhibited a slower increase in the dose of dopaminergic medications than those in the non-gastrectomy group (estimated monthly LED changes, 4.83 in the gastrectomy group and 7.25 in the non-gastrectomy group; p <0.001; Table 4).

In addition, we performed comparative analysis of longitudinal LED changes using the matched Parkinson's disease groups. The gastrectomy group showed a slower increase in the dose of dopaminergic medications than those in the non-gastrectomy group (29 in the gastrectomy group, 4.96; 204 in the non-gastrectomy group, 7.00; p <0.001; Table 4).

In the subgroup analysis, the total gastrectomy group (4.28) also showed a slower increase in LED compared to the non-gastrectomy group (7.02, p <0.001), while the subtotal gastrectomy group (8.01) showed a comparable increase in LED with the non-gastrectomy group (7.09, p = 0.200; Table 5).

Table 4. Longitudinal changes in levodopa-equivalent doses across time in patients with Parkinson's disease with a follow-up duration >2 years.

	Estimated beta	Standard error	*p*-value
Before PSM (29 vs. 463)			
PD-G+ [a]	4.8346	0.4618	<0.001
PD-G-	7.2486	0.1638	<0.001
Difference	-2.4140	0.4876	<0.001
After PSM (29 vs. 204)			
PD-G+ [a]	4.9569	0.4699	<0.001
PD-G-	7.0019	0.2699	<0.001
Difference	-2.0450	0.5359	<0.001

The estimate (β) is the change in LED per month (Δ LED), i.e., positive value indicates the dose-up of PD medications. A linear mixed model included four fixed effects (three between-subjects effects, namely the PD subgroup according to a previous history of gastrectomy, age at PD onset, and baseline UPDRS-III scores; and one within-subject effect, namely time). The effect of the PD subgroup on the change in LED across time was tested using the time x PD subgroup interaction term, and the PD-G+ group exhibited a slower rate of dose-up of Parkinson's disease medications than the PD-G- group. Abbreviations: LED, levodopa-equivalent dose; PD, Parkinson's disease; PD-G+, PD with a previous history of gastrectomy; PD-G-, PD without a previous history of gastrectomy; PSM, propensity score matching.

[a] Of 53 patients in the gastrectomy group, 29 patients were followed up at least two years.

Table 5. Comparisons between the gastrectomy subgroups and non-gastrectomy group.

	Total gastrectomy			Subtotal gastrectomy		
	PD-G+	PD-G-	*p*-value	PD-G+	PD-G-	*p*-value
Striatal DAT availability						
Whole striatum	1.968 (0.177)	1.823 (0.044)	0.430	1.953 (0.109)	1.830 (0.047)	0.303
Anterior caudate	1.971 (0.209)	1.911 (0.052)	0.783	2.001 (0.122)	1.924 (0.053)	0.564
Posterior caudate	1.303 (0.176)	1.283 (0.043)	0.909	1.177 (0.098)	1.294 (0.042)	0.273
Anterior putamen	2.453 (0.223)	2.208 (0.055)	0.289	2.366 (0.139)	2.214 (0.060)	0.315
Posterior putamen	1.845 (0.173)	1.416 (0.043)	0.018	1.700 (0.119)	1.416 (0.051)	0.031
Ventral striatum	1.871 (0.214)	2.008 (0.053)	0.535	2.088 (0.126)	2.015 (0.054)	0.596
Δ LED						
Estimated beta (SE)	4.2775 (0.5508)	7.0226 (0.2487)	<0.001	8.0077 (0.6730)	7.0866 (0.2589)	0.200

The values are expressed as estimated mean (standard error) for the DAT availability. The estimate (β) is the change in LED per month (Δ LED), i.e., positive value indicates the dose-up of PD medications. Abbreviations: PD, Parkinson's disease; PD-G+, PD with a previous history of gastrectomy; PD-G-, PD without a previous history of gastrectomy; DAT, dopamine transporter; LED, levodopa-equivalent dose; SE, standard error.

D. Comparison of neuropsychological assessment between the groups

The gastrectomy group tended to have lower K-MMSE scores (25.12 ± 3.27 in the gastrectomy group and 26.11 ± 3.48 in the non-gastrectomy group; p = 0.095). The gastrectomy group also showed poorer performances on the attention/working memory (-1.06 ± 1.22), frontal/executive (-1.08 ± 0.95), and memory function (-1.06 ± 0.98) domains than the non-gastrectomy group (-0.39 ± 0.87, p = 0.013; -0.56 ± 0.81, p = 0.029; -0.51 ± 0.82, p = 0.023, respectively), while the levels of cognitive performances in the language (p = 0.417) and visuospatial function (p = 0.417) domains were comparable between the groups (Table 6).

Table 6. A detailed neuropsychological test of patients with Parkinson's disease

	PD-G+ (n = 24)	PD-G- (n = 107)	*p*-value
Neuropsychological test			
Attention/working memory	-1.06 ± 1.22	-0.39 ± 0.87	0.013
Frontal executive function	-1.08 ± 0.95	-0.56 ± 0.81	0.029
Language	-0.90 ± 1.74	-0.54 ± 1.40	0.417
Memory	-1.06 ± 0.98	-0.51 ± 0.82	0.023
Verbal memory	-1.34 ± 1.17	-0.62 ± 0.92	0.009
Visual memory	-0.78 ± 1.09	-0.41 ± 1.01	0.318
Visuospatial	-1.00 ± 2.53	-0.32 ± 1.39	0.417

The values are expressed as mean ± standard deviation. A Bonferroni-Holm correction for multiple comparisons was used for the results of a detailed neuropsychological test. Abbreviations: PD-G+, Parkinson's disease with a previous history of gastrectomy; PD-G-, Parkinson's disease without a previous history of gastrectomy.

E. Comparison of cortical thickness between the groups

There were no significant differences in demographic characteristics between the groups (14 with a prior gastrectomy and 56 without; see Table 7). Figure 6 shows the cortical thickness difference between the gastrectomy and non-gastrectomy groups. On the t-map, red and blue color indicate greater and lesser cortical thinning in the gastrectomy group compared to the non-gastrectomy group, respectively. On the p-map, the patients in the gastrectomy group tended to exhibit greater cortical thinning in the right lateral temporal region (RFT-corrected p <0.05). No areas were found in which the non-gastrectomy group exhibited greater cortical thinning than did the gastrectomy group. The areas showing different cortical thickness between the two groups in the cluster- and vertex-levels are listed in Table 8.

Table 7. Demographic characteristics of patients with Parkinson's disease who underwent brain MRI scans

	PD-G+ (n = 14)	PD-G- (n = 56)	*p*-value
Age (years)	70.64 ± 7.78	69.17 ± 8.69	0.567
Female, No. (%)	4 (28.6%)	25 (44.6%)	0.275
Age at PD onset (years)	68.71 ± 7.80	67.79 ± 8.93	0.724
PD duration (months)	22.29 ± 30.19	19.13 ± 15.80	0.587
UPDRS-III	26.29 ± 10.59	25.92 ± 11.11	0.913
CCSIT	4.75 ± 2.09	5.86 ± 2.63	0.178
RBD	6 (42.9%)	18 (32.1%)	0.534
BDI	17.90 ± 10.13	13.02 ± 8.03	0.094
K-MMSE	25.38 ± 3.89	25.80 ± 3.09	0.680

The values are expressed as mean ± standard deviation or number (percentage). The propensity score was computed for each patient using a logistic regression model including the following variables: age at onset, sex, and PD duration. Abbreviations: PD, Parkinson's disease; PD-G+, PD with a previous history of gastrectomy; PD-G-, PD without a previous history of gastrectomy; UPDRS-III, Unified PD Rating Scale Part III; CCSIT, the cross-cultural smell identification test; RBD, rapid eye movement behavior disorder; BDI, Beck Depression Inventory; K-MMSE, the Korean version of the Mini-Mental State Examination.

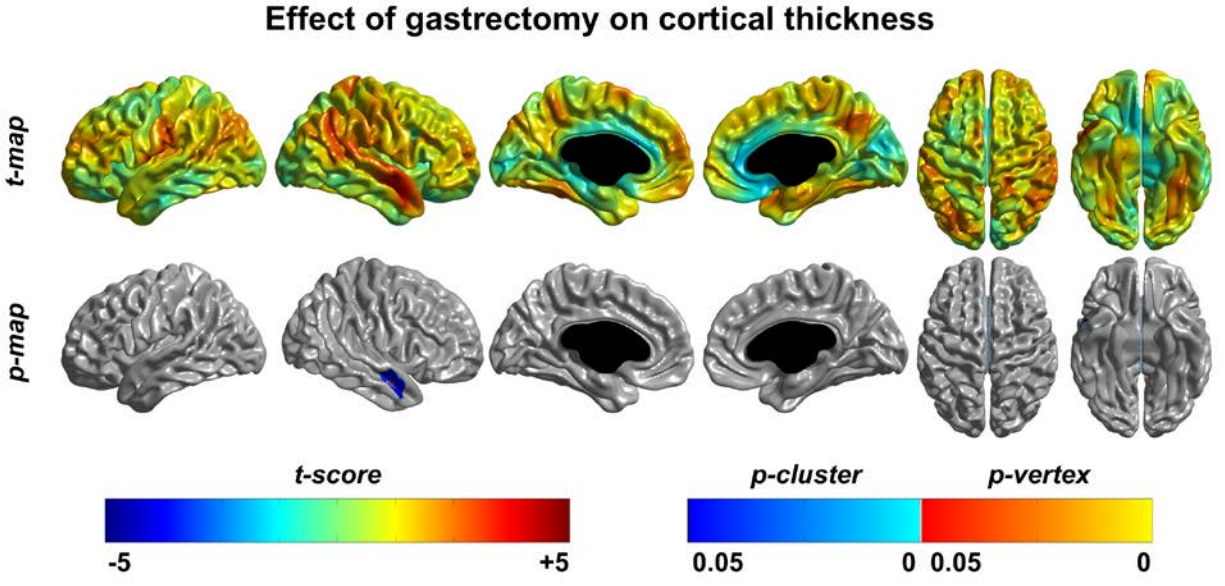

Figure 6. Comparison of cortical thickness between the groups of patients with Parkinson's disease with and without a history of gastrectomy. The t-map shows differences in cortical thickness between the groups. The red and blue color indicate greater and lesser cortical thinning in the gastrectomy group compared to the non-gastrectomy group, respectively. The p-map indicates the regions of significant cortical thinning in the gastrectomy group compared to the non-gastrectomy group (RFT-corrected p <0.05).

Table 8. Differences in cortical thickness between the Parkinson's disease groups according to a previous history of gastrectomy

	Region	*t*-score	*p*-cluster	*p*-vertex	x	y	z
Right	Middle Temporal	4.28	0.048	NS	50.6	-2.7	22.4
	Superior Temporal	4.45	0.048	0.037	49.5	-1.4	-18.8

Abbreviations: NS, not significant.

IV. DISCUSSION

The present study investigated whether vagotomy itself could have a protective effect on the survival of nigral dopaminergic neurons in a parkinsonian animal model. Additionally, this study investigated whether gastrectomy and vagotomy prior to parkinsonian symptom onset could alleviate the neurodegenerative load in patients with de novo Parkinson's disease. The major findings were as follows: (1) Decreases in the number of TH-positive neurons as well as immunoblotting band density of TH in the SN were comparable between the parkinsonian animal models according to the vagotomy procedure. (2) Transition to an amoeboid morphology as well as an increase in Iba-1 expression in the SN in the parkinsonian model group with vagotomy were at a level between those in the control group and the parkinsonian model group without vagotomy. (3) The patients in the gastrectomy group had less severely decreased DAT availability in the posterior putamen compared with the patients in the non-gastrectomy group. (4) The gastrectomy group had slower longitudinal changes in LED than the non-gastrectomy group. (5) However, the patients in the gastrectomy group showed poorer cognitive performance on the attention/working memory, frontal/executive, and memory function domains than those in the non-gastrectomy group. These findings suggest that vagotomy has protective effects on striatal dopaminergic denervation and longitudinal requirement of dopaminergic medications in subsequent Parkinson's disease, but may be unfavorable to cognitive impairment in patients with Parkinson's disease. Blockade of the spread of α-synuclein from the gut to the brain could account for a substantial proportion of the neuroprotective effect of vagotomy,

while the modulation of inflammatory response might also contribute to some extent.

Ample evidence has suggested that α-synuclein possesses prion-like properties.[6] Cell-to-cell transmission of α-synuclein appears to be mediated through various routes including exocytosis, exosome-mediated secretion, receptor-mediated secretion or via tunneling nanotubes,[37] and may be suggested to be a cellular pathomechanism underpinning with clinical progression of Parkinson's disease.[38] Based on a neuropathological staging system of Parkinson's disease,[3] Braak and colleagues proposed a dual-hit hypothesis: LB pathology would start in the olfactory and gastrointestinal systems and progress to the neuro-anatomically connected structures.[5] Ample evidence has demonstrated that LB pathology arising from the gastrointestinal system propagated to the brainstem via the vagal nerve in patients with Parkinson's disease. Thus, LB pathology was detected in the enteric nervous system with the greatest involvement in the lower esophagus and stomach in patients with premotor Parkinson's disease,[39-41] even though the scarcity of cases with isolated LB in the gut raises a criticism of gastrointestinal onset in Parkinson's disease.[4, 42, 43] Animal studies have shown that the vagal nerve is a key connecting structure of α-synuclein spreading between the gastrointestinal tract and brain,[8-11, 44, 45] and vagotomy prevented α-synuclein propagation. Pan-Montojo et al. reported the spread of gastric LB to the brain in a rotenone-induced Parkinson's disease mouse model,[8] which was prevented by vagotomy in their subsequent study.[9] Other animal studies also have shown that the vagal nerve is a key connecting pathway between the gastrointestinal tract

and brain for α-synuclein transfer.[10, 11, 44, 45] In particular, Kim et al.[11] demonstrated that pathological α-synuclein preformed fibrils spread sequentially from the intestine to the dorsal motor nucleus, the caudal portions of the hindbrain, and the SN. Recently, two nationwide registry-linkage cohort studies in Denmark and Sweden have reported that vagotomy was associated with a reduced risk of developing Parkinson's disease.[12, 13] Epidemiological data from the Korean National Health Insurance database also demonstrated that gastrectomy was associated with a decreased incidence of Parkinson's disease.[46] Taken together, the interventions blocking the progression of LB pathology originating in the stomach through a vagal connection would be a promising disease-modifying strategy in patients with Parkinson's disease.

In this study, we hypothesized that vagotomy itself would have a protective effect against the neurodegenerative processes underlying Parkinson's disease, not simply by blocking the caudo-rostral spread of α-synuclein, but by modulating the inflammatory and/or oxidative stress responses. To test it, we constructed a parkinsonian animal model by intraperitoneal injection of MPTP, rather than by direct injection of α-synuclein in the gut or vagal nerve.[8, 10, 11, 44] Our experimental data did not find any difference in the survival of nigral TH-positive neurons or TH expression in the SN depending on the vagotomy procedure, which might be due to a small number of experimental animals or a relatively short interval between MPTP administration and sacrifice. Meanwhile, mice in the parkinsonian model group with vagotomy showed less microglial activation assessed by Iba-1 expression than those in the parkinsonian model group without vagotomy. These finding suggest that vagotomy could alleviate

the neurodegenerative load via regulating the vagal tone and inflammatory reflex.[47] To apply these experimental data to clinical practice, it is necessary to establish vagal nerve stimulation settings that can achieve similar effects to vagotomy,[48] which is too invasive to perform in patients with Parkinson's disease. Actually, it remains elusive whether vagotomy or vagal nerve stimulation would have more anti-inflammatory properties in the brain.[14, 49-53]

Interestingly, the present study demonstrated that patients with Parkinson's disease undergoing gastrectomy had less severely decreased DAT availability in the posterior putamen compared with those without gastrectomy. In addition, the gastrectomy group had slower longitudinal changes in dopaminergic medications than the non-gastrectomy group. These findings seem to support the clinical application of vagal nerve modulation. The protective effects of gastrectomy on dopaminergic neuronal loss and its related disease progression would be associated with the elimination of a seeding source of α-synuclein aggregates in the stomach. Cell-to-cell transmission of α-synuclein aggregates is not a single discontinuous process, and the secondary secretion of co-aggregates of the seed and endogenous α-synuclein is required for the spread of LB pathology.[54] Moreover, the rate of cell-to-cell transmission seems to be related to α-synuclein concentration.[37, 55] Thus, a removal of potential seeded aggregates in the stomach would prevent or reduce the continuous transfer of pathogenic α-synuclein into the brainstem. In addition, given that vagal nerve-preserving gastrectomy is not conventionally performed for patients with gastric cancer,[56] a high proportion of truncal vagotomy in combination with gastrectomy could block the propagation of LB pathology as suggested in

previous studies.[12, 13] Alternatively, vagotomy may influence the vagal modulation of innate inflammatory processes[57] or the microbiota-gut-brain axis,[58, 59] which is regarded as an important trigger underlying Parkinson's disease pathogenesis.[60, 61] The neurochemical effects of the microbiota through the bidirectional communication of the gut-brain axis was not observed in vagotomized mice,[58, 59] identifying the vagal nerve as a major communicating pathway.[62] Accordingly, modulation of the vagal signal would be a useful therapeutic adjunct in patients with Parkinson's disease.

If the dual-hit hypothesis is valid, the olfactory bulb would be a main entry site for prion-like propagation[63] in patients in the gastrectomy group relative to the non-gastrectomy group. Rey et al.[64, 65] reported that α-synuclein injected into the olfactory bulb was transferred to the interconnected olfactory structures via an axonal transport as well as cell-to-cell transmission.[64] Later, they found that α-synucleinopathy triggered in the olfactory bulb propagated along the anatomical pathways over the course of 12 months.[65] The secondary and tertiary olfactory structures were affected by 1 month and 3 months, respectively. At the 12-month time point, more widespread cortical associative and secondary cortical brain regions including the secondary visual and somatosensory cortices and the anterior cingulate area were affected. In addition, α-synuclein aggregates were observed in the brainstem including the SN, locus coeruleus, and raphe nuclei in the later period. Thus, it can be expected that propagation of LB pathology would not follow the predictable anatomical pattern according to the Braak staging system in the gastrectomy group, with an earlier cortical involvement of LB pathology via the olfactory system. In the present study, a

subgroup analysis of cortical thinning pattern demonstrated that the gastrectomy group exhibited greater cortical atrophy in the lateral temporal region compared to the non-gastrectomy group. Additionally, the gastrectomy group had a poorer cognitive performance than the non-gastrectomy group. Even though an alteration in pattern and burden of cortical LB pathology after vagotomy is unknown, it is speculated that these patterns of cortical thinning and cognitive dysfunction may be a consequence of an earlier involvement of LB pathology in the cerebral neocortex of Parkinson's disease patients with gastrectomy. However, a further study with a larger sample size would be needed to clarify this issue.

In terms of the level of cognitive performance, the patients in the gastrectomy group appear to be a phenotype of dementia with Lewy bodies spectrum rather than classical Parkinson's disease.[66, 67] Although a clear distinction between Parkinson's disease with dementia and dementia with Lewy bodies has not been established, some evidence has suggested that the pattern of LB pathology progression in dementia with Lewy bodies would differ from that in Parkinson's disease; the LB pathology of the patients with dementia with Lewy bodies progressed first in the amygdala, which is accessible from the nose, and subsequently extended into the limbic cortex and neocortex.[68] Recently, Toledo and colleagues also have suggested that LB pathology of dementia with Lewy bodies would originate in the olfactory bulb and amygdala, while LB pathology with an origin in the caudal brainstem regions would be important in the cases of classical Parkinson's disease.[43] A longitudinal cognitive assessment would be needed to investigate whether the patients in the gastrectomy group

have a higher risk of dementia, similar to the dementia with Lewy bodies phenotype. Accordingly, taking the cognitive aspect into consideration, therapeutic approaches that target both the olfactory vector[69] and the gut-brain axis would be needed to modulate propagation of LB pathology.

Our study has some limitations. First, we cannot completely exclude the possibility that LB pathology had already accumulated in the brainstem when the subjects underwent gastrectomy. The premotor phase of Parkinson's disease from the onset of nigral neuronal loss was estimated to be 3 to 7 years in a series of postmortem and PET studies,[70-72] while some studies suggested that the prodromal phase spanned up to 20 years.[41] The patients in our study had previously undergone gastrectomy a mean of 5.83 years prior to the onset of parkinsonian motor symptoms. Thus, a prior gastrectomy might prevent the initial Parkinson's disease pathogenesis in some cases and merely alleviate the pathological burden in other cases. A further longitudinal study of patients with Parkinson's disease with a history of gastrectomy spanning at least 20 years is needed to draw firm conclusions. In addition, Helicobacter pylori infection, which is associated with an increased risk of gastric cancer[73] and Parkinson's disease,[74, 75] might have affected the disease course in the gastrectomy group. Second, our assumption that LB pathology progresses continuously according to the Braak staging system may not always be right. Approximately 7% of patients with Parkinson's disease have been reported not to exhibit LB pathology in the dorsal motor nucleus of the vagus,[76, 77] which suggested that a small fraction of patients with Parkinson's disease do not follow the Braak staging system. In addition, a neurodegenerative evolution of Parkinson's

disease may occur in a stepwise fashion,[78] that is to say that a single triggering event in the Braak stage 1 structures does not guarantee the full development of Parkinson's disease. Third, the subgroups for the quantitation of DAT availability, cortical thickness analyses, cognitive assessment, and longitudinal assessment of disease progression were not identical; however, there were no significant differences in demographic characteristics between the subgroups. Fourth, a longitudinal change in LED might not accurately reflect the disease progression, even though a consensus for assessing Parkinson's disease progression has not been established. However, LED appears to be indirectly associated with parkinsonian disability.[79] Additionally, a gastrectomy may lead to rapid and effective absorption of dopaminergic medications due to reduced gastric emptying time, thus influencing the drug dosage in the gastrectomy group.[80] However, considering comparable changes in LED between the subtotal gastrectomy group and the non-gastrectomy group, LED changes could not fully explained by gastrectomy-associated pharmacokinetics. Nevertheless, our results should be cautiously interpreted. Fifth, in experimental study, we did not perform behavioral tests since there was no difference in the number of TH-positive neurons between the two parkinsonian model groups, which might be due to a relatively short interval between the injection of MPTP and sacrifice.

V. CONCLUSION

The present study demonstrated that patients with Parkinson's disease

undergoing gastrectomy and vagotomy have a unique motor and cognitive phenotype, suggesting the disease-modifying effects of the modulation of α-synuclein aggregates seeds as well as inflammatory responses. Vagotomy has protective effects on the nigrostriatal degeneration and disease progression in subsequent Parkinson's disease, but may be unfavorable to cognitive performance.

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ABSTRACT(IN KOREAN)

파킨슨 질환에서 미주신경차단술의 도파민 신경세포 조절에 대한 효과 규명

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정석종

배경: 파킨슨병의 병태 생리로서 위장관에서 기원한 루이소체 병리가 미주신경을 통해 뇌로 전파되며, 미주신경차단술을 통해서 이를 조절할 수 있다는 여러 근거들이 제시되고 있다. 본 연구에서는 미주신경차단술이 파킨슨 동물 모델에서 흑색질 도파민 신경세포의 생존에 보호 효과가 있는지 확인하고자 한다. 더불어 파킨슨병 환자에서 파킨슨 증상 발생 전에 위절제술 및 미주신경차단술을 시행한 과거력이 있을 경우 신경퇴행성 진행이 경감되는지 확인하고자 한다.

방법: 파킨슨 동물 모델을 구축하기 위해 5주령 수컷 C57BL/6J 마우스에 5일간 1-methly-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)을 복강내 주사하였다. 일부 마우스에서는 MPTP 주사

전에 왼쪽 경부 미주신경차단술을 시행하였다. MPTP 주사 6주 경과 후, 흑색질에서 도파민 신경세포 (tyrosine hydroxylase, TH) 및 미세아교세포 (ionized calcium binding adaptor molecule-1, Iba-1)에 대한 면역조직화학 검사와 웨스턴 블롯팅 분석 및 염증 사이토카인 발현 분석을 진행하였다.

더불어 3개의 대학 병원으로부터 파킨슨 증상 발생 전에 위절제술 및 미주신경차단술을 시행받은 51명의 파킨슨병 환자 (위절제술 그룹)를 모집하였고, 비교 분석을 위하여 204명의 위절제술 과거력이 없는 파킨슨병 환자 (비위절제술 그룹)을 매칭하였다. 두 그룹간에 기저핵 도파민 운반체 밀도 및 인지기능 수준을 비교하였고, 선형 혼합 모형을 이용하여 2년 이상의 추적 기간 동안 파킨슨 증상 조절을 위한 도파민 약물 증량 속도를 비교하였다.

결과: 파킨슨 동물 모델에서 흑색질 TH 양성 신경세포 수나 TH 발현 밀도는 미주신경차단술을 시행한 그룹과 시행하지 않은 그룹 간에 차이가 없었다. 반면, 흑색질에서 Iba-1 발현은 미주신경차단술을 시행하지 않은 그룹에서만 현저히 증가하였다. Interleukin-1β와 interleukin-6 발현은 파킨슨 동물 모델에서 모두 증가하였는데, 특히 미주신경차단술을 시행하지 않은 그룹에서

더 현저하게 증가하였다.

파킨슨병 환자 임상 데이터를 보면, 위절제술 그룹과 비위절제술 그룹 간에 인구통계적 특성은 차이나지 않았다. 위절제술 그룹에 속한 파킨슨병 환자들이 뒤쪽 조가비핵에서의 도파민 운반체 밀도가 좀더 보존되어 있었고 (p = 0.006), 추적 기간 동안 파킨슨 증상 조절을 위한 도파민 약물 용량도 적게 필요하였다 (p <0.001). 하지만 위절제술 그룹은 비위절제술 그룹에 비해 주의집중력/작업기억 (p = 0.013), 전두엽/실행능력 (p = 0.029) 및 기억력 (p = 0.023) 영역에서 더 낮은 인지수행 능력을 보였다.

결론: 본 연구 결과는 미주신경차단술이 파킨슨병 환자에서 흑색질 도파민 신경세포 사멸 및 병의 진행에 보호 작용을 할 수 있으나, 인지기능에서는 부정적일 수도 있음을 시사한다. 미주신경차단술의 신경 보호 효과는 위장관에서 뇌로 알파 시누클레인이 전파되는 것을 막음으로써 작용할 가능성이 높으며, 염증 반응 조절 등이 그 기전에 관여할 것으로 보인다.

핵심되는 말: 도파민 신경세포; 미주신경차단술; 신경보호; 위절제술; 파킨슨병

PUBLICATION LIST

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