의학박사 학위논문

성인에서 발생한 거대 결장 국소 신경절세포감소증: 전향적 코호트 연구

Focal hypoganglionosis, adult-onset megacolon: A detailed phenotyping and prospective cohort study

울산대학교대학원
의 학 과
윤 지 영

성인에서 발생한
거대 결장 국소 신경절세포감소증:
전향적 코호트 연구

지도교수 명 승 재

이 논문을 의학박사 학위 논문으로 제출함

2022 년 8 월

울산대학교 대학원
의 학 과
윤 지 영

윤지영의 의학박사학위 논문을 인준함

심사위원　　명승재　(인)
심사위원　　정기욱　(인)
심사위원　　김지훈　(인)
심사위원　　서명숙　(인)
심사위원　　송은미　(인)

울 산 대 학 교 대 학 원

2022 년　8 월

ABSTRACT

OBJECTIVES: It is still controversial whether adult-onset megacolon with focal stenosis is a unique disease entity. We describe a cohort of 29 patients with a putative new GI neuromuscular disease, focal hypoganglionosis adult-onset megacolon (FHAM) at our institution from 2017 to 2020.

METHODS:

This prospective cohort study assessed the radiologic, endoscopic, histopathologic phenotyping, and treatment outcomes of patients with FHAM. stool samples were compared with those of 13 asymptomatic volunteers using 16S rRNA gene sequencing. Data of community controls (19,948 adults aged 40-60 years undergoing health screening) were analyzed to identify the risk factors. Experts in the UK re-reviewed the clinical features and pathological specimens according to the international guidance for GI neuromuscular pathology.

REUSLTS:

The median age of FHAM patients at symptom onset was 59 years (range 32.0–74.9) with mean symptom onset only 1 year before diagnosis. All patients had focal stenotic regions with proximal bowel dilatation (mean diameter of 78.8 mm; 95% CI, 72–86). Comparison with community controls showed no obvious risk factors. Ten patients underwent surgery. All exhibited significant hypoganglionosis: 5.4 (IQR, 3.7–16.4) myenteric ganglion cells/cm in stenotic regions compared to proximal 278 (190–338) and distal 95 (45–213) colonic cell densities. Hypoganglionosis was associated with $CD3^+$ T cells along the myenteric plexus. Colectomy was associated with significant symptom improvements compared to ongoing medical treatment (changes in Global Bowel Satisfaction scale score, -5.4 [surgery] vs. -0.3 [medical] points; $P < 0.001$). Bacterial richness and diversity were more significantly increased in patients with FHAM than in asymptomatic volunteers. Verrucomicrobia at the phylum level and Frisingicoccus, Ruthenibacterium, and Agathobaculum at the genus level were significantly more prevalent in patients with FHAM than in asymptomatic volunteers ($P < 0.05$).

CONCLUSIONS: FHAM appears to be a new enteric neuropathy characterized by hypoganglionosis that may be secondary to inflammation. Bowel resection appears to benefit patients. FHAM is also associated with a rich and diverse fecal microbiota, with a high abundance of the genus,

Verrucomicrobia and the genera, Frisingicoccus, Ruthenibacterium, and Agathobaculum.

***Keywords*:** hypoganglionosis; megacolon; constipation; treatment outcome.

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

INTRODUCTION

A megacolon is characterized by an enlarged diameter of the colon, which is associated with chronic processes[1,2]. Contrary to the conventional megacolon, some patients show a dilated colon with a stricture-like lumen with decreased distensibility on imaging. We and others have previously described small numbers of patients with a segmental megacolon consequent on finding a short stenotic region of the colon characterized by reduced diameter and distensibility but without complete obstruction. Such patients have significant symptoms of digestive dysmotility[3-6], and seem to be particular to Asia, mainly from Korea and Japan. The stenotic region was previously described as a transition zone[5] on the basis of the histological finding of hypoganglionosis (reduced density of myenteric ganglia akin to Hirschsprung disease) although this was probably a misnomer since the hypoganglionosis does not reflect a 'transition' between normal and aganglionic segments. The transition zone seemed to appear as a stricture associated with subacute obstruction with decreased distensibility, as visualized by computed tomography (CT) or colonoscopy, because there was a significantly lower number of ganglion cells in this area than in the proximal part of the colon. The etiology and management of this disease remain uncertain. We investigated these patients and renamed the disease as focal hypoganglionosis with adult-onset megacolon (FHAM), as it better represents its clinical characteristics, which differ from those of a typical megacolon and Hirschprung disease (figure 1). We described a prospective cohort of 29 patients from Korea with adult-onset regional megacolon secondary to a focal stenosis of the colon that we referred to this disease entity as 'FHAM'. To our knowledge, this is the first report detailing the disease's clinical, radio-physiological and pathological findings, as well as longitudinal clinical outcomes and prognosis.

In addition, many studies have reported that the gut microbiota plays a key role in health and affects various activities of host physiology, including gut motility. Dysbiosis (i.e., imbalances in the microbial community) of the intestinal microbiota is associated with many diseases, such as autoimmune and allergic diseases, obesity, inflammatory bowel disease (IBD), and diabetes.[7-12] Gut microbiota disturbances can contribute to gut dysfunction, but it is unclear if the changes in the microbiota occur prior to or because of colon motility disorders. Previous studies have suggested that

methane-producing intestinal microbiota lead to the development of constipation by reducing bowel motility.[13] Studies have shown that patients with chronic constipation have more methane-producing bacteria than healthy subjects.[14,15] Other studies have reported that the dysbiosis contributes to the development of chronic constipation via the regulation of serotonin transporter in the intestine.[16] Moreover, a previous study on idiopathic megacolon showed an improved megacolon after fecal microbiota transplantation (FMT).[17] Therefore, dysbiosis plays a key role in megacolon. However, its exact role in megacolon, especially in FHAM, is not known yet. Thus, we aimed to investigate the composition of the intestinal microbiota of patients with FHAM and asymptomatic volunteers.

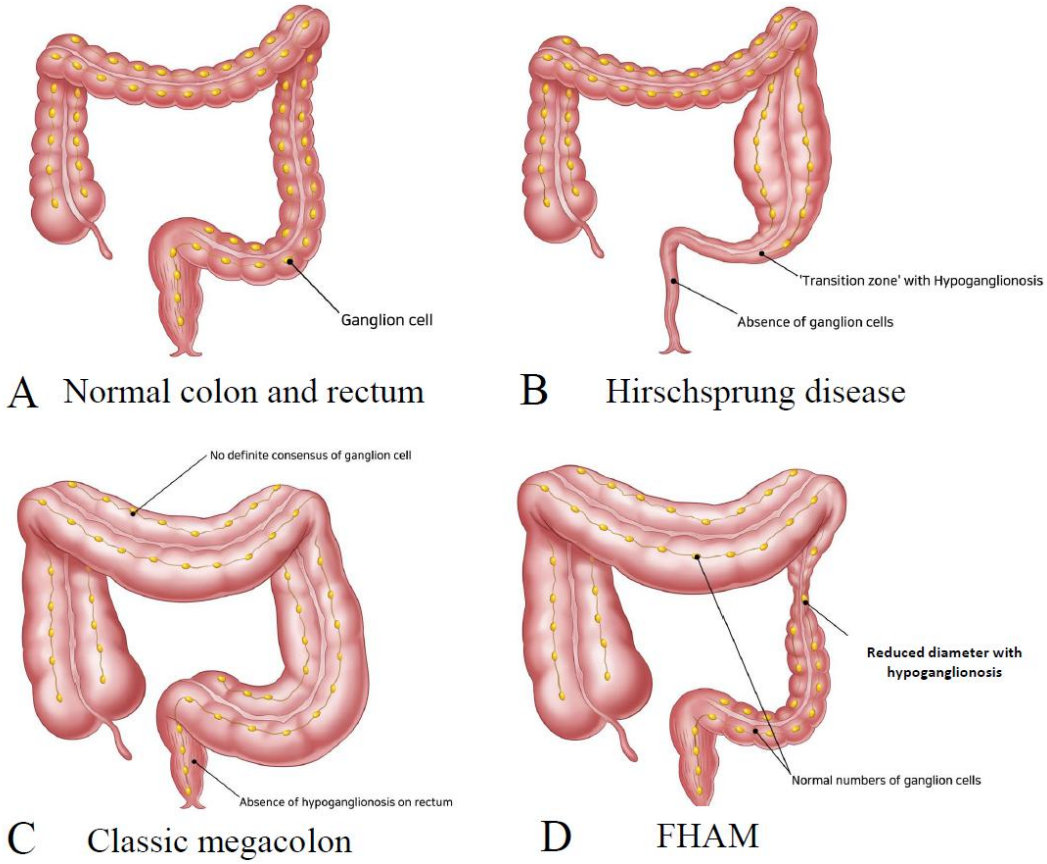


Fig 1. Schematic view of ganglion cells (yellow dots). (A) In a normal bowel, the ganglion cells are present throughout the large intestine. (B) In HSCR, ganglion cells do not develop appropriately in the rectum. The transition zone between the normoganglionic bowel and aganglionic segment in HSCR shows hypoganglionosis. (C) No definite consensus of ganglion cells could be drawn in megacolon, but it shows the absence of hypoganglionosis on rectal biopsy. (D) In FHAM, the hypoganglionic segment, referred to as FFN, was located between segments with abundant ganglionic cells.

HSCR, Hirschsprung's disease; FHAM, focal hypoganglionosis and adult-onset megacolon; FFN, focal functional narrowing.

METHODS

1. Study Population

All 29 prospectively recruited patients with FHAM visited a single tertiary-care center in Seoul between January 2017 and March 2020. The study design was approved by the institutional review board of the Asan Medical Center, Seoul, South Korea (IRB number, 2017-0021), and written informed consent was obtained from all subjects before enrolment.

To date, there have been no established criteria for the diagnosis of FHAM. Thus, we diagnosed FHAM according to the following criteria based on previous studies[3,5,6,18,19]. (1) chronic refractory constipation and recurrent abdominal pain and distension suggestive of sub-occlusive episodes, (2) colonic dilatation of > 5 cm and/or presence of intestinal air-fluid levels detected on axial CT, and (3) an area of focal stenosis observed on CT and/or colonoscopy, without obvious structural cause.

Because the etiology of FHAM is unknown, a control population was included to identify associations with relevant diseases that might be risk factors for disease development, such as focal ischemia or connective tissue disease. Between January and December 2019, a total of 19,948 asymptomatic adults, aged 40–60 years, underwent health screening tests, including colonoscopy and/or CT, at the Health Screening and Promotion Center of Asan Medical Center, Seoul, Korea. The clinical characteristics of the control population were compared with patients with FHAM.

A fecal microbiome analysis was performed once after the patients were enrolled in the study. Stool samples of FHAM were compared with those of 13 asymptomatic volunteers.

2. Study Protocol

Data collection

Multiplanar axial CT and colonoscopy were used to confirm focal stenosis and proximal bowel dilatation without mechanical obstruction. Focal functional narrowing (FFN) was defined by an abrupt decrease in the colonic diameter within the limits of the junctional area between the dilated and non-dilated colon. A minimum colon diameter of > 5.0 cm suggested colonic dilatation, indicative of megacolon[3,5,19]. Several gastrointestinal (GI) function tests including High-resolution anorectal manometry (HRAM), balloon expulsion test (BET), colon transit (CTT) test, high-resolution

esophageal manometry, and Gastric emptying scan (GES) were performed to exclude pan-GI dysmotility. The GI function tests were performed based on previous studies[20,21].

Longitudinal data, including sex, date of birth, symptom onset, diagnosis, weight and height at enrollment, past medical and surgical history, symptom information, and use of medications (including bulk laxatives [e.g., psyllium, polycarbophil], osmotic laxatives [e.g., magnesium hydroxide, lactulose, polyethylene glycol], stimulant laxatives [e.g., senna, bisacodyl], and pro kinetics), were prospectively updated in a registry linked to electronic medical records.

The patients were asked to complete structured questionnaires based on the Rome III criteria[22,23] to capture longitudinal bowel and abdominal symptoms, including stool frequency, Bristol Stool Form Scale score (1 [separate hard lumps] to 7 [liquid consistency with no solid pieces]), and Global Bowel Satisfaction scale score (GBS), with higher scores indicating greater satisfaction)[23]. GBS was calculated before and after medical or surgical treatment, alongside recourse to medications (including laxatives or prokinetics) and change in bowel frequency per week as crude indicators of treatment success[24,25].

Histopathological examination

For patients who underwent surgery, all resected colon specimens were opened, photographed, and fixed with 10% neutral buffered formalin on a corkboard. Dilated and non-dilated segments were identified and maximal and minimal internal circumferences were measured. The region connecting the dilated and non-dilated areas was referred to as the focal stenosis and sections were taken contiguously along its long axis. Myenteric cell bodies, identified by their characteristic microscopic morphology, such as Nissl substance, large vesicular nuclei, and single prominent nucleoli, were counted per ganglion on haematoxylin and eosin (H&E) staining. Calretinin immunostaining was performed when no unequivocal ganglion cells were identifiable with H&E staining[26]. The number of ganglion cells in the myenteric plexus was counted per centimetre. Given that luminal dilatation could decrease the ganglion cell number in proportion to the luminal circumference, we multiplied the ganglion cell number per centimetre by the mean internal circumference in each intestinal segment, and the result was defined as ganglion cell density. Representative sections from all pathological specimens were

reviewed independently by two expert pathologists (JK in South Korea, and JM in the UK) based on international diagnostic criteria from the London classification of GI neuromuscular pathology[26,27] , including CD3 immunostaining, which was performed for cases suspected of lymphocytic ganglionitis.

DNA extraction, 16S rRNA gene amplification, and pyrosequencing

The fecal samples, which were taken at the patients' homes, were collected immediately and stored at -80 ℃ until analysis. Samples were analyzed using 16S rRNA gene pyrosequencing. Bacterial DNA from the fecal samples was extracted using a Fast-DNA SPIN extraction kit (MP Biomedicals, Santa Ana, CA, USA). The 16S rRNA gene targeted V3–V4 of the variable region, which was amplified using the primer pair. The amplifications were carried out under the following conditions: an initial denaturation step performed at 95 °C for 3 min followed by 25 cycles of denaturation (95 °C for 30 sec), annealing (55 °C for 30 sec) and extension (72 °C for 30 sec), and a final elongation of 5 min at 72 °C. Then, a secondary amplification for attaching the Illumina NexTera barcode was performed with the i5 forward primer.

The polymerase chain reaction (PCR) products, confirmed by using 1% agarose gel electrophoresis, were purified with the QIAquick PCR purification kit (Qiagen, Valencia, CA, USA). The quality and product size were assessed on the Bioanalyzer 2100 (Agilent, Palo Alto, CA, USA) using a DNA 7500 chip. Equimolar concentrations of each amplicon were pooled, and the sequencing was carried out at Chunlab, Inc. (Seoul, Korea) with the Illumina MiSeq Sequencing system (Illumina, USA), according to the manufacturer's instructions.

Analysis of sequencing data

The raw sequence files from the pyrosequencing were analyzed according to a previously described procedure.[28] The sequences for each sample were sorted according to a unique barcode in the demultiplexing step, and low quality reads (average quality score <25 or read length <300 bp) were removed. Primer sequencers based on the profile of the 16S rRNA were trimmed by a pairwise sequence alignment and the HMM-search program of the HMMER 3.0 package.[29] The EzBioCloud database is used for the taxonomic assignment using USEARCH (8.1.1861_i86linux32).[30] UCHIME and the non-

chimeric 16S rRNA database from EzBioCloud are used to detect and remove the chimera sequences. The sequence data are then clustered using CD-HIT and UCLUST.[29,31] The compositions and proportions of the bacterial species were calculated using the CLcommunity software (ChunLab, Inc., Seoul, Korea).

3. Statistical Analysis

Continuous variables were presented as means ± standard deviations or median [IQR], and categorical variables were presented as numbers and percentages. The differences in characteristics were analyzed using X^2 test or Fisher's exact test for categorical variables and t-test or Mann-Whitney U tests for continuous variables. A linear mixed model was used to compare changes in questionnaire responses over time between patients undergoing medical and surgical treatment. In the surgical group, similar comparisons were made before and after surgery. All analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC, USA) and R version 3.6.1 (R foundation for statistical computing, Vienna, Austria). A *P*-value of < 0.05 was considered statistically significant.

RESULTS

Demographic and clinical characteristics

The baseline clinical characteristics of the 29 patients with FHAM (male:female, 12:17) are summarized in Table 1. Patient median age at symptom onset was 59 years (range, 32.0–74.9) and the median time from symptom onset to FHAM diagnosis was 1.3 years (range, 0.8–8.0). All patients had constipation; most of them had symptoms characteristic of sub-occlusive episodes (e.g., abdominal pain and distension with or without nausea and vomiting), and ten (34.5%) patients had undergone previous abdominal surgeries, including laparoscopic appendectomy, uterine myomectomy, hysterectomy, and cholecystectomy. Table 1 also shows the baseline characteristics of the community control population. There were no significant differences between the two groups in terms of sex, history of abdominal surgery, and past medical history including hypertension, diabetes mellitus, ischemic heart disease,

vascular disease, hyperthyroidism, other autoimmune diseases, and neuromuscular disease. The weight and body mass index in the FHAM group were significantly lower than those in the community control group ($P < 0.001$), with concomitant chronic GI symptoms, which were present in all patients with FHAM but only in approximately 5% of the community control population.

No patient with FHAM had a family history of inflammatory bowel disease or GI neuromuscular disorder nor a history of premature birth, developmental problem, or radiotherapy. Within the FHAM cohort, there was no evidence that patients were receiving any medication that could be consistently associated with development of FHAM, nor were there any unusual dietary habits or occupational exposures that were common to the cohort[32].

Table 1. Clinical and demographic characteristics of patients with FHAM versus community controls

	FHAM (n = 29)	Health check-up aged 40–60 years (n = 19,948)	p-value
Basic demographics			
Men, n (%)	12 (41.4)	11783 (59.1)	1.000
Weight, median (IQR)	55.0 (47.4–61.0)	66.2 (56.9–74.6)	**< 0.001**
Height, median (IQR)	161.3 (155.6–165.4)	166.9 (160.4–173.0)	**0.002**
BMI at enrolment, kg/m^2 (IQR)	22.0 (18.8–23.3)	23.6 (21.2–25.3)	**< 0.001**
Age at symptom onset (years), median (range)	59.1 (32.0–74.9)	NA	NA
Duration from onset to diagnosis (years), median (range)	1.3 (0.1–8.0)	NA	NA
Gastrointestinal disease, n (%)			
Inflammatory bowel disease	0 (0)	0 (0)	NA
Abdominal or GI tuberculosis	0 (0)	0 (0)	NA
Previous abdominal surgery	10 (34.5)	4628 (23.2)	0.224
Previous abdominal or pelvic radiotherapy	0 (0)	0 (0)	NA
Past medical history, n (%)			
Hypertension	11 (37.9)	5508 (27.6)	0.302
DM	6 (20.7)	2279 (11.4)	0.136
Ischemic heart disease	0 (0)	781 (3.9)	0.629
Peripheral/cerebral vascular disease	0 (0)	740 (3.7)	0.626
Hyperthyroidism	1 (4.2)	492 (2.5)	0.516
Other autoimmune diseases	0 (0)	639 (3.2)	0.107
Connective tissue disease	0 (0)	0 (0)	NA
Neuromuscular disease	0 (0)	45 (0.2)	1.000
Main presenting symptoms, n (%)			
Abdominal pain	24 (82.8)	524 (2.6)	**< 0.001**
Constipation	29 (100)	1218 (6.1)	**< 0.001**
Diarrhoea	4 (13.8)	1004 (5.0)	0.084

Nausea/vomiting	12 (41.4)	453 (2.3)	**< 0.001**
Abdominal distension	20 (69.0)	1397 (7.0)	**< 0.001**
Weight loss	19 (65.5)	NA	NA

FHAM, focal hypoganglionosis adult megacolon; IQR, interquartile range; CT, computed tomography; NA, not available; BMI, body mass index; HTN, hypertension; DM, diabetes mellitus; *abdominal surgery included laparoscopic appendectomy, uterine myomectomy, hysterectomy, and cholecystectomy.

Diagnostic findings

Results of a panel of blood test showed no abnormalities in complete blood count, urea and electrolytes, liver function, and C-reactive protein level. CT and colonoscopy results are summarized in Table 2. The most common site of maximal dilatation was the splenic flexure (44.8%, 13/29), and the mean diameter was 78.8 mm [95% confidence interval (CI), 71.9–85.8 mm], as measured on CT. Typical plain and tomographic radiological findings of a patient with FHAM are shown in Figures 2A and 2B. The colon was considerably dilated with feces and air, and FFN was observed in all patients as an area of transition in diameter but without obstructive lesions. Overall, 13, 7, 5, and 4 patients had FFN in the splenic flexure, descending colon, sigmoid colon, and transverse colon, respectively. CT also excluded possible cases of mesenteric ischemia (all vessels patent) or adjacent peritoneal or solid organ disease (e.g., chronic pancreatitis). Figure 2C shows a typical endoscopic image of a patient with FFN in the sigmoid colon. Overall, 58.6% (17/29) of the FFN lesions were identified by colonoscopy.

Functional test results

Table 2 summarizes the GI functional test results. The rectoanal inhibitory response (RAIR) was intact in all patients. On BET, five patients failed to eject the balloon within 1 min. HRAM showed an abnormal pattern of rectoanal coordination in 21 patients. CTT test was performed in 23 patients, with a mean result of 58.8 ± 15.7 hours. Thus, 19 patients were defined as having delayed colonic transit on the basis of published control values[21,33,34]. Esophageal manometry was performed in 13 patients; 8 patients showed normal motility, 5 demonstrated ineffective esophageal motility, and none had classic achalasia. GES was performed in 18 patients and all of them had normal liquid and solid gastric emptying times

Table 2. Diagnostic test results in patients with FHAM

No	Sex	Age (years)	Age at onset (years)	CT location	Maximum Diameter (mm)	Colonoscopy-Confirmed	Anorectal physiology* †		CTTS (hours)	GES
							BE	HRAM		
1	F	34.0	33.1	SF	76	Yes	N	ABN	46.8	N
2	F	65.6	65.0	DC	63	Yes	N	ABN	64.8	N
3	F	48.7	45.9	DC	65	No	N	ABN	61.2	N
4	M	44.4	42.2	SF	126	Yes	N	ABN	67.2	N
5	M	75.8	74.9	SC	66	No	Delayed	ABN	70.8	NP
6	F	62.8	55.9	SF	69	Yes	N	ABN	68.4	N
7	M	76.5	68.4	SF	95	Yes	N	ABN	63.6	NP
8	F	69.6	61.8	SF	61	Yes	Delayed	N	58.8	N
9	F	65.5	60.6	SF	57	No	N	N	74.4	N
10	M	61.7	61.4	DC	107	Yes	N	N	NA	NP
11	M	42.5	42.3	SC	71	Yes	N	ABN	70.8	N
12	F	36.1	33.2	TC	95	No	N	ABN	68.4	N
13	F	65.5	51.9	SF	88	Yes	N	ABN	72	N
14	M	73.6	73.2	DC	87	No	Delayed	N	55.2	N
15	M	67.9	66.6	SC	69	No	Delayed	N	26.4	N
16	F	64.3	63.4	SC	55	No	N	N	69.6	N
17	F	46.9	46.7	TC	51	Yes	N	ABN	56.4	N
18	F	32.9	32.0	SF	72	No	N	ABN	50.4	N
19	F	59.5	59.1	TC	62	Yes	N	N	NA	N
20	F	46.4	45.3	SF	87	No	N	ABN	38.4	N
21	F	57.3	53.3	SF	89	Yes	N	ABN	58.8	NP
22	M	78.4	74.1	SF	95	Yes	N	ABN	69.6	NP
23	M	76.4	72.4	DC	61	No	N	ABN	10.8	N
24	F	53.7	51.5	SC	127	Yes	N	ABN	NA	NP
25	M	63.2	62.1	SF	85	No	N	ABN	58	NP

26	F	57.3	56.5	SF	78	Yes	N	N	NA	NP
27	M	68.5	62.2	TC	73	No	N	ABN	NA	NP
28	F	72.3	62.5	DC	74	Yes	N	ABN	NA	NP
29	M	58.6	57.4	DC	83	Yes	Delayed	ABN	71.8	NP

FHAM, focal hypoganglionosis adult megacolon; BE, balloon expulsion; HRAM, high-resolution anorectal manometry; CTTS, Colon transit time study; GES, gastric emptying study; ABN, abnormal pattern of recto-anal coordination; TC, transverse colon; SF, splenic flexure; DC, descending colon; SC, sigmoid colon; NP, not performed; N, normal.

* according to the London classification[20]

† Recto-anal inhibitory reflex present in all patients

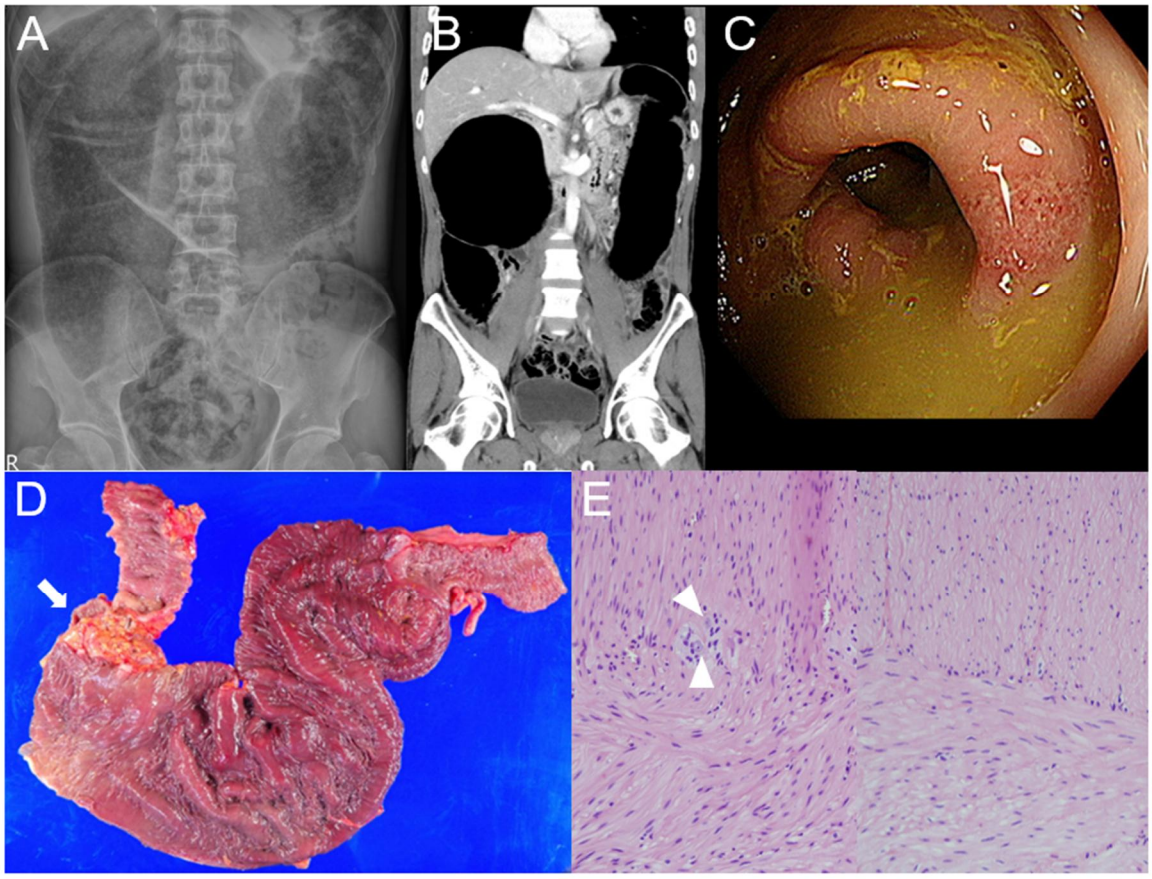


Figure 2. Example of patient with FHAM; Plain abdominal radiography (a) and computed tomography (b) of a typical patient with focal hypoganglionosis and adult-onset megacolon (patient #7). The colon shows marked dilatation without any definite obstructive lesion. FFN observed at colonoscopy (c). Gross specimen of subtotal colectomy showing dilated proximal and non-dilated distal colonic segments, and FFN connecting them (arrow) (d). Myenteric plexus of the dilated segment shows abundant ganglion cells (arrowhead), left and myenteric plexus in FFN (e) shows decreased number of ganglion cells (3–7 ganglion cells/cm), right (H&E, 20× objective lens). FHAM, focal hypoganglionosis adult-onset megacolon; FFN, focal functional narrowing; H&E, hematoxylin and eosin.

Histopathological findings

Ten patients underwent colonic resection with total colectomy in seven patients (for distally located FFN) and subtotal colectomy in three patients (more proximally located FFN). These patients had a generally more severe phenotype than those on medical treatment (see **Clinical course** section). The pathological characteristics of each patient are summarized in Table 3. Findings of gross inspection and H&E examination of the specimens are shown in Figures 2D and 2E. No abnormalities signifying evidence of inflammatory bowel disease, vasculitis, or parasitic infection were observed in the mucosa, muscularis propria, or vasculature. There was no evidence of fibrosis consistent with classical strictures. Post-operative histologic examination showed FFN with median length 25cm (interquartile range [IQR], 11-28cm). Myenteric ganglion cell density in the FFN, adjusted for the colonic circumference, was reduced when compared to that in the proximal dilated segments (median, 5.4 vs. 278.0 ganglia/cm) and the distal segments (presumed normal colon; median, 95.0) (Table 3) in all but one patient (#10), in whom the distal colon also appeared hypoganglionic. Inflammatory cell infiltration in the plexus was easily identified using H&E in some patients who underwent colectomy; CD3 immunostaining confirmed the localization of CD3-positive T cells mainly along the myenteric plexus. T cell infiltration was multifocal, with variable density, and was distributed primarily in the FFN area (Figures 3A and 3B).

Table 3. Histopathological findings in 10 FHAM patients who underwent colectomy

Patient No	FFN (cm)	Adjusted ganglion cell density*			Other findings
		Proximal	FFN	Distal	
4	28	152.6	13.8	13.8	LG
7	25	327.7	4.6	286.0	DN
10	24	370.0	3.9	3.9	LG
12	75	217.8	7.3	66.7	LG
24	15	156.1	3.4	126.9	EG
25	40	189.9	25.4	203.1	DN
26	10	287.5	5.4	85.9	DN
27	50	190.5	2.8	23.9	DN
28	30	348.9	41.5	95.4	DN
29	15	278.2	7.14	140	DN
Total†	25 (11–28)	278 (190–338)	5.4 (3.7–16.4)	95.0 (45.3–213.0)	

* Ganglion cell count/cm x internal circumference (cm)

† Data are expressed as medians (interquartile range).

FHAM, focal hypoganglionosis adult megacolon; FFN, focal functional narrowing; LG, lymphocytic ganglionitis; EG, eosinophilic ganglionitis; DN, degenerate neurons

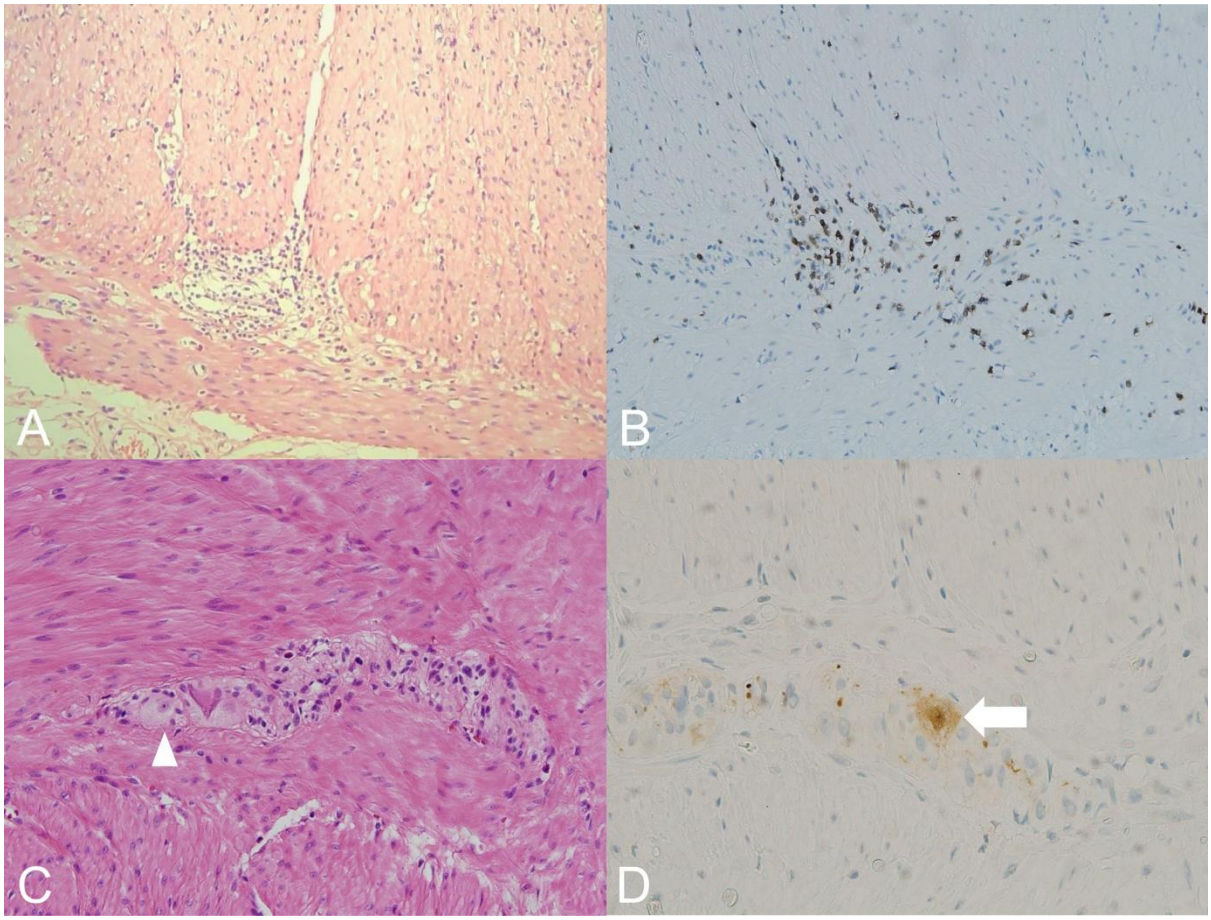


Figure 3. Key morphologic features for pathological analysis; Lymphocytic ganglionitis (a) and (b). A few mature lymphocytes are observed along the myenteric plexus (H&E, 10× objective lens) (a). CD3-positive T-cells are found mainly along the myenteric plexus (20× objective lens) (b). Ganglion cells (arrowhead) (H&E, 20× objective lens) (c). Ganglion cell with calretinin immunostaining (arrow) (d). Calretinin immunostaining reveals a rare ganglion cell in a hypoganglionic segment (40× objective lens). H&E, hematoxylin and eosin.

Clinical course

Among the 29 patients with FHAM in this study, 19 (65.5%) were managed with medical treatment, while the remaining 10 (34.5%) underwent surgery because of symptoms refractory to medical treatment. The latter patients had greater symptom burden and larger megacolon diameter than the former (surgical vs. medical groups: 90.0 mm [IQR, 79.2–104.0 mm] vs. 69.0 mm [IQR, 61.5–81.5 mm], $P = 0.002$) (Table 4). The mean interval from symptom onset to surgery was 30 ± 25 months.

Patients who were medically treated continued to have sub-occlusive episodes, with a mean annual frequency of two emergency department visits or hospitalization during the follow-up period. These patients often required decompression of the dilated lumen using endoscopy, Gomco suction pump (Microvention, California, USA) via Levine or rectal tube, as well as high-dose laxative therapy with polyethylene glycol. This was reflected in unchanged GBS and bowel frequency per week in the medical group during follow-up, compared to surgically managed patients who showed significant improvements in GBS (-0.3 vs. -5.4, $P < 0.001$) and bowel frequency per week (−5.4 vs +26, $P < 0.001$) (Figures 4A and 4B). The mean change in stool form was not significantly different between the two groups (+1.1 vs. +1.8, $P = 0.293$) (Figure 4C). Figures 4D and 4E present longitudinal data showing the benefits of surgery over time, with sustained changes in both GBS score and bowel movement frequency up to the 18 months of follow-up. Surgery also markedly reduced the recourse to medication (Figure 5).

Table 4. Baseline characteristics and clinical course of FHAM patients according to treatment.

	Medical treatment group (n = 19)	Surgery group (n = 10)	*p*-value
Basic demographics			
Men, n (%)	6 (31.6)	6 (60.0)	0.280
Weight, median (IQR)	55.0 (47.3–61.3)	56.0 (51.8–60.6)	0.963
Height, median (IQR)	160.7 (156.5–163.9)	165.2 (155.1–167.5)	0.324
BMI at enrolment, kg/m^2 (IQR)	21.9 (18.9–23.7)	22.1 (18.9–22.7)	0.783
Age at symptom onset, years, median (range)	59.1 (32.0–74.9)	59.2 (33.2–68.4)	0.818
Duration from symptom onset to diagnosis, years, median (range)	1.1 (0.2–7.7)	1.8 (0.1–8.0)	0.748
Past history, n (%)			
Hypertension	8 (42.1)	3 (30.0)	0.813
Diabetes mellitus	8 (42.1)	7 (70.0)	0.299
Hyperthyroidism	1 (4.2)	0 (0.0)	1.000
Previous abdominal surgery	4 (21.1)	6 (60.0)	0.092
Main presenting symptoms, n (%)			
Abdominal pain	14 (73.7)	10 (100.0)	0.205
Constipation	19 (100.0)	10 (100.0)	NA
Diarrhoea	1 (5.3)	3 (30.0)	0.204
Nausea/vomiting	5 (26.3)	7 (70.0)	0.061
Abdominal distension	11 (57.9)	9 (90.0)	0.176
Weight loss	11 (57.9)	8 (80.0)	0.436
Maximum diameter on CT, median (IQR)	69.00 (61.5–81.5)	90.0 (79.2–104.0)	**0.002**
Location of FFN, n (%)			0.205
Transverse–descending colon	14 (73.7)	10 (100.0)	
Sigmoid colon	5 (26.3)	0 (0.0)	
CTT (hours), median (IQR)	60.0 (51.6–69.6)	67.2 (63.6–68.4)	0.433

FHAM, focal hypoganglionosis adult megacolon; IQR, interquartile range; BMI, body mass index; CT, computed tomography; HTN, hypertension; DM, diabetes mellitus; NA, not available; FFN, focal functional narrowing; CTT, colon transit time

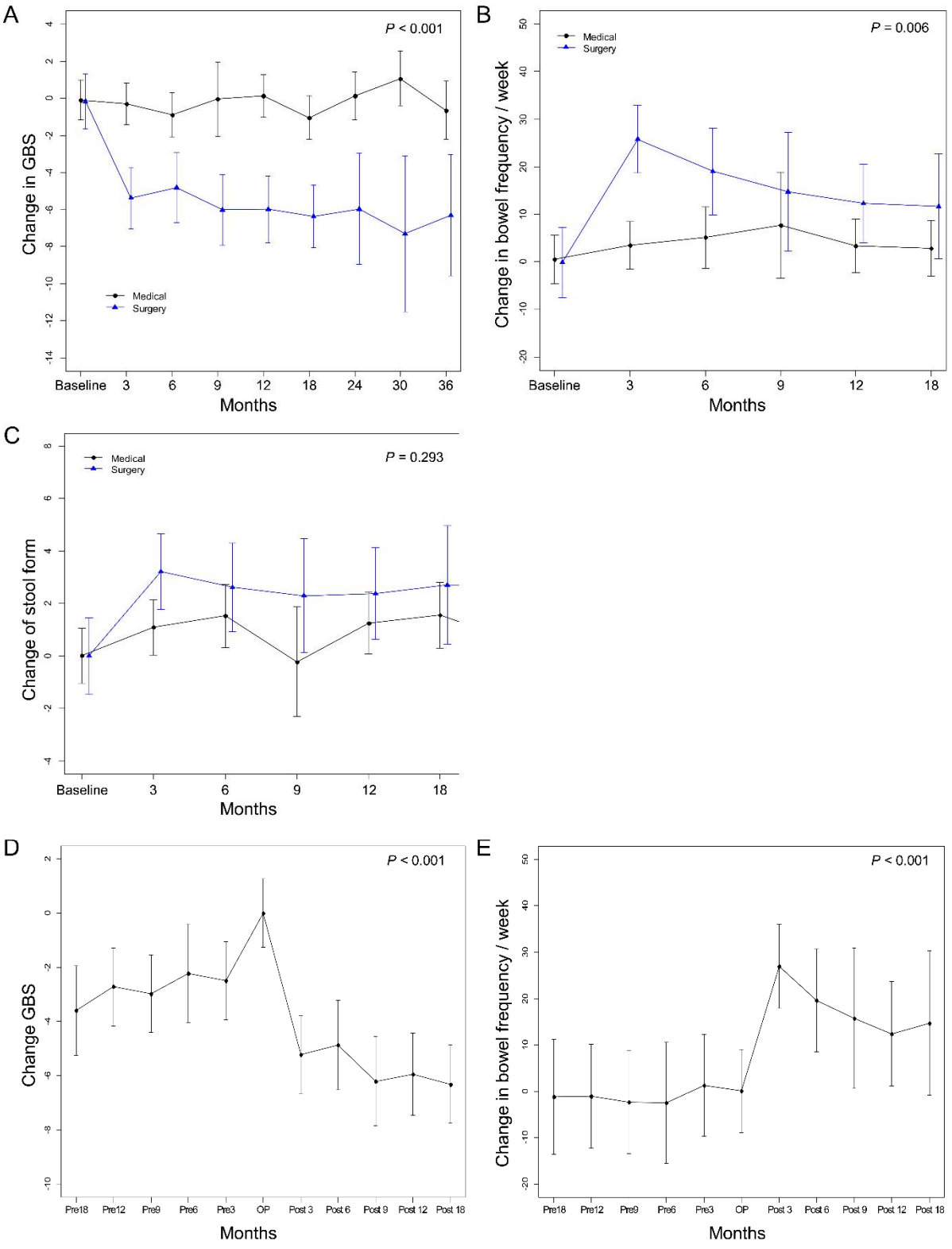


Figure 4. Longitudinal change of treatment outcomes; GBS score (0, dissatisfaction; 10, satisfaction) (a) and bowel movement frequency (b) per week in patients undergoing medical or surgical treatment.

Patients who underwent surgery showed significant improvement after 3 months. Longitudinal change in stool form in patients undergoing medical or surgical treatment (c). The mean change of stool form was not significantly different between the two groups. Improvements in GBS (d) and bowel movement frequency (e) clearly equated with timing of surgery and were sustained for 18 months. $P < 0.001$ for both regression lines. GBS, Global Bowel Satisfaction.

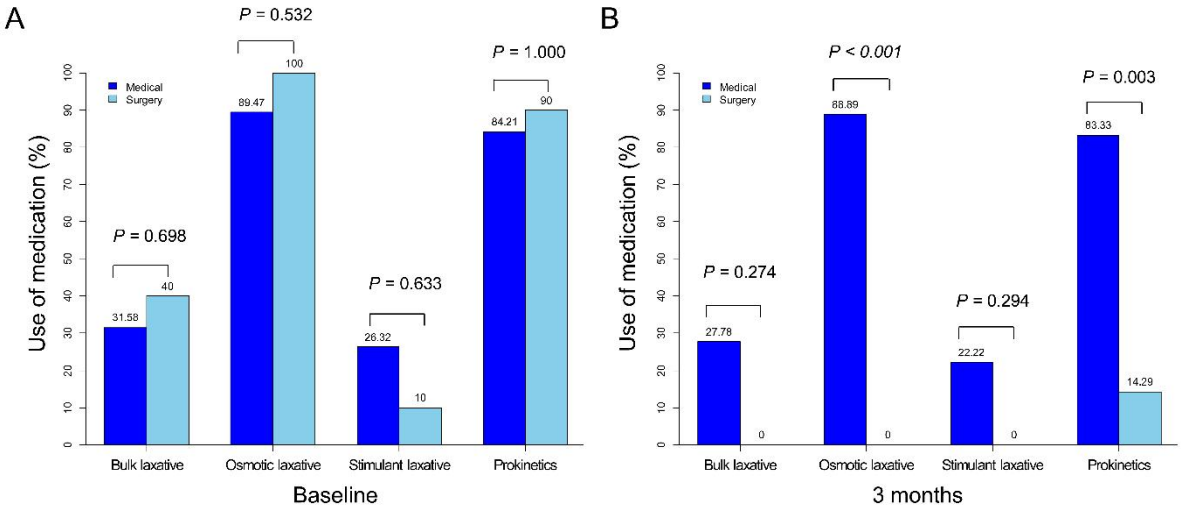


Figure 5. Comparison of medication use between the medically and surgically managed groups (A) at baseline and (B) at 3 months follow-up. There were no significant differences between the two groups at baseline in terms of laxative and prokinetic use. After 3 months, there were significant differences between the groups in terms of laxative and prokinetic use.

Fecal microbiome analysis

We next analyzed the fecal microbiome changes in both patients with FHAM and asymptomatic volunteers. The microbiome analyses showed that the operational taxonomic unit (OTU) of patients with FHAM was greater than that of asymptomatic volunteers (median: 890.5, 95% confidence interval (CI): 569.0-1182.0 (patients with FHAM) versus median: 351.0, CI: 293.0-644.0 (asymptomatic volunteers); $p<0.01$). The alpha diversity indices, such as Chao1, Observed OTUs, and Shannon, increased significantly in the FHAM group than in asymptomatic volunteers (Fig. 6A). These results indicate that the FHAM group had increased bacterial richness and diversity compared with asymptomatic volunteers. Applying the total microbiota profiles (all phyla together) or per phylum analysis, no disease-specific separation was observed by principal coordinates analysis (PCoA) (Fig. 6B). The patients with FHAM and asymptomatic volunteers showed comparable microbiota relative abundance at the phylum level, including Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria. However, Verrucomicrobia was significantly more prevalent in the FHAM group than in the asymptomatic volunteers (Fig. 7A; $p<0.05$). The most discriminative species were Butyricicoccus, Prevotella, and Agathobacter in FHAM patients, and Frisingicoccus, Ruthenibacterium, and Agathobaculum in asymptomatic volunteers at the genus level (Fig. 7B; all $P<0.05$).

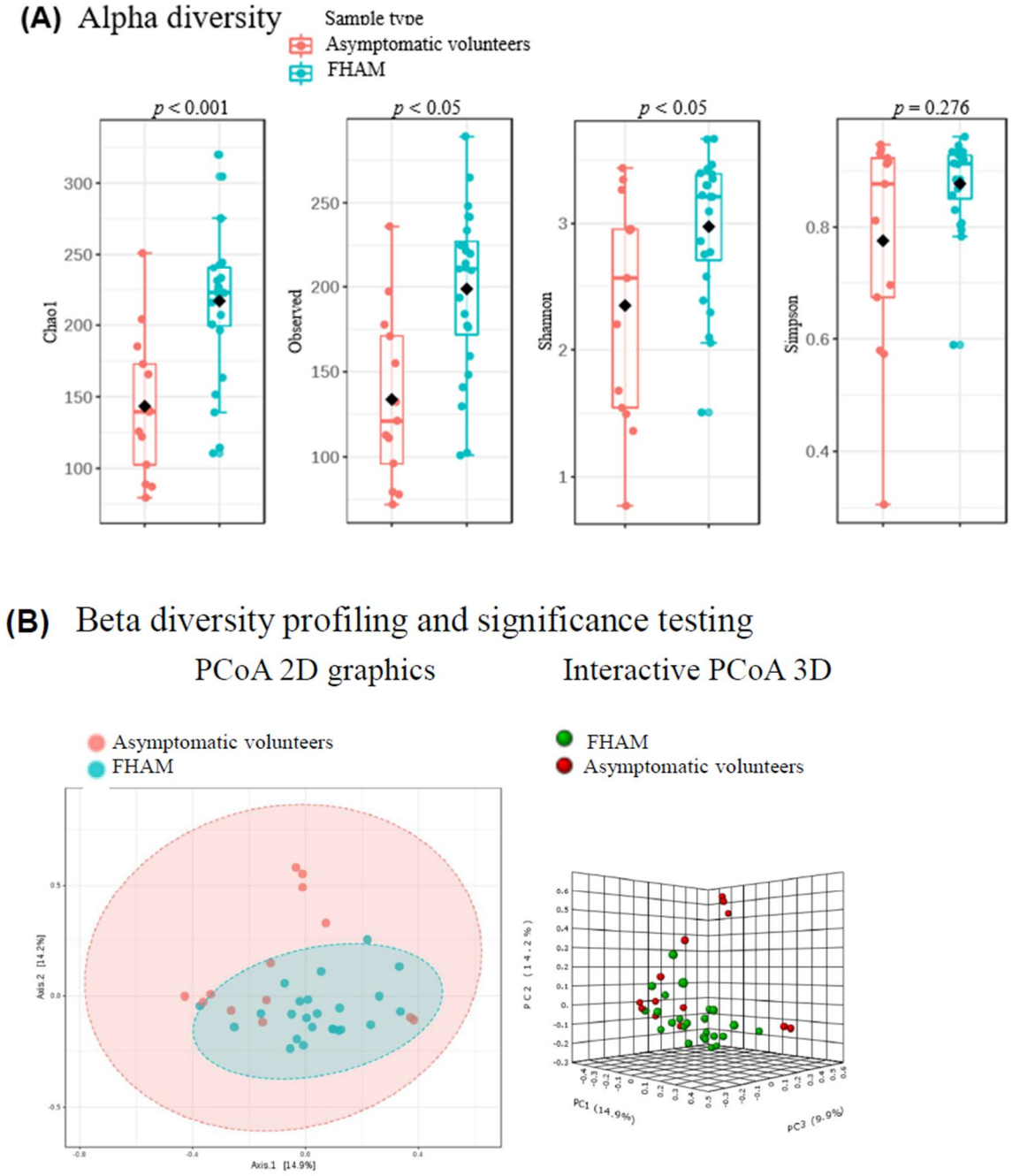
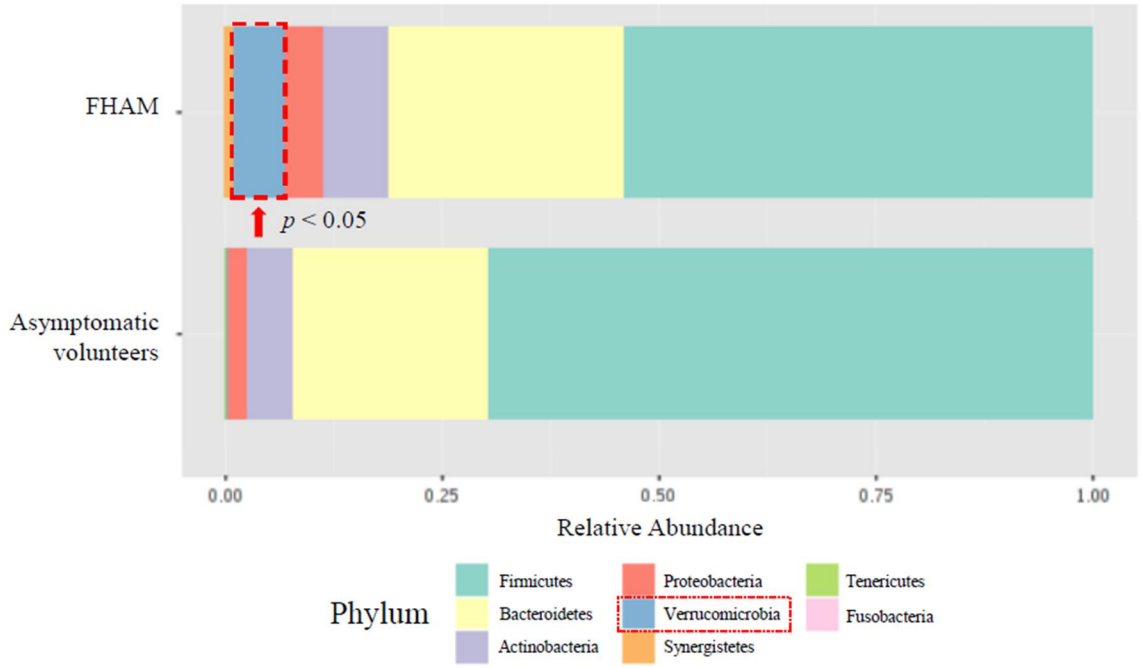


Figure 6. (A) Comparison of the alpha diversity of the microbiota between patients with focal hypoganglionosis with adult-onset megacolon (FHAM) (blue) and asymptomatic volunteers (red). Four indices were used to represent the richness (Chao, Observed OTUs) and sample diversity (Shannon and Simpson indices). (B) Principal coordinates analysis (PCoA) focused on the fecal bacterial communities by performing a principal component analysis in two distinct groups. The spatial distance showed the

similarity degree of the bacterial taxons among samples. PCoA showed that no disease-specific separation was observed. asymptomatic volunteers: red dots, n = 13; patients with FHAM: blue dots; n = 29.

(A) Classical univariate statistical comparisons at Phylum level



(B) Classical univariate statistical comparisons at Genus level

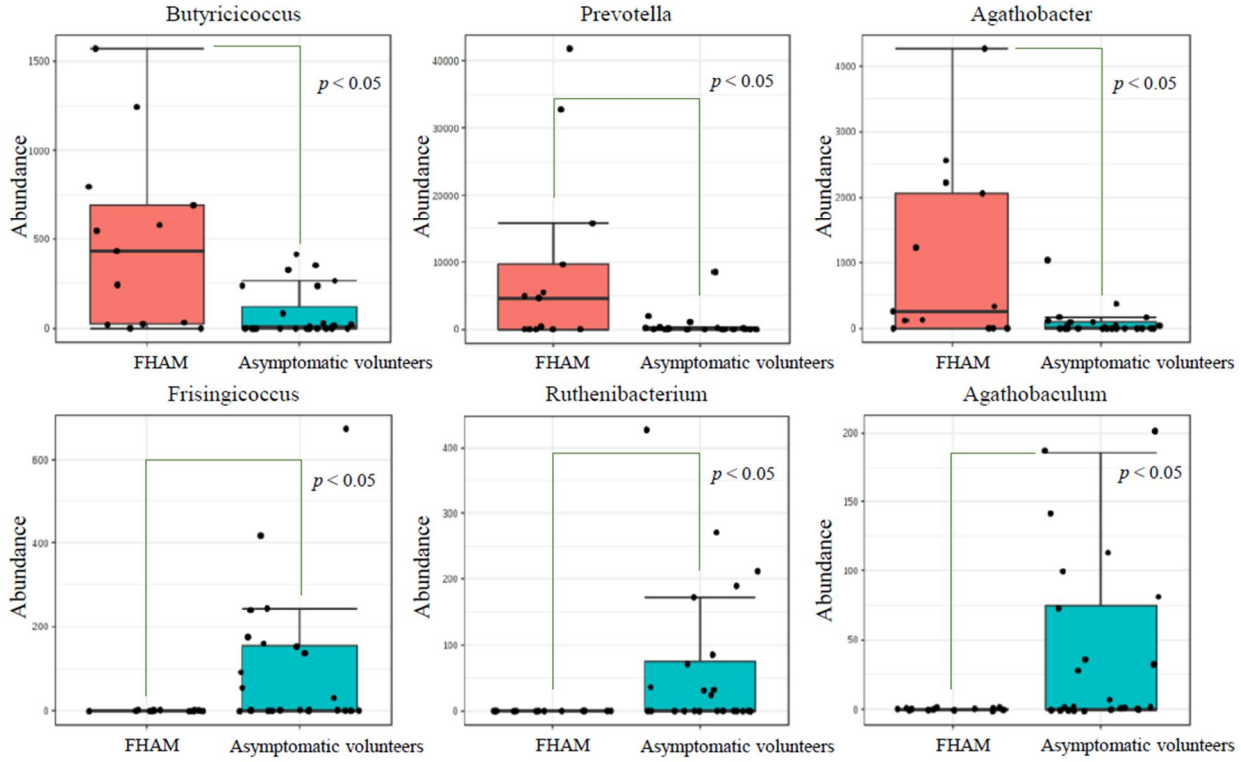


Figure 7. (A) Relative abundance. Patients with FHAM and asymptomatic volunteers showed comparable microbiota relative abundance at the phylum level, including Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria. However, Verrucomicrobia was significantly more prevalent in the FHAM group than in asymptomatic volunteers. (B) Classical univariate statistical comparisons at the genus level. The most discriminative species were Butyricicoccus. Prevotella, and Agathobacter for patients with FHAM, and Frisingicoccus, Ruthenibacterium, and Agathobaculum for asymptomatic volunteers at the genus level.

DISCUSSION

The current study describes a cohort of patients presenting to a single university hospital with chronic dilatation of a segment of the colon (megacolon) proximal to a short (1-7cm) area of reduced luminal diameter for which we have used the general term 'stenosis'. This disease is found roughly equally in males and females, with symptom onset usually in late adulthood (median age, 59 years). The stenotic segment is most frequently at the splenic flexure and can be readily observed through endoscopy or axial imaging in CT. In a subgroup of patients with severe symptoms undergoing colonic resection, there was gross loss of neurons in the stenotic region, as well as histopathological findings sufficient to diagnose hypoganglionosis, according to the London classification[26,27]. Moreover, patients with FHAM are associated with a rich and diverse fecal microbiome, including a high abundance of the phylum, Verrucomicrobia and the genera, Frisingicoccus, Ruthenibacterium, and Agathobaculum.

In this paper, we offer a new description of FHAM, acknowledging that while this term is new, it is not the first description of the disease. Three papers (all from Korea) have previously described FHAM, with 39 cases of "*colonic pseudo-obstruction with transition zone*"[5], 13 cases of "*colonic pseudo-obstruction with intractable constipation"*[6], and 24 with "*hypoganglionosis and adult-onset Hirschsprung's disease"*[18]. The newly proposed nomenclature—FHAM—corrects previous errors in the use of terms, such as pseudo-obstruction, transition zone, and Hirschsprung's disease (HSCR), noting that the correct term for chronic (rather than acute) dilatation of the colon is "megacolon" and that "transition" is a misnomer when there is no aganglionosis, which would "transition" to normoganglionic bowel. This study also considerably adds to the systematic description of the disease phenotype in terms of clinical characteristics and diagnostic findings from 29 patients, and the surgical (with corresponding surgical outcomes) and histopathological findings from ten patients.

By including almost 20,000 community controls, this study also attempted to further elucidate potential risk factors for the etiology of FHAM. On this basis, as well as based on the clinical, endoscopic, radiological, and histopathological phenotyping findings, FHAM cannot be explained by any obvious known disease or risk factor[35]. Thus, the FFN (the stenotic region) is categorically not attributable to any standard textbook etiology of colonic stricture, such as tumors, inflammatory bowel

disease, diverticular disease, endometriosis, radiation, intussusception, adhesions, or ischemia. There was no history of birth prematurity. Notably, while ischemia is supported by the distribution in and around the splenic flexure in many patients, i.e., in the watershed area of colonic vascularization, no patient had any history of cardiovascular or peripheral vascular disease, risk factors for arteriosclerosis, or evidence of mesenteric vascular disease on axial imaging. Histopathological examination similarly showed no evidence of typical changes associated with chronic ischemia or vasculitis, and we have avoided the use of the term "stricture" based on the absence of mural inflammation or fibrosis.

However, independent histopathological review at a reference center did confirm the very rare diagnosis of hypoganglionosis made by a combination of neuronal cell counting (per cm length of intestine) by an experienced pathologist (JHK) in Seoul and confirmed by documentation of gross reductions in ganglion cells in all representative sections sent to London (JM). It is accepted that the absolute absence of ganglion cells, as in HSCR[27], or subtle reductions in neuronal number, as seen in some functional conditions[36,37], require the specific use of neuronal immunomarkers, such as Hu C/D, synaptophysin, bcl-2, or NeuN, to count neurons reliably[26], although errors may still arise, according to our understanding of normality in the different intestinal regions[38]. However, the London Classification technical guidelines[26] provide the caveat that gross reductions can be diagnosed from H&E-stained sections, provided these are evaluated on sections at multiple levels through the block by pathologists familiar with the appearance of neurons after H&E staining. Indeed, it is clear from the presented quantitative data that when the proximal dilated segment (corrected for distension effect), functionally narrowed zone, and normal caliber distal segment were compared for neuronal counts, these were an order of 10-50 times different in density.

Lymphocytic ganglionitis is also a London Classification-recognized[27] diagnosis that is observed in a variety of GI neuromuscular diseases, characterized by normal caliber and chronic intestine dilation[39]. A significant number of lymphocytes were readily evident on H&E staining, which were confirmed as T-lymphocytes by CD3 immunostaining as per international recommendations[26]. These findings, combined with the absence of any abnormality indicating leiomyopathies in the muscularis propria, point to, but do not prove a causal sequence for, a disease characterized by inflammation,

neuronal degeneration, and neuronal loss. This combination of findings has been previously observed in a small number of patients with several GI neuromuscular diseases, such as intestinal pseudo-obstruction[39,40]. However, to our knowledge, these findings, in combination with a single focal stenotic area, are limited only to achalasia[41,42].

The central pathological finding of achalasia is myenteric neuronal loss leading to tonic contraction of the lower esophageal sphincter, absence of peristalsis, and later, proximal esophageal dilatation with obstruction to normal swallowing[43]. The observation of T-lymphocyte infiltration in early achalasia with progression to aganglionosis in advanced disease suggests that neuronal loss is intrinsically due to inflammation, with theories that this may be autoimmune or infectious in etiology[43]. In this study, unlike achalasia, there was no predominance of autoimmune diseases in patients, compared to community controls. Further comparison is also limited by the recognition that the pathophysiology of non-relaxation of a native sphincteric region (that is normally tonically contracted) is clearly different from that of a colon segment where the bowel is tonically relaxed. We have also tried to avoid comparisons with HSCR, in which the grossly shrunken aganglionic segment is considered to be a developmental consequence, due to overgrowth of the extrinsic motor innervation[44]. The characteristic serosal nerve trunks, observed best with calretinin immunostaining in aganglionic HSCR resections[45], were not evident on calretinin-stained resected colonic tissues in the current study. Figure 1 summarizes the findings schematically in comparison with HSCR and classical megacolon.

Similarly, with the absence of any obvious autoimmune etiology, we were unable to establish any other environmental risk factors (e.g., specific occupational or dietary exposures) in the population studied. Given the apparent distinct geographical distribution (Japan and Korea), parasitic disease was considered. However, histopathological findings bore no similarity to rare granulomatous inflammation and polyposis observed with *Schistosoma japonicum* infection that can occasionally lead to stricture formation[46]. Moreover, no patient had travelled to areas endemic for Chagas' disease; in fact, Chagasic megacolon has never been reported in Korea, and only one congenital case was reported in Japan in the child of immigrant South American parents[47]. While other specific geographical diseases leading to megacolon have been described, e.g. East African megacolon[48], affected patients had massive dilatation

of the whole colon without a stenotic segment and the pathology was an obvious leiomyopathy. A viral etiology has been sought for other GI neuromuscular diseases, including achalasia[43], but was not studied in FHAM patients.

The follow-up period of up to 3 years suggests that surgery is safe and associated with good outcomes. Besides reinforcing surgery as a good option in selected patients with severe symptoms[5], these data add further weight to the focal nature of the described disease and to the concept that the regional megacolon in this instance represents a form of pre-stenotic dilatation of the colon rather than the "dominantly" affected area of a more diffuse disease. Surgery is generally considered impotent in GI neuromuscular diseases associated with dilatation in more diffuse neuropathic and myopathic disease processes even when these appear localized[49].

It was gradually realized that establishing and maintaining a beneficial balance between the intestinal microbiota and the human body are necessary for the normal functioning of the intestine. The dysbiosis will contribute to the pathogenesis of functional gastrointestinal disorders. Therefore, it is essential to study the role of intestinal microbiota from patients with FHAM in gut motility. It remains unclear whether the microbiota participates in the pathogenesis of FHAM. In this study, we extracted and analyzed the microbiota in the fecal samples obtained from patients with FHAM. Our study also showed that a specific genus of bacteria is correlated with FHAM, which could not be observed in asymptomatic volunteers. The present investigation is the first microbiome analysis of FHAM, which will serve as a cornerstone in the exploration of the pathogenesis of FHAM.

Bacterial richness and diversity were more increased in the FHAM group than in the asymptomatic volunteers. The intestinal microbiota in normal adults are predominantly composed of three phyla, which are as follows: Bacteroidetes, Firmicutes, and Actinobacteria.[50] Other Proteobacteria, such as Verrucomicrobia, are also among the major intestinal microbiota. Another study have found that Bacteroidetes were more abundant in the colonic mucosa of patients with chronic constipation.[51] Moreover, Firmicutes including Firmicutes-Coprococcus, Firmicutes-Faecalibacteriu, Firmicutes-Lactococcus, and Firmicutes-Roseburia were independently significantly useful for predicting CTT.[21] Slow CTT is inversely correlated with colonic serotonin content, associated with a decreased relative

abundance of Firmicutes and an increased relative abundance Bacteriodetes, and associated with altered fecal content of short-chain fatty acids and bile acids.[16] Accumulated evidence show gut microbes can interact with the human host through modulation of 5-HT signaling. Yano et al. reported that the intestinal microbiota could change the level of serotonin transporter (SERT), a transmembrane transport protein that re-uptakes excessive 5-HT from effective location to terminate its physiological effects and regulate the gastrointestinal function.[52] Some specific bacteria have been reported to regulate the expression of SERT in intestinal epithelial cells, such as *Listeria monocytogenes* [53] and *Escherichia coli*.[54] Similarly, in the FHAM group, Bacteroidetes were more abundant, and Firmicutes were decreased. Verrucomicrobia was significantly increased in our study. We also found a significantly higher abundance of Butyricicoccus, Prevotella, and Agathobacter and significantly lower abundance of Frisingicoccus, Ruthenibacterium, and Agathobaculum at the genus level in patients with FHAM than in the healthy controls. Butyricicoccus was also found to be significantly higher in Parkinson's disease patients with constipation in one study.[55] Moreover, as a microbially produced metabolite, butyrate has been shown to be metabolized by the microbiota in the intestine.[56] In our study, we found that Butyricicoccus were significantly higher in the FHAM group than in the healthy controls. Therefore, it is possible that these butyrate-producing bacteria change the metabolism of the host, thereby affecting the gastrointestinal motility. This hypothesis is supported by animal experiments that have demonstrated that butyric acid may inhibit colonic smooth muscle contraction and cause slow transit constipation, owing to the colonic absorption of water, which makes the feces dry and hard.[57] Prevotella was also found to be significantly high in elderly patients with constipation.[58] Another study by Zhu et al. showed a significantly decreased abundance of Prevotella in constipated patients [59]. However, Prevotella and Bacteroides have been found to be directly and inversely related with CTT, which has been suggested for irritable bowel syndrome (IBS).[60] These findings indicate that Bacteroides and Prevotella may be positively related to functional constipation and FHAM.

One study suggested that FMT could increase the bowel movements and improve constipation in patients.[61,62] Another pilot study suggested that FMT might be beneficial for the treatment of CIPO.[17] Therefore, the microbiota dysbiosis was suspected as the cause of megacolon, rather than the result of

the dilated colon. Our study is the first to evaluate the fecal microbiota dysbiosis in FHAM. We cannot definitely conclude that the main cause of FHAM is[17] microbiota dysbiosis. However, some genes associated with chronic constipation are found in FHAM. Our study is the first one to evaluate the fecal microbiota dysbiosis in this patient group. Further research is needed to determine whether the fecal microbiome composition normalizes after antibiotic therapy or surgical treatment in patient with FHAM. The microbiota plays a key role in the development and promotion of normal gut neuromotor function.

This study has several limitations. First, the collection of patients' fecal material was done after symptom development. Therefore, we cannot guarantee that this finding could be the result of fecal stasis owing to the dilated colon, rather than the cause. However, patients showed persistent symptoms since the onset of disease. Moreover, they did not undergo FMT or antibiotic treatment. Furthermore, a fecal microbiome analysis was performed only once after their enrollment in the study. Other study in patients with constipation with repeat tests shows the reproducibility of our results without change.[14] Second, the number of patients analyzed is relatively small. However, FHAM is a very rare disease.[3,18,63] Therefore, this population size might still be clinically meaningful

CONCLUSION

In conclusion, FHAM is characterized by chronic refractory constipation and recurrent episodes of abdominal pain/bloating, colon dilation of >5.0 cm, and/or presence of intestinal air-fluid levels on radiologic studies. Moreover, the FFN causing obstruction as detected by CT and/or lower endoscopy could be found. FHAM is a new enteric neuropathy, characterized by hypoganglionosis that may be secondary to inflammation, and bowel resection appears to benefit patients. Moreover, FHAM is associated with a rich and diverse fecal microbiota, including a high abundance of Frisingicoccus, Ruthenibacterium, and Agathobaculum and a low abundance of Butyricicoccus, Prevotella, and Agathobacter. This finding could be related to chronic refractory constipation or might be owing to hypoganglionosis. Further studies are required to establish its etiology.

REFERENCES

1. Wang XJ, Camilleri M. Chronic Megacolon Presenting in Adolescents or Adults: Clinical Manifestations, Diagnosis, and Genetic Associations. *Dig Dis Sci* 2019; 64: 2750-2756.

2. Cuda T, Gunnarsson R, de Costa A. Symptoms and diagnostic criteria of acquired Megacolon - a systematic literature review. *BMC Gastroenterol* 2018; 18: 25.

3. Lee KJ, Jung KW, Myung SJ, Kim HJ, Kim NY, Yoon YH *et al.* The clinical characteristics of colonic pseudo-obstruction and the factors associated with medical treatment response: a study based on a multicenter database in Korea. *J Korean Med Sci* 2014; 29: 699-703.

4. Ohkubo H, Masaki T, Matsuhashi N, Kawahara H, Yokoyama T, Nakajima A *et al.* Histopathologic findings in patients with idiopathic megacolon: a comparison between dilated and non-dilated loops. *Neurogastroenterol Motil* 2014; 26: 571-580.

5. Song EM, Kim JW, Lee SH, Chang K, Hwang SW, Park SH *et al.* Colonic Pseudo-obstruction With Transition Zone: A Peculiar Eastern Severe Dysmotility. *J Neurogastroenterol Motil* 2019; 25: 137-147.

6. Do YS, Myung SJ, Kwak SY, Cho S, Lee E, Song MJ *et al.* Molecular and Cellular Characteristics of the Colonic Pseudo-obstruction in Patients With Intractable Constipation. *J Neurogastroenterol Motil* 2015; 21: 560-570.

7. Clemente JC, Ursell LK, Parfrey LW, Knight R. The impact of the gut microbiota on human health: an integrative view. *Cell* 2012; 148: 1258-1270.

8. Castaner O, Goday A, Park YM, Lee SH, Magkos F, Shiow STE *et al.* The Gut Microbiome Profile in Obesity: A Systematic Review. *Int J Endocrinol* 2018; 2018: 4095789.

9. Lane ER, Zisman TL, Suskind DL. The microbiota in inflammatory bowel disease: current and

therapeutic insights. *J Inflamm Res* 2017; 10: 63-73.

10. Li B, Selmi C, Tang R, Gershwin ME, Ma X. The microbiome and autoimmunity: a paradigm from the gut-liver axis. *Cell Mol Immunol* 2018; 15: 595-609.

11. Pascal M, Perez-Gordo M, Caballero T, Escribese MM, Lopez Longo MN, Luengo O *et al.* Microbiome and Allergic Diseases. *Front Immunol* 2018; 9: 1584.

12. Tilg H, Moschen AR. Microbiota and diabetes: an evolving relationship. *Gut* 2014; 63: 1513-1521.

13. Sahakian AB, Jee SR, Pimentel M. Methane and the gastrointestinal tract. *Dig Dis Sci* 2010; 55: 2135-2143.

14. Attaluri A, Jackson M, Valestin J, Rao SS. Methanogenic flora is associated with altered colonic transit but not stool characteristics in constipation without IBS. *Am J Gastroenterol* 2010; 105: 1407-1411.

15. Lee KM, Paik CN, Chung WC, Yang JM, Choi MG. Breath methane positivity is more common and higher in patients with objectively proven delayed transit constipation. *Eur J Gastroenterol Hepatol* 2013; 25: 726-732.

16. Cao H, Liu X, An Y, Zhou G, Liu Y, Xu M *et al.* Dysbiosis contributes to chronic constipation development via regulation of serotonin transporter in the intestine. *Sci Rep* 2017; 7: 10322.

17. Gu L, Ding C, Tian H, Yang B, Zhang X, Hua Y *et al.* Serial Frozen Fecal Microbiota Transplantation in the Treatment of Chronic Intestinal Pseudo-obstruction: A Preliminary Study. *J Neurogastroenterol Motil* 2017; 23: 289-297.

18. Do MY, Myung SJ, Park HJ, Chung JW, Kim IW, Lee SM *et al.* Novel classification and pathogenetic analysis of hypoganglionosis and adult-onset Hirschsprung's disease. *Dig Dis Sci* 2011; 56: 1818-1827.

19. Han EC, Oh HK, Ha HK, Choe EK, Moon SH, Ryoo SB *et al.* Favorable surgical treatment outcomes for chronic constipation with features of colonic pseudo-obstruction. *World J Gastroenterol* 2012; 18: 4441-4446.

20. Carrington EV, Heinrich H, Knowles CH, Fox M, Rao S, Altomare DF *et al.* The international anorectal physiology working group (IAPWG) recommendations: Standardized testing protocol and the London classification for disorders of anorectal function. *Neurogastroenterol Motil* 2020; 32: e13679.

21. Metcalf AM, Phillips SF, Zinsmeister AR, MacCarty RL, Beart RW, Wolff BG. Simplified assessment of segmental colonic transit. *Gastroenterology* 1987; 92: 40-47.

22. Longstreth GF, Thompson WG, Chey WD, Houghton LA, Mearin F, Spiller RC. Functional bowel disorders. *Gastroenterology* 2006; 130: 1480-1491.

23. Song KH, Jung HK, Min BH, Youn YH, Choi KD, Keum BR *et al.* Development and Validation of the Korean Rome III Questionnaire for Diagnosis of Functional Gastrointestinal Disorders. *J Neurogastroenterol Motil* 2013; 19: 509-515.

24. Rao S, Lembo AJ, Shiff SJ, Lavins BJ, Currie MG, Jia XD *et al.* A 12-week, randomized, controlled trial with a 4-week randomized withdrawal period to evaluate the efficacy and safety of linaclotide in irritable bowel syndrome with constipation. *Am J Gastroenterol* 2012; 107: 1714-1724; quiz p.1725.

25. Corsetti M, Tack J. FDA and EMA end points: which outcome end points should we use in clinical trials in patients with irritable bowel syndrome? *Neurogastroenterol Motil* 2013; 25: 453-457.

26. Knowles CH, De Giorgio R, Kapur RP, Bruder E, Farrugia G, Geboes K *et al.* Gastrointestinal neuromuscular pathology: guidelines for histological techniques and reporting on behalf of the Gastro 2009 International Working Group. *Acta Neuropathol* 2009; 118: 271-301.

27. Knowles CH, De Giorgio R, Kapur RP, Bruder E, Farrugia G, Geboes K *et al.* The London Classification of gastrointestinal neuromuscular pathology: report on behalf of the Gastro 2009 International Working Group. *Gut* 2010; 59: 882-887.

28. Jeon YS, Chun J, Kim BS. Identification of household bacterial community and analysis of species shared with human microbiome. *Curr Microbiol* 2013; 67: 557-563.

29. Eddy SR. Accelerated Profile HMM Searches. *PLoS Comput Biol* 2011; 7: e1002195.

30. Edgar RC. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 2010; 26: 2460-2461.

31. Fu L, Niu B, Zhu Z, Wu S, Li W. CD-HIT: accelerated for clustering the next-generation sequencing data. *Bioinformatics* 2012; 28: 3150-3152.

32. Shafiee NH, Razalli NH, Mokhtar NM, Tan E, Ali RAR. An evaluation of dietary adequacy among patients with constipation-predominant irritable bowel syndrome in Malaysia. *Intest Res* 2021.

33. Song EM, Lee HJ, Jung KW, Kim MJ, Hwang SW, Park SH *et al.* Long-Term Risks of Parkinson's Disease, Surgery, and Colorectal Cancer in Patients With Slow-Transit Constipation. *Clin Gastroenterol Hepatol* 2020.

34. Arhan P, Devroede G, Jehannin B, Lanza M, Faverdin C, Dornic C *et al.* Segmental colonic transit time. *Dis Colon Rectum* 1981; 24: 625-629.

35. Mahajan R, Gupta Y, Singh A, Dhiman P, Midha V, Kakkar C *et al.* Clinical profile and outcomes of opioid abuse gastroenteropathy: an underdiagnosed disease entity. *Intest Res* 2020; 18: 238-244.

36. Knowles CH, Lindberg G, Panza E, De Giorgio R. New perspectives in the diagnosis and management of enteric neuropathies. *Nat Rev Gastroenterol Hepatol* 2013; 10: 206-218.

37. Chikkamenahalli LL, Pasricha PJ, Farrugia G, Grover M. Gastric Biopsies in Gastroparesis: Insights into Gastric Neuromuscular Disorders to Aid Treatment. *Gastroenterol Clin North Am* 2020; 49: 557-570.

38. Knowles CH, Veress B, Kapur RP, Wedel T, Farrugia G, Vanderwinden JM *et al.* Quantitation of cellular components of the enteric nervous system in the normal human gastrointestinal tract--report on behalf of the Gastro 2009 International Working Group. *Neurogastroenterol Motil* 2011; 23: 115-124.

39. Lindberg G, Törnblom H, Iwarzon M, Nyberg B, Martin JE, Veress B. Full-thickness biopsy findings in chronic intestinal pseudo-obstruction and enteric dysmotility. *Gut* 2009; 58: 1084-1090.

40. Lindberg G. Pseudo-obstruction, enteric dysmotility and irritable bowel syndrome. *Best Pract Res Clin Gastroenterol* 2019; 40-41: 101635.

41. Goldblum JR, Whyte RI, Orringer MB, Appelman HD. Achalasia. A morphologic study of 42 resected specimens. *Am J Surg Pathol* 1994; 18: 327-337.

42. Clark SB, Rice TW, Tubbs RR, Richter JE, Goldblum JR. The nature of the myenteric infiltrate in achalasia: an immunohistochemical analysis. *Am J Surg Pathol* 2000; 24: 1153-1158.

43. Rieder E, Fernandez-Becker NQ, Sarosiek J, Guillaume A, Azagury DE, Clarke JO. Achalasia: physiology and diagnosis. *Ann N Y Acad Sci* 2020; 1482: 85-94.

44. Tam PK, Boyd GP. Origin, course, and endings of abnormal enteric nerve fibres in Hirschsprung's disease defined by whole-mount immunohistochemistry. *J Pediatr Surg* 1990; 25: 457-461.

45. Hwang S, Kapur RP. Advances and Pitfalls in the Diagnosis of Hirschsprung Disease. *Surg Pathol Clin* 2020; 13: 567-579.

46. Strickland GT. Gastrointestinal manifestations of schistosomiasis. *Gut* 1994; 35: 1334-1337.

47. Imai K, Misawa K, Osa M, Tarumoto N, Sakai J, Mikita K *et al.* Chagas disease: a report of 17 suspected cases in Japan, 2012-2017. *Trop Med Health* 2019; 47: 38.

48. Kaschula RO, Moore SW, Rode H, Cywes S. Degenerative leiomyopathy in African children: a review of current perspectives. *East Afr Med J* 1993; 70: 37-39.

49. Stanghellini V, Cogliandro RF, De Giorgio R, Barbara G, Morselli-Labate AM, Cogliandro L *et al.* Natural history of chronic idiopathic intestinal pseudo-obstruction in adults: a single center study. *Clin Gastroenterol Hepatol* 2005; 3: 449-458.

50. Arumugam M, Raes J, Pelletier E, Le Paslier D, Yamada T, Mende DR *et al.* Enterotypes of the human gut microbiome. *Nature* 2011; 473: 174-180.

51. Zhao Y, Yu YB. Intestinal microbiota and chronic constipation. *Springerplus* 2016; 5: 1130.

52. Yano JM, Yu K, Donaldson GP, Shastri GG, Ann P, Ma L *et al.* Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. *Cell* 2015; 161: 264-276.

53. Latorre E, Pradilla A, Chueca B, Pagan R, Layunta E, Alcalde AI *et al.* Listeria monocytogenes Inhibits Serotonin Transporter in Human Intestinal Caco-2 Cells. *Microb Ecol* 2016; 72: 730-739.

54. Esmaili A, Nazir SF, Borthakur A, Yu D, Turner JR, Saksena S *et al.* Enteropathogenic Escherichia coli infection inhibits intestinal serotonin transporter function and expression. *Gastroenterology* 2009; 137: 2074-2083.

55. Ilie OD, Ciobica A, McKenna J, Doroftei B, Mavroudis I. Minireview on the Relations between Gut Microflora and Parkinson's Disease: Further Biochemical (Oxidative Stress), Inflammatory, and Neurological Particularities. *Oxid Med Cell Longev* 2020; 2020: 4518023.

56. Louis P, Flint HJ. Formation of propionate and butyrate by the human colonic microbiota. *Environ Microbiol* 2017; 19: 29-41.

57. Bach Knudsen KE, Lærke HN, Hedemann MS, Nielsen TS, Ingerslev AK, Gundelund Nielsen DS *et al.* Impact of Diet-Modulated Butyrate Production on Intestinal Barrier Function and Inflammation. *Nutrients* 2018; 10.

58. Guo M, Yao J, Yang F, Liu W, Bai H, Ma J *et al.* The composition of intestinal microbiota and its association with functional constipation of the elderly patients. *Future Microbiol* 2020; 15: 163-175.

59. Zhu L, Liu W, Alkhouri R, Baker RD, Bard JE, Quigley EM *et al.* Structural changes in the gut microbiome of constipated patients. *Physiol Genomics* 2014; 46: 679-686.

60. Wolf PG, Parthasarathy G, Chen J, O'Connor HM, Chia N, Bharucha AE *et al.* Assessing the colonic microbiome, hydrogenogenic and hydrogenotrophic genes, transit and breath methane in constipation. *Neurogastroenterol Motil* 2017; 29: 1-9.

61. Tian H, Ding C, Gong J, Ge X, McFarland LV, Gu L *et al.* Treatment of Slow Transit Constipation With Fecal Microbiota Transplantation: A Pilot Study. *J Clin Gastroenterol* 2016; 50: 865-870.

62. Ge X, Zhao W, Ding C, Tian H, Xu L, Wang H *et al.* Potential role of fecal microbiota from patients with slow transit constipation in the regulation of gastrointestinal motility. *Sci Rep* 2017; 7: 441.

63. Taguchi T, Masumoto K, Ieiri S, Nakatsuji T, Akiyoshi J. New classification of hypoganglionosis: congenital and acquired hypoganglionosis. *J Pediatr Surg* 2006; 41: 2046-2051.

국문요약

OBJECTIVES: 기존의 알려진 거대결장과 달리 일부 환자는 영상 검사나 대장 내시경 시 국소적인 협착이 관찰되며 이 협착은 보통 구불 결장 만곡부 이하 부위에서 관찰되고 이 협착 부위의 근위부가 늘어나 거대 결장이 관찰된다. 이전 연구에서는 성인 환자, 특히 한국인과 일본인을 중심으로 한 아시아인에서 국소적으로 좁아진 이행대가 있는 신경절 저하증으로 기술되었다. 우리는 이러한 특징을 가진 환자를 조사하고 이 질병을 성인에서 발생한 거대 결장 국소 신경절세포감소증 (Focal hypoganglionosis, adult-onset megacolon, FHAM)으로 기술하였는데, 이는 전형적인 거대 결장과는 다른 임상적 특징을 더 잘 나타내기 때문이다. 본 연구에서는 이러한 특징을 가진 환자들을 대상으로 영상학적, 내시경적, 병리학적, 그리고 임상 경과를 조사하였고 대변 검체를 모아 장내 미생물의 특징을 조사하였다.

METHODS: 2017 년 1 월부터 2020 년 3 월까지 서울 아산 병원에 내원한 환자들 중 거대 결장 국소 신경절 세포 감소증의 특징을 보이는 환자들을 포함하였다. 현재까지 성인에서 발생한 거대 결장 국소 신경절세포감소증에 대해 확립된 진단 기준은 없어 이전 연구를 기반으로 만성 불응성 변비 및 재발성 복통 및 폐쇄성을 암시하는 팽만, 5cm 를 넘는 대장의 팽창, 명백한 구조적 원인 없이 CT 및/또는 대장 내시경에서 관찰된 국소 협착이 관찰되는 환자들을 대상으로 하였다. 본원에서 상기 기준을 만족하는 29 명의 환자들이 연구 기간 동안 포함되었다. 성인에서 발생한 거대 결장 국소 신경절세포감소증의 병인이 알려져 있지 않기 때문에 질병 발병의 위험 요인을 알아보기 위해 2019 년 1 월부터 12 월까지 40-60 세의 서울 아산 병원 검강검진센터에서 대장 내시경 및 복부 CT 를 포함한 건강 검진을 받은 성인 19,948 명이 대조군으로 포함되어 대조군의 임상적 특징을 성인에서 발생한 거대 결장 국소 신경절세포감소증 환자들과 비교하였다. 포함된 성인에서 발생한 거대 결장 국소 신경절세포감소증 환자들을 대상으로 소화기 기능 검사를 시행하였고 복용하는 약물, 응급실 내원, 복통, 대변 회수, 대변 형태 등 임상 경과에 대해 약물 치료 군과 수술 치료 군을 비교하였다. 수술 받은 환자들에 대해서는 병리학적 분석을 시행하였고 신경절 세포를 확인하였다. 또한 신경절 염증이 의심되는 경우에 CD3 염색을 추가로 시행하였다. 성인에서 발생한 거대 결장 국소 신경절세포감소증 환자들의 대변을 채취하여 장내 미생물을 확인하였고 비교를 위해 무증상의 정상인 13 명의 대변과 비교 분석하였다.

RESULTS: **총 29 명의** 거대 결장 국소 신경절 세포 감소증의 환자들이 포함되었고 증상이 발현한 나이 중앙값은 59.1 세였다. 19,948 명의 건강 검진을 받은 대조군과 비교하였을 때 특이 위험 인자는 확인되지 않았고 체중은 거대 결장 국소 신경절 세포 감소증 환자들이 유의하게 체중 감소를 보였다. 복부 CT 와 대장 내시경 검사에서 장이 늘어난 근위부 결장과 정상 직경을 보이는 원위부 결장을 잇는 국소적으로 좁아진 부위를 확인할 수 있었고 이 부위를 국소 기능적 협착부위로 (focal functional narrowing, FFN) 명명하였다. 이러한 국소 기능적 협착부위는 비만곡부에서 44.8%로 가장 높은 빈도를 보였다. 또한 늘어난 결장의 평균 직경은 78.8 mm 로 (95% 신뢰도 71.9-85.8)이었다. 29 명의 환자 중 10 명이 결장전절제술 또는 결장아전절제술을 시행 받았고 병리 분석을 시행하였다. 절제된 검체에서는 염증성 대장 질환, 혈관염, 기생충 감염 등을 의심할만한 소견은 관찰되지 않았고 일반적인 협착에서 볼 수 있는 섬유화의 소견도 관찰되지 않았다. 국소 기능적 협착부위에서는 협착 부위의 근위부와 원위부에 비해 저신경절증을 확인할 수 있었다 (중앙값 5.4 vs 278.0 ganglia/cm, 국소 기능적 협착 부위 vs 근위 결장). H & E 염색에서 신경절 주변으로 염증 세포 침윤이 일부 환자에서 관찰되었고 이 환자들은 CD 3 염색을 통해 신경절을 따라서 있는 CD 3 양성 T 세포를 확인할 수 있었다. 10 명의 수술 받은 환자들은 19 명의 약물 치료만 유지했던 환자들에 비해 수술 후 만족도와 배변 회수에서 유의한 호전을 보였고 사용 약물의 수도 감소 하였다. **29 명의** 거대 결장 국소 신경절 세포 감소증 환자들에 대해 장내 미생물 분석을 시행하였고 무증상 대조군에 비해 알파 다양성이 유의하게 증가되어 있었다. 또한 Butyricoccus, Prevotella, Aganthobacter 가 우세하였다.

CONCLUSIONS: 거대 결장 국소 신경절 세포 감소증은 만성 불응성 변비 및 재발성 복통 및 폐쇄성을 암시하는 팽만, 5cm 를 넘는 결장의 팽창, 명백한 구조적 원인 없이 CT 및/또는 대장 내시경에서 관찰된 국소 협착이 관찰되는 특징이 있는 질환으로 저신경절증이 동반되어 있다. 결장 절제술이 예후에 도움이 되며 장내 미생물의 분포도 대조군과 우세한 미생물 종류에 있어서 차이를 보인다.

Keywords: hypoganglionosis; megacolon; constipation; treatment outcome.